Journal of Plant Development Sciences

(An International Monthly Refereed Research Journal)

Volume 9 **Number 6** **June 2017**

Contents

RESEARCH ARTICLE

RESEARCH ARTICLE

VARIATION IN MORPHOMETRIC CHARACTER OF WILD POMEGRANATE (*PUNICA GRANATUM* L.) IN HIMACHAL PRADESH

Jyoti Dhiman, Tara Gupta and Thiyam Jefferson Singh*

Dr. Y. S. Parmar University of Horticulture and Forestry Nauni, Solan-173 230 (HP)
Email : lampardleo@gmail.com

Received-04.06.2017, Revised-15.06.2017

Abstract: The present study was aimed to document the morphometric variability in Wild Pomegranate (*Punica granatum* L.)" was carried out at two sites namely Tatool (S_1) and Narag (S_2) in the Department of Forest Genetic Resources, Dr. Y. S. Parmar University of Horticulture & Forestry Nauni, Solan (HP) during 2013-2014.. The phenological studies inclusive of phenophases, vegetative characters and reproductive characters observed earlier in Tatool (S_1) followed by the second site: Narag (S_2). Leaf bud swell was first observed in Tatool (S_1) with the begining of 2nd week of February followed by Narag (S_2) by the 4th week of February. Leaf bud burst was first observed in Tatool from 2nd week of March followed by Narag from 3rd week of March. Leafing first appeared from 4th week of March at Tatool and from 5th week of March at Narag. There was significant variation observed in leaf morphometeric characters within trees. Maximum values for leaf size and leaf area was observed for those leaves borne on lower position of trees in both the sites. Three different types of flowers were present namely hermaphrodite, male and intermediate flower. The reproductive bud appears on axillary and terminal position of the tree in cymose inflorescence. Anthesis of flowers was observed to take place between 10 am and 2pm. There were remarkable variation in different vegetative, reproductive, leaf morphometric and flower characteristics studied in the two sites which paved the way for further improvement programmme in wild pomegranate.

Keywords: *Punica granatum* L., Variation, Morphometric characters, Phenophases

INTRODUCTION

Wild Pomegranate (*Punica granatum* L.), vern. Daru is one of the oldest known edible wild fruits and is capable of growing in different agro-climatic conditions ranging from the tropical to sub-tropical land (Levin, 2006 and Jalikop, 2007). It is native to Turkey, Iran and also spread to the Himalayas in Northern India (Mars, 2000). Wild pomegranate with narrow petals, friable seeds, fruits resistant to cracking is found naturally in Northern India which is locally known as "Daru". In North India, Pomegranate flowers during spring season, but in Central and South India it flowers almost throughout the year. Flowering occurs about 1 month after bud break on newly developed branches of the same year, mostly on spurs or short branches. The flowers are borne mostly in clusters of two – three flowers, either terminally or auxiliary and inflorescence reported to be a cyme (Nath and Randhawa, 1959). The period of full bloom lasts about one month, and flowering and fruit set occurs in about 3 or 4 distinct waves. (El Sese, 1988). In Northern India, a major use of the wild fruits is for the preparation of "Anardana", the juice sacs (aril) being dried in the sun for 10–15 days and then sold as a condiment. The wild pomegranate generally cultivated through seeds which tends to create heterozygosity and variations, which makes the fruit selection a significant tool in pomegranate breeding programs (Jalikop and Kumar, 1998). So keeping in view the immense scope of improvement and breeding of wild pomegranate (Daru), on the basis of its reproductive peculiarities, fruit variations and also the socio-economic importance; it is imperative to take up the research work on the reproductive aspect.

MATERIAL AND METHOD

The study of morphometric variation in Wild Pomegranate (*Punica granatum* L.) was carried out in the Department of Tree Improvement and Genetic Resources, College of Forestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh during 2013-2014. Two sites were selected for study of phenological, morphological and breeding system of wild *Punica granatum* L. The sites selected were Tatool (S_1) district Solan and Narag (S_2) district Sirmour (Table-1). From each selected site, five medium sized tree were selected and marked. On each tree five branches were selected and marked with metallic tags and numbered from 1 to 5.

RESULT

Different tree phenotypic characters viz; height, crown spread and girth were observed for different trees selected at both the sites i.e. Tatool (S_1) and Narag (S_2). (Table 2).

Tatool (S_1) was found to initiate phenological events earlier than Narag (S_2). Leaf bud swell was first observed in Tatool (S_1) with the begining of 2nd week of February followed by Narag (S_2) by the 4th week of February. Leaf bud burst was first observed in Tatool from 2nd week of March followed by Narag from 3rd week of March. Leafing first appeared from 4th week of March at Tatool and from 5th week of March at Narag. Initiation of leaf fall recorded from

*Corresponding Author

4th week of October at Tatool and 5th week of October at Narag. Duration of number of days registered at Narag to all vegetative characters was greater than the duration for all those similar characters recorded at Tatool. Similarly, the duration between onset and completion of almost all the reproductive characters was found to be comparatively greater from Narag site (S_2) than Tatool site (S_1). Reproductive bud swell at Tatool (S_1) appeared from 3rd week of March, bud bursting from 3rd week of April and flowering initiation from 5th week of April. Average no. of days registered for flowering at Narag (45.82) was greater than recorded at Tatool (42.26) (Table 4). Maximum duration was recorded for fruit development among all reproductive characters under studied in Narag site. Fruit initiation was observed during 1st week of May at Tatool and 2nd week of May at Narag. Fruit ripening completed within 3 to 4 months after fruit initiation.

Variation in leaf morphological characters (Figure 1) was observed only with regards to the leaf shape and two types of leaf shape viz: lanceolate and elliptical lanceolate were found to exist. Leaf morphometeric traits studied within tree were of great significance as it showed great variability in leaf size within tree. Leaf size viz; leaf length (5.12 cm), leaf width (1.62 cm) and leaf area (5.05 cm) were observed maximum at lower portion of tree followed by upper portion and minimum were observed at middle portion of tree (Table 5, 6).

The floral bud during the period of their development were divisible into ten distinct stages and took 20 to 27 days to develop into a complete flower. Anthesis took place between 6 am to 6.00 pm, having shown the maximum anthesis between 12.00 noon to 2.00 pm at both the sites (Tale 7). Temperature also played an important role in opening of flowers at these sites.

The maximum anther dehiscence was observed during mid day hours i.e. 12 noon to 2 pm at both sites and the type of dehiscence was longitudinal (Table 8).

DISCUSSION

Tree phenotypic characters

Phenotypic characters are of first and foremost importance for species improvement. In the present study, the height of selected trees were found to vary from 4.0 m to 6.4 m, tree diameter range 9.39 cm to 17.80 cm and crown spread range observed was 3.90 cm to 7.62 cm (Table 2). These characters were taken as a criteria for selection of trees. These findings are consistent with the findings of Joshi and Joshi (2001), where height range of 5-10 m has been reported in this species from Kathmandu, Nepal.

Phenological characters

Phenology defined as the study of timing of recurring biological events, the cause of their timing with regards to biotic and abiotic forces and the inter relation among phases of same or different species (Lieth 1974). In the present investigations, such phenophases of the trees including the vegetative bud swell, bud burst, leafing and reproductive bud phases and flowering have been discussed below:

Vegetative Characters

The pattern of genetic variation among these phenological traits is large in forest tree species. Phenological traits are not inherited in a simple Mendalian mode, but rather exhibit an additive variation typical of quantitative traits (Tsarouhas, 2002). A close appraisal of the Table 3 clearly indicated that bud swell started to appear in 2nd week of February and continued till 1st week of March at Tatool (S_1), whereas at Narag (S_2) it continues from 4th week of February to 3rd week of March. The occurrence of all the vegetative parameters were observed earlier at Tatool site, followed by Narag site. The variations in these events can be ascribed owing to the difference in locality factors of the sites viz; Sothern (warmer) and western aspect of Tatool (S_1) and Narag (S_2) respectively. The present findings receive support from Gunaga Rajesh (2000) working on Teak, where early commencement of leaf flushing among southern and central provenance was shaped by early onset of monsoon in those provenance compared to northern provenances. The vegetative bud swell took 19.08 average number of days at Tatool, whereas 23.88 days at Narag. Followed by the mean duration of 12.76 days and 17.84 days for vegetative bud burst at Tatool and Narag respectively. Which in agreement with the findings of Wani (2005) on *Bauhinia variegate*, where the climate variables such as wind, rain, air, humidity, temperature and light intensity were found to be important determinants governing the time and the duration of different phenological events in life history of a species.

Reproductive Characters

Flowering initiation at Tatool (S_1) started from 5th week of April and continued till 2nd week of June followed by 1st week of May to 4th week of June at Narag (S_2) (Table 4). Similar observation was given by Pratap (1997) for blooming period of flowering in pomegranate. According to his examination it bloom between late spring and early summer (May – June). The flowering in wild pomegranate appearing from the middle of April till the end of May was also recorded (Rana *et al*. 2007), although two off season bloom of much less intensity were also observed during July and November. Flowering habit of pomegranate depends upon climatic conditions, it flowers almost all the year round but once in a year in the sub tropics (Stover and Mercure, 2007).

Fruit development initiated after two weeks of flowering and fruit ripening indices recorded during the 4th week of September i.e. nearly five months after flowering initiation and continued till the end of October at Tatool (S_1). The observed variations in

the reproductive phases at the two sites can be explained on the basis of varying temperature conditions of the sites as influenced by the locality factors mainly the aspects viz; Southern and Western aspect of Tatool and Narag respectively. These findings are parallel to the results represented by Morton (1987) for this species, where the fruit ripen was recorded about 6 to 7 months after flowering. Similarly Adsule and Patil (1995) also reported that fruit ripening between 135 to 170 days after anthesis in pomegranate. Fruits are harvested when the fruit rind colour turns slightly yellow and fruit gives a metallic sound when tapped.

Morphological characters

The morphological characters are most important to observe variations within and among different trees and the ways these are influenced by different environmental conditions.

Leaf Morphological Characters

In the present study the leaf morphological characters viz; leaf shape, leaf arrangement, leaf apex shape was observed. The leaves were observed in lanceolate shape, with acute apex and opposite - sub opposite type of arrangement (Table: 5). These results are in conformity with the studies carried out by Lama S D. (2001) and Joshi and Joshi, (2001) in this species in Nepal. They reported that pomegranate leaves are entire, lanceolate to broadly oblanceolate and elliptical lanceolate. Singh (2012) recorded leaf shape and leaf tip of pomegranate germplasm under Indian arid ecosystem in which leaf shape was observed to be acute, cuspidate, mucronate and obtuse.

Leaf Morphometeric Characters

The maximum leaf length, leaf breadth and leaf area was observed from the leaf samples collected from lower portion of the tree, followed by upper and middle portion. This revealed significant variation in leaf morphometeric characters within tree. Maximum leaf length recorded was 5.77 cm for lower position and shortest length observed was 4.24 cm (Table 6) from upper position, similar findings have been reported Pratap (1997) and Bista *et al.* (2001). Maximum leaf area also observed in lower position of tree i.e. 5.67 cm^2 followed by middle position of tree (4.49 cm^2). The parallel results to these finding was given by Wani *et al.* (2012). He reported leaf area of wild pomegranate genotypes ranged between 4.48 cm^2 to 14.04 cm^2. The variation recorded in leaf size at different position of tree i.e. within tree supported by Poething (1997). The findings express that plant produces different types of leaf during their development, the first few true leaves produced are usually smaller, simpler and anatomically different from leaves produced later in development. Present findings are also consistent with reports of Esau (1965) and Byrne *et al. (*2001) as they have explained the change in shape and size of successive leaves on a plant on the basis of physiological changes associated with increasing age of plant alongwith the interaction between shoot apical meristem and developing leaf primordial, under a variety of environmental factors. Verwijst and Wen (1996) found supporting results in *Salix*, they observed leaf length, leaf width ratio changed with leaf size and varied between different types of shoots. Ferris *et al.* (2001) and Taylor *et al.* (2001) supported result by concluding that elevated CO_2 promote individual leaf size.

Floral biology

Present study revealed that flower bud appeared on current year shoot in axil of leaves either in cluster or solitarily, in cymose inflorescence. Three types of flowers were observed *viz*; hermaphrodite, male and intermediate on the basis of pistil length in comparision to filament and stamen length. These results were supported by Lawrence (1951), Watson and Dallwitz (1992). They characterised pomegranate flowers in three types on the basis of the length of pistil and the flower shape. The vase shaped flowers were considered as hermaphrodite and bell shaped as male flowers. Similarly the criterion to project flower types have been also given by (Nath and Randhawa, 1959) while working on pomegranate cultivars and reported the presence of functionally unisexual male flowers. The hermaphrodite or perfect flowers have long style, protruding distinctly through staminal column.

Floral Bud Development

In general the period between initiation and flowering is correlated with growth habit of the tree which is in turn governed by climatic range of species (Sedgley and Griftin, 1989).

On the basis of shape, size and colour of the floral buds these were assorted into ten distinct stages (Plate 1). Similar observations have been reported by different workers while describing reproductive biology of various species viz; Nalawadi *et al.* (1973) grouped the flower bud development into ten stages in pomegranate, Parmar (1961) assembled *Grewia asiatica* into seven stages, Nath and Randhawa (1959) aggregated pomegranate into eight stages. Nalawadi (1973) and Josan (1979) reported the time required for completion of flower bud development in Indian cultivars is between 20 and 27 days.

Anthesis

The knowledge of Anthesis play most significant role in determining the time of pollination, breeding success, visiting rate of pollinating agents in any species. In the present study, maximum anthesis that took place between 12 noon to 2 pm (Table7), and rate of anthesis decreasing towards evening hours. These finding are supported by Nath and Randhawa (1959) and Josan *et al.* (1979) with their results of anthesis in pomegranate flowers under Delhi conditions. Almost similar timing had been observed in different tree species by various workers Chauhan *et al.* (2004) reported maximum anthesis between 11:30 am to 1:30 pm in *Dalbergia sisso*.

Srivastva (1983) also brought in light that in palash anthesis took place between 5 am to 6 pm. Balalia and Chauhan (1994) in *Delonix regia* recorded maximum flower open in between 6am to 7am. Josan *et al*. (1979) reported that time taken by the pomegranate flowers to complete anthesis was 3 to 5 hours.

Anther Dehiscence

The present investigation revealed that the anther dehiscence takes place in the longitudinal fashion. The maximum anther dehiscence was observed between 12 noon to 2 pm (Table 8) at both the sites. These results are in consistent with finding of Sareen and Vashisht (1982) in *Delonix regia* he reported anther dehisced by longitudinally slits and dehiscence occurring between 10.30 am to 11.30 am and 3.30 to 4.00 pm. These observation also supported by the studies conducted by Srivastava (1983) while working on *Butea monosperma* (Palash) reported that the anthers dehisced between 5.00 to 6.00 am.

Table 1. Two selected study sites for phenological, morphological, breeding system and pollination mechanism of wild *Punica granatum* L.

Sites	Aspect	Latitude	Longitude	Code
Tatool	Southern	30˚86´N	77˚14´N	**S_1**
Narag	Western	30˚87´N	77˚18´N	**S_2**

Table 2. Phenotypic characters of selected trees

Sites	Characters	Mean	Range	S E($\bar{X}$)	CV
Tatool (S_1)	**Tree Height (m)**	5.28	4.00 - 5.90	1.68	5.28
	Crown Spread (m) (N-S)	4.12	3.90 - 4.30	0.33	3.60
	Crown Spread (m) (E-W)	4.24	4.00 - 4.60	0.51	5.43
	Diameter (cm)	9.65	9.39 -11.43	1.27	1.87
Narag (S_2)	**Tree Height (m)**	6.14	5.90 - 6.40	0.46	3.38
	Crown Spread (m) (N-S)	5.89	4.60 - 7.13	2.47	18.76
	Crown Spread (m) (E-W)	6.02	4.30 - 7.62	3.38	25.10
	Diameter (cm)	13.96	9.62 - 17.80	25.32	25.44

Table 3. Temporal variation for vegetative characters in wild *Punica granatum* L.

Sites	Treatment No. (Trees)	Vegetative characters							
		Bud swell		Bud burst		Leafing		Leaf fall	
		Initiation	Completion	Initiation	Completion	Initiation	Completion	Initiation	Completion
Tatool (S_1)	**T_1**	2nd week of February	1st week of March	2nd week of March	3rd week of March	4th week of March	1st week of July	4th week of October	4th week of November
	T_2	3rd week of February	1st week of March	2nd week of March	4th week of March	4th week of March	1st week of July	4th week of October	4th week of November
	T_3	3rd week of February	1st week of March	2nd week of March	4th week of March	4th week of March	1st week of July	4th week of October	4th week of November
	T_4	3rd week of February	2nd week of March	2nd week of March	4th week of March	4th week of March	1st week of July	Last week of October	4th week of November
	T_5	3rd week of February	2nd week of March	2nd week of March	3rd week of March	4th week of March	1st week of July	Last week of October	Last week of November
Narag (S_2)	**T_1**	4th week of February	2nd week of March	3rd week of March	4th week of March	Last week of October	3rd week of July	Last week of October	1st week of December
	T_2	4th week of February	3rd week of March	3rd week of March	Last week of October	1st week of April	3rd week of July	1st week November	2nd week of December
	T_3	4th week of February	3rd week of March	4th week of March	1st week of April	1st week of April	3rd week of July	1st week November	2nd week of December
	T_4	4th week of February	3rd week of March	4th week of March	1st week of April	1st week of April	3rd week of July	1st week November	2nd week of December
	T_5	4th week of February	3rd week of March	4th week of March	1st week of April	2nd week of April	4th week of July	1st week November	2nd week of December

Table 4. Days taken to flowering and fruit initiation at different sites in wild *Punica granatum* L.

Sr No.	Treatment No. (Trees)	Sites			
		Flowering Duration (days)		Fruiting Duration (days)	
		Tatool (S_1)	Narag (S_2)	Tatool (S_1)	Narag (S_2)
1	**T_1**	41.80	44.00	121.20	127.80
2	**T_2**	39.50	45.40	121.60	128.20
3	**T_3**	43.80	46.50	122.80	131.80
4	**T_4**	45.60	47.80	123.00	134.20
5	**T_5**	40.60	45.40	122.20	134.30
Mean		**42.26**	**45.82**	**122.16**	**131.26**

$CD_{0.05}$		
Site	0.46	0.63
Tree within site$_1$	1.02	1.40
Tree within site$_2$	1.02	1.40

Table 5. Variability for Leaf length and leaf breadth

Position of branch	Leaf morphometeric characters					
	Leaf Length (cm)			Leaf Breadth(cm)		
	Tatool (S_1)	Narag (S_2)	Mean	Tatool (S_1)	Narag(S_2)	Mean
Upper	5.01	5.41	**5.21**	1.23	1.57	**1.4**
Middle	4.27	4.21	**4.24**	1.29	1.42	**1.36**
Lower	**5.82**	**5.72**	**5.77**	**1.73**	**1.86**	**1.79**
Mean	**5.03**	**5.12**		**1.42**	**1.62**	

CD site	0.06	0.06
Position	0.08	0.08

Table 6. Leaf morphometric characters i.e. Leaf area and petiole length

Position of branch	Leaf morphometeric characters					
	Leaf Area (cm^2)			Leaf Petiole Length (mm)		
	Narag	Tatool	Mean	Narag	Tatool	Mean
Upper	4.67	4.30	**4.49**	6.50	5.20	**5.90**
Middle	4.62	4.29	**4.46**	6.00	5.90	**5.90**
Lower	5.84	5.49	**5.67**	6.60	6.50	**6.50**
Mean	**5.05**	**4.70**		**6.30**	**5.90**	

CD site	0.01	NS
Position	0.01	NS

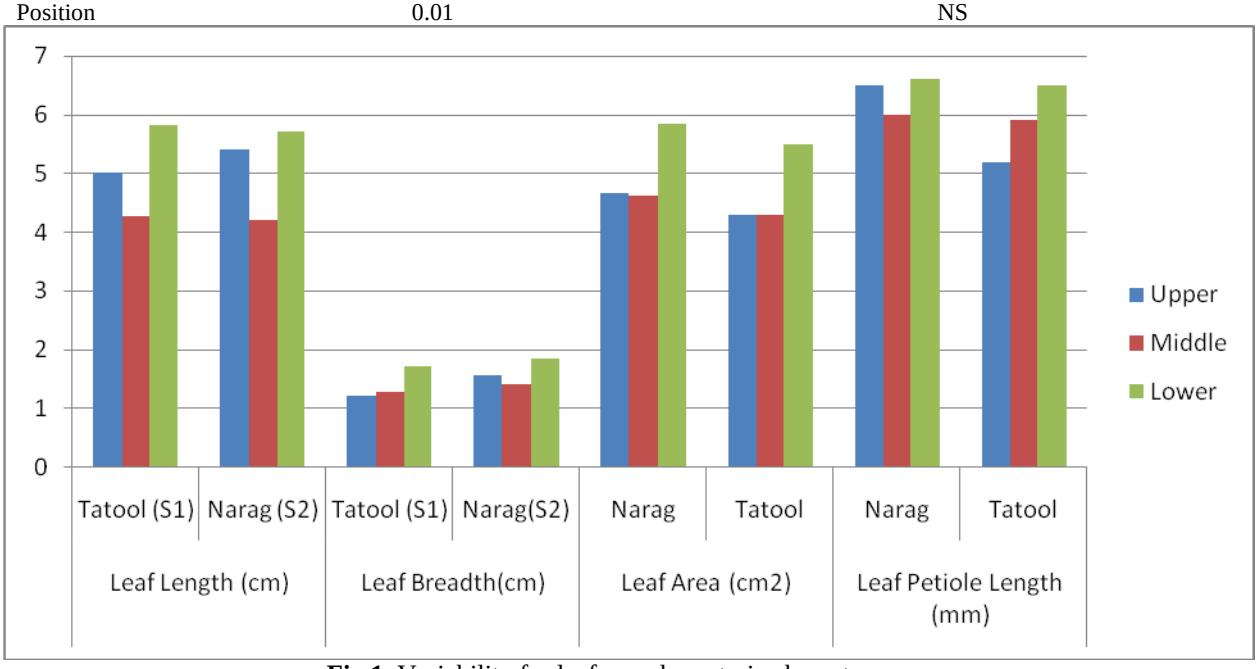


Fig 1. Variability for leaf morphometeric characters

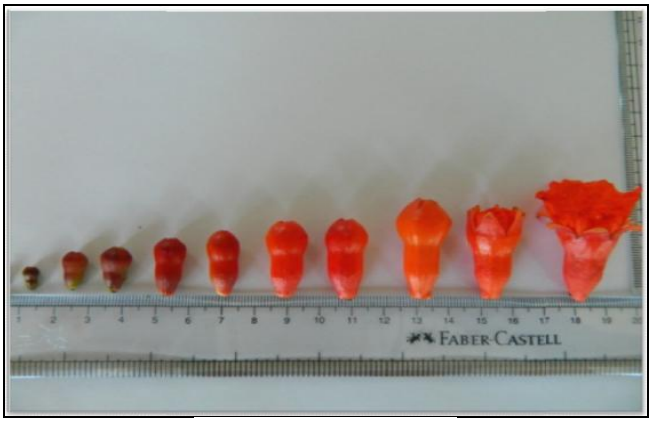
Table 7. Time of Anthesis in *Punica granatum* L.

Sites	Treatment No. (Trees)	Anthesis between different timing (%)						
		6PM to 6am	6am to 8am	8am to 10am	10am to 12 noon	12 noon to 2 pm	2 pm to 4 pm	4pm to 6 pm
Tatool (S_1)	T_1	0.00	7.10	14.30	30.32	33.95	13.14	1.19
	T_2	0.00	7.80	14.22	30.25	32.58	13.9	1.25
	T_3	0.00	7.50	14.20	30.18	32.73	14.00	1.39
	T_4	0.00	7.40	14.11	30.26	34.48	12.3	1.45
	T_5	0.00	7.90	14.24	30.12	32.60	13.92	1.22
Mean		**0.00**	**7.54**	**14.21**	**30.23**	**33.27**	**13.45**	**1.30**
Narag (S_2)	T_1	0.00	7.00	12.81	30.59	34.50	14.00	1.10
	T_2	0.00	6.90	13.98	31.30	34.24	14.20	1.22
	T_3	0.00	6.35	13.88	29.76	34.50	13.98	1.53
	T_4	0.00	6.28	13.90	31.60	34.98	12.11	1.13
	T_5	0.00	6.80	13.84	30.98	33.71	13.17	1.50
Mean		**0.00**	**6.67**	**13.68**	**30.85**	**34.39**	**13.49**	**1.30**

Table 8. Anther dehiscence at different timing in *Punica granatum* L.

Sites	Treatment no. (trees)	Per cent anther dehiscence at different timing						
		6pm to 6am	6am to 8am	8am to 10am	10am to 12 noon	12noon to 2 pm	2 pm to 4 pm	4pm to 6 pm
Tatool (S_1)	T_1	0.00	4.92	14.76	26.23	42.63	8.19	3.27
	T_2	0.00	4.56	14.52	26.54	42.83	8.23	3.22
	T_3	0.00	4.34	14.34	26.13	43.5	8.41	3.28
	T_4	0.00	4 .81	14.81	26.18	42.52	8.24	3.44
	T_5	0.00	4.9	14.67	26.17	42.33	8.09	3.84
Mean		**0.00**	**4.70**	**14.62**	**26.25**	**42.76**	**8.23**	**3.41**
Narag (S_2)	T_1	0.00	4.86	14.51	26.04	43.12	8.21	3.26
	T_2	0.00	4.12	14.46	26.56	43.18	8.36	3.32
	T_3	0.00	4.09	13.86	26.81	43.80	8.44	3.00
	T_4	0.00	4 .31	13.90	27.10	43.10	9.02	2.57
	T_5	0.00	4.29	14.37	26.99	42.42	9.32	2.61
Mean		**0.00**	**4.33**	**14.22**	**26.7**	**43.12**	**8.67**	**2.52**

A) Flower in bud stage



B) Floral bud stages

Plate1. Stage of flower growth development

REFERENCES

Lawrence, H M. (1951). Taxonomy of Vascular Plants. Macmillon and Company, New York.

Nath, N. and Randhawa, G.S. (1959). Studies on floral biology in the pomegranate (Punica granatum L.). Flowering habit, Flowering season, bud development and sex ratio in flowers. Indian Journal of Horticulture, 16(2) :61-68.

Esau, K. (1965). The leaf. In: Plant anatomy (Esau K., ed.). John Wiley and sons. New York, London, Sidney. 467-480.

Nalawadi, U.G., Farooqi, A.A., Dasappa, M.A., Reddy, N., Sulikeri, G.S. and Nalini, A.S. (1973). Studies on the floral biology of pomegranate(Punica granatum L.). Mysore Journal of Agricultural Sciences, 7: 213-225.

Lieth, H. (1974). Phenology and seasonality modeling. Springer-Verlag New York, 8: 473 p.

Levin, G.M. (1978). Pattern formation in ecological communities. Pages 433-466. In J. H. Steele, editor. Spatial pattern in plankton communities. Plenum, New York, USA.

Josan, J.S., Jawanda, J.S. and Uppal, D.K. (1979). Studies on floral biology of pomegranate: anthesis, dehiscence, pollen studies and receptivity of stigma. Punjab Journal of Horticulture, 19 (1 & 2): 66-70.

Sareen, T.S. and Vasisht, S. (1983). Breeding system of Delonix regia Raf. (syn. Poinciana regia Bojer). In: Improvement of forest Biomass. ed. Khosla P K , Pragati Press, Delhi, 33-40.

Srivastava, S.C. (1983). Floral biology of palas : Butea Monosperma (LAM.) Taub. Indian Journal of Forestry, 16(1): 42-46.

Morton, J. (1987). Pomegranate. In: Fruits of warm climates. eds. Julia F. Morton and Miami F L. 352-355.

El Sese, A.M. (1988). Physiological studies on flowering and fruiting habits of some pomegranate cultivars under assuit conditions. Assuit Journal of Agricultural Sciences, 19(4): 320-336.

Sedgley, M. and Griffin, A.R. (1989). Sexual reproduction of tree crops. Acadamic press New York. 378 .

Watson, L. and Dallwitz, M.J. (1992). Families of flowering plants : Descriptions, illustrations, identification and information retrieval, Available online:http://deltainteky.com/angiosperms/www/punicaceae .html.

Balalia, R. and Chauhan, S.V.S. (1994). Phenology and reproductive biology of some leguminous trees. Journal of Tree Sciences, 13(2): 121-123

Adsule, R.N. and Patil, N.B. (1995). Pomegranate. In: Salunkhe D K and Kadam S S (eds), Handbook of Fruit Science and Technology: Production, Composition, Storage and Processing. Marcel Dekker Inc., New York. 455 p.

Verwijst, T. and Wen, F.A. (1996). Leaf allometery of Salix viminalis during the first growing season. Tree physiology, 16:655-660.

Poething, R.S. (1997). Leaf morphogenesis in flowering plants. The plant cell, 9:1077-1087.

Pratap, U. (1997). Bee flora of Hindukush Himalayas. Inventory and Management. ICIMOD. KTM. Nepal. 297.

Jalikop, S.H. and Kumar, P.S. (1998). Use of a gene marker to study the mode of pollination in pomegranate (Punica granatum L.). Journal of Horticulture Science, 65:221-223.

Gunaga, R. and Surendran, T. (2000). Leaf morphological variation in teak (Tectona grandis SL.f.) clones. Evergreen, 48: 8-9.

Mars, M. (2000). Pomegranate plant material : Genetic resources and breeding, a review. Option Mediterraneennes Serie A, Seminaires Mediterraneens, 42: 55-62.

Bista, M.S., Adhikari, M.K. and Rajbhandari, K.R. (2001). Flowering plants of Nepal (Phanerogams). Bulletin of Plant Resources. (No. 18), Department of Plant Resources, National Herbarium and Plant Lab, Lalipur, Nepal. 399 pp.

Byrne, M., Timmermans, M., Kinder, C. and Martienssen, R. (2001). Development of leaf shape. Current Opinion in Plant Biology, 4: 38-43.

Ferris, R., Sabbati, M. and Taylor, G. (2001). Leaf area is stimulated in Populus by free air CO_2 enrichment, through increased cell expansion and production. Plant, cell and environment, 24: 305-315.

Joshi, K.K. and S.D. Joshi. (2001). Genetic heritage of medicinal and aromatic plants of Nepal Himalayas. Buddha Academic Publishers and Distributors Pvt. Ltd., Kathmandu, Nepal.

Lama, S.D. (2001). Genetic heritage of medicinal and aromatic plants of Nepal Himalayas. Buddha Academic Publishers and Distrbutors Pvt. Ltd., Kathmandu Nepal.

Taylor, G., Ceulemans, R. and Ferris, R. (2001). Increased leaf area expansion of hybrid poplar in elevated CO_2. From controlled environment to open top chambers and to face. Environmental Pollution, 115:463-472.

Tsarouhas, V. (2002). Genome mapping of quantitative trait loci in Salix with an emphasis on freezing resistance. Doctoral thesis. Swedish University of Agricultural Sciences Uppsala, Swedan.

Chauhan, R., Chauhan, S. and Khujaria, H.N. (2004). Reproductive biology and variability studies in Dalbergia sissoo Roxb. Advances in Forestry Research in India, 28:24-37.

Jalikop, S.H. (2007). Linked dominant alleles of inter – locus interaction results in a major shift in pomegranate fruit acidity of Ganesh and Kabul yellow, Euphytica, 158: 201-207.

Rana, J.C., Pradeep, K. and Verma, V.D. (2007). Naturally occurring wild relatives of temperate fruits of western Himalayan region of India: An analysis. Biodiversity Conservation, 16: 3963-3991.

Stover, E. and Mercure, E.W. (2007). Pomegranate: a new look at the fruit of paradise. Hortscience, 42:1088-1092.

Singh, N., Singh, S. and Meshram, D. (2012). Pomegranate: In vitro propagation and biohardening. Lambert Academic publishing, Saarbrucken, Germany, 160.

Wani, A., Bhat, M.Y., Lone, Abid, A., Bandy, F.A., Khan, I.A. and Ganai, A. (2012). Variation in some promising selection of wild pomegranate (Punica granatum L.) in Central Kashmir. Applied Biological Research, 14(2):211-214.

RESEARCH ARTICLE

DEVELOPMENT OF SUGARCANE PLASTID TRANSFORMATION SYSTEM USING PARTICLE BOMBARDMENT

Ravindra R. Kale[1,2]*, Pallavi Wadyalkar[1,3], Prashant G. Kawar[1,4], V.S. Ghole[1,5] and K. Harinath Babu[1*]

[1]*Vasantdada Sugar Institute, Manjari (Bk), Pune – 412307, Maharashtra, India*
[2]*Institute of Biotechnology, PJTSAU, Rajendranagar, Hyderabad, Telangana, India*
[3]*B1-402, Tirupathi Campus, Phase-2, Rd No.2, Tingrenagar, Vishrantiwadi, Pune-411015*
[4]*ICAR-DOFR, College of Agriculture Campus, Shivajinagar, Pune 411005 Maharahstra, India*
5National Institute of Virology, Pashan, Pune, Maharashtra, India
Email: khbabu_63@yahoo.com

Received-30.03.2017, Revised-01.05.2017

Abstract: Chloroplast transformation has number of advantages over nuclear transformation like high-level of transgene expression, transgene containment and lack of gene silencing. The present work carried out to develop a chloroplast transformation protocol for sugarcane. Embryogenic calli of sugarcane variety Co86032 used as target tissue for transformation. Chloroplast specific transformation vector pZE39 having *NPTII* and *GFP* genes flaked by *trnG* and *pzbZ* of chloroplast sequence used for transformation. Selection of transformants were carried out at callus, shoot and rooting stages with Geneticin ranging from 25 to 75 mg/l during different selection cycles. Most of the regenerated shoots turned albino during selection. Among different bombardment parameters tested, rupture discs pressure at 1350 psi, distance between target tissue and stopping screen at 8 cm and expose of target tissue to light for 8 days before bombardment found prominent in producing more number of green and resistant plants on selection medium. Molecular analysis revealed that out of 146 plants tested, 44 plants are found PCR positive. Four of eleven PCR poitive plants showed positive by Southern hybridization and five of ten plants are showed positive signals for GFP. This is the first report on an attempt to develop a chloroplast transformation protocol for sugarcane.

Keywords: Chloroplast transformation, Co86032, NPTII, GFP, Particle bombardment

INTRODUCTION

The modern sugarcane cultivars were developed from initial hybrids having chromosome number (2n) ranging between 100 and 130 as a result of hybridization and repeated clonal selection (Christy *et al*., 2009). Though classical plant breeding has been main approach for sugarcane development, various problems associated with sugarcane development are; complexity of the sugarcane genome, it's narrow genetic base, high polyploidy and heterozygocity, poor fertility and time required for a new variety to reach commercialization (Lima *et al*., 2002; Christy *et al*., 2009).Biotechnology offers an alternative and excellent opportunity for sugarcane crop improvement. But it also has certain constrains such as transgene inactivation, somaclonal variation, low transformation efficiency and long time required for regeneration and commercialization. Tissue culture forms a major part of biotechnological technique. The advantages of tissue culture methods are aid in the mutation, propagation and breeding study, production of virus free plants of sugarcane (Nickell 1967 and Leu 1972).

Different transformation techniques like Electroporation (Rathus and Birch 1992), Polyethyleneglycol treatment (Chen *et al*., 1987), Particle bombardment (Franks and Birch 1991), and *Agrobacterium* mediated transformation (Arencibia *et al*., 1998) were used for sugarcane transformation. Transformation of sugarcane for various traits like resistance to lesser cornstalk bore (Nutt *et al*., 1999), increased total sucrose concentration (Wu and Birch 2007), transformation with Cry1Aa3 for resistanct to early shoot borer (Kalumke et al, 2009), *cry1Ab* and aprotinin genes for resistant to early shoot borer (Arvinth *et al*., 2010) and salt tolerance (Rani *et al*., 2012) has been carried out. The most regular method of transformation used is nuclear transformation have various concerns like low expression levels, inconsistent expression profile among transgenic population and transgene flow (Aziz *et al*., 2005) Plastid or chloroplast transformation is an alternative method to overcome problems related with nuclear transformation. Chloroplast in plants also known as plastid, carry out photosynthesis as well as other important activities like evolution of oxygen, amino acid and fatty acid synthesis, sequestration of carbon, and starch production (Wani *et al*., 2010). As plastid transformation has a number of merits over nuclear transformation like high-level transgene expression, multi-gene engineering, transgene containment and lack of gene silencing and position effect, (Lee *et al*., 2006) this approach effectively used for genetic improvement of various plants. Plastid transformation of different crops like carrot, alfalfa, Brassicacea, cotton, soybean, tobacco, rice, tomato, egg plant, cabbage have been carried out for various agronomic traits and also to produce therapeutic proteins (Cosa *et al*., 2001; Skarjinskaia *et al*., 2003;

*Corresponding Author

Dufourmantel *et al*., 2004; Wurbs *et al*., 2007; Singh *et al*., and Wei *et al*., 2011). Many pharmaceutical proteins like next-generation antibiotics, antibody fragments, blood coagulation factors also expressed in plastid successfully (Oey *et al.*, 2009; Verma *et al.*, 2010; Gisby *et al.*, 2011 and Lentz et *al.*, 2012). Khan and Maliga developed a marker system for chloroplast transformation to differentiate between individual transformed and wild type plastids which facilitate the extension of plastid transformation to non-green plastids in embryogenic cells of cereal crops. There are no published reports on genetic engineering system for sugarcane chloroplast.

In present work we have tried to develop a chloroplast transformation system for sugarcane using a chloroplast specific transformation vector pZE39 that targets gene at *trnG* and *psbZ* region of chloroplast genome. The confirmation of transgene was done by PCR, Southern blot hybridization and GFP analysis. This system will be useful in transfer of gene in sugarcane for important traits like stress resistance, valuable products production and can be use effectively in developing elite variety of sugarcane.

MATERIAL AND METHOD

Plant materials

Sugarcane variety Co86032, a commercially released and popular in state of Maharashtra, India, was selected for chloroplast transformation. Leaf segments of 6-8 cm below the apical dome was selected from 6-8 months old field grown sugarcane. These segments were sterilized with ethanol (70%) for 1-2 min and then with $HgCl_2$ (0.1%) for 10 min followed by thrice washing with sterile distilled water. Outer layers were removed aseptically and leaf segments of ~2 mm thick were inoculated on MS salts and vitamins medium (Murashige and Skoog 1962) supplemented with 3 mg/l 2, 4-D, 0.5 mg/l Kinetin, 500 mg/l Proline and 3 % Maltose (3MES) for callus induction and proliferation. Cultures were incubated in dark at 26 $\pm$ 2 ^{o}C. Embryogenic calli developed after 6-8 weeks were taken for transformation experiments.

Transformation vector

The plasmid transformation vector pZE39 was constructed by placing the expression cassettes containing the green fluorescent protein gene (*GFP*) and the neomycin phosphotransferase II gene (*NPTII*) (Fig.1). This cassette is flanked by trnG and psbZ sequences of sugarcane chloroplast genome and kindly provided by Dr. Ralph Bock, Max Planck Institute, Germany, used in transformation experiments. This vector consists of *gfp* as the reporter gene derived by *CrPrrn-G10L* promoter followed by terminator *Cr.3atpA* and the selectable marker gene *nptII* with *Nt.PrrnG10-L* promoter and *Nt.TrbcL* terminator. The entire vector pZE39 transfrred into *E. coli* (DH5α) for isolation of plasmid DNA to use in transformation experiments.

Gene coating and Plastid transformation parameters

About 10 µl plasmid DNA (1 µg/µl) of pZE39 was mixed with 50 µl gold suspension. While agitating this suspension on vortex mixer, 50 µl of $CaCl_2$ (0.25 mM) followed by 20 µl of Spermidin (100 mM) were added and vigourously agetated the suspension for 30 min at 4 ^{o}C in cold room. After 30 min incubation, suspension was centrifuged for few seconds and discarded the supernatant. The pellet was washed thrice with 100% ethanol and finally dissolved in 36 µl of absolute alcohol that served for 6 bombardments.

Embryogenic calli of Co86032 was used as target tissue for bombardment placed on osmotic medium, 3MES supplemented with 0.2 M each of sorbitol and mannitol for osmotic treatment to the callus for 4 h prior to bombardment. Embryogenic calli of 2-3 mm diameter were arranged in a circle of 3 cm diameter in 9 cm petri dishes on osmotic medium [Fig 2]. These calli were bombarded with 0.6 mm diameter gold particles coated with plasmid DNA of chloroplast specific transformation vector pZE39. Bombardment was carried out using the PDS 1000/He biolistic system (Bio-Rad, Richmond, USA). Different parameters like rupture disc pressures of 1100, 1350 and 1550 psi, distance between rupture disc and target tissue for bombardment at 6 and 8 cm and expose of calli to light before taking for bombardment for 3, 4, 5, and 8 days were tested.

Selection and regeneration of transformants

The bombarded calli were incubated in dark on the same medium for overnight and then next day transferred to 3MES for next 5 days to recover from bombardment shock. After 5 days, they were transferred to 3MES with geneticin 25 mg/l for selection of transformed cels. These calli were subcultured at 15 days intervals each for 3-4 times and freshly grown geneticin resistance calli were transferred to 3MES medium with higher concentration of geneticin (50 mg/l) for 2-3 rounds selection. Geneticin resistant actively proliferating calli transferred to regeneration medium, basal MS medium supplemented with kinetin (0.5 mg/l), NAA (0.5 mg/l), geneticin (50 mg/l) [SRM] and incubated at 26 $\pm$ 2 °C with 16 h light and 8 h dark photoperiodism for 3 cycles of 21 days interval. The green shoots at height of 7-10 cm were excised and transferred to rooting medium (MS + 0.5 mg/l NAA + 40 mg/l geneticin). Juvenile rooted plants that were survived on selection medium were transferred to soil pots containing sterilized mixture of soil, sand manure (1:1:1) and grown in greenhouse for further growth of the plant.

Confirmation of transformants

PCR analysis

Sugarcane DNA was extracted from the isolated chloroplasts of both the transformed and untransformed plants for PCR analysis as per method described by Aljanabi (1999). PCR amplification was done using gfp specific primers; forward: 5'- CGT AAGGGGAAGG GGAAAAC-3' and reverse: 5'- CCATGTGTA ATCCCAGCAGC- 3' to obtain 886 bp PCR amplicon. PCR conditions were worked out using following conditions for *gfp* gene primers for pZE39 vector. One cycle of initial denaturation at 94 °C for 5 min followed by 35 cycles: 94 °C for 30 sec, 56 °C for 30 sec, and 72 °C for 45 sec, and final extension at 72 °C for 10 min. Finally the reaction was held at 4 °C till reaction terminates. PCR product was run on 1% agarose gel for observation of bands.

Southern blot hybridization analysis

Genomic and chloroplast DNA of the PCR positive putative transformants was extracted to perform Southern blot hybridization for identification of integratin and copy number of the transgene. Ten µg of DNA was digested with restriction enzyme XhoI at 1.5 U/µg DNA concentration (37 °C, overnight) and applied to 0.8% (w/v) agarose gel and electophoresed at 15 V for 12 h. The separated DNA was transferred to positively-charged Nylon Membrane (PALL,USA) overnight and cross-linked with UV at 1200 jouls for 90s. GFP gene specific PCR product of size 886 bp used as probe labeled with (^{32}P)-dCTP (BRIT, India) using Random primer DNA labeling kit and used for hybridization. The DNA-fixed membrane was hybridized using ULTRAHyb Hybridization Buffer (Ambion, USA) at 42 °C for 16 h with the probe in a hybridization oven. Blots were washed and exposed to X-ray film (Fuji X-ray film Super HR-U, 16.5 cm x 21.6 cm). These X-ray film cassettes were incubated at -70 °C for 48 h to develop autoradiogram. These exposed X-ray films were developed and photographed for recording the bands.

GFP expression detection

Confocal microscopy was used to determine the expression of GFP protein in chloroplast of transplastomic plants. Subcellular localization of GFP was verified by laser scanning confocal microscopy (PALM Robo Axio Observer D1, Zeiss make) at IISER, Pune. The system utilizes excitation at 395 nm, and emission at 509 nm. The sterile transgenic leaves of different lines were collected and a transverse section mounted on slide. In this study, the strong signal of green fluorescence emission from transformed cells was observed.

RESULT

Development of embryogenic calli and optimization of antibiotic concentration for selection of transformants

MS medium containing 2,4-Dichloro phenoxyacetic acid (3 mg/l), maltose (3%), kinetin (2 mg/l), proline (500 mg/l) induced about 80% of embryogenic callus (data not shown). Embryogenic calli for transformation experiment developed on media as described Wadyalkar et al (2011). Antibiotic sensitivity test for Geneticin on embryogenic calli was performed. Geneticin 25 mg/l showed that most of the callus was fresh and very little browning was observed. Browning was started increasing from 50 mg/l to 75 mg/l and complete browning was found in 100 mg/l. Hence, geneticin at the concentration of 50 mg/l where it inhibited 60-70% of the callus growth was selected for transformantion.

Before hardening stage, selected plants were transferred to lower dose of selection (40 mg/l) for gradual removal of antibiotic pressure for further acclimatization of regenerated plants. Shoots with 3-4 cm height were excised and successfully rooted on MS media supplemented with 0.5 mg/l NAA with selection agent geneticin at 40 mg/l concentration. The survival percentage of regenerated plantlets on rooting medium was 30%, after transfer to soil pots was 27% where as survival rate of plants in green house was 40%.

Optimization of particle bombardment for chloroplast transformation

Embryogenic calli developed from leaf roll discs was used in transformation experiments. Effect of light on callus prior to transformation was studied. Embryogenic calli was exposed to fluorescent cool light for 3, 4, 5, and 8 days before taking for bombardment. The embryogenic calli exposed to light for different days was arranged on osmotic medium in plats were bombarded with *gfp* & *nptII* gene construct. Different bombardment parameters tested are distance between rupture disc and target tissue and pressure of rupture discs. After bombardment, bombarded calli was transferred on osmotic free medium and incubated for 5 days to recover from bombardment shock. Bombarded calli were exposed to selection of transformants for 3-4 rounds of selection pressure. Each selection round of three weeks on callus proliferation medium with 50 mg/l Geneticin. Selection of transformed calli was followed by regeneration of shoots and roots with selection. A total of 1476 plants were regenerated after bombardment on continuous selection pressure. However, most of the plants (1027) that were regenerated were turned into albinos.

In the present experiments, effect of light on callus prior to transformation was studied. Embryogenic calli was exposed to fluorescent cool light at various days were 3, 4, 5, 7 and 8 days. Callus exposure to light might have advantage in converting proplast to chloroplast hence more number of green plants (49) were regenerated at 8 days light incubation as compare to 3 days (7 plants).

After transformation for recovery, calli transformed to medium without selection for 7 days followed by further transfer of calli on selection medium having geneticin at concentration 25 mg/l with further

selection at increasing concentration to 50 mg/l and 75 mg/l in subsequent selection cycles.

In the present investigation, rupture disc pressure at 1350 psi was found more beneficial as compare to 1100 and 1550 psi. Around 50 plants were regenerated under continuous selection at this pressure. It appears that 1100 psi is less effective in transferring the particles and 1550 psi is high pressure that might damage the tissue. Distance between stopping screen (SS) to target tissue (TT) also has correlation. Lower the pressure (1100 psi) and shorter the distance (6 cm) yielded more number of green plants (5) as compared to 8 cm (3 plants). This pressure might not be enough to penetrate the particles. However, at the higher pressure (1350) shorter the distance has yielded less number of green plants (4) as compared to longer distance of 8 cm (49 plants). Higher pressure and shorter the distance might have deleterious effect on the cells.

Confirmation of transgene integration and GFP detection

Integration of transgenes confirmed with the help of gene specific PCR producing 926 bp for *nptII* and 886 bp for *gfp* products (Fig. 3 A and B). Out of total 146 plants tested, 44 plants are found PCR positive. Southern blot hybridization analysis was performed using total genomic DNA isolated from transformed and untransformed sugarcane plants. Digestion of total DNA carried with XhoI restriction enzyme (Fig. 3 C). DNA digested by XhoI was analyzed by Southern blot using an 886 bp probe resulting in a hybridization signals of size 1.5 kb for four transplastomic lines indicating integration of gene (Fig. 3 D). Four out of eleven PCR confirmed plants showed positive signals for Southern blot hybridization. Plastid based GFP accumulation was analysed in five out of ten lines after observation under confocal laser scanning microscopy. A distinct plastid localized-GFP fluorescence observed in leaf section under confocal laser scanning microscope (Fig. 4).

In the present study, chloroplast specific transformation vector pZE39 which contain as cassette with *NPTII* and *GFP* gene flanked by trnG and psbZ chloroplast specific sequences was used. After several round of selection well survived plants processed for further and transfer to green house. DNA isolated from plants growing in green house for analyses by PCR using gene specific primers for GFP. After PCR analysis, 13 out of 35 clones showed PCR amplification. The eleven PCR positive plants were subjected to further confirmation by Southern hybridization. A GFP specific probe of 886 bp labeled with ^{32}P used in hybridization process. Four out of eleven plants showed signals for southern hybridization. While in gfp protein detection study 5 out of ten PCR positive plants showed presence of GFP signals in plants.

DISCUSSION

The present work carried out in terms to develop a suitable chloroplast transformation protocol for sugarcane. This is the first report on chloroplast transformation in sugarcane. The optimization of antibiotic concentration for transformants selection has been carried out for geneticin antibiotic. Among the various concentrations tested 50 mg/l geneticin found effective at initial stage of selection at calli level. While it is increased further upto 75 mg/l for selection of regenerated plants. The geneticin concentration at 45 mg/l for transformants selection has been used (Bower and Birch, 1992).

Transformation efficiency observed low with other chloroplast transformation vector with flaking sequences from tobacco or Arabidopsis used for potato, tomato and *Lesquerella* (Sidorov *et al*., 1992; Skarjinskaia *et al*., 2003 and Ruf *et al*., 2007). The vector used in current research work contains flanking sequence trnG and psbZ from sugarcane genome itself, which helps to reduced chance of low transformation efficiency. It was reported that in carrot transformation use of carrot chloroplast species-specific vector showed higher transformation efficiency (Kumar *et al*., 2004). In case of solanaceous crop chloroplast transformation hinder by use of non-green tissue (Bogorad, 2000). There are number of proplastid structure present in non-green tissues which have different gene regulation mechanism than mature chloroplast. Upon transformation transformed proplastid has to develop into mature chloroplasts and transformants has to survive on selection.

In our study effect of light on callus prior to transformation was also studied. Embryogenic calli was exposed to fluorescent cool light at various days were 3, 4, 5, 7 and 8 days. Callus exposure to light might have advantage in converting proplast to chloroplast hence more number of green plants (49) were regenerated at 8 days light incubation as compare to 3 days (7 plants).

However it was reported that development of dark-grown tobacco suspension cell model can be used to investigate the transformation potential of undeveloped plastids (Langbecker *et al*.,2004). Results indicate that the rate-limiting steps for nuclear and plastid transformation are different and must be optimized separately and it also indicated that plastid size, subcellular localization and developmental stage are apparently not the rate limiting factors for successful and efficient plastid transformation.

After transformation for recovery, calli transformed to medium without selection for 7 days followed by further transfer of calli on selection medium having geneticin at concentration 25 mg/l with further selection at increasing concentration to 50 mg/l and 75 mg/l in subsequent selection cycles. Callus proliferation for four days after bombardment

followed by 4-6 subculture of 2 weeks on selection medium produce more number of transformed plants. Callus proliferation for four days after bombardment followed by 4-6 subculture of 2 weeks on selection medium produce more number of transformed plants (Bower and Birch, 1992).

Application of chloroplast transformation system in crop like sugarcane would be a most advantages in its development programme. With using parameters mentioned, sugarcane transformation for particular trait can be carried out in future. With lot of advancement in chloroplast transformation technique it is still need to develop a stable and more effective system for agronomically important crops.

ACKNOWLEDGEMENT

We are grateful to the Department of Biotechnology (GOI), New Delhi for providing financial assistance to this work through project (No.BT/PR13389/NDB/53/28/2009) and to Dr. Ralph Bock, Max Planck Institute, Germany for providing chloroplast transformation vector.

Table 1. Details of bombardments using different parameters for embryogenic calli and regenerating tiny shoot buds

Days light incubation (Prior to bombardment)	Rupture disc pressure (psi)					
	1100		1350		1550	
	Distance between Stopping Screen to Target Tissue (cm)					
	6	8	6	8	6	8
3	22	17	54	64	6	6
4	NT	60	NT	107	NT	NT
5	6	6	37	37	6	6
7	6	6	21	21	5	5
8	18	26	10	122	NT	NT

NT= Not tested

Table 2. Number of plants regenerated using different parameters from embryogenic calli and regenerating tiny shoot buds

Days light incubation (Prior to bombardment)	Rupture disc pressure (psi)					
	1100		1350		1550	
	Distance between Stopping Screen to Target Tissue (cm)					
	6	8	6	8	6	8
3	0	0	4	3	0	0
4	NT	0	NT	0	NT	NT
5	0	0	0	0	1	0
7	0	0	0	8	0	0
8	5	3	0	35	NT	NT

NT= Not tested

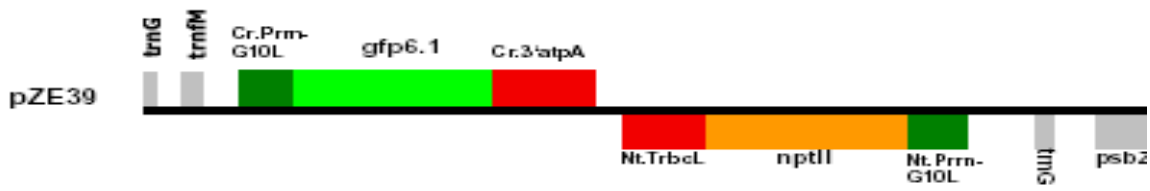


Fig.1

Fig. 1 Physical map of chloroplast specific transformation vector pZE39

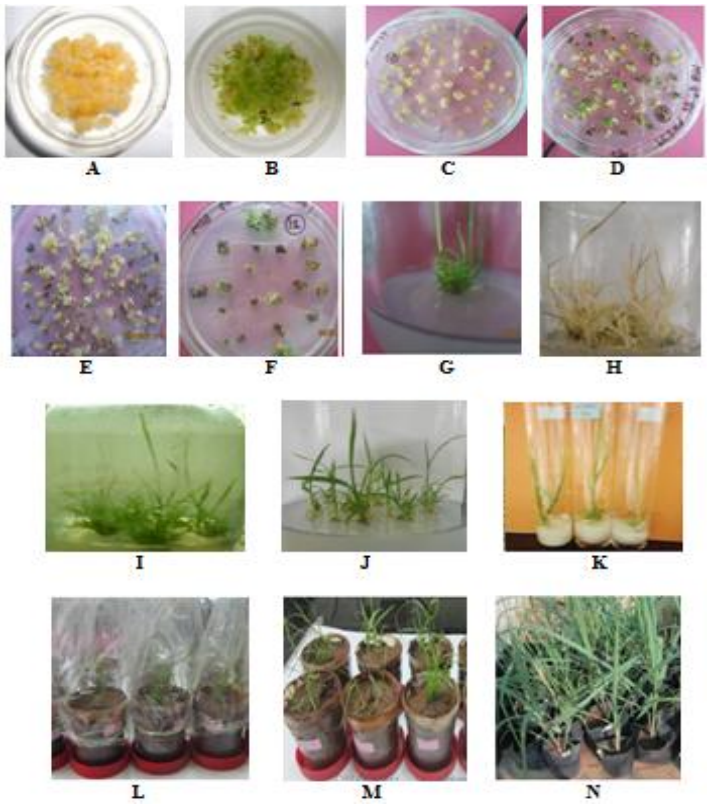


Fig. 2

Fig. 2 Stages of selection of chloroplast transformed materials from callus to the regenerated plantlets & hardening of putative transformed plants. **A** & B –Callus and tiny regenerating shoot buds on osmotic medium used for bombardment**,** C & D - Bombarded callus and tiny regenerating shoot buds on osmotic free medium for one week**,** E - Bombarded callus on 3 cycles of selection (50mg/l geneticin)**,** F - Tiny regenerating shoot buds on 2 cycles of selection (50mg/l geneticin)**,** G –Regeneration and plantlets growth on $4^{th}/3^{rd}$ selection cycles (75 mg/l geneticin), H – Albino plants produced during selection. I- Resistant plants on shooting media, J- Resistant plants separated from bunch and put on solid rooting medium, K- Resistance plants separated from bunch and put on liquid rooting medium L-Resistance plants survived on selection medium transferred to soil pots covered with polypropylene bags, M- Plants in soil pots for hardening, N- Hardened antibiotic resistance plants in green house.

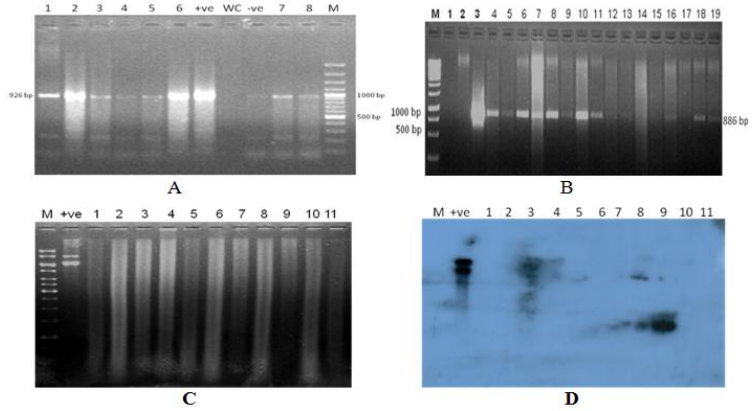


Fig. 3

Fig. 3 PCR and Southern-blot analysis of putative transplastomic plants. A- PCR using *Npt*II gene specific primers 1 to 8 are plant numbers; +ve = positive control plasmid DNA; M- ladder 100bp; WC-Water Control, -ve = control plant DNA, B- PCR analysis using *gfp* gene specific primers. M= 1 kb ladder, 1= water control, 2= untransformed plant DNA, 3= plasmid pZE39, and 4 to 19 = chloroplast transformed plants DNA, C- transgenic plant DNA digested with XhoI, D- Southern blot hybridized with GFP probe, M- 1 kb marker, +ve- Plasmid as positive control, 1 to 11- PCR positive transgenic plants derived after repetitive subculture in liquid medium under Genetcin selection.

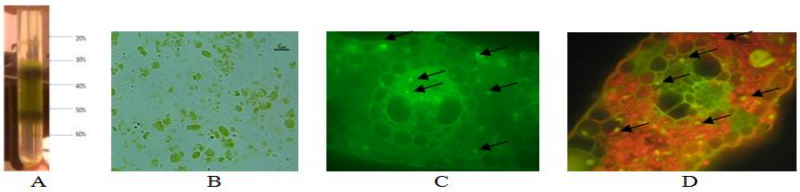


Fig. 4

Fig. 4. Chloroplast isolation and localization of GFP protein in leaf sections. A- speprated chloroplast layer in sucrose gradient (20-60%), B- Isoalted chloroplast under microscope (400X). was observed from transplastomic line. C- The emission of green fluorescence excited by a 395 nm laser, D- The merged image is of chlorophyll and GFP fluorescence (marked with arrows).

RERFERENCES

Aljanabi, S.M., Forget, L. and Dookun, A. (1999). An improved and rapid protocol for the isolation of polysaccharide- and polyphenol-free sugarcane DNA. *Plant Molecular Biology Reports,*17 : 1-8.

Arencibia, A., Carmon, E., Tellez, P., Chan, M. T., Yu, S.M., Trujillo, L. and Oramas, P. (1998). An efficient protocol for sugarcane (*Saccharum* spp.) transformation mediated by *Agrobacterium* tumefaciens. *Transgenic research,* 7 : 213-222.

Arvinth, S., Arun, S., Slevakesavan, R.K., Srikanth, J., Mukunthan, N., Kumar, P.A. (2010). Premachandran M N and Subramonian N, Genetic transformation and pyramiding of aprotinin-expressing sugarcane with cry1Ab for shoot borer (*Chilo infuscatellus*) resistance, *Plant Cell Reports,* 29 : 383-395.

Aziz, A.M., Sikriwal, D., Singh, S., Jarugula, S., Kumar, P.A. and Bhatnagar, R. (2005). Trasnformation of an edible crop with the *pag*A gene of *Bacillus anthracis, The FASEB Journal,* 19 : 1501-1503.

Bock, R. (2014) . Genetic engineering of the chloroplast: novel tools and new applications, *Current Opinion in Biotechnology,* 26 : 7-13.

Bower, R. and Birch, R.G. (1992). Transgenic sugarcane plants via microprojectile bombardment, *Plant Journal,* 2 : 409-416.

Chen, W.H., Gartland, K.M.A., Davey, M.R., Sotak, R., Gartland, J.S., Mulligan, B.J., Power, J.B. and Cocking, E.C. (1987). Transformation of sugarcane protoplasts by direct uptake of a selectable chimeric gene, *Plant Cell Reports,* 6 : 297-301.

Christy, L.A., Arvinth, S., Saravanakumar, M., Kanchana, M., Mukunthan, N., Srikanth, J., Thomas, G., Subramonian, N. (2009). Engineering sugarcane cultivars with bovine pancreatic Trypsin inhibitor (aprotinin) gene for protection against top borer (*Scirpophaga excerptalis* Walker), *Plant Cell Reports,* 28 : 175-184.

Cosa, B.D., Moar, W., Lee, S., Miller, M. and Daniell, H. (2001). Overexpression of the Bt cry2Aa2 operon in chloroplast leads to formation of insecticidal crystals, *Nature Biotechnology,* 19 : 71-74.

Dufourmantel, N., Pelissier, B., Garcon, F., Peltier, G., Ferullo, J.M. and Tissot, G. (2004). Generation of fertile transplastomic soybean, *Plant Molecular Biology,* 55 : 479-489.

Franks, T. and Birch, R.G. (1991). Gene transfer into intact sugarcane cells using microprojetile bombardment, *Australian Journal of Plant Physiol,* 18 : 471-480.

Gisby, M.F., Mellors, P., Madesis, P., Ellin, M., Laverty, H., O'kane, S., Ferguson, M.W.J. and Day, A. (2011). A synthetic gene increases TGFβ3 accumulation by 75-fold in tobacco chloroplast enabling rapid purification and folding into a biologically active molecule, *Plant Biotechnology Journal,* 9 : 618-628.

Kalunke, R.M., Kolge, A.M., Babu, K.H. and Prasad, D.T. (2009). *Agrobacterium* mediated transformation of sugarcane for borer resistance using cry 1Aa3 gene and one-step regeneration of transgenic plants, *Sugar Tech,* 11 : 355-359.

Khan, M.S. and Maliga, P. (1999). Fluorescent antibiotic resistance marker for tracking plastid transformation in higher plants, *Nature Biotechnlogy,* 17 : 910-915.

Kumar, S., Dhingra, A. and Daniell, H. (2004). Stable transformation of the cotton plastid genome and maternal inheritance of transgenes, *Plant Molecular biology,* 56 : 203-216.

Langbecker, C.L., Ye, G., Broyles, D.L., Duggan, L.L., Xu, C.W., Hajdukiewicz, P.T.J., Armstrong, C.L. and Staub, J.M. (2004). High-Frequency Transformation of Undeveloped Plastids in Tobacco Suspension Cells, *Plant Physiology,* 135 : 39-46.

Lee, S.M., Kang, K., Chung, H., Yoo, S.H., Xu, X.M., Lee, S.B., Cheong, J.J., Daniell, H. and Kim, M. (2006). Plastid Transformation in the Monocotyledonous Cereal Crop, Rice (*Orzya sativa*) and Transmission of Transgenes to their progeny, *Molecular Cells,* 21(3) :401-410.

Lentz, E.M., Garaicoechea, L., Alfano, E.F., Parreno, V., Wigdorovitz, A. and Bravo-Almonacid, E.F. (2012). Translation fusion and redirection to thylakoid lumen as strategies to improve the accumulation of a camelid antibody fragment in transplastomic tobacco, *Planta,* 236 : 703-714.

Leu, L.S. (1972). Freeing Sugarcane from mosaic virus by apical meristem culture and tissue culture, *Report of Tiwan Sugar Experimental Station,* 57 : 57-63.

Lima, M.L., Garcia, A.A., Oliveria, K.M., Matsuoka, S., Arizono, H., Jr deSouza, C.L. and De Souza, A.P. (2002). Analysis of genetic similarity detected by AFLP and coefficient of parentage among genotypes of sugarcane (*Saccharum* spp.), *Theorotical and Applied Genetics,* 104 : 30-38.

Liu, C.W., Lin, C.C., Chen, J.J. and Tseng, M.J. (2007). Stable chloroplast transformation in cabbage (*Brassica oleracea* L.var. capitata L.) by particle bombardment, *Plant Cell Reports,* 26 : 1733-1744.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiologia Plantarum,* 15 : 473-497.

Nickell, L.G. (1967). Tissue and cell culture for sugarcane research, *Proc.ISSCT* 12 : 887-892.

Nutt, K.A., Allsopp, P.G., Mcghie, T.K., Shepherd, K.M., Joyce, P.A., Taylor, G.O., Mcqualter, R.B. and Smith, G.R. (1999). Transgenic Sugarcane with increased resistance to canegrubs, *Proceedings of Australian Society of Sugar Cane Technology,* 21 : 171-176.

Oey, M., Lohse, M., Scharff, L.B., Kreikemeyer, B. and Bock, R. (2009). Plastid production of protein antibiotics against pneumonia via a new strategy for high-level expression of antimicrobial proteins, *Proceedings of National Academy of Science USA,* 106 : 6579-6584.

Rani, K., Sandhu, S.K. and Gosal, S.S. (2012). Genetic augmentation of sugarcane through direct gene transformation with Osgly II gene constructs. *Sugar Tech* 14 : 229-236.

Rathus, C. and Birch, R.G. (1999). Stable transformation of callus from electroporated sugarcane protoplasts, *Plant Science,* 82 : 81-89.

Ruf, S., Karcher, D. and Bock, R. (2007). Determining the transgene containment level provided by chloroplast transformation, *PNAS,* 104 (17) : 6998-7002.

Sidorov, V.A., Kasten, D., Pang, S.Z., Hajdukiewicz, P.T.J., Staub, J.M. and Nehra, N.S. (1999). Technical advance: stable chloroplast transformation in potato: use of green fluorescent protein as a plastid as a plastid marker, *Plant Journal,* 19 : 209-216.

Singh, A.K., Verma, S.S. and Bansal, K.C. (2010). Plastid ttrasnformation in egg plant (*Solanium melongena* L.), *Trasngenic Research,* 19 : 113-119.

Skarjinskaia, M., Svab, Z. and Maliga, P. (2003). Plastid transformation in *Lesquerella fendleri,* an oilseed Brassicacea, *Transgenic Research,* 12 : 115-122.

Verma, D. and Daniell, H. (2007). Chloroplast vector systems for biotechnology applications, *Plant Physiology,* 145 : 1129-1143.

Verma, D., Moghimi, B., LoDuca, P.A., Singh, H.D., Hoffman, B.E., Herzog, R.W. and Daniell, H. (2010). Oral delivery of bioencapsulated coagulation factor IX prevents inhibitor formation and fatal anaphylaxis in hemophilia B mice, *Proceedings of National Academy of Science USA,* 107 : 7101- 7106.

Wadyalkar, P., Dhamangaonkar, S., Snehal, N., Nerkar, G., Kawnar, P., Devarumath, R.M., Ghole, V.S. and Babu, K.H. (2011). Improved protocols for embryogenic callus induction and direct regeneration of sugarcane (*Saccharum officinarum* L.) cv. Co 86032, in *Proceedings of 4th International Sugar Conference and Expo,*780-784.

Wani, S.H., Haider, N., Kumar, H. and Singh, N.B. (2010). Plant Plastid Engineering, *Current Genomics,* 11 : 500-512.

Wei, Z., Liu, Y., Lin, C., Wang, Y., Cai, Q., Dong, Y. and Xing, S. (2011). Transformation of alfalfa chloroplast and expression of green fluorescent protein in a forage crop, *Biotechnology Letters,*1-8.

Wu, L. and Birch, R. (2007). Doubled sugar contains in sugarcane plants modified to produce a sucrose isomer, *Plant Biotechnology Journal,* 5 : 109-117.

Wurbs, D., Ruf, S. and Bock, R. (2007). Contained metabolic engineering in tomatoes by expression of carotenoid biosynthesis genes from the plastid genome, *The Plant Journal,* 49 : 276-28.

RESEARCH ARTICLE

SCREENING COTTON GENOTYPES AGAINST *BEMISIA TABACI* IN SOUTH WESTERN PUNJAB

Sanjeev Kumar Kataria*, Paramjit Singh and Bhawana

PAU, Regional Station, Bathinda – 151001
Email:k.sanjeev@pau.edu

Received-02.05.2017, Revised-06.06.2017

Abstract: Field evaluation of 47 genotypes was carried out for screening against whitefly (*Bemisia tabaci*) in South-western region of Punjab. The population of whitefly was recorded on each genotype after 60, 90, 120 days of sowing during 2015 and 2016. Damage index was calculated and these genotypes were categorized into 6 categories - most resistant, resistant, moderately resistant, susceptible, moderately susceptible and highly susceptible. The genotypes with highest damage index were categorized highly susceptible whereas the genotypes with lowest damage index were categorized as most resistant. During the present study, some of the genotypes were found resistant. In relation to climate, population of whitefly was reported highest during the month of August-September.

Keywords: Cotton, Genotypes, Whitefly, *Bemisia tabaci*, Damage index

INTRODUCTION

Cotton, a cash crop, is a natural fibre grown worldwide (Riaz *et al.*, 2013). It provides basic raw material for textile industry. The crop is of great economic importance as it plays a crucial role in agricultural and industrial development and is the main source of foreign income through export of its raw materials and refined products (Tuteja, 2014). Therefore, adequate production of cotton to fulfill the fibre needs of the increasing world population has become a necessity (Farooq *et al.*, 2013). Cotton productivity is influenced by many abiotic and biotic factors. Abiotic factors are mainly concerned with environment whereas biotic factors are related with insect-pest and diseases. During the whole growing period of the crop, attack of 1326 insect-pests has been estimated, which results in heavy quantitative and qualitative losses to the crop (Manjunath, 2004). For the control of the insect-pests, farmers depend on the use of chemical control (Arif *et al*., 2007) leading to increase in cost of production, reducing the population of natural enemies of the pest, development of pesticide resistant races of the pest and environmental pollution. One of the most hopeful ways to improve cotton productivity and quality is to grow resistant varieties, which is very efficient, inexpensive, economical and environment friendly approach (Pedigo, 1989).

Host plant resistance acts as an effective tool for controlling the insect pests by enabling the plant to avoid, tolerate or recover from insect-pest attack (Pedigo, 1996). Over the years, many Bt and non-Bt varieties and hybrids of cotton have been introduced. These commercialized transgenic Bt cotton cultivars though resistant to bollworm attack but are highly vulnerable to sucking insect-pests (Kranthi *et al*., 2005). Among sucking pests, whitefly (*Bemisia tabaci* Genn. Homoptera: Aleyrodidae) plays an important role by sucking a large amount of plant juices (Oleveira *et al.*, 2001). It also causes indirect loss by secreting honeydew, closing respiratory pores, enhancing growth of sooty mold fungus and reducing the process of photosynthesis. Most importantly, it act as a vector of some dangerous plant viruses, which are acquired and transmitted between plants through this insect. Some of these viruses are Tomato leaf curl virus (TLCV), Cotton leaf curl virus (CLCV), Cucurbit yellow stunting disorder virus (CYSDV) and Sweet potato mild mottle virus (SPMMV) (Makkouk *et al.*, 1979; Byrne and Bellows, 1991; Ioannou, 1994; Ismail *et al.*, 2004).

Therefore, this pest is responsible for huge loss of quality and quantity of plants. A large number of Bt cotton hybrids developed by different private seed companies are being cultivated by the farmers in our country. But there is a great need for screening of these Bt cotton hybrids against disease/pest attack before recommending them to the farmers. With this purpose, 47 genotypes of cotton were tested against the attack of *Bemisia tabaci* in South-Western region of Punjab.

MATERIAL AND METHOD

The study was conducted at Regional Station of Punjab Agricultural University, Bathinda, Punjab, India for two consecutive crop seasons *i.e.* 2014-15 and 2015-16. The crop was sown at a site having 74º 18' E longitude, 30º 58' N latitude and 211m altitude. The site is characterized by semi-arid climate with three distinct seasons including hot and dry summer (April to June), hot and humid monsoon (July to September) and cold winters (November to January). The cotton seed was provided by different private seed companies. Sowing of 47 varieties of cotton was done in the month of May at a spacing of 67.5cm (row to row) and 75cm (plant to plant) in a plot size of 6.0m in a Completely Randomized Block Design

*Corresponding Author

(CRBD) following package of practices recommended by PAU.

The population of whitefly was recorded at three different time periods: 60, 90 and 120 days after sowing. The data was taken from three plants/ plot with three fully opened leaves of the upper canopy/plant before 10 a.m. Statistical analysis was done as per procedure laid down for completely randomized block design (CRBD). Analysis of variance, critical difference (CD) and variance components were calculated for interpretation of results following the study done by Panse and Sukhtame (1989). The recorded data was subjected to analysis with CPCS1 software developed by Department of Mathematics and Statistics, Punjab Agricultural University, Ludhiana. The mean and critical differences were calculated for finding the significance of the recorded data. The genotypes were categorized into six categories on the basis of rating scale of 0-5 which was calculated according to the formula given by Ahmad (1993) given below:

Rating 0 = $\bar{x}$(A) < ($\bar{X}$ - σ)

Rating 1 = ($\bar{X}$ - σ) < $\bar{x}$(A) < ($\bar{X}$ - $\frac{\sigma}{2}$)

Rating 2 = ($\bar{X} - \frac{\sigma}{2}$) < $\bar{x}$(A) < ($\bar{X}$)

Rating 3 = ($\bar{X} + \frac{\sigma}{2}$) > $\bar{x}$(A) > ($\bar{X}$)

Rating 4 = ($\bar{X}$+σ) > $\bar{x}$(A) > ($\bar{X} + \frac{\sigma}{2}$)

Rating 5 = $\bar{x}$(A) > ($\bar{X}$ + σ)

Where $\bar{x}$(A) is the mean population of whitefly, $\bar{X}$ is the overall mean of whitefly population for all the 47 genotypes and σ is the standard deviation.

The reaction of different genotypes was noted down according to the table given below:

S. No.	Category code	Rating	Reaction
1	R1	0	Most resistant
2	R2	1	Resistant
3	R3	2	Moderately resistant
4	S3	3	Moderately susceptible
5	S2	4	Susceptible
6	S1	5	Most susceptible

Weightage percentage of resistance was calculated by considering minimum and maximum population of whitefly as 0 to 100 and applying the following formula:

$$WPR = \frac{MaLAF - LAFFC}{MaLAF - MiLAF}$$

where WPR is the weightage percentage of resistance, $MaLAF$ is the maximum whitefly population, $LAFFC$ is the population of whitefly in the concerned genotype and $MiLAF$ is the minimum whitefly population.

RESULT AND DISCUSSION

Field evaluation of 47 genotypes was carried out for screening against whitefly (*Bemisia tabaci*) in South-western region of Punjab. The population of whitefly was recorded on each genotype after 60, 90, 120 days of sowing during 2015 and 2016. Damage index was calculated according to the formula given by Ahmad (1994). Categorization of different genotypes was done on the basis damage index and is given in table 1 & 2. During 2015, the genotypes *viz*. Ankur 3228, Cotton H. Gold star BGII, KDCHH 541, Super 971 in which damage index was less than 32.17 after 60 days of sowing were categorized as most resistant and the genotypes *viz*. 6539-2, KSCH 211, PCH 877, RCH 314, RCH 653, SO7H 878, SWCH 4713 having damage index more than 76.59 after 60 days of sowing were categorized as most susceptible. The genotypes ABCH 244, Ankur 3244, JKCH 8935, JKCH 8940, MRC 7365, NSPL 2223, RCH 314, RCH 791, SO7H 878, SWCH 4713 in which damage index was less than 85.27 after 90 days of sowing were categorized as most resistant and the genotypes *viz*. ABCH 243, Ankur 3228, Cotton H. Solar-BGII, JKCH 0109, KSCH 213, MRC 7041, PCH 877, RCH 653, RCH 773, SWCH 4755 having damage index more than 138.09 after 90 of sowing were categorized as most susceptible genotypes. The genotypes in which damage index was less than 8.90 after 120 days of sowing were categorized as most resistant and the genotypes having damage index more than 12.02 after 120 days of sowing *viz*. 6539-2, KSCH 211, PCH 877, RCH 314, RCH 653, SO7H 878, SWCH 4713 were categorized as most susceptible.

During 2016, the data for population of whitefly was recorded on 60, 90, 120 days after sowing. After 60 days, the population of whitefly was found comparatively lower on Ankur 3028, Ankur Jassi, JKCH 8940, MH 5302, PRCH 7799 and these genotypes were found most resistant with a damage index ranging from <4.58. The genotypes having damage index >12.07 fall under the category of most susceptible genotypes and these were 6539-2, Ankur3224,Ankur 3244, JKCH 0109, RCH 314, RCH 791, SWCH 4707 and SWCH 4744. After 90 days, the population of whitefly was found comparatively lower on ABCH 243, Cotton H.Solar-75BGII, JKCH 8940, KDCHH 516, NAMCOT 616, PRCH 333, PRCH 7799, SWCH 4713 and these were found comparatively resistant with a damage index of <8.62 and three genotypes *viz*. Cotton H. Gold Star, RCH 653, RCH 773 (DI >11.13) with high population of whitefly were categorized as most

susceptible. After 120 days, Ankur Jassi, MH 5302, MRC 7365, NSPL 2223, PCH 877, RCH 791, SWCH 4707, SWCH 4755 were found most resistant as population of whitefly was lower and damage index was also lower (DI < 8.62). The genotypes namely Ankur 3244, Cotton H. Solar-77 BGII, JKCH 1050, JKCH 1947, JKCH 8935, JKCH TARZAN, KDCHH 541, MRC 7041, Super 971, SWCH 4713 were categorized as most susceptible due to higher population of whitefly recorded after 120 days of sowing and consequent higher damage index of > 37.31. .

During these investigations, it was concluded that different genotypes possess differential response to *Bemisia tabaci* and no genotype escaped from its attack. However, differences in resistance level between different cultivars were found which may be due to the genetic makeup of the plant or certain morphological or physiological characters or due to some bio-chemical composition of the plant leaves. These genotypes can further be evaluated for mechanism of resistance. Present investigations are parallel to the study done by some of the workers. Mumtaz *et al*. (1997) identified resistant genotype among seven varieties tested against whitefly. Robert *et al*. (1997) observed differential response of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to *Gossypium* genotypes. Various research workers namely Karar *et al.* (2013) worked on onion genotypes, Babar *et al.* (2013) and *Shahid et al.* (2015) on cotton cultivars, Karar *et al.* (2015) on mango cultivars and found that there is a variation in resistance level among different crops. Nath *et al.* (2000), Shad *et al*. (2001) and Amjad *et al.* (2009) reported differences of resistance levels in different crop genotypes against sucking pest complex. Vikas *et al.* (2007) also worked on cotton genotypes for screening their resistance to whitefly. Ali and Aheer (2007) observed varietal resistance of cotton genotypes against sucking insect-pests. Javaid *et al.* (2012) also carried out experiments for screening 10 cotton genotypes against whitefly and find differences in resistance/susceptibility.

Karar *et al*.(2016) observed difference in resistance of cotton genotypes. Variation in resistance level among different cotton varieties against sucking pests has also been reported by earlier workers (Ali *et al*., 1999, Nath *et al*., 2000). Acharya and Singh (2007) also did work on screening the cotton germplasm against whitefly and categorized them into four categories *viz*. resistant/tolerant, moderately resistant, moderately susceptible and susceptible based on the criteria of population. Khan (2011) also studied the varietal performance of nine varieties of cotton for screening against sucking pests. Seo *et al.* (2006) carried out field experiments for evaluation of cotton germplasm against whitefly but none of the genotype was found highly resistant which is in contrast to the present study in which differential response of different cultivars was observed and some of the genotypes were found resistant. The differences in resistance reaction to sucking pests of cotton in some new genotypes may be due to physico-morphic plant characters as reported by Raza and Afzal (2000) and Bashir *et al*. (2001). Though a lot of work has been done on screening of genotypes against *Bemisia tabaci* based on the population of whitefly and came to a conclusion that there are some differences in resistance level but none of them has calculated the resistance level with respect to damage index. In the present study, the resistant/ susceptible reaction of different cultivars was observed with respect to the damage index and some of the genotypes found resistant and gave quite promising results.

The weightage percentage of resistance was also calculated and its range varied from 0 to 85.93 for 60 days, from 0 to 63.09 for 90 days and 0 to 47.00 for 120 days in the year 2015 and from 0 to 94.38 for 60 days, 0 to 52.08 for 90 days and 0 to 42.20 for 120 days for the year 2016.

The comparison of data on population of *Bemisia tabaci* taken for different months suggested that the climate also affects the resistance/susceptibility of cotton genotypes to the attack of whitefly. During 2015, it was observed that the damage caused by *Bemisia tabaci* to cotton crop was highest in the month of August as compared to that in July and September. The damage index varied from 32.17 to 76.59 in July, 85.27 to 138.09 in August and from 8.90 to 12.02 in September. During 2016, damage caused by *Bemisia tabaci* was highest in September as compared to that in July and August. The damage index varied from 4.58 to 12.07 in July, 8.62 to 11.13 in August and from 28.31 to 37.31 in September. Some of the workers did a lot of work on the population build-up of whitefly in relation to climate. Idris (1990) found that susceptibility of cotton to *Bemisia tabaci* was highest in August-September in Pakistan. Javaid *et al*. (2012) also found the month of August as most favorable period for the growth of whitefly. However, Sharma and Rishi (2004a) observed high incidence of this insect in October-November in Northern India.

Hegde *et al*. (2004) also observed peak population of whitefly during October. These observations are quite contradictory to Anitha and Nandihalli (2008) who observed peak incidence of whitefly during last week of April under South Indian conditions. Variation in peak population of whitefly may be due suitable climatic conditions. High rainfall before seed setting results in higher population of whitefly due to abundant food supplies (Sharma, 2002). Further investigations may be carried out during field studies to observe the peak period for the build-up of whitefly population and prepare simulation models and hence suitable measures can be suggested for the management of the pest population prior to the damage caused by this deadly/harmful pest.

Selection and identification of resistant germplasm against whitefly and other insect-pest of cotton is pre-requisite for improving the quality and raising the yield attributes. Resilient genotypes outline one of the most critical components of integrated pest management. Cultivation of resistant cultivars is helpful in the reduction of use of pesticides, reduction in soil-water contamination; bring health benefits to farm workers and protection of natural enemies of insect-pests and non-targeted animals. The present findings also suggest the use of resistant varieties but these varieties should be tested against insect-pests and diseases before recommending them to the farmers so that loss could not occur at the farmer's end. Moreover, cultural practices and other measures can be modified according to the climatic factors for combating the pest population of whitefly. Different simulation models can be prepared for forecasting the outbreaks of pest population and farmers and other field workers can be guided accordingly.

In the present study, the difference in resistance levels of different cotton cultivars has been observed during their field performance which is suspected to be caused by difference in their genotypes. Some of the genotypes *viz.* Ankur 3228, Cotton H. Gold star BGII, KDCHH 541, Super 971 , ABCH 244, Ankur 3244, JKCH 8935, JKCH 8940, MRC 7365, NSPL 2223, RCH 314, RCH 791, SO7H 878, SWCH 4713 6539-2, KSCH 211, PCH 877, RCH 314, RCH 653, SO7H 878, SWCH 4713 during 2015 and genotypes Ankur 3028, Ankur Jassi, JKCH 8940, MH 5302, PRCH 7799, ABCH 243, Cotton H. Solar-75BGII, JKCH 8940, JKCH TARZAN, NAMCOT 616, PRCH 333, PRCH 7799, Ankur Jassi, MH 5302, MRC 7365, NSPL 2223, PCH 877, RCH 791, SWCH 4707 and SWCH 4755 during 2016 are found to have lower damage index leading to resistant reaction against cotton whitefly (*Bemisia tabaci*). The resistant germplasm from these genotypes can further be used in breeding program for developing new cotton strains which are resistant to this notorious pest *Bemisia tabaci.*

Table 1. Categorization of different genotypes with reference to their damage index during 2015

60 DAS		90 DAS		120 DAS	
DI	**GENOTYPES**	**DI**	**GENOTYPES**	**DI**	**GENOTYPES**
<32.17	6, 9, 21, 42	<85.27	3, 7,17, 18, 26, 30, 34, 39, 40, 44	<8.90	14, 15, 23, 32, 39, 40
32.17-43.61	2, 11, 12, 14, 16 , 18, 19, 20, 25, 28, 41, 43, 45, 46	85.27-98.47	4, 9, 10, 29, 32, 35, 47	8.90-9.68	5, 10, 13, 16, 18, 22, 26, 28, 30, 31, 34, 37, 42, 44
43.61-54.60	3, 8, 10, 15, 24, 27, 33, 35, 38,39	98.47-111.68	1, 5, 8, 19, 28, 33, 38, 41, 42	9.68-10.46	3, 4, 35, 36
54.60-65.60	4, 5, 7, 13, 17,23, 26, 30, 32	111.68-124.88	12, 20, 21, 22, 24,43	10.46-11.24	6, 12, 19, 20,21, 27, 29, 33, 38, 43
65.60-76.59	29, 37, 47	124.88-138.09	13, 15, 16, 27, 45	11.24-12.02	1, 2, 7, 9, 11, 24, 47
>76.59	1, 22, 31, 34, 36, 40, 44	>138.09	2, 6, 11, 14, 23, 25, 31, 36, 37, 46	>12.02	8, 17, 25, 41, 45, 46

Table 2. Categorization of different genotypes with reference to their damage index during 2016

60 DAS		90 DAS		120 DAS	
DI	**GENOTYPES**	**DI**	**GENOTYPES**	**DI**	**GENOTYPES**
<4.58	4, 8, 18, 24, 33	<8.62	2, 11, 18, 19, 27, 32, 33, 44	<28.31	8, 24, 26, 30,31,39, 43, 46
4.58_6.45	6, 15, 16, 17, 20, 25, 27, 29, 32, 40,46	8.62-9.25	15, 20, 45	28.31-30.56	1, 3, 4, 10, 29, 38
6.45-8.33	3, 9, 19, 21, 22, 26, 30, 37	9.25-9.88	6, 13,16, 17, 31,34, 38,39, 43, 46, 47	30.56-32.81	5, 11, 14, 20, 23, 27, 28,32, 33, 35, 40, 41, 45, 47
8.33-10.20	2, 10, 11, 13, 23, 28, 31, 35, 36, 38, 41, 42, 44	9.88-10.50	4, 5, 7, 8, 10, 14, 21, 22, 23, 42	32.81-35.06	34, 36
10.20-12.07	12	15.50-11.13	1, 3, 12, 24, 25, 26, 28, 29, 30, 35, 40, 41	35.06-37.31	2, 6, 9 , 13, 18, 22, 37
>12.06761	1, 5, 7, 14, 34, 39, 43, 45	>11.13	9, 36, 37	>37.31	7 , 12, 15, 16, 17, 19, 21, 25, 42, 44

*****DAS refers to days after sowing

Table 3. Population of whitefly at 60, 90, 120 days after sowing (DAS) during 2015

Code No.	Entry	60 DAS	90 DAS	120 DAS
1	6539-2	109.17(10.49)	107.16(10.39)	11.33(3.51)
2	ABCH 243	40.17(6.41)	139.17(11.84)	11.83(3.59)
3	ABCH 244	44.67(6.70)	84.17(9.22)	10.17(3.34)
4	ANKUR 3028	60.50(7.84)	88.83(9.47)	11.83(3.58)
5	ANKUR 3224	56.83(7.60)	102.67(10.17)	9.17(3.18)
6	ANKUR 3228	29.67(5.53)	138.17(11.78)	11.17(3.49)
7	ANKUR 3244	56.83(7.60)	80.66(9.02)	11.33(3.50)
8	ANKUR JASSI	51.67(7.25)	104.00(10.24)	13.83(3.80)
9	Cotton H. Gold Star BGII	26.83(5.26)	90.83(9.57)	11.83(3.59)
10	Cotton H. Solar -65 BGII	51.33(7.23)	121.67(11.07)	9.67(3.26)
11	Cotton H. Solar -75 BGII	43.00(6.61)	149.33(12.26)	11.67(3.56)
12	Cotton H. Solar -77 BGII	38.17(6.26)	123.33(11.14)	11.16(3.49)
13	DPC 3083	61.50(7.90)	125.17(11.21)	9.50(3.23)
14	JKCH 0109	39.67(6.38)	146.33(12.13)	8.16(3.00)
15	JKCH 1050	52.50(7.31)	133.17(11.59)	8.00(3.10)
16	JKCH 1947	40.67(6.45)	133.67(11.60)	9.50(3.22)
17	JKCH 8935	54.83(7.47)	59.66(7.74)	12.50(3.28)
18	JKCH 8940	33.00(5.80)	82.67(9.13)	9.67(3.27)
19	JKCH TARZAN	43.33(6.64)	103.83(10.23)	10.83(3.42)
20	KDCHH 516	34.33(5.90)	114.00(10.72)	12.00(3.59)
21	KDCHH 541	21.83(4.74)	121.17(11.05)	10.67(3.41)
22	KSCH 211	87.00(9.38)	114.33(10.73)	9.50(3.23)
23	KSCH 213	64.33(8.08)	145.33(12.08)	8.67(3.10)
24	MH 5302	49.17(7.08)	121.33(11.05)	11.33(3.50)
25	MRC 7041	40.50(6.43)	149.17(12.26)	13.83(3.86)
26	MRC 7365	63.50(8.02)	76.67(8.80)	9.50(3.23)
27	NAMCOT 616	47.00(6.88)	132.33(11.54)	11.17(3.49)
28	NAMCOT 617	32.67(5.80)	99.83(10.04)	9.00(3.12)
29	NCS 855	73.16(8.61)	91.83(9.61)	10.50(3.39)
30	NSPL 2223	58.50(7.71)	71.83(8.53)	9.33(3.21)
31	PCH 877	82.33(9.13)	161.67(12.74)	9.17(3.18)
32	PRCH 333	54.67(7.45)	98.17(9.96)	7.33(2.89)
33	PRCH 7799	51.17(7.22)	111.50(10.60)	10.50(3.38)
34	RCH 314	111.67(10.61)	80.83(9.00)	9.67(3.27)
35	RCH 602	46.50(6.88)	89.17(9.49)	9.83(3.29)
36	RCH 653	97.83(9.94)	160.16(12.69)	9.83(3.29)
37	RCH 773	73.33(8.62)	139.67(11.86)	9.50(3.23)
38	RCH 776	50.50(7.17)	102.17(10.15)	11.17(3.49)
39	RCH 791	53.83(7.40)	74.50(8.63)	8.83(3.13)
40	SO7H878	86.00(9.33)	74.17(8.67)	7.50(2.90)
41	Super 931	34.83(5.93)	111.33(10.60)	12.33(3.64)
42	Super 971	20.00(4.58)	110.33(10.54)	9.33(3.221)
43	SWCH 4707	42.67(6.60)	117.00(10.86)	11.00(3.44)
44	SWCH 4713	103.83(10.23)	83.67(9.14)	9.17(3.18)
45	SWCH 4744	39.66(6.36)	126.50(11.30)	12.83(3.71)
46	SWCH 4755	40.00(6.39)	159.33(12.67)	13.17()3.74

47	VICH 309	71.17(6.49)	96.33(9.86)	11.83(3.58)
	CD(p=0.05)	(1.07)	(1.15)	NS
	CV(%)	7.28	5.47	8.76

*****Figures in parentheses are transformed values

Table 4. Population of whitefly at 60, 90, 120 days after sowing (DAS) during 2016

Code No.	**Entry**	**60 DAS**	**90 DAS**	**120 DAS**
1	6539-2	13.00(3.72)	11.11(3.48)	29.89(5.56)
2	ABCH 243	12.33(3.63)	8.33(3.05)	36.22(6.07)
3	ABCH 244	7.33(2.86)	10.83(3.45)	30.11(5.53)
4	ANKUR 3028	5.33(2.49)	10.22(3.33)	29.44(5.50)
5	ANKUR 3224	13.00(3.71)	10.33(3.36)	31.00(5.62)
6	ANKUR 3228	6.00(2.62)	9.67(3.26)	35.33(6.01)
7	ANKUR 3244	23.67(4.97)	10.22(3.33)	43.44(6.64)
8	ANKUR JASSI	5.00(2.44)	10.22(3.35)	26.89(5.27)
9	Cotton H. Gold Star BGII	7.00(2.81)	13.44(3.78)	35.78(6.06)
10	Cotton H. Solar -65 BGII	9.67(3.27)	10.33(3.33)	29.67(5.53)
11	Cotton H. Solar -75 BGII	9.67(3.26)	8.33(3.05)	31.22(5.67)
12	Cotton H. Solar -77 BGII	10.33(3.36)	10.56(3.40)	38.00(6.23)
13	DPC 3083	9.33(3.17)	9.78(3.28)	36.44(6.11)
14	JKCH 0109	11.00(3.46)	10.22(3.34)	31.56(5.66)
15	JKCH 1050	5.33(2.50)	8.89(3.12)	38.78(6.30)
16	JKCH 1947	7.00(2.82)	9.33(3.20)	38.33(6.27)
17	JKCH 8935	6.00(2.62)	9.78(3.26)	40.78(6.46)
18	JKCH 8940	1.33(1.52)	8.44(3.05)	35.56(6.04)
19	JKCH TARZAN	7.33(2.87)	8.00(2.99)	38.00(6.24)
20	KDCHH 516	5.33(2.51)	9.11(3.17)	31.22(5.64)
21	KDCHH 541	7.67(2.89)	10.44(3.39)	37.67(6.20)
22	KSCH 211	7.33(2.85)	10.33(3.37)	35.89(6.07)
23	KSCH 213	5.33(2.51)	9.89(3.26)	30.11(5.56)
24	MH 5302	4.00(2.23)	11.00(3.46)	25.56(5.12)
25	MRC 7041	6.33(2.67)	11.11(3.48)	42.44(6.59)
26	MRC 7365	7.33(2.82)	10.78(3.40)	27.22(5.30)
27	NAMCOT 616	6.33(2.66)	6.44(2.70)	31.89(5.70)
28	NAMCOT 617	8.33(3.04)	11.11(3.43)	30.89(5.63)
29	NCS 855	6.33(2.70)	10.67(3.41)	29.00(5.48)
30	NSPL 2223	7.33(2.88)	10.22(3.32)	27.33(5.28)
31	PCH 877	9.33(3.20)	9.33(3.18)	25.11(5.06)
32	PRCH 333	5.00(2.43)	7.00(2.82)	32.00(5.73)
33	PRCH 7799	4.33(2.30)	8.22(3.03)	32.78(5.80)
34	RCH 314	12.33(3.64)	9.67(3.27)	33.33(5.83)
35	RCH 602	10.67(3.41)	10.67(3.41)	32.22(5.76)
36	RCH 653	8.33(3.02)	12.11(3.61)	32.89(5.73)
37	RCH 773	8.00(2.99)	11.22(3.49)	36.44(6.10)
38	RCH 776	8.67(3.10)	9.44(3.22)	28.89(5.41)
39	RCH 791	10.00(3.31)	9.67(3.26)	25.67(5.10)
40	SO7H878	7.67(2.93)	11.00(3.44)	31.00(5.62)
41	Super 931	8.67(3.02)	10.67(3.40)	31.67(5.87)
42	Super 971	9.33(3.20)	10.22(3.34)	37.44(6.18)
43	SWCH 4707	14.00(3.71)	9.33(3.20)	27.89(5.37)
44	SWCH 4713	11.33(3.50)	7.89(2.97)	37.44(6.18)
45	SWCH 4744	16.33(4.14)	9.22(3.18)	30.67(5.62)
46	SWCH 4755	7.33(2.89)	9.67(3.22)	27.56(5.24)
47	VICH 309	3.33(2.06)	9.67(3.24)	32.33(5.77)
	CD(p=0.05)	(0.69)	NS	(0.86)
	CV(%)	14.12	10.42	9.13

*****Figures in parentheses are transformed values

Table 5. Weightage Percentage of Resistance (WPR) of different cultivars during 2014-15 and 2015-16.

		2014-15			2015-16		
Code No.	Entry	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS
1	6539-2	2.24	33.72	18.08	45.08	17.34	31.19
2	ABCH 243	64.03	13.92	14.46	61.98	38.02	16.62
3	ABCH 244	60.00	47.94	26.46	69.03	19.42	30.69
4	ANKUR 3028	45.82	45.05	14.46	81.71	23.96	32.23
5	ANKUR 3224	49.11	36.49	33.69	45.08	23.14	28.64
6	ANKUR 3228	73.43	14.54	19.23	74.65	28.05	18.67
7	ANKUR 3244	49.11	50.11	18.08	0.00	23.96	0.00
8	ANKUR JASSI	53.73	35.67	0.00	83.10	23.96	38.10
9	Cotton H. Gold Star BGII	75.97	43.82	14.46	70.43	0.00	17.63
10	Cotton H. Solar -65 BGII	54.03	24.74	30.08	59.15	23.14	31.70
11	Cotton H. Solar -75 BGII	61.49	7.63	15.62	59.15	38.02	28.13
12	Cotton H. Solar -77 BGII	65.82	23.71	19.31	56.36	21.43	12.52
13	DPC 3083	44.93	22.58	31.31	60.58	27.23	16.11
14	JKCH 0109	64.48	9.49	41.00	47.91	23.96	27.35
15	JKCH 1050	52.99	17.63	42.15	77.48	33.85	10.73
16	JKCH 1947	63.58	17.32	31.31	76.05	30.58	11.76
17	JKCH 8935	50.90	63.10	9.62	74.65	27.23	6.12
18	JKCH 8940	70.45	48.86	30.08	94.38	37.20	18.14
19	JKCH TARZAN	61.20	35.78	21.69	69.03	40.48	12.52
20	KDCHH 516	69.26	29.49	13.23	77.48	32.22	28.13
21	KDCHH 541	80.45	25.05	22.85	67.60	22.32	13.28
22	KSCH 211	22.09	29.28	31.31	69.03	23.14	17.38
23	KSCH 213	42.39	10.11	37.31	63.37	26.41	28.38
24	MH 5302	55.97	24.95	18.08	83.10	18.15	41.16
25	MRC 7041	63.73	7.73	0.00	73.26	17.34	2.30
26	MRC 7365	43.14	52.58	31.31	69.03	19.79	37.34
27	NAMCOT 616	57.91	18.15	19.23	73.26	52.08	26.59
28	NAMCOT 617	70.74	38.25	34.92	59.15	17.34	28.89
29	NCS 855	34.49	43.20	24.08	73.26	20.61	33.24
30	NSPL 2223	47.61	55.57	32.54	69.03	23.96	37.09
31	PCH 877	26.27	0.00	33.69	60.58	30.58	42.20
32	PRCH 333	51.04	39.28	47.00	78.88	47.92	26.34
33	PRCH 7799	54.18	31.03	24.08	81.71	38.84	24.54
34	RCH 314	0.00	50.00	30.08	36.63	28.05	23.27
35	RCH 602	58.36	44.84	28.92	60.58	20.61	25.83
36	RCH 653	12.39	0.93	28.92	64.81	9.90	24.29
37	RCH 773	34.33	13.61	31.31	71.82	16.52	16.11
38	RCH 776	54.78	36.80	19.23	63.37	29.76	33.49
39	RCH 791	51.80	53.92	36.15	47.91	28.05	40.91
40	SO7H878	22.99	54.12	45.77	67.60	18.15	28.64
41	Super 931	68.81	31.14	10.85	63.37	20.61	27.09
42	Super 971	82.09	31.76	32.54	57.75	23.96	13.81
43	SWCH 4707	61.79	27.63	20.46	40.85	30.58	35.80
44	SWCH 4713	7.02	48.25	33.69	61.98	41.29	13.81
45	SWCH 4744	64.48	21.75	7.23	45.08	31.40	29.40
46	SWCH 4755	64.18	1.45	4.77	74.65	28.05	36.56
47	VICH 309	36.27	40.42	14.46	85.93	28.05	25.58

REFERENCES

Acharya, V.S., Singh, A.P. (2007). Screening of cotton genotypes against whitefly, *Bemisia tabaci. Journal of Cotton Research and Development*. 21(1): 111-114.

Ahmad, M. (1993). Relative resistance in different clones of *Populus deltoides* to poplar defoliator *Clostera cuperata* (Lepidoptera: Notodontidae). *Ann. For* : 1: 1-12.

Ali, A., Aheer, G.M. (2007). Varietal resistance against sucking insect-pests of cotton under Bahawalpur ecological conditions. *Journal of Agricultural Research*, 45: 1-5.

Ali, A., Aheer, G.M., Saeed, M. (1999). Physico-morphic factors influencing resistance against sucking insect pests of cotton. *Pakistan Entomologist*. 21(1-2): 53-55.

Amjad, M., Bashir, M.H., Afzal, M., (2009). Comparative resistance of some cotton cultivars

against sucking insect pests. *Pak. J. Life Soc. Sci*., 7: 144-147.

Anitha, K.R., Nandihalli, B.S. (2008). Seasonal incidence of sucking pests in okra ecosystem. *Karnatka Journal of Agriculture Science*, 21: 137-138.

Arif , M.J., Abbas, G., Saeed S. (2007). Cotton in danger, p:4 Dawn, *The Internet Edition*, March 24th 2007.

Babar, T.K., Karar, H., Hasnain, M., Shahazad, M.F., Muhammad Saleem, M., Ali, A. (2013). Performance of some transgenic cotton cultivars against sucking insect pests. *Pak. J. Agri.Sci*., 50(3): 367-372.

Bashir, M.H., Afzal, M., Sabri, Raza, A.M. (2001). Relationship between sucking pests and physico-morphic plant characters towards resistance/susceptibility in some new genotypes of cotton. *Pakistan Entomologist*, 22(1-2): 75-78.

Butter, N.S., Vir, B,K. (1989). Morphological basis of resistance in cotton to the whitefly *Bemisia tabaci. Phytoparasitica* 17(4); 251-261.

Butter, N.S., Vir, B.K. (1990). Sampling of whitefly *Bemisia tabaci* Genn. in cotton. *Journal of Research,* PAU, 27(4): 615-619.

Byrne, D.N., Bellows, Jr. T.S. (1991). Whitefly biology. *Annual Review of Entomology*, 36: 341-357.

Farooq, J, Anwar, M., Riaz, M., Mahmood, A., Farooq, A., Iqbal, M. S., Iqbal, M. S. (2013). Association and path analysis of earliness, yield and fibre related traits under cotton leaf curl virus(CLCuV) intensive conditions in G. *hirsutum* L. *Plant Knowledge Journal*. 2(1): 43-50.

Hegde, M.M., Srinivas, M., Biradar, D.P., Udikeri, S.S., Khadi, B.M. (2004). Seasonal incidence of sucking pests and their natural enemies on cotton at Siruguppa. Paper presented. *Int. Symp. Strat. Sust. Cotton Prodn.- A Global vision*, Karnatka, India, pp. 23-25.

Idris, A.M. (1990). Cotton leaf curl virus disease in the Sudan. *Mededehngen van de Faculteit LtndboHwwetenschappen Riijksuniversiteit Genetics* 55: 263-267.

Ioannu, N. (1994). Diseases of tomato caused by whitefly transmitted *gemini* viruses. *Horticultural Abstracts*, 64: 54-73.

Ismail, I.D., Raie, S.U., Akil, A.K. (2004). A survey of Sweet Potato Virus Diseases in Syrian coastal region (Lattakia) using Tissue Blot Immunobinding Assay. Tishreen University Journal For Studies and Scientific Research-*Agriculture Science Series*, 26: 125-132.

Javaid , M., Arif, M.J., Gogi, M.D., Shahid, M.R., Iqbal, M.S., Bibi, R., Shehzad, M.A. (2012). Relative resistance in different cultivars of Pakistani cotton against cotton whitefly, *Bemisia tabaci*. *Academic Journal of Entomology* 5(3): 143-146.

Karar, H, Shahid, M., Ahamad, S. (2016). Evaluation of innovative cotton genotypes against insect pest prevalence, fibre trait, economic yield and virus incidence in Pakistan. *Cercetari Agronomice* in Moldova. Vol. XLIX, No. 1(165): 29-39.

Karar, H., Abbas, G., Hameed, A., Shahzad, M.F., Ahmad, G., Ali, A. Saleem, M. (2013). Relative susceptibility of onion(*Allium cepa*) genotypes of Pakistan to onion thrips (*Thrips tabaci*) (Thysanoptera: Thripidae). *Pak. J. Agri. Sci*., Vol. 50(3): 351-357.

Karar, H., Arif, M.J., Arshad M., Ali, A., Abbas, Q. (2015). Resistance/ susceptibility of different mango cultivars against mango mealybug(*Drosicha magiferae* G.). *Pak. J. Agri. Sci*., 52(2): 367-377.

Khan, S.M. (2011). Varietal performance and chemical control used as tactics against sucking pests of cotton. *Sarhad J. Agric*. 27(2): 255-261.

Kranthi, K.R., Kranthi, S., Ramesh, K., Nagrare, V.S., Barik, S. (2005). Advances in cotton IPM (Technical Bulletin). *Central Institute for Cotton Research* Post Bag No., Shankar Nagar, PO, Nagpur (MS), India.

Makkouk, K. M., Shebab, S., Madjalini, S. E. (1979). Tomato yellow leaf curl: incidence, yield losses and trasmission in Lebanon. *Phytopathology*, 96: 263-267.

Manjunath, T.M. (2004). Bt cotton in India. In: the technology wins as the controversy wanes. Available at *www.monsanto.co.uk/newsukshowlib.html?wid+8478*.

Mumtaz, M.K., Pervaz, A., Ahmad, A., Arshad, A., Ali, A. (1997). Abiotic factors affecting the population fluctuation of leaf curl virus incidence in different genotypes of cotton. *Journal of Animal and Plant Science*, 7(3-4): 119-121.

Nath, P., Chaudhary, Sharma, P.D., Kaushik, H.D. (2000). Studies on the incidence of important insect pests of cotton with special reference to desi cotton. *Indian J. Entom*. 62(4): 391-395.

Oliveira, M.R.V., Henneberry, T.J., Anderson, P. (2001). History, current and collaborative research projects for *Bemisia tabaci. Crop Protection*, 20: 709-723.

Panse, V.G., Sukhtame, P.V. (1989). In Sukhtame PV, Amble VN (eds) *Statistical methods for agricultural workers*. Indian Council for Agricultural Research publication, New Delhi, pp. 100-109.

Pedigo, I.P. (1989). *Entomology and Pest Management*. Prentice Hall of India (Pvt.) Ltd. New Delhi-110001, 413 p.

Pedigo, L.P. (1996). Plant resistance to insects. *In*: *Entomology and Pest Management*. Prentice hall of India Private Limited, New Delhi, pp. 413-424.

Raza, A.M., Afzal, M. (2000). Physio-morphic plant characters in relation to resistance against sucking insect-pests in some new genotypes. *Pakistan Entomologist*, 22(1-2): 73-78.

Riaz, M., Naveed, M., Farooq, J., Farooq, A., Mahmood, A., Rafiq, Ch. M., Nadeem M., Sadiq A. (2013). AMMI analysis for stability, adaptability

and GE interaction studies in cotton (Gossypium hirsutum L.). *J. Anim. Plant Sci*.,23(3) : 865-871.

Robert, L., Meagher, Jr., Wayne C. Smith, Wendy, J., Smith (1997). Preference of Gossypium genotypes to *Bemisia argentifolii* (Homoptera: Aleyrodidae). *Journal of Economic Entomology*. 90(4): 1046-1052.

Seo, Y.S., Zhou, Y.C., Turini, T.A., Cook, C.G., Gilbertson, R.L., Natwick, E.T. (2006). Evaluation of cotton germplasm for resistance to the whitefly and Cotton Leaf Crumple (CLCr) Disease and etiology of CLCr in California's Imperial valley. *Plant Disease*. 90(7): 877-884.

Shad, S.A., Waseem, A., Rizwan, A. (2001). Relative response of different cultivars of cotton to sucking insect pests at Faisalabad. *Pak. Entomol*. 23(1/2): 79-81.

Shahid, M.R., Farooq,J., Mahmood, A., Iqbal, M.S., Mahmood, K., Abbas, H.G. (2015). Economic yield, Fibre trait and sucking insect pest incidence on advanced genotypes of cotton genotypes of cotton in Pakistan. *Cercetari Agronomice* in Moldova, Vol. XLVIII, No. 1(161), Iasi, Romania.

Sharma, P. (2002). Molecular approaches for detection and diagnosis of cotton leaf curl *gemini* virus and its mode of dissemination in the fields. *PhD Thesis, Deptt. Plant Pathology*, CCS HAU, Hissar, 150 pp.

Sharma, P. Rishi, N. (2004a). Influence of weather variables on the incidence of Cotton leaf curl virus disease. *Indian Journal of Plant Protection* 32: 174-175.

Shivanna, B. K., Nagaraja, D.N., Manjunatha, M., Naik, M.I. (2009). Seasonal incidence of sucking pests on transgenic Bt cotton and correlation with weather factors. *Karnatka J. Agric. Sci*. 22 (3-spl. Issue): 666-667.

Sukhija, H.S., Butter, N.S., Singh, J. (1986). Determination of economic threshold of whitefly *Bemisia tabaci* on *American* cotton in Punjab. Tropical Pest Management 32(2): 134-136.

Tuteja, O.P. (2014). Studies on heterosis for yield and fibre quality traits in GMS hybrids of upland cotton (*Gossypium hirsutum* L.). *J.cotton Res. Dev*.28(1) :1-6.

Vikas, J., Arora, R., Kumar, R. (2007). Screening of cotton genotypes for resistance to sucking pests. *Annals of Plant Protection Sciences*. 15(1): 26-29.

RESEARCH ARTICLE

ENVIRONMENTAL EFFECT ON PHENOLOGY AND GROWTH PARAMETERS OF RICE CROP IN CHHATTISGARH PLAIN REGION

S.K. Chandrawanshi*, S.R. Patel[1], P.R. Bobade[1], Y. Mahesh[1], D.K. Kaushik[1], Pritpal Singh[1] and Swati Gupta[1]

[1]Department of Agrometeorology, College of Agriculture, Indira Gandhi Krishi Vishwadhyalaya Raipur (Chhattisgarh)
**Agricultural Meteorological Cell, Department of Agriculutral Engineering, N.M. College of Agriculture, Navsari Agricultural University, Navsari- 396 450 (Gujarat)*
Email: sandeepagromet@gmail.com

Received-04.02.2017, Revised-12.04.2017

Abstract: The results of phenological studies revealed that the days taken from sowing to seedling, tillering, panicle initiation, booting, panicle emergence, 50 percent flowering, milking, dough and maturity varied with different varieties. The results of growth characters revealed that plant height, number of filled grains per panicle, test weight, number of panicle per m^2, grain yield and harvest index at maturity were recorded maximum in Mahamaya as compared to Karma Mahsuri and MTU-1010. Whereas, the length of panicle and sterility percentage were recorded maximum in Karma Mahsuri while straw yield is maximum in MTU-1010. The results of dry matter accumulation, crop growth rate and relative growth rate showed that these were maximum in Karma Mahsuri, Mahamaya and MTU-1010.

Keywords: Environment, Phenology, Growth parameter, Rice crop

INTRODUCTION

Chhattisgarh popularly known as "Rice Bowl of India" occupies an area around 3610.47 thousand hectares with the production of 5.48 Mt and productivity of 1517 kg ha^{-1} (Anonymous, 2010). The major causes of low productivity of rice in Chhattisgarh are inappropriate adoption of agronomical practices, limited irrigation (28.0%), lack of improved varieties suitable to different ecosystems and lack of extension services. Chhattisgarh farmers are mainly depends on climate for rice cultivation. The ultimate source of all the energy for physical and biological processes occurring on the earth is radiation received from the sun that is why it is commonly called solar radiation. Agriculture is the exploitation of solar energy under adequate supply of nutrients and water by maintaining plant growth.

Crop growth is a result of many physical and physiological processes, each of which is affected by environmental factors. The main factors which have strong influence on crop growth and yield are air temperature, duration and quantity of light, radiation from the sun, cloudiness and precipitation**.** In Chhattisgarh, rice is mainly grown under rainfed condition. Rice is also grown under tube well and canal irrigation.

Anonymous (1997) reported that the total dry matter accumulation above ground (g/m^2) had significant influence at all the stages of irrigated ecosystem over rainfed ecosystem. Dry matter accumulation above the ground was recorded significantly higher under July 15 as compared to 25 July and August 4 planting. Kumar and Subramaniam (1991) found that transplanting done during June-July produced taller plants at harvest in comparison to delay transplanting. However, delay in planting from mid June caused considerable reduction in plant height.

Om *et al*., (1996) reported that dry matter production was highest in July 25 transplanting up to 60 and 40 DAT in 1993 and 1994, respectively. At later growing stages, this trend was observed in June 25 planting. At the last two crop growth stages non significant differences were observed between June 25 and July 5; and June 15 July 5 in 1993, while dry matter production varied significantly with each date of transplanting in 1994.

MATERIAL AND METHOD

Crop growth rate (g/ m^2 /days)

Crop growth rate represents the dry matter accumulation by a unit area of crop per unit time (Leosold and Kriedemann 1975) and is computed with the following formula:

$$CGR = \frac{W2 - W1}{t2 - t1} = g/\ m2\ /day$$

Where,

W2-W1- Difference in over dry biomass at the time interval (t2-t1)

t2-t1- time interval in days

Relative growth rate (g/g/day)

Relative growth rate is an index of the amount of growing material per unit dry weight of plant per unit time (Leosold and Kriedemann 1975). The relative growth rate was computed by using following formula:

$$RGR = \frac{\ln W2 - \ln W1}{t2 - t1} = g/g/\ day$$

*Corresponding Author

Where,
Ln- is the logarithm at base e (natural log)
W2-W1- Difference in over dry biomass at the time interval (t2-t1)
t2-t1- time interval in days

Statistical analysis

All the data were tabulated and analysed statistically as per the procedure suggested by Panse and Sukhatme (1967) and Chandel (1984). The F test was used for judging the significance of the treatment mean at 5% level. Whenever F test showed significant difference the differences between treatment means were further tested by using critical difference (CD) value. To compare different mean value of treatments, critical difference (CD) values were calculated as follows:

$$SEm \pm = \sqrt{\frac{Ems}{n}}$$

Where,
Sem ± Standard error of mean
Ems = Error mean square
n = Number of observations on which the mean values is based
(ii) CD (5%) = Sem x√ 2 x t at 5 % for Error d.f. (2.02).

RESULT AND DISCUSSION

Phenological studies

The data regarding different rice varieties sown on three different dates (10 days interval) are shown in Table 1. The data showed that Karma Mahsuri (V_1) sown on 10th June (D_1) required 50 days for tillering, 72 days for panicle initiation, 86 days for booting, 97 days for panicle emergence, 105 days for 50 percent flowering, 113 days for milking, 122 days for dough and 138 days for maturity. Whereas, the duration taken by the same variety sown on 20th June (D_2) were 49 days for tillering, 71 days for panicle initiation, 86 days for booting, 96 days for panicle emergence, 102 days for 50 percent flowering, 109 days for milking, 118 days for dough and 134 days for maturity and for crop sown on 30th June (D_3) the duration were 48 days for tillering, 68 days for panicle initiation, 84 days for booting, 92 days for panicle emergence, 99 days for 50 percent flowering, 106 days for milking, 115 days for dough and 130 days for maturity. Thus, timely sowing on 10th June (D_1) Karma Mahsuri variety took 8 days more to mature as compared to delayed sowing on 30th June.
Similarly, Mahamaya (V_2) sown on 10th June (D_1) required 44, 65, 79, 89, 96, 104, 113 and 129 days for tillering, panicle initiation, booting, panicle emergence, 50 percent flowering, milking, dough and maturity respectively. The same variety sown on 20th June (D_2) required 42, 63, 78, 88, 95, 102, 110 and 126 days to complete for tillering, panicle initiation, booting, panicle emergence, 50 percent flowering, milking, dough and maturity respectively. The same variety sown 30th June (D_3) required 41, 63, 76, 85, 91, 98, 106 and 122 days to complete for tillering, panicle initiation, booting, panicle emergence, 50 percent flowering, milking, dough and maturity respectively. Hence, Mahamaya sown on 10th June took 7 days more to mature as compared to delayed sowing crop on 30th June.
Among the variety MTU-1010 (V_3) sown on 10th June (D_1) required 40, 59, 71, 81, 88, 95, 103 and 120 days the days to complete for tillering, panicle initiation, booting, panicle emergence, 50 percent flowering, milking, dough and maturity respectively. Similarly, the variety sowing on 20th June (D_2) 38, 56, 68, 78, 85, 91, 98 and 116 days required tillering, panicle initiation, booting, panicle emergence, 50 percent flowering, milking, dough and maturity respectively, and for MTU-1010 (V_3) sown on 30th June (D_3) 37, 54, 66, 76, 83, 88, 95 and 113 days required for tillering, panicle initiation, booting, panicle emergence, 50 percent flowering, milking, dough and maturity respectively. Thus MTU-1010 sowing on 10th June took 7 days to mature as compared to delayed sowing on 30th June. This might be due to the early sowing of crop that was sown in optimum temperature which took more number of days for 50 per cent flowering and maturity as compared to late sown crop. Apart from these phenophase other phenological events also decreased by 5 to 8 days under delayed sown condition. These statements agree with the finding of Dakhore (2003).

Plant height (cm)

The plant height was taken at 15 days intervals from 20 days after transplanting (DAT) to maturity. The initial plant height at 20 DAT was highest in MTU-1010 (46.3cm) followed by Mahamaya (44.9 cm) and Karma Mahsuri (42.6 cm) as shown in Table 2, Fig 1. It was observed that the plant height increased rapidly from 20 DAT to 80 DAT that is till the crop entered at reproductive stage. Thereafter the increase in height was marginal. At maturity Mahamaya attained highest plant height of 118.7 cm, followed by MTU-1010 (114.4 cm) whereas; Karma Mahsuri attended the lowest height of (107.7 cm).
Regarding the effect of different date of sowing the plant height at 20 DAT was highest 48.1 cm with 10th June sown, followed by 20th June 43.3 cm and lowest height 30th June 42.3 cm. It was observed that the plant height increased rapidly from 20 DAT to 80 DAT that till the crop entered reproductive stage. Thereafter the increase in height was marginal. At maturity the crop sown on 10th June attended maximum height of 115.9 cm, followed by 20th June 113.1 cm whereas, 30th June attended the lowest height of 111.9 cm. It was observed that the plant height increased with advancement in crop age and reached to maximum at maturity. The increase in plant height taken place slowly after 80 days after transplanting. Maximum rate of increased in plant

height was observed between 50 to 80 days after transplanting when the varieties are in panicle emergence stage. In general, highest plant height was observed with Mahamaya as compared to MTU-1010 and Karma Mahsuri. It was observed that the average plant height varied among the varieties because of the genotypic characters of these varieties.

The plant height increased at faster rate up to 35 days after transplanting. The plant height again increased rapidly further from 35 to 80 days after transplanting. Thereafter increase in plant height was only marginal. Finally at maturity the plant height of 115.9 cm was obtained in 10th June sowing, while 111.9 cm was obtained in 30th June sowing in general plant height was highest in D_1 as compared to D_2 and D_3 in all the observations. Similar results were found by Kumar and Subramanian (1991).

Number of tillers/hill

The number of tillers per hill of different varieties at 15 days intervals from 20 DAT to maturity was showed in Table 3. It was found that the number of tillers per hill 20 and 35 days after transplanting was statistically similar in all the varieties. However, Karma Mahsuri are produced highest number of tillers as compared to rest two varieties at 35 days after transplanting. It also observed that the number of tillers per hill increased gradually from 20 DAT and reached at its maximum at 65 DAT that is the crop till entered in elongation stage. Thereafter the number of tillers per hill decreased at 80 DAT and at maturity. Karma Mahsuri attained highest number of tillers/hill (8.3) followed by (7.3) with MTU-1010, Lowest number of tillers/hill attainded by was Mahamaya (7.1).

Among the different dates of sowing the number of tillers/hill at 20 DAT was highest on 20th June (6.3) followed by 10th June (5.5) and lowest number of tillers/hill observed at 30th June which was (4.9). With 10th June sown attained maximum tillers/hill 8.4 followed by 20th June (7.3) and 30th June (7.0). It was observed that the number of tillers/hill increased with advancement in crop growth and reached to maximum at 65 DAT. Maximum rate of increase in a number of tillers/hill was observed between 35 to 50 days after transplanting. In general, highest number of tillers/hill was observed in variety Karma Mahsuri and MTU-1010 on 10th June sown as compared to 20th June and 30th June sown. The lowest number of tillers/hill was recorded in variety Mahamaya. It also observed that the average number of tillers/hill was varied among the varieties because of the genotypic characters of these varieties. Similar results were also observed by Singh and Singh (1999).

Dry matter production (g/m^2)

The dry matter production is the best measure and index of the total performance of the varieties to weather conditions. The performance of different varieties to different sowing dates was given in Table 4. The dry matter production of the different varieties at 15 days interval from 20 DAT to maturity is shown through Fig 2. It can be seen that the initial dry matter production at 20 DAT was highest with Mahamaya (46.2 g/m^2) followed by MTU-1010 that is (44.2 g/m^2) and Karma Mahsuri (40.1 g/m^2). The dry matter production increased rapidly from 20 DAT to 110 DAT that is the crop till entered dough stage. Thereafter increase in dry matter was marginal. At maturity Karma Mahsuri attainded highest dry matter of (1550.3 g/m^2) followed by Mahamaya (1432.7 g/m^2) and the lowest dry matter was with MTU-1010 that is (1383.8 g/m^2). Under different dates of sowing dry matter production at 20 DAT in 10th June sowing was (46.8 g/m^2) followed by 20th June sowing (44.1 g/m^2) and the lowest dry matter was in 30th June sowing (39.6 g/m^2). It was observed that the dry matter production increased gradually from 20 DAT to 50 DAT and 110 DAT that is the crop till entered dough stage. Thereafter, the increased in dry matter was marginal. At maturity the crop sown on 10th June produced highest dry matter (1514.9 g/m^2) followed by 20th June (1461.1 g/m^2) and 30th June (1390.8 g/m^2). The rate of increase in dry matter production was highest during 35 to 110 DAT due to highest photosynthesis and active vegetative phase of the crop.

The dry matter production and its partitioning to different plant organs decreased considerably in delayed sown crop 30th June as compared to the crop 10th June. The biomass increased continuously right from sowing to maturity, whereas the leaf biomass increased up to 96 days after transplanting i.e. milking stage / dough stage and slightly towards maturity. However the rate of leaf dry matter accumulation was maximum between 35-50 days after transplanting. Similarly the decrease in leaf dry matter at maturity may be due to the senescence of older leaves and translocation of older leaves and translocation of food material from leaf to grain. The stem dry weight was increased up to maturity as the stem dry matter was taken including the panicle. It is well known that after the gram filling the weight of panicles increased and this may be the reason of increasing stem dry matter up to maturity. Apart from this the food materials also moved from source to sink at the time of maturity. On the other hand the total dry matter increased up to maturity. The increase in total dry matter was also reported by Anon (1997), similar result were obtained by Om *et al.*, (1996).

Crop growth rate

The data presented in Table 5 showed crop growth rate of different varieties and the values of crop growth rate were fluctuating during different stages. The highest crop growth rate were observed 80-95 days in Karma Mahsuri (23.43) followed by MTU-1010 (20.31) and Mahamaya (17.98) during panicle emergence stage. Among the different sowing dates crop growth rate was found highest in 10th June sowing as compared to 30th June sowing in all observations between 50 to 65 days after

transplanting. The crop growth was increased with advancement of crop growth and reached at in maximum between 80-95 days after transplanting. Highest crop growth rate associated with timely sowing was mainly due to highest leaf area index. This accumulated dry matter at a faster rate per unit time and thereby reducing tiller mortality and senescence of older leaf

Relative growth rate

The values of relative growth rate (RGR) were highest for the crop sown on 10th June as compared to the crop sown on 30th June. In 10th June sowing the relative growth rate was negligible up to 20-50 days after transplanting, thereafter, increased sharply up to 50-65 days after transplanting. It was slightly decreased in relative growth rate during 65 to 110 days after transplanting and reached in maximum of (0.192 g/g/day) during 0-20 days after transplanting. In 20th June sowing the RGR was showed fluctuated trend during in different growth stages the relative growth rate was maximum of (0.189 g/g/day) in between 0 to 20 days after transplanting. In 30th June sowing the relative growth rate was minimum (0.184 g/g/day) in between 0 to 20 days after transplanting, shows Table 6.

CONCLUSION

It was found that timely sown crop took more number of days from sowing to maturity as compared to delayed sowing. Days to attain maturity with all the variety Karma mahsuri, Mahamaya and MTU-1010 were maximum under first date of sowing (138 days, 129 days and 120 days respectively) followed by second date of sowing (134 days, 126 days and 116 days respectively) and late sowing (130 day, 122 day and 113 days respectively). The duration of the late sown rice was shortened by 8 days due to delayed sowing in both Karma mahsuri and Mahamaya whereas, it was shortened by 7 days in MTU-1010. The highest plant height was observed in timely sowing Mahamaya followed by Karma mahsuri and MTU-1010. Higher number of tillers/hill was recorded with Karma mahsuri as compared to Mahamaya and MTU-1010. Among the different dates of sowing higher plant height and number of tillers/hill were observed under 10 June sowing which was dominant over 20 and 30 June sowing. Finally, sowing done on 10 June attained the higher plant height and number of tillers/hill as compared to 20 and 30 June sowing. The dry matter accumulation was maximum with Karma mahsuri as compared to Mahamaya and MTU-1010. Under different dates of sowing the dry matter accumulation was recorded higher with 10 June sowing at 65-85 days after transplanting over 20 and 30 June sowing. The crop growth rate and relative growth rate was higher with MTU-1010 as compared to Karma mahsuri and Mahamaya while under dates of sowing it was recorded maximum under 10 June sowing at 95-110 days after transplanting over 20 and 30 June sowing.

Table 1. Effect of different date of sowing on phenology of rice varieties

Sowing Dates	Crop growth stages								
	Seedling	Tillering	Panicle Initiation	Booting Stage	Panicle Emergence	50% Flow.	Milking	Dough	Maturity
V_1-Karma mahsuri									
D_1-10 June	25	50	72	86	97	105	113	122	138
D_2-20 June	25	49	71	86	96	102	109	118	134
D_3-30 June	25	48	68	84	92	99	106	115	130
V_2-Mahamaya									
D_1-10 June	25	44	65	79	89	96	104	113	129
D_2-20 June	25	42	63	78	88	95	102	110	126
D_3-30 June	25	41	63	76	85	91	98	106	122
V_3-MTU-1010									
D_1-10 June	25	40	59	71	81	88	95	103	120
D_2-20 June	25	38	56	68	78	85	91	98	116
D_3-30 June	25	37	54	66	76	83	88	95	113

Table 2. Plant height (cm) of rice varieties at 15 days interval under different sowing dates

Sowing dates	**Days after transplanting**							
	20	35	50	65	80	95	110	Maturity
V_1-Karma mahsuri	42.6	58.1	81.3	98.7	105.5	106.5	107.6	107.7

V_2 -Mahamaya	44.9	59.7	80.6	94.8	111.5	112.5	116.2	118.7
V_3 -MTU-1010	46.3	60.9	80.1	107.4	113.0	113.2	114.5	114.5
S Em±	0.3	1.4	0.9	1.6	1.0	0.8	1.0	1.0
CD (P=0.05)	1.0	NS	NS	4.8	2.9	2.4	3.0	3.0
D_1 -10 June	48.1	58.7	85.1	104.4	114.2	115.2	115.9	115.9
D_2 -20 June	43.3	67.2	79.9	100.9	110.7	111.1	113.1	113.1
D_3 -30 June	42.3	52.8	76.9	95.6	105.3	106.7	106.8	111.9
S Em±	0.3	1.4	0.9	1.6	1.0	0.8	1.0	1.0
CD (P=0.05)	1.0	4.3	2.7	4.8	2.9	2.4	3.0	3.0
VXD								
S Em ±	0.6	2.5	1.5	2.8	1.7	1.4	1.7	1.7
CD (P=0.05)	1.7	NS	4.6	8.3	4.9	4.2	5.1	5.2
CV (%)	2.3		3.3	4.8	2.6	2.2	4.0	2.6

Table 3. Number of tillers/hill of rice varieties at 15 days interval under different sowing dates

Sowing dates	**Days after transplanting**							
	20	35	50	65	80	95	110	Maturity
V_1- Karma mahsuri	5.5	10.1	11.8	12.1	9.1	8.8	8.6	8.3
V_2- Mahamaya	5.6	9.2	9.5	9.0	7.7	7.8	7.9	7.1
V_3- MTU-1010	5.6	8.5	9.7	10.5	7.6	7.7	7.3	7.3
S Em±	0.2	0.4	0.6	0.4	0.4	0.3	0.2	0.3
CD (P=0.05)	NS	NS	1.7	1.2	1.3	0.9	0.6	0.8
D_1 -10 June	5.5	9.4	12.0	11.9	9.2	9.3	8.9	8.4
D_2 -20 June	6.3	10.0	9.8	8.5	8.1	7.8	8.1	7.3
D_3 -30 June	4.9	8.3	9.2	11.2	7.2	7.2	7.7	7.0
S Em±	0.2	0.4	0.6	0.4	0.4	0.3	0.2	0.3
CD (P=0.05)	0.6	1.3	1.7	1.2	1.3	0.9	0.6	0.8
VXD								
S Em±	0.3	0.4	1.0	0.7	0.7	0.5	0.3	0.5
CD (P=0.05)	0.6	0.7	NS	NS	2.2	1.6	1.0	1.6
CV (%)	10.6	14.0			15.6	11.3	10.4	9.4

Table 4. Dry matter production (g/m^2) of rice varieties at 15 days interval under different sowing dates

Sowing dates	**Days after transplanting**							
	20	**35**	**50**	**65**	**80**	**95**	**110**	**Maturity**
V_1-Karma mahsuri	40.1	169.7	313.3	594.7	912.3	1263.7	1482.0	1550.3
V_2-Mahamaya	46.2	207.1	409.9	650.7	883.3	1176.4	1354.6	1432.7
V_3-MTU-1010	44.2	210.2	391.5	587.8	809.5	1114.2	1383.8	1383.8
S Em±	1.4	11.2	12.8	15.3	14.5	22.4	22.8	26.4
CD (P=0.05)	4.2	33.7	38.4	45.9	43.6	67.3	68.5	79.3
D_1 -10 June	46.8	177.4	353.9	581.6	858.2	1269.8	1504.2	1514.9
D_2 -20 June	44.1	239.0	441.2	665.3	902.7	1189.7	1401.5	1461.1
D_3 -30 June	39.6	170.5	319.6	586.3	844.3	1094.8	1349.2	1390.8
S Em±	1.4	11.2	12.8	15.3	14.5	22.4	22.8	26.4
CD (P=0.05)	4.2	33.7	38.4	45.9	43.6	67.3	68.5	79.3
VXD								
S Em ±	2.4	19.5	22.2	26.5	25.2	38.9	39.6	45.8
CD (P=0.05)	7.3	58.4	66.5	79.5	75.4	116.5	118.6	137.3
CV (%)	9.7	17.2	10.3	7.5	5.0	5.7	7.2	5.5

Table 5. Crop growth rate (g/m^2/day) of rice varieties at 15 days interval under different sowing dates

Sowing dates	**Days after transplanting**							
	0-20	20-35	35-50	50-65	65-80	80-95	95-110	Maturity
V_1- Karma mahsuri	2.01	8.64	9.57	18.76	21.17	23.43	14.55	4.55
V_2- Mahamaya	2.31	10.72	13.52	16.06	15.51	19.54	11.88	5.20
V_3 - MTU-1010	2.21	11.06	12.09	13.08	14.78	20.31	17.98	0.00
S Em±	0.17	0.71	0.45	0.55	0.78	0.95	1.11	1.41
CD (P=0.05)	0.21	NS	1.36	1.66	2.33	2.85	3.33	2.00
Date of sowing								
D_1 10 June	2.34	8.71	11.77	15.18	18.44	27.44	15.63	0.71
D_2 20 June	2.21	12.99	13.48	14.94	15.83	19.14	14.12	3.97
D_3 30 June	1.98	8.73	9.94	17.78	17.20	16.70	16.96	2.77
S Em±	0.17	0.71	0.45	0.55	0.78	0.95	1.11	1.41
CD (P=0.05)	0.21	2.14	1.4	1.66	NS	2.85	NS	2.00
VXD								
S Em±	0.12	1.24	0.79	0.96	1.35	1.65	1.92	2.45
CD (P=0.05)	0.47	3.71	2.36	2.88	NS	4.94	5.72	NS
CV (%)	9.73	21.13	11.52	10.68		12.95	38.61	

Table 6. Relative growth rate (g/g/day) rice varieties at 15 days interval under different sowing dates

Sowing dates	Days after transplanting							
	0-20	20-35	35-50	50-65	65-80	80-95	95-110	Maturity
V_1- Karma mahsuri	0.185	0.096	0.041	0.043	0.029	0.022	0.011	0.004
V_2- Mahamaya	0.192	0.100	0.046	0.031	0.020	0.019	0.009	0.004
V_3 - MTU-1010	0.189	0.104	0.041	0.027	0.021	0.021	0.014	0.003
S Em±								
	0.002	0.003	0.002	0.002	0.001	0.001	0.002	0.001
CD (P=0.05)	NS	NS	NS	0.005	0.004	NS	NS	0.003
Date of sowing								
D_1 10 June	0.192	0.089	0.046	0.033	0.026	0.026	0.011	0.005
D_2 20 June	0.189	0.113	0.041	0.027	0.020	0.018	0.011	0.007
D_3 30 June	0.184	0.097	0.042	0.040	0.024	0.017	0.014	0.005
S Em±	0.002	0.003	0.002	0.002	0.001	0.001	0.002	0.001
CD (P=0.05)	0.005	0.010	NS	0.005	0.004	0.003	NS	0.003
VXD								
S Em±	0.003	0.006	0.004	0.003	0.002	0.002	0.003	0.002
CD (P=0.05)	0.009	0.018	NS	0.008	0.007	0.006	0.010	NS
CV (%)	2.738	10.579		13.611	17.074	16.980	51.033	

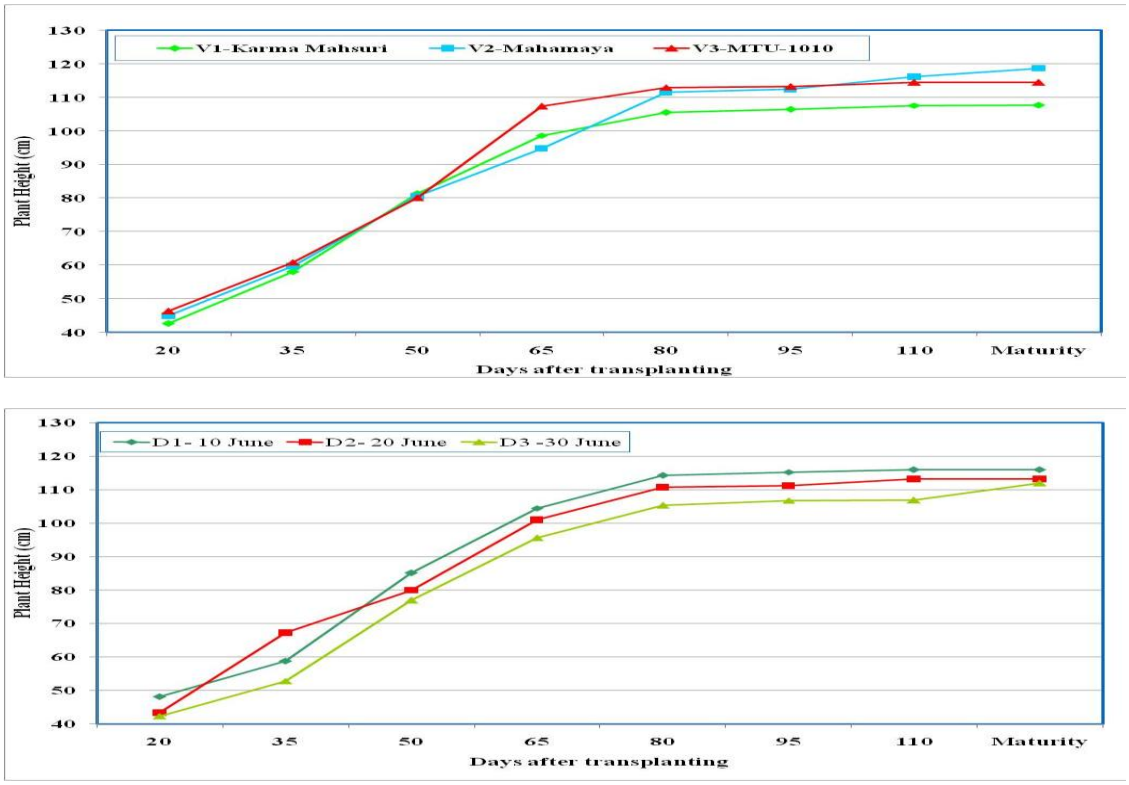
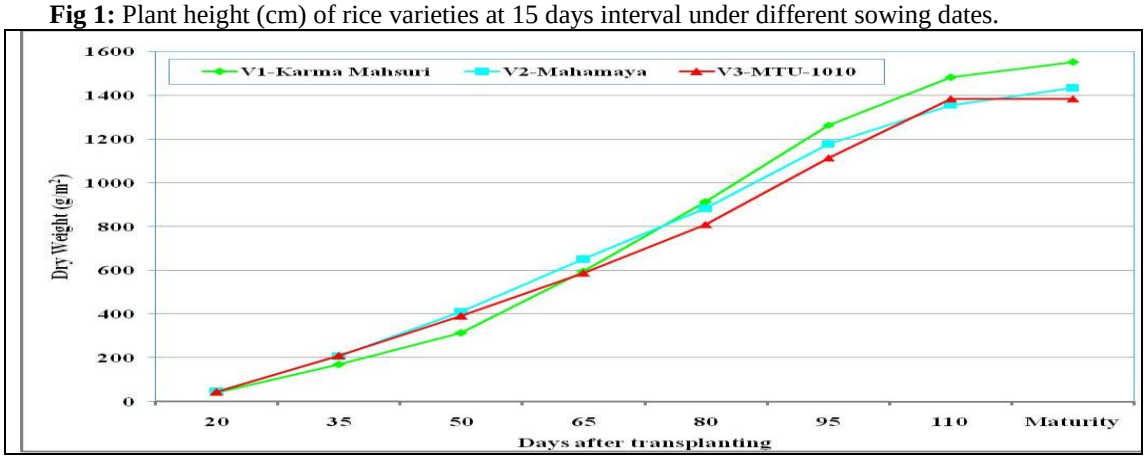


Fig 1: Plant height (cm) of rice varieties at 15 days interval under different sowing dates.



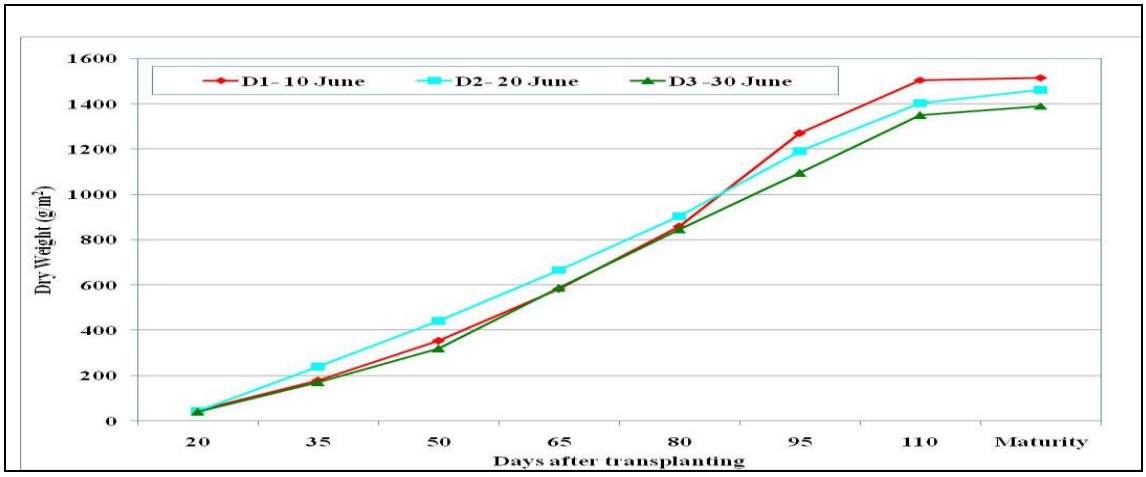


Fig 2: Dry matter production (g/m^2) of rice varieties at 15 days interval under different sowing dates.

REFERENCES

Anonymous (2010). State level Agricultural statistics, Directorate of Agriculture, Raipur (C.G.).

Anonymous (1997). Annual Progress Report (AICRPAM). Department of Agricultural meteorology, N.D. University of Agricultural and Technology. Kumarganj, Faizabad (U.P.): 17-18.

Chandel, S.R.S. (1984). A hand book of Agricultural Statistics. Achal Prakashan Mandir, Kanpur (U.P.) India. Pp: 149-318.

Dakhore, K. K. (2003). Effect of light and thermal regimes on growth, development and yield of rice crop under Raipur condition. M.Sc. (Ag.) thesis submitted to IGKV, Raipur.

Kumar, K.V. and Subramanian, S. (1991). Influence of meteorological parameters on growth and yield of lowland rice. *Oryza*, **28**(1): 49-54.

Leosold, A.C. and P.E. Kriedemann (1975). The dynamics of growth, In plant growth and development. Tata Mcg raw Hill publishing co. Ltd. New Delhi Publication second edition pp (77-105).

Om Hari, Katyal, S.K. and Dhiman, S.D. (1996). Effect of transplanting schedule on leaf area index and dry matter production in different rice hybrids. *Haryana Journal of Agronomy*, **12**(2): 146-150.

Panse, V.G. and Sukhatme P.V. (1967). statistical method for Agricultural workers. Second Edition Indian Council of Agricultural Research, New Delhi.

Singh, R.S. and Singh, S.B. (1999). Effect of age of seedlings, N-levels and time of application on growth and yield of rice under irrigated condition. *Oryza*, **36**(4): 351-354.

RESEARCH ARTICLE

EFFECT OF GLYPHOSATE HERBICIDE ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF *VIGNA MUNGO* L.

Megha Singhal, **Ashok Kumar* and Kuldeep Kumar**

Department of Botany, CCS University Campus, Meerut
Email: dr.ashokbotany@gmail.com

Received-12.05.2017, Revised-06.06.2017

Abstract: A field experiment was conducted to evaluate the effect of Glyphosate on different physiological and biochemical parameters of *Vigna mungo* L. The results obtained from this study revealed that the low amount of application of glyphosate (50 ppm and 100 ppm) of glyphosate have stimulatory effect on plant growth but adversely affect the growth parameters at higher concentration (>100 ppm). At higher concentration glyphosate decrease the protein, chlorophyll and leghaemoglobin contents of plants and interrupt the *Rhizobium*-legume symbiosis. Hence, the present study can conclude that glyphosate in the limited amount (50ppm and 100ppm) can enhance the productivity of plant *Vigna mungo* L.

Keywords: Glyphosate, *Vigna mungo* L., *Rhizobium*, Herbicides, Weed control methods

INTRODUCTION

Legumes belong to an important family Fabaceae (legume family) of flowering plants (angiosperms) with more than 650 genera and 18000 species. It is a large and economically important family. The name Fabaceae comes from the genus *Faba* now included into *Vicia*. Leguminosae is an older name still considered valid (Burkitt *et al.*, 1985), and refers to the typical fruit of these plants, called legumes.

Legumes are second only to cereals as a source of nutrition for humans and animals (Erdman and Fordyce, 1989).Legumes as food has high protein contents and used as food worldwide. On a worldwide scale, legumes provide 22% protein, 32% fat and oil, and 7% carbohydrates in terms of human nutrition. In terms of livestock nutrition, they provide 38% protein, 16% lipids and 5% carbohydrates. The beneficial effect of legumes in agriculture has been recognized even before the principles of crop rotation were established (Herridge, 1982)

India is the largest producer and consumer of *Vigna mungo* L. It occupies a unique position in Indian agriculture. Among the pulses, it stands fourth in production and acreage (Deepalakshmi, *et al.*, 2004). India produces about 1.5 million tons of Urd annually from about 2.5 million hectares of area with an average productivity of 400 kg per hectare. It contains the perfect combination of all nutrients, which include proteins (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins (Karamany, 2006).

Weeds are one of the major biological constraints in crop production and therefore, their control is important. Pynenburg *et al.* (2011) found that seed yield in common bean (*Phaseolus vulgaris*) gets reduced up to 85% as a result of season-long weed competition. Weeds reduce the yield of legumes crops by competing with legume plants for nutrition, water and space. To overcome such type of problems we use different mechanical, physical and chemical strategies to check or inhibit the growth of weeds. Weed control by chemicals is still the predominant component of weed management.

Herbicides are unique in that they are designed to kill plants. Sufficient high doses kill both crop and weed, while small doses have no effect upon crop and weed. The action of herbicides is usually determined by its chemical and physical properties, its effect on plant metabolism, the types of plant and the environment. Herbicides target key enzyme in the plant metabolic pathway, which disrupts plant food production and eventually kills it. The time and methods of application of herbicide are determined by its mode of action (Tu *et al.*, 2001).

Glyphosate [N-(phosphonomethyl) glycine] is commercially-available products as a white crystalline powder, which is commonly used as a broadleaf herbicide (weed killer), It is often available in liquid formulations (U.S. Environmental Protection Agency, 2005).

Glyphosate is a post-emergent, systemic and non-selective (or broad-spectrum) herbicide used in both agricultural and non-agricultural areas. Recommended application rates do not exceed 5.8 kg active ingredient per hectare. It is used to kill all plant types including grass perennials, and woody plants. It is mainly absorbed into the plant through the leaves and then transported throughout the plant where it acts on the plant's enzyme system. It acts as a potent inhibitor of the shikimic acid pathway for biosynthesis of aromatic amino acids. It is a competitive inhibitor of 5-enolpyruvilshikimate-3-phosphate (EPSP) synthase with respect to phosphoenolpyruvate (PEP) and noncompetitive with respect to shkimate-3-phosphate (S3P) (Coruzzi and Last, 2002).

Glyphosate is moderately persistent in soil, with an estimated average half-life of 47 days (Wauchope *et al.*, 1992; WSSA, 1994). It is strongly absorbed to most soils, even those with lower organic and clay contents, and it is highly soluble in water. Field and

*Corresponding Author

laboratory studies show that it does not leach appreciably, and has the low potential for runoff (except as adsorbed to colloidal matter) (Wauchope *et al.*, 1992).

MATERIAL AND METHOD

The present investigation carried out on the effect of Glyphosate herbicide on *Vigna mungo* (L.) plants which was undertaken with a view to have data from field experiments on the beneficial/adverse effects of Glyphosate herbicide with special emphasis on changes in physio and biochemical properties of *Vigna mungo* (L.). The details of the investigation are described as follow.

Geographical Situation

Meerut district is situated between 290 01N latitudes and 770 45E longitudes at an altitude of 237 meters above sea level. The C.C.S. University is situated at the distance of about 10km from Meerut city railway station and near about 12km on Delhi-Dehradun highway. The total geographical area of Meerut district is 2564 km2. The district falls under the western plain zone of Uttar Pradesh, sub-region of upper Gangetic plain.

Experimental site

Field experiments were conducted during the Kharif season in the month of March to June in 2016 to evaluate the response of Glyphosate herbicide on the Physio- chemical properties and yield of *Vigna mungo* L. The seeds of *Vigna mungo* L. were grown in the field of Botany Department, C.C.S. University, Meerut. The experiments were designed in 10 plots of equal size: (1 control and nine treated with glyphosate herbicide).

Material used

1. IARI certified seeds of PU-31 variety of *Vigna mungo* (L.).
2. Glyphosate herbicide (Round up) brought from IARI New Delhi.

Other Details (Experimental Details):

1. Total no. of blocks- 10
2. Control block- 1
3. Total no. of treated plots- 9
4. Plot size (area of plot) – 500 × 500 cm.

Fifty healthy seeds of *Vigna mungo* L. were sown in every plot. All plots were irrigated with tap water. The seed germination percentages were calculated after counting the difference between germinated (coming out of soil) and non-germinated seeds (remaining inside soil, non emergent).

Figure-A

Figure-B

Figure-C

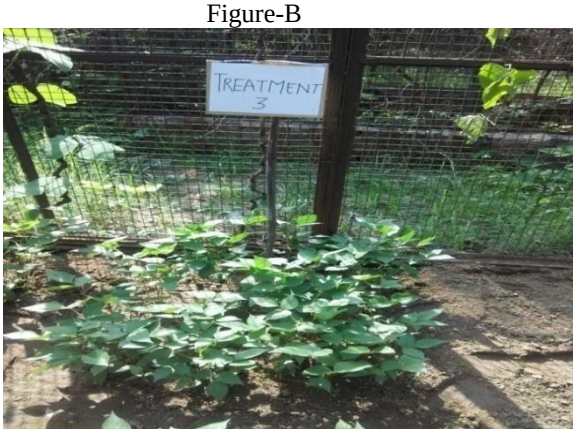


Figure-D

Figure-E

Figure-F

Germination %

The seed germination percentage was calculated by the given formula-

$$\text{Germination percentage} = \frac{\text{Seeds germinated} \times 100}{\text{Total seeds}}$$

Nodulation: Plants from nine plots for each treatment and control were removed 30 days after seeding (DAS) and were observed for the extent of nodulation for observation.

Determination of protein

The protein was estimated by the method adopted by Bradford (1976).The following formula was used for the measurement of protein content-

$$\text{Protien}\left(\frac{\text{Mg}}{\text{g}}\right) = \frac{\text{O.D.} \times \text{Factor} \times \text{Dilution (if any)} \times 1000}{100 \times \text{Total volume/volume of replicate}}$$

Leghaemoglobin content

Leghaemoglobin quantities of the nodules were measured spectrophotometrically as haemochromogen according to the method of **Bergersen (1980).** Leghaemoglobin content was calculated by using the following formula-

$$\text{LB: Protein} = \frac{\text{LB/g fresh weight of nodule X 100}}{\text{Protein/g/ fresh weight of nodules}}$$

Estimationof chlorophyll content

Chlorophyll content was estimated by using **Arnon's method.**For calculation the following formula was used -

$$\text{Chl. a (mg/g f } wt.) = \frac{12.7\ (A663) - 2.69\ (A645) \times V}{1000 \times W}$$

$$\text{Chl. b (mg/g f } wt.) = \frac{22.9\ (A645) - 4.89\ (A663) \times V}{1000 \times W}$$

$$\text{Total Chl. (mg/g f } wt.) = \frac{20.2\ (A645) - 8.02\ (A663) \times V}{1000 \times W}$$

$$\text{Carotenoid} = \frac{7.6\ (A445) - 8.02\ (A663) \times V}{1000 \times W}$$

Where,

V = final volume of chlorophyll extract

A = absorbance at specific wavelength

W = fresh weight of tissue extract

Estimation of total nitrogen (snell and snell, 1954)

Total nitrogen was estimated by the method as suggested by Snell and Snell, (1967).

RESULT AND DISCUSSION

Germination Percentage

It was noticed that application of glyphosate herbicide increases the germination percentage of seeds in 50 and 100 ppm treatments as compared to control and 150ppm (Fig. 1). According to Cavusoglu *et al.* (2011) 100, 250 and 500 mg l-1 doses of glyphosate caused 24%, 40% and 60%, reduction in seed germination and the root length of *Allium cepa*. Similar results were also reported by Zaidi *et al.* (2005) that Glyphosate and metribuzin herbicide (2 g a.i. kg–1) applied pre-emergence completely reduced the germination percentage. Failure to germinate at a higher concentration of metribuzin herbicide could be attributed to the rupturing of seed testa and damage to the cell membranes, leading to the efflux of nutrients and other cellular contents. Similar evidence of phytotoxic activity of the pre-emergent application of herbicides on germination and plant growth of soybean has previously been reported (Rennie and Dubetz, 1984).

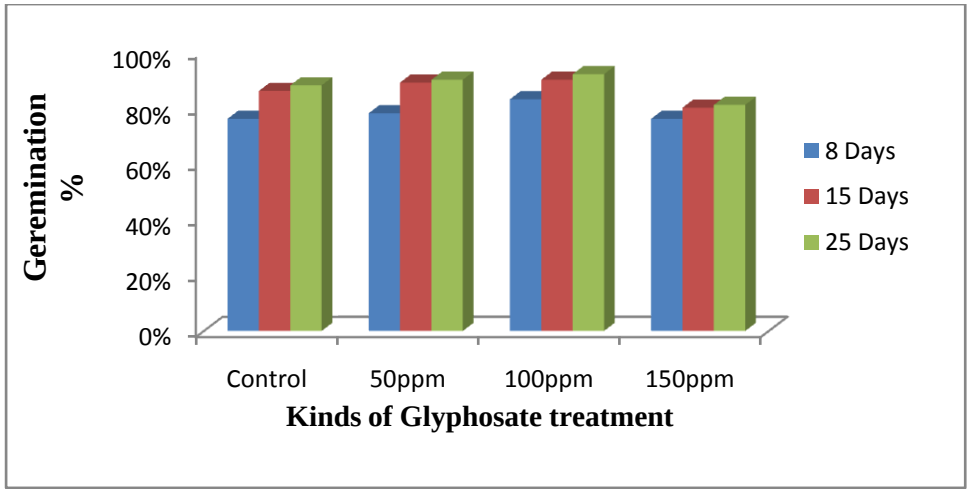


Figure-1. Effect of glyphosate on the germination percentage of *Vigna mungo* (L.) after 30 days of sowing

Nodulation: Maximum no. of nodules were found in the case of 100ppm solution treatment as compare to control and other treatments. Besides number, weight (dry and fresh) of nodules was also got affected by the treatments. Maximum weight of nodules (dry and fresh) was found in 100ppm solution treatment. Application of 50ppm and 100ppm solution caused a significant increase in nodule number and weight of nodules(Fig 2&3). Eberbach and Douglas, (1983) in their study found the lack of inhibitory effect on nodulation observed with glyphosate could be due to its rapid inactivation in soils or its rapid translocation, along with photosynthate, to a distant metabolic sink.

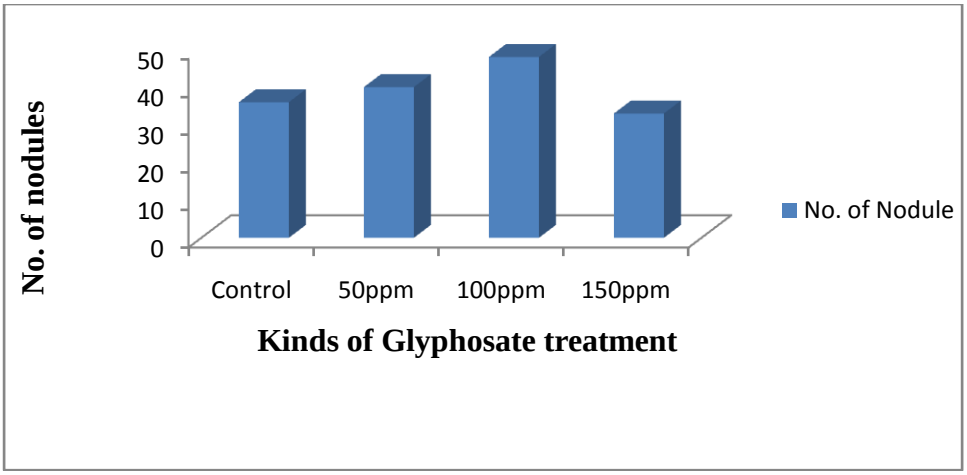


Figure-2. Effect of glyphosate on the Nodule number of *Vigna mungo* (L.) after 30 days of sowing

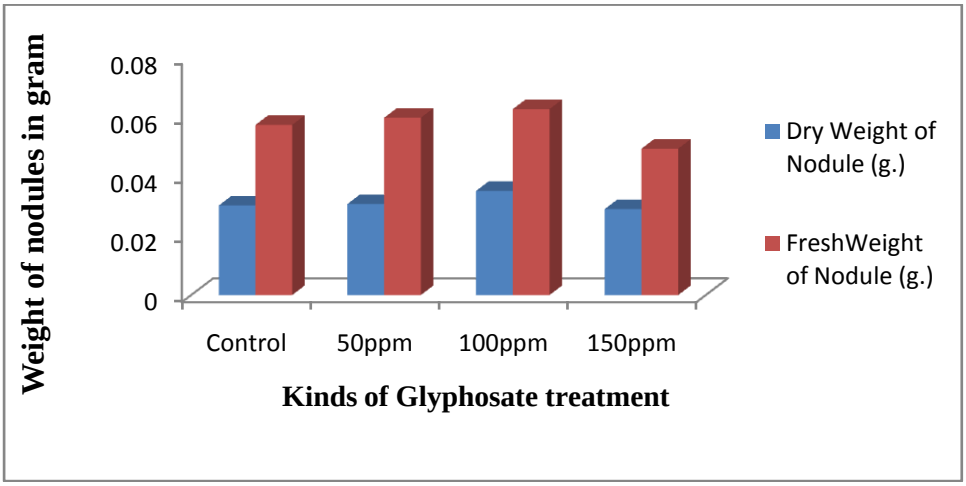


Figure-3. Effect of glyphosate on the Nodule fresh and dry weight of of *Vigna mungo* (L.) after 30 days of sowing

Plant Growth

The plant growth is measured by the length of its root and shoots. Maximum root and shoot length were measured at 100 ppm concentration as compare all other treatments (50 and 150ppm) and control.Application of glyphosate above the 100 ppm level caused a decrease in the plant length (Fig-4). Similarly, Shaban *et al.*, (1987) reported glyphosate was decreasing the plant height of faba bean. They suggested that glyphosate may increase the level of ethylene. Others (Stenley *et al.*, 1973) reported that ethylene inhibits cell division of meristematic tissues and noticed that plants exposed to ethylene-induced inhibition of stem height, so as a result, plant height got decreased when treated with glyphosate.

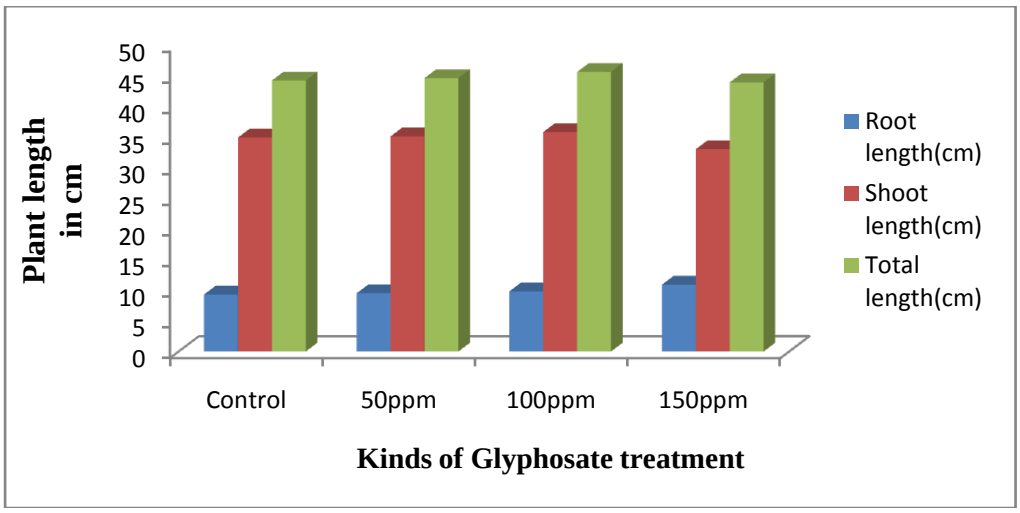


Figure-4. Effect of glyphosate on the growth of *Vigna mungo* (L.) after 30 days of sowing

The fresh and dry weight of root and shoot

Maximum fresh and dry weight of shoot and root of plant. were observed in 50ppm and 100ppm treatment as compared to control and 150ppm solution treatments. Wyszkowska (2002) also referred Treflan and Glyphosate as decreasing the fresh weight of bean plants compared with control and wheat plants compared with control respectively. These results are in agreement with reports which determined that herbicide application to the soil adversely affect physiological characteristics in crop plants. Treflan and Glyphosate caused a significant reduction in shoot dry weight in both bean and wheat plants. Treflan and Glyphosate application to wheat plants caused marked decrease in root fresh weight While, Ridomil increased root fresh weight (Vaughn, and Lehnen. 1991). Fresh and dry weights adversely affected as the duration and concentration of Glyphosate increased.

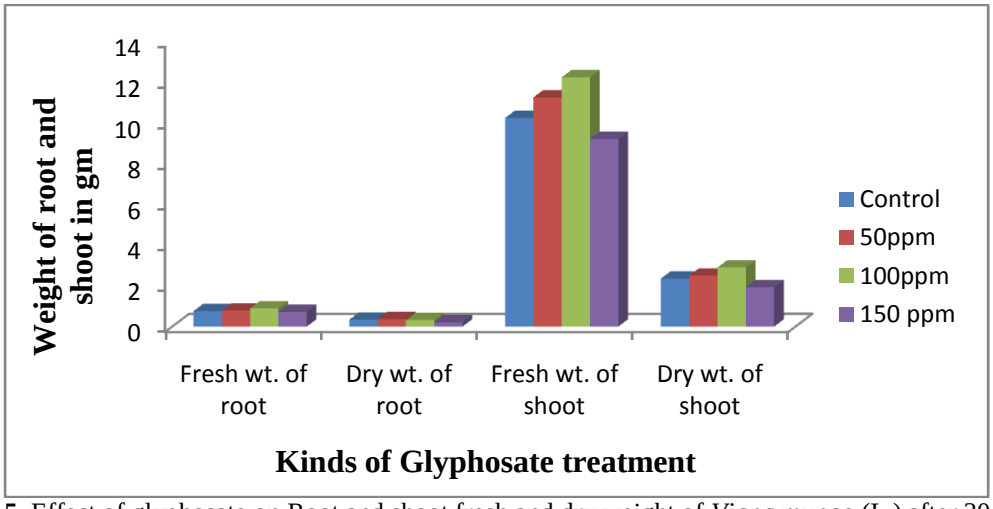


Figure-5. Effect of glyphosate on Root and shoot fresh and dry weight of *Vigna mungo* (L.) after 30 days of sowing

Chlorophyll

Impact of glyphosate was found to increase the level of chlorophyll in first and second treatment and decrease in third treatment as compared to control(Fig-6). Huang *et al.* (2012) also observed the similar results when they applied the different

concentration of glyphosate. Their result showed that Chl a & chl b content were remarkably decreased by increasing the concentration and treatment time of glyphosate. The glyphosate metabolite AMPA (Aminomethylphosphonic acid) can temporarily reduce chlorophyll content (causing yellowing or chlorosis) and photosynthesis in GR(Growth resistant) soybeans. Glyphosate may prevent chlorophyll synthesis indirectly by decreasing the Mg content in leaves, as shown by Cakmak *et al.* (2009), which leads to a decreased chlorophyll content and photosynthetic rate (Zobiole *et al.*, 2012). Indeed, the incorporation of Mg by Mg-chelatase in the porphyrin structure is a necessary step leading to the synthesis of chlorophyll molecules.

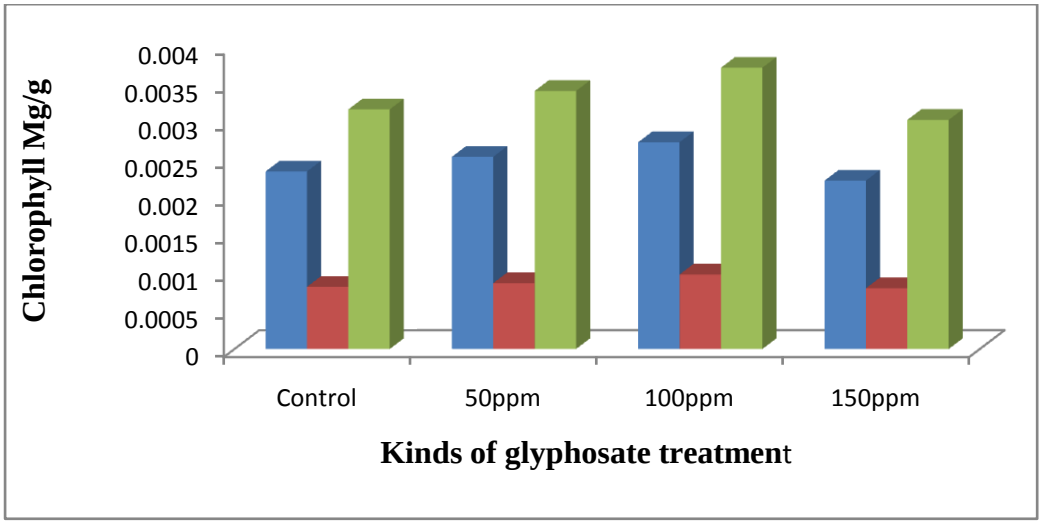


Figure-6. Effect of glyphosate on Chlorophyll content of *Vigna mungo* (L.) after 30 days of Sowing

Nitrogen content

It was noticed that application of glyphosate in limited amount increases the nitrogen content but when concentration increased nitrogen content decreased gradually (Fig-7). Glyphosate has also been observed to induce a similar reduction at higher concentration in Nitrogenase activity & nodulation of soybeans while not affecting legume growth or total plant nitrogen content with low amount of glyphosate applied(Eberbach,1998).Increase the concentration of Glyphosate may induce nutritional disturbances by immobilizing certain nutrients in plants or interfering with their uptake and translocation (Cakmak *et al.* 2009). Glyphosate can also influence N metabolism through direct effects on the rhizobial symbiont or indirectly by affecting the physiology of the host plant. Zaidi *et al.*(2005) observed that nitrogen contents declined with increasing dose rates for Glyphosate and other herbicides individually at both 35 and 60 DAS.

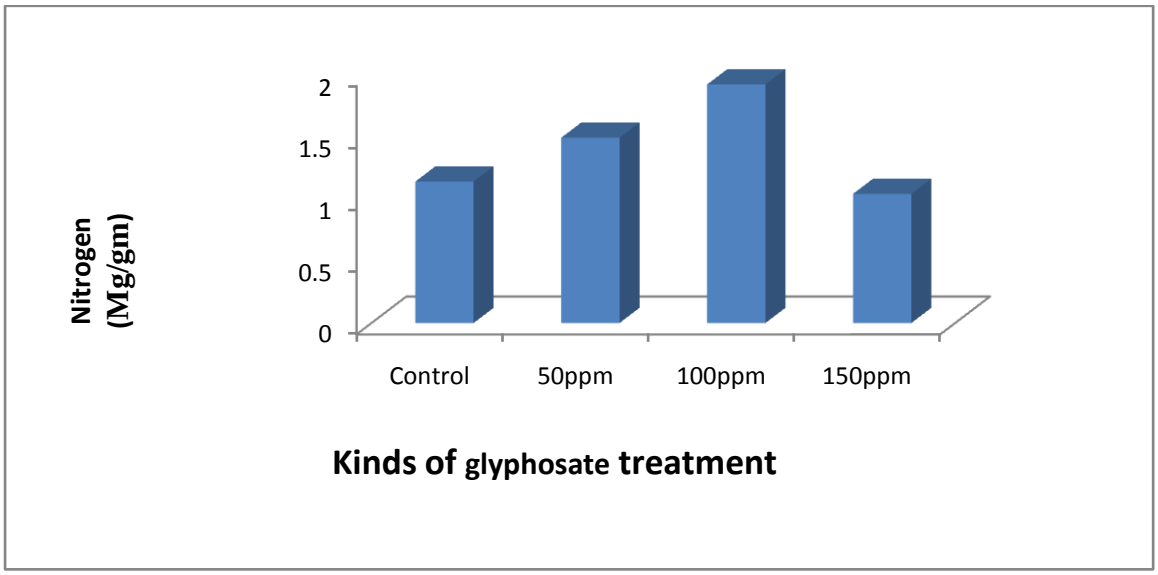


Figure-7. Effect of glyphosate on Nitrogen content of *Vigna mungo* (L.) after 30 days of sowing

Protein content

In this experiment, it was observed that addition of the low amount of glyphosate increases the protein content as compared to control and treatment III but as the concentration of glyphosate increased the protein content of plants significantly reduced (Fig-8). Eberbach(1998) observed that results obtained with plants that resist the phytotoxic effect of herbicides suggest that the resistance observed in the plants may be due to the ability of cells to rapidly detoxify the herbicide at a certain level. After a critical level, the detoxifying capacity of microorganism lost and reduction in growth occur. Reduction in protein content may be due to the higher amount of glyphosate and its direct involvement in inhibition of amino acid synthesis pathway. Glyphosate inhibits an enzyme pathway, the shikimic acid pathway, preventing plants from synthesizing three aromatic amino acids (Phenylalanine, Tryptophan, and Tyrosine). These amino acids are essential for growth and survival of most plants. EPSP (5-enolpyruvylshikimate3phosphate) is the key enzyme inhibited by glyphosate synthase (Cox, 2008). In addition, glyphosate has been proposed to interfere with ALA biosynthesis by controlling the conversion of alpha-ketoglutarate to ALA (ð-aminolevulinic acid) or the condensation of glycine with succinyl-CoA to form ALA and CO2 (Kitchen, 1980). Zaidi et al (2005) also observed the similar result that grain protein is significantly reduced with increased in the concentration of glyphosate.

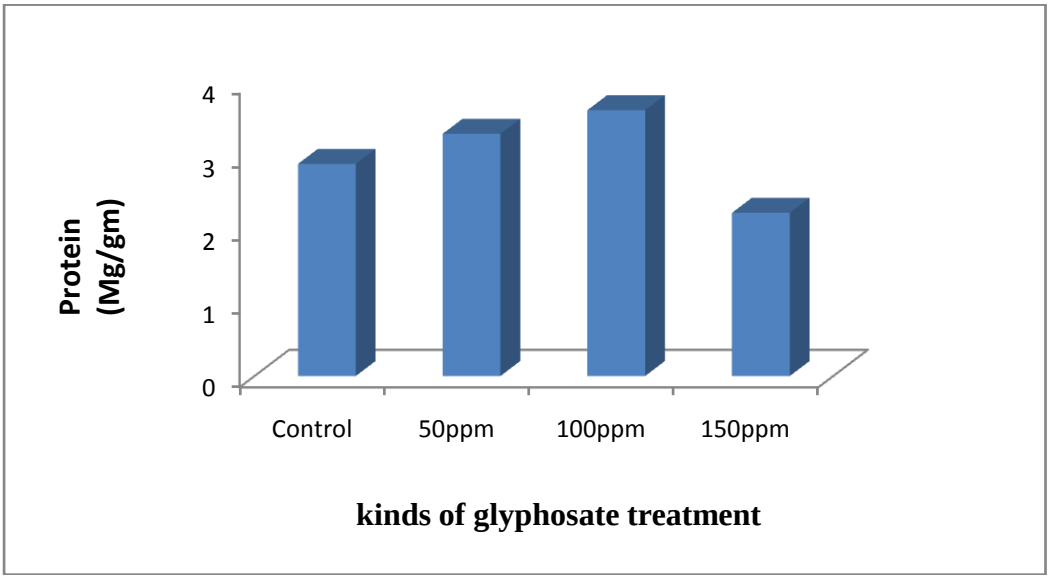


Figure-8. Effect of glyphosate on Protein content of *Vigna mungo* (L.) after 30 days of sowing

Leghaemoglobin content

Impact of glyphosate was found to increases the level of leghaemoglobin in first and second treatment and decreases in third treatment as compare to control (Fig-9).Glyphosate application rapidly stimulates soil microbial activity as measured by C&N mineralization by Haney et al(2000).Glyphosate that reaches the soil surface should be quickly degraded by soil microorganisms without adversely affecting them but this activity is restricted to a limited concentration of this herbicide and high concentration adversely affected the microorganism activity.

Aside from plants, microorganisms also possess EPSPS enzymes and are therefore susceptible to glyphosate (Fischer *et al.*, 1986) at higher concentration. For example, the soybean N-fixing symbiont *Bradyrhizobium japonicum* possesses a GS EPSPS (Glyphosate sensitive 5-enolpyruvylshikimate3phosphate synthase) and accumulates shikimate and hydroxybenzoic acids, such as protocatechuic and gallic acids, upon exposure to glyphosate. This leads to growth inhibition and induces death at high glyphosate concentrations (de María *et al.*, 2006).So glyphosate might impair with nitrogen fixation efficiency of symbiotic bacteria and their symbiosis with host plant which can disturb the formation of leghaemoglobin but further studies are required to explore the impact of Glyphosate on biological nitrogen fixation and leghaemoglobin content of plants.

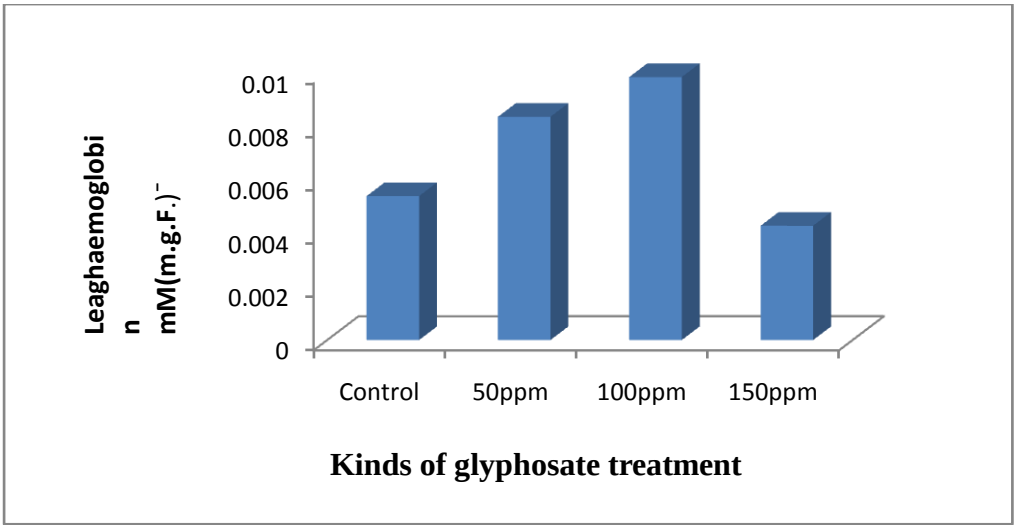


Figure-9. Effect of glyphosate on Leghaemoglobin content of *Vigna mungo* (L.) after 30 days of sowing

CONCLUSION

It can be concluded from the present study that application of glyphosate to certain limit increases the availability of many chemicals/nutrients in soil by killing certain weeds but when the concentration of Glyphosate is increased it adversely affects *Rhizobium* population and interrupt the legume-*rhizobium* symbiosis and biological nitrogen fixation efficiency of legumes. However, Glyphosate can be used to remove the weeds at low concentration but further study is required to explore the actual effect of glyphosate on plant metabolism and growth. Glyphosate can be replaced by using eco-friendly bioherbicides to overcome its adverse effect on soil microflora and plants growth.

REFERENCES

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. *plant physiology*. **24**: 1-15.

Bergersen, F. J., Turner, G. L. (1980). *Journal of General Microbiology*, **118**:235-52.

Breadford, M. M.(1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*: **72**:248-254.

Burkitt, D.P., Trowell,H.C.(1985): Refined carbohydrates in foods and disease, some implications of dietary fiber. *Academic Press New York*.

Cakmak, I., Yazici, A., Tutus, Y., Ozturk, L. (2009) Glyphosate reduced seed and leaf concentrations of calcium, magnesium, manganese, and iron in non-glyphosate resistant soybean. *Eur J Agron*: **31**:114–119

Cavusoglu, K., Yapar, K., Oruc, E., Yalcin, E. (2011). Protective effect of Ginkgo biloba L. leaf extract against glyphosate toxicity in Swiss albino mice. *J Med Food*: **14**: 1263-1272.

Coruzzi G., Last, R.L. (2000). Amino acids. In: Biochemistry and Molecular Biology of Plants. *American Society of Plant Physiologists*; pp. 358–401.

Cox, C.(1998). Glyphosate (Roundup). *J. Pest. Reform.*

Deepalakshmi, A.J., Anandakumar, C.R. (2004). Creation of genetic variability for different polygenic traits in blackgram (Vigna mungo L. Hepper) through induced mutagenesis. *Legume Res* ;**27**: 188-192.

Eberbach, P. (1998). Applying non-steady-state compartmental analysis to investigate the simultaneous degradation of soluble and sorbed glyphosate (N-(phosphonométhyl)glycine) in four soils. *Pesticide Sci*., **52**: 229-240

Eberbach, P.L., Douglas, L.A. (1983) Persistence of glyphosate in a sandy loam soil, Soil Biol. *Biochem.;* **15**: 485–487.

Erdman, J. W. and Fordyce, E. J. (1989) Soy products and the human diet. *Am. J. Clin. Nutr*. **49**: 725-737.

Haney, R.L., Senseman, S.A., Hons, F.M. and Zuberer, D.A. (2000). chemicals in soil. Effect of Roundup on soil microbial activity and biomass. *Weed Sci*.; **9**: 48:89

Herridge, D.F. (1982). Relative abundance of ureides and nitrate in plant tissues of soybean as a quantitative assay of nitrogen fixation. *Plant Physiology*; **70**: 1-6.

Huang, J. (2012). Effects of glyphosate on photosynthesis, chlorophyll fluorescence and physicochemical properties of cogongrass (Imperata cylindrical L.). *Plant Omics Journal* **5:** 177– 183.

Karamany, M.F.E.L. (2006). Double purpose (forage and seed) of mungbean production 1-effect of plant density and forage cutting date on forage and seed yields of mungbean (Vigna radiata (L.) Wilczeck). *Res J Agri Biol Sci* ;**2:** 162–165.

Maria, D., Becerril, N., Garcia-Plazaola, J.I., Hernandez, A., Felipe, M.R., Fernandez-Pascual, M.(2006). New insights on glyphosate mode of

action in nodular metabolism: role of shikimate accumulation. *J. Agric. Food Chem*. **54**: 2621–2628.

Pynenburg, G. M. (2011) The interaction of annual weed and white mold management systems for dry bean production in Canada. *Can. J. Plant Sci.;* **91**(3): 587-598.

Rennie, R.J.; Dubetz, S. (1984). Effect of fungicides and herbicides on nodulation and N2 fixation in soybean fields lacking indigenous *Rhizobium japonicum*. *Agronomy Journal*; **76**: 451-454.

Shaban, S.A., EI-Hattab, A.H .. Hassan, E.H. and Abo-EI Suoud, M.R. (1987). Recovery of faba bean (vicia faba I.) plants as affected by glyphosate. *J. Agron. and Crop Sci*.;**158**: 294-303.

Snell, F.D . and Snell ,C.T.(1967). Colorimetric method of analysis including photometric methods. *Van Nostrand, Inc; Princerton*, New Jersey. **4**: 217-23.

Stanley P. and Burg ,S. P.(1973). Ethylene in Plant Growth. Proceed. *Nati. Acad. Sci.*, **70**(2): 591–597

Tu, C.M. (1994). Effects of herbicides and fumigants on microbial activities in soil. *Bulletin of Environmental Contamination and Toxicology*; **53**:12-17

Vaughn, K.C. and Fuerst, E.P (1985). Structural and physiological studies of paraquat resistant Conyza. *Pestic. Biochem. Physiol.;* **24** : 86-94

Wauchope, R.D. Buttler ,T.M. Hornsby, A.G., Augustijn-Beckers P.W.M., and Burt J.P. (1992). Pesticide Information Profiles. *Extension Toxicology Network.*

WSSA. (1994).Glyphosate In: Herbicide handbook. *Weed science society of America*, Champaign.7th edition, pp-149-152.

Wyszkowska, J. and Kucharski. J. (2004). Biochemical and Physicochemical Properties of Soil Contaminated with Herbicide Triflurotox 250 EC. *Polish J.Envir. Studies*; **13**(2): 223-231.

Zaidi,A , Khan, S., Rizvi, P. Q.(2005). Effect of herbicides on growth, nodulation and nitrogen content of greengram. *Agronomy for Sustainable Development, Springer*; **25** (4):497-504.

Zobiole, L. H. S., Kremer, R. J., Oliveira, R. S. and Constantin, J. (2012). Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean. *J. Plant Nutr. Soil Sci.* **175**:319–330.

RESEARCH ARTICLE

DEVELOPMENT AND PARASITIZATION OF *PHENACOCCUS SOLENOPSIS* TINSLEY (HEMIPTERA: PSEUDOCOCCIDAE) ON *BT* COTTON BY *AENASIUS BAMBAWALEI* HAYAT (HYMENOPTERA: ENCYRTIDAE)

S.R. Pawar*, H.R. Desai, G.R. Bhanderi and C.J. Patel

Main Cotton Research Station, Navsari Agricultural University, Surat-395007 (Gujarat), India
Email: grbhanderi@yahoo.co.in

Received-25.05.2017, Revised-26.06.2017

Abstracts: Studies on development and parasitization potential of *Aenasius bambawalei* Hayat on *Bt* cotton mealybug was carried out at the room temperature of 20.63 ± 0.60 ºC and humidity of 64.81 ± 3.02 per cent during January to February 2011 at bio-control laboratory of Main Cotton Research Station, Surat. The parasitoid, *A.bambawalei* preferred III instar nymphs (av. 51.48 ± 21.55 % parasitism) and newly emerged female adult (av. 38.15 ± 11.81 % parasitism) more compared to II instar nymphs of mealybug (av. 4.93 ± 4.96 % parasitism) for parasitism. The developmental period of *A. bambawalei* (oviposition of egg inside to adult emergence) was 10.29 ± 0.86, 10.49 ± 0.80 and 10.56 ± 0.97 days when female adult parasitoid exposed to II Instar nymphs, III instar nymphs and female adult mealybugs, respectively. Maximum parasitoid recovered on 10 days after exposure in both of the preferred stages of mealybug. *Aenasius bambawalei* was solitary endoparasitoid. Female was found parasitizing the mealybug by inserting ovipositor from the ventral side of the mealybug body. On dissection of the parasitized mealybug, white legless larva without appendages prior to mummy formation of parasitized mealybug and brownish black exarate type pupa within mummified body of mealybug observed under microscope. The single female adult of *A. bambawalei* parasitized on an average of 125 ± 13.2 mealybugs. Maximum parasitism (60.00 %) observed by 7-day old age female wasp when exposed to its preferred host (III instar mealybug). The longevity of female adult of *A. bambawalei* was 11 to 16 (av.13.8 ± 1.76) and of male was 1 to 2 (1.20 ± 0.45) days.

Keywords: *A. bambawalei*, Nymphs, Parasitoid, Parasitism, Mummified, Ovipositor, Exarate

INTRODUCTION

Mealybugs are soft bodied insects belonging to the family Pseudococcidae of the order Hemiptera. About 5000 species of mealybug have been recorded from 246 families of plants throughout the world. Among these, 56 species have been reported from 15 genera of family Malvaceae, including cotton and many other plants of economic importance (Ben Dov, 1994). The first report of invasive species of mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) in 2005, was found causing serious damage to cotton was from Gujarat where it was thought to be undescribed. Solenopsis mealybug, *P. solenopsis* (Central and South zone of India) and papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (South zone), mirid bugs *viz.*, *Creontiades biseratense* Distant, *Hyalopeplus linefer* Walker and *Compylomma livida* Reuter (South zone) and flower bud maggot / gall midge, *Dasineura gossypii* Fletcher (Karnataka in South Zone) have emerged as major pests in cotton and posed serious challenges (Jhala *et al.*, 2008; Dharajothi *et al.* 2010, Kumar *et al.*, 2010, Udikeri *et al.*, 2010). The mealybug feeds on phloem tissue, sucking plant sap and causing leaves to distort yellow and even die. It also produces large amount of honeydew. The pest has spread quickly to cotton growing areas posing a serious problem. The pest is hard to kill as it inhabits concealed locations and even in exposed conditions, the congregation of individuals, protection of late age nymphs and adults by loose, cottony waxy substance on body secreted by the Mealybug and oviposition in waxy ovisacs act as barriers to proper penetration and action of insecticides. Management of this mealybug is difficult due to its wide host range, presence of waxy coating on the body and high reproductive potential (Dhawan *et al.*, 2007). Hence, the biological control with parasitoids is of great importance, since, they have proved their value in checking so many homopteran pests (Anil *et al.*, 2008). In past, natural control has played major role in limiting this pest to a minimal level. The present study aimed to study parasitoid potential for the management of mealybug *P. solenopsis.*

MATERIAL AND METHOD

Host preference and development of *A. bambawalei*

The developmental period and extent of parasitization studied during the peak activity of *A. bambawalei* in the field. For the purpose, twigs having maximum dark brown/ reddish mummies brought to the laboratory from field. These twigs were kept under the glass jar (diameter: 14.5 cm and height: 20 cm) tied with muslin cloth with rubber band and observed for parasitoid emergence. Adults of parasitoids emerged out were observed critically for the separation of male and female parasitoids. Males were small, while the females were larger with shiny black body. To determine the total developmental period of parasitoid, *A. bambawalei*, newly emerged pairs were collected in the glass tube (5 x 1 cm) and were expose to different stages of the

*Corresponding Author

mealybug for studying the developmental period and preference of parasitism. For the purpose, cotton seedlings raised in polythene bags under protected condition of polycarbonate house (free from such parasitism) were used and were allowed for infestation of mealybug by releasing female adults of mealybug. Counted number of individuals of different stages of mealybug (2nd and 3rd instar nymphs and freshly emerged female adults) were allowed to retain on the seedlings separately and rests were removed with fine camel hair brush. Such infested seedlings kept under glass jar covered with fine muslin cloth tied with rubber band. Newly emerged pairs of *A. bambawalei* exposed for 24 hours to different stages of mealy bug separately and allowed for parasitization. In the glass jar, honey streak on a paper strip provided as food for the released parasitoids. The developmental period of *A. bambawalei* worked out from date of exposure to date of emergence of adult from mummified cocoons formed after effective parasitism.

Parasitizing potential of *A. bambawalei*

To study the fecundity, longevity and parasitizing potential of *A. bambawalei*, counted number of 3rd instar mealy bug, being preferred stage for parasitization was used. Each pair of parasitoids was offered counted number of third instar nymphs of mealybug on cotton seedlings daily until death. Number of mealybug parasitized by the individual female observed daily for parasitism and estimated for total parasitization during its entire life span. The record of death of male and female parasitoids maintained.

RESULT AND DISCUSSION

Host preference and development period of *A. bambawalei*

Adult parasitoids (pair) were exposed to different stages of host mealybug (II & III instar nymphs and freshly formed female adults) for studying the preference and development period of *A. bambawalei* within the body of the mealybug, *P. solenopsis*. As far as parasitism of *A. bambawalei* to different stages of mealybug is concerned (Plates 1and2), maximum parasitism (Table1) was observed on III instar nymphs (av. 51.48 ± 21.55 %) and it was followed by newly emerged female adult (38.15 ± 11.81 %), while it was minimum (av. 4.93 ± 4.96 %) on II instar nymphs. Thus, *A. bambawalei* mostly preferred III instar nymphs followed by newly emerged female adult as compared to II instar nymphs of mealybug for parasitism. Maximum parasitoids were emerged on 10th day of exposure as the highest emergence of 2.31, 24.3 and 20.61 out of 5.04,

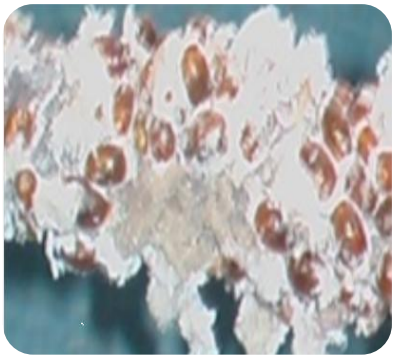
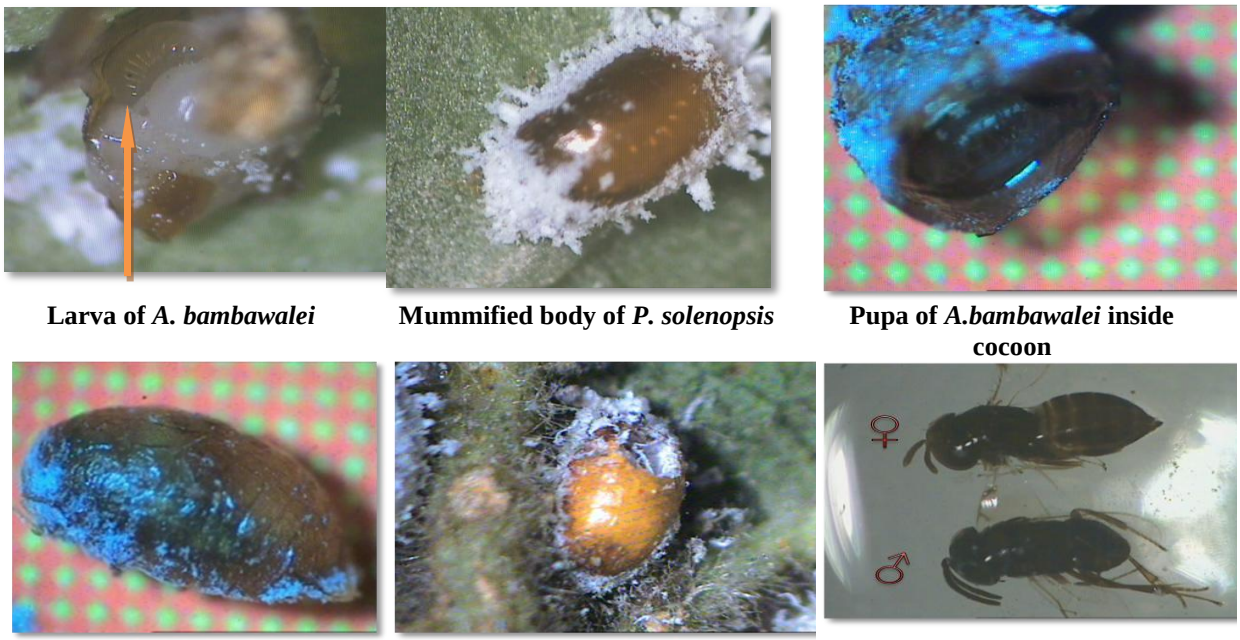


Plate 1: Parasitization of *A. bambawalei* to third instar nymphs of *P. solenopsis*

Plate 2: Parasitization of *A. bambawalei* to female adults of *P. solenopsis*

51.05 and 38.59 per cent of total parasitoids emerged from II instar, III instar and adult mealybug, respectively.

The developmental period of *A. bambawalei* (oviposition of egg inside to adult emergence) was found to be varied from 9 to 12 (av. 10.29 ± 0.86), 9 to 13 (av. 10.49 ± 0.80) and 9 to 13 (av. 10.56 ± 0.97) days when female adult parasitoid exposed to II Instar nymphs, III instar nymphs and adult mealybugs, respectively (Table 1). During entire study, only single adult found to emerge from the single parasitized mealybug in all the stages exposed which clearly indicated the solitary nature of parasitoid. While dissecting the intact body of mealybug within 4 days of exposure for parasitism, white legless larva without appendages observed under microscope while that of from mummified body, on dissecting, brownish black pupa of *A. bambawalei* was clearly observed and it was exarate type (Plate 3). Female found to insert ovipositor from the ventral side of the mealybug body.



Larva of *A. bambawalei* **Mummified body of *P. solenopsis*** **Pupa of *A.bambawalei* inside cocoon**

Exarate pupa of *A. bambawalei* **Emergence hole on cocoon** **Adult from emergence hole**

Plate 3: Development of *A. bambawalei* on *P. solenopsis*

Earlier Ashfaq *et al.* (2010) reported that the mummy formation did not differ significantly when unparasitized third instar mealy bug nymphs and adults analyzed separately. Further, they reported that adult eclosion started 11 days after exposure and continued until 13 days after exposure. The result of the study showed that a significantly higher number of adults emerged on 11th day. Similarity of the present data in this regard with that of above mentioned workers, confirmed the ongoing discussion.

Parasitism potential of *A. bambawalei*

The data presented in Table 2 revealed that the fecundity of *A. bambawalei* was recorded to be 96 to 145 (av. 125.00 ± 13.20) in five different sets having pairs of *A. bambawalei*. 7-day-old age female wasp when exposed to its host III instar mealybug (Figure 1) observed maximum parasitism (60.00 per cent). The longevity of female adult of *A. bambawalei* was 11 to 16 (av. 13.8 ± 2.17) days and of male was 1 to 2 (av. 1.20 ± 0.45) days. The parasitoid development recorded by Pala Ram (2009) which showed that *A. bambawalei* was solitary endoparasitoid taking 12 to 14 days to complete its development in the host and the mating took place soon after the adult emergence. The female oviposited an egg into the mealybug body through ventral surface of the host, which turned into a mummy after 5 to 6 days of oviposition. They also concluded that the third instar mealy bugs were most preferred stage for parasitization and single female parasitized 38 to 163 (fecundity) mealybugs during its lifetime at the rate of 2 to 17 mealybugs per day. The present findings are somewhat similar with these observations.

Table 1. Preference and developmental duration of *A. bambawalei* on *P. solenopsis* reared on *Bt* cotton

Date of release of parasitoid	Number of mealybugs exposed	Number of parasitoid emerged on different days after exposure							Total	Per cent parasitism
		8	9	10	11	12	13	14		
A. Host: II instar nymph										
03-Jan	45	0	0	2	1	0	0	0	3	6.67
03-Jan	50	0	1	3	2	1	0	0	7	14.00
03-Jan	45	0	0	0	0	0	0	0	0	0.00
04-Jan	43	0	0	1	1	0	0	0	2	4.65
04-Jan	47	0	1	2	1	0	0	0	4	8.51
05-Jan	55	0	1	1	1	0	0	0	3	5.45
05-Jan	49	0	0	0	0	0	0	0	0	0.00
06-Jan	45	0	0	0	0	0	0	0	0	0.00
06-Jan	50	0	1	2	1	1	0	0	5	10.00
07-Jan	47	0	0	0	0	0	0	0	0	0.00
Total	**476**	**0**	**4**	**11**	**7**	**2**	**0**	**0**	**24**	**5.04**
Parasitism (%)		**0.00**	**0.84**	**2.31**	**1.47**	**0.42**	**0.00**	**0.00**	**5.04**	**4.93 ± 4.96**
Developmental duration (days)			**9 to 12 (10.29 ± 0.86)**							
B. Host: III instar nymph										
03-Jan	55	0	1	9	4	3	0	0	17	30.91
03-Jan	39	0	3	17	11	6	2	0	39	100.00
03-Jan	59	0	1	12	9	4	1	0	27	45.76
04-Jan	40	0	0	11	7	3	4	0	25	62.50

Date of release of parasitoid	Number of mealybugs exposed	Number of parasitoid emerged on different days after exposure							Total	Per cent parasitism
		8	9	10	11	12	13	14		
04-Jan	45	0	2	12	9	3	1	0	27	60.00
05-Jan	58	0	3	19	10	4	1	0	37	63.79
05-Jan	48	0	0	10	5	2	0	0	17	35.42
06-Jan	46	0	0	9	5	1	1	0	16	34.78
06-Jan	44	0	3	11	6	3	0	0	23	52.27
07-Jan	51	0	0	8	4	2	1	0	15	29.41
Total	**485**	**0**	**13**	**118**	**70**	**31**	**11**	**0**	**243**	**50.10**
Parasitism (%)			**2.68**	**24.3**	**14.4**	**6.39**	**2.27**		**51.05**	**51.48 ± 21.55**
Developmental duration (days)			**9 to 13 (10.49 ± 0.80)**							

Table 1. Preference and developmental duration of *A. bambawalei* on *P. solenopsis* reared on *Bt* cotton (Continued)

Date of release of parasitoid	Number of mealybugs exposed	Number of parasitoid emerged on different days after exposure							Total	Per cent parasitism
		8	9	10	11	12	13	14		
C. Host: Female adult (newly emerged)										
03-Jan	45	0	2	6	4	2	0	0	14	31.11
03-Jan	55	0	3	18	8	3	1	0	33	60.00
03-Jan	47	0	1	12	9	3	1	0	26	55.32
04-Jan	49	0	0	10	5	2	0	0	17	34.69
04-Jan	47	0	1	7	3	1	0	0	12	25.53
05-Jan	55	0	2	14	7	2	1	0	26	47.27
05-Jan	49	0	0	8	6	1	0	0	15	30.61
06-Jan	45	0	0	6	7	1	0	0	14	31.11
06-Jan	50	0	1	9	4	1	0	0	15	30.00
07-Jan	53	0	2	12	5	0	0	0	19	35.85
Total	**495**	**0**	**12**	**102**	**58**	**16**	**3**	**0**	**191**	**38.59**
Parasitism (%)			**2.42**	**20.61**	**11.72**	**3.23**	**0.61**		**38.59**	**38.15 ± 11.81**
Developmental duration (days)			**9 to 13 (10.56 ± 0.97)**							

Table 2. Parasitism potential of *A. bambawalei* on *P. solenopsis* (III instar) reared on cotton at MCRS, Surat

Age	No. of parasitized mealybug at different age of female parasitoid (out of 25 III instar mealybug exposed)																	Total fecundity	Longevity (days)	
Pair No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		Female	Male
1	8	10	12	14	13	15	18	12	11	9	7	6	5	3	1	1	-	145	16	1
2	8	9	11	13	14	16	13	11	9	7	5	3	2	-	-	-	-	121	13	2
3	11	12	13	11	12	14	15	13	11	8	6	4	2	-	-	-	-	132	13	1
4	10	9	10	8	9	12	17	16	13	9	6	5	3	2	1	1	-	131	16	1
5	9	11	10	12	11	13	12	7	6	4	1	-	-	-	-	-	-	96	11	1
Total	**46**	**51**	**56**	**58**	**59**	**70**	**75**	**59**	**50**	**37**	**25**	**18**	**12**	**5**	**2**	**2**	**-**	625	-	-
Average (± SD)	9.2	10.2	11.2	11.6	11.8	14	15	11.8	10	7.4	5	3.6	2.4	1	0.4	0.4		125 ± 13.8	13.8 ± 2.17	1.20 ± 0.45

Parasitism (%)	36.8	40.8	44.8	46.4	47.2	56	60	47.2	40	29.6	20	14.4	9.6	4	1.6	1.6		500		

Note: Each set of exposed mealybugs were critically observed for adult emergence daily after 8th day of exposure

Fig. 1: Parasitizing efficiency of *A. bambawalei* at different age after emergence

ACKNOWLEDGEMENT

We acknowledge with thanks to Dr. Sushila Joshi, Insect Identification Service, Division of Entomology, New Delhi for prompt identification of mealybug parasitoid, *Aenasius bambawalei* Hayat (08.02.2010) as well as to Research Scientist (Cotton) for providing necessary facilities under the RKVY project, "Community Approach for Implementation of IPM for Mealybug in Cotton"

REFERENCES

Anil, K., Kurtadikar, J. S., Wadnerkar, D. W. and Nemade, P. W. (2008). Studies on the safety of pesticides to grapevine mealybug predator, *Cryptolaemus montrouzieri* Aiyar. *Pestology*, **32** (4):17–27.

Ashfaq, M., Ghulam, S. S., Ali, R. N., Shahida P. A. and Shahid M. (2010). Report of a parasitic wasp (Hymenoptera: Encyrtidae) parasitizing cotton mealybug (Hemiptera: Pseudococcidae) in Pakistan and use of PCR for estimating parasitism levels. *Biocontrol Sci. and Tech.*, 20(6): 625-630.

Ben-Dov, Y. (1994). *A systemic catalogue of the mealybugs of the World*. Intercept Limited, UK, 686 p.

Dharajothi, B., Surulivelu, T., Sonai Rajan, T., Manjula, R. and Kumaran, N. (2010). Occurrence and seasonal dynamics of mealy bug, mirid bug and predators on cotton in Coimbatore district of Tamil Nadu. National Conference on Paradigm Shift in Cotton Research and Cultivation held on October 19-21, 2010 at Surat. Compendium of Abstracts. pp-12.

Dhawan, A. K., Singh, K., Saini, S., Mohindru, B., Kaur, A., Singh, G. and Singh, S. (2007). Incidence and Damage Potential of Mealybug, *Phenacoccus solenopsis* Tinsley, on Cotton in Punjab. *Indian J. Ecol*., **34**(2): 166-172.

Jhala, R. C., Bharpoda, T. M. and Patel, M. G. (2008). Occurrence of Mealy Bugs, *Phenacoccus solenopsis* Tinsley and *Phenacoccus solani* Ferris (Hemiptera: Pseudococcidae) on Cotton in Gujarat. *Insect Environ.*, **13**(4): 149-150.

Kumar, V., Maisuria, I. M., Patel, C. J., Desai, H. R., Sheth, D. B. and Seema Bhaduriya (2010). *Eco-friendly Management of Mealybug in Cotton*, Main Cotton Research Station, NAU, Athwa Farm, Surat 395 007.

Pala, Ram., Saini, R. K. and Vijaya (2009). Preliminary studies on field parasitization and biology of solenopsis mealy bug parasitoid, *Aenasius bambawalei* Hayat (Encyrtidae: Hymenoptera). *J. Cotton Res. Dev.*, **23**(2): 313-315.

Udikeri, S. S., Patil, S. B., Guruprasad, G. S., Vandal, N., Kundari, V., Kranthi S. and Sharma, R. M. (2010). Potential threat of mirid bugs and flower bud maggots for *Bt* cotton cultivation: status / report and case studies from Karnataka. National Conference on Paradigm Shift in Cotton Research and Cultivation held on October 19-21, 2010 at Surat. Compendium of Abstracts. pp-6.

RESEARCH ARTICLE

IN VITRO BIO-EFFICACY OF ENTOMOPATHOGENIC FUNGI *BEAUVERIA BASSIANA* (BALS.) VUILL., AGAINST GRAM POD BORER, *HELICOVERPA ARMIGERA* HUBNER ON CHICKPEA

Yaspal Singh Nirala*, V.K. Dubey, J.L. Ganguli and R.K.S. Tiwari

Department of Entomology, College of agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012, Chhattisgarh, India
Email: ypsnirala@gmail.com

Received-15.06.2017, Revised-26.06.2017

Abstract: The present study was conducted at Bio-control lab, Department of Entomology, College of agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur during 2015-16 and 2016-17. The results of *in vitro* experiments revealed that the 2-3rd instars larvae of *Helicoverpa armigera* susceptible to different doses of *Beauveria bassiana*. Mortality of larvae was started after 2-3 day of treatment. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 0.00-75.00% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 0.00-45.00%, T_2 (*B. bassiana* @2500g/ha) 0.00-32.00% and T_1 (*B. bassiana* @2000g/ha) 0.00 to 15.00 % but superior than control T_7 (0.00 %) in both the year.

Keywords: Chickpea, *Helicoverpa armigera*, *Beauveria bassiana*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) belongs to the family Leguminosae and commonly known as Chana, Bengal gram or Garbanzo, is mainly used for human consumption and also a small proportion forms the part of animal and poultry feed. It has one of the highest nutritional compositions as of any dry edible legume and is not reported to contain any specific major anti-nutritional factors. In India, during 2014-15 Bengal gram production area was 8250.5 (In ' 000 Hectare), production 7331.8 (In ' 000 Tonne) tones and productivity 889 (In Kg./Hectare). In Chhattisgarh during 2014-15 the production area was 280.6 (In ' 000 Hectare), production 267.6 MT (In '000 MT) and productivity 1035 (In Kg./Hectare) (Anonymous, 2016). The potential seed yield of about 5 t/ha has been reported in chickpea. However, the realized seed yield hovers around 850 kg/ha which has stagnated over the years. Series of biotic and abiotic stresses reduce the yield and yield stability leaving room only marginal for improvements and the key biotic constraint is the pod borer, *Helicoverpa armigera* Hubner.

The gram pod borer, *Helicoverpa armigera* Hubner, (Lepidoptera: Noctuidae) is a major threat to intensive agriculture (Sigsgaard, 2002). It is one of the most destructive and cosmopolitan insect pests of field crops worldwide and is highly polyphagous causing severe damage to a wide range of food, oil, fodder, vegetables, horticultural, ornamental, aromatic and medicinal plants (Neoliya, 2007, Kontsedalov *et al.* 2012). Yield losses due to this pest in chickpea may range from 70 to 95% (Prakash *et al*., 2007). Due to its wide host range, production of multiple generations per year, high fecundity, migratory behavior and pronounced resistance to many insecticides, the control up to desired level has become difficult (Mccaffery *et al.* 1998). Moderate to high level of resistance to conventional insecticides such as (chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids) as well as to neonicotinoids pesticides and Insect Growth Regulator (IGR's) has been reported in field populations of *H. armigera* (Nauen and Bretschneider 2002). Chemical control is the most commonly used method in insect pest management. Due to adverse effects on non-target organisms, toxicity to mammals and birds and the risk of environmental pollution, chemical control measure should be replaced by the other environmentally friendly control methods to refrain from consumption and other ways it can be replaced (Fields, 1998). Therefore, with the current urgent and conflicting goals of reduced pesticide usage while maintaining adequate agricultural production, microbial control agents with selectivity and a low environmental impact could become ideal components of integrated pest management programs (IPM) in this century (Lacey and Goettel, 1995). The entomopathogenic fungus has been used for biological control of pests to reduce pesticide usage. More than 700 fungal species from about 90 genera are pathogenic to insects (Hong, 2003). Under natural conditions, these pathogens are frequent and often cause natural mortalities of insect populations.

The entomopathogenic hyphomycetes fungi have great potential as biological control agents against insect pests and are used as an important component in integrated pest management systems, particularly *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metch) Sorok, *Verticillium lecanii* Zimmeman and *Nomuraea rileyi* (Farlow) Samson have been found to be promising in the control of several agriculturally important insect pests and are facultative pathogens (Lingappa *et al.*, 2005). Among the entomopathogenic fungi *B. bassisna* (Balsamo) Vuill, (Deuteromycetes:

*Corresponding Author

Hyphomycetes) belonging to sub division Deuteromycotina grows naturally in soils throughout the world and acts as a parasite on various arthropod species, causing white muscardine disease. Over 200 species of insects in nine orders, mainly lepidoptera, coleoptera and hemiptera have been recorded as hosts (Li and Yang, 1988). *B. bassiana* are characterized by having conidiophores consisting of whorls and dense clusters of sympodial, short and globose or flask shaped conidiogenous cells with apical denticulate rachis and one celled conidia. This fungal biopesticide receives more attention because of its more eco-safe, eco-friendly environmental control measures. Its spores may be formulated and applied in a similar way as chemical pesticides and, therefore, could be adopted as a new technology. This includes oil based, dust, powder formulations and ultralow volume application, cheap to produce and may provide low cost control (Langle, 2006).

MATERIAL AND METHOD

Rearing of the insects

Gram pod borer, *Helicoverpa armigera* (Hubner) larvae were collected from nearby fields and brought into the laboratory for further rearing. Larvae were reared individually in plastic tube/plastic disposal cup with cap/plastic petriplates (size,9cm diameter)/glass petriplates (size, 9 cm diameter) on artificial diet (Krishnareddy and Hanur, 2015, Ahmed *et al.* 1998) to get pure line culture. Freshly emerged larvae from pure line culture were used for experimental purpose (Namasivayam, *et al.* 2015).

Experimental Protocols

The experiment was conducted with 7 treatments including control and in each replication 10 larvae (2^{nd} - 3^{rd} instars) were used. The experiment was replicated four times. Commercial formulation of 1.15% WP ($1x10^8$ cfu/g min) of *B. bassiana,* was used in this experiment. The observations were recorded at 24 hrs interval upto eight (8) days. All experiments were conducted in BOD having optimum temperature and humidity ($25\pm 2\ ^0$C and 70 ± 10% RH). Calculated amount (ml) of each fungal suspension ($1x10^8$ cfu/g) was taken in a petridish/plastic beaker 1000 ml size 15cm length and 12cm diameter and 2^{nd}- 3^{rd} instars larvae was treated by leaf dip- sprays method. One to two pieces of chickpea leaves cut into 5 cm-7 cm pieces were dipped in fungal conidial suspension for contaminated both sides of the leaf surfaces then left on a laboratory bench to allow to dry for 2–3 min under aseptic conditions in laminar air flow.

Ten larvae were then placed on the conidia treated leaves in each petridish then also spray conidial suspension on larvae using a potters sprayer (agriculture hand sprayer) and allowed to feed for 24 h, after which the remains of the leaf material and frass were carefully removed. The larvae were then provided with unsprayed leaves. The control larvae (10 larvae per exposure) were sprayed sterile distilled water (Tefera and Pringle, 2003, Elizabeth *et al.,* 2008, Senthamizhlselvan, 2010). The dead larvae were surface sterilized by sodium hypochlorite and placed on petridish lined with moist filter paper. These petridishes were incubated at 25 ± 2°C and 70 ± 10% RH to encourage fungal growth and sporulation in order to confirm infection of microbial agents on the larvae which showed mycelial growth were considered to have died of infection and only those counts were used to compute the pathogenicity of microbial agents. Slides was prepared by taking spores from dead larvae and observed under microscope to study its morphology for confirmation.

RESULT AND DISCUSSION

First Year

In vitro bio-efficacy of *B. bassiana* against *H. armigera* Hubner on Chickpea year 2015-16 presented (table 1 Fig 1) revealed that at 1 day after treatment among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 62.00 % which is not on par with T_4 (*B. bassiana* @5000g/ha) 0.00%. Among the doses of *B. bassiana* show 0.00 % mortality similar to control T_7 0.00 %. At 2^{nd} day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 72.50 % which is not on par with T_4 (*B. bassiana* @5000g/ha) 5.00%. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 5.00% show the maximum mortality but another treatment T_3, T_2 and T_1 showed similar to control T_7 0.00%. At 3^{rd} day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 85.00 % which is not on par with T_4 (*B. bassiana* @5000g/ha) 15.00 %. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 15.00% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 0.00 % and T_2 (*B. bassiana* @2500g/ha) 0.00 % and T_1 (*B. bassiana* @2000g/ha) 0.00 %. In this day the percent mortality of treatment T_2 and T_1 was similar to control T_7 (0.00%).

At 4^{th} day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 90.00 % which is not on par with T_4 (*B. bassiana* @5000g/ha) 25.00%, T_3 (*B. bassiana* @3000g/ha) 15.00% and T_2 (*B. bassiana* @2500g/ha) 15.00%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 10.00 % but superior than control. At 5^{th} day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 100.00 % show the superior

mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 30.00% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 15.00% and T_2 (*B. bassiana* @2500g/ha) 15.00%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 10.00 % but superior than control. At 6th day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 60.00% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 30.00% and T_2 (*B. bassiana* @2500g/ha) 20.00%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 15.00 % but superior than control T_7 (0.00 %).

At 7th day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 75.00% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 40.00% which is on par with T_2 (*B. bassiana* @2500g/ha) 30.00%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 15.00 % but superior than control T_7 (0.00 %). At 8th day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 75.00% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 45.00% which is on par with T_2 (*B. bassiana* @2500g/ha) 32.00%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 15.00 % but superior than control T_7 (0.00 %).

Second year

In vitro bio-efficacy of *B. bassiana* against *H. armigera* Hubner on Chickpea year 2016-17 presented (table 2 Fig 2) revealed that at 1 day after treatment among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 57.00 % which is not on par with T_4 (*B. bassiana* @5000g/ha) 0.00%. Among the doses of *B. bassiana* show 0.00 % mortality similar to control T_7 0.00%. At 2nd day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 77.50 % which is not on par with T_4 (*B. bassiana* @5000g/ha) 5.00%. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 5.00% show the maximum mortality but another treatment T_3, T_2 and T_1 showed similar to control T_7 0.00%. At 3rd day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % show the superior mortality over the other treatment, followed by T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 90.00 % which is not on par with T_4 (*B. bassiana* @5000g/ha) 15.00%. Among the doses of *B. Bassiana* T_4 (*B. bassiana* @5000g/ha) 15.00% show the maximum mortality which is on par with T_3 (*B. bassiana* @3000g/ha) 5.00% and T_2 (*B. bassiana* @2500g/ha) 5.00%. The lower percent mortality recorded T_1 (*B. bassiana* @2000g/ha) 0.00 % similar to control T_7 (0.00%).

At 4th day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 90.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 25.00% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 17.50% which is on par with T_2 (*B. bassiana* @2500g/ha) 15.00%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 10.00 % but superior than control. At 5th day the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 90.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 30.00% show the maximum mortality which is not on par with T_3 (*B. bassiana* @3000g/ha) 17.50%, T_2 (*B. bassiana* @2500g/ha) 15.00% and T_1 (*B. bassiana* @2000g/ha) 12.50 % but superior than control. At 6th day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 55.00% show the maximum mortality which is not on par with T_3 (*B. bassiana* @3000g/ha) 30.00% and T_2 (*B. bassiana* @2500g/ha) 22.50%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 12.50 % but superior than control T_7 (0.00 %).

At 7th day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 67.50% show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 40.00% which is on par with T_2 (*B. bassiana* @2500g/ha) 32.50%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 12.50 % but superior than control T_7 (0.00 %). At 8th day among the treatment T_6 (Profenophos 50% EC @1000ml/ha) 100.00 % and T_5 (Neem oil Azadirechtin 0.03%@5000ml/ha) 100.00 % show the superior mortality over the other treatment, which is on par with each other. Among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) 72.50% show

the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha) 45.00% which is on par with T_2 (*B. bassiana* @2500g/ha) 32.50%. Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) 12.50 % but superior than control T_7 (0.00 %).

Earlier similar work was done by Prasad, *et al.* (1990) recorded five entomopathogenic fungi for their infectivity to 2nd -instar larvae of *Heliothis armigera* [*Helicoverpa armigera*] by spraying them with conidial suspensions. Among them, *B. bassiana* (Bapatla isolate) was found to be the most virulent, recording the lowest LC_{50} of 2.17×10^5 conidia/ml. Tefera *et al.* (2003) also reported the effects of exposure methods, conidial concentrations, and temperature on mortality, mycosis and sporulation in second instar *Chilo partellus* cadavers infected by *B. bassiana* in laboratory. Larvae directly sprayed with conidia, exposed to conidia-treated leaves and dipped into conidial suspension resulted in high mortality (98–100%). The longest LT_{50} (3.5 days) and days to mortality (2.6 days) were observed in the treated-leaves exposure method. The shortest LT_{50} (1 day) and time for mortality (1 day) were recorded for the dipping method.. Exposure of larvae to treated-leaves resulted in high sporulation.

Prasad *et al.* (2010) also worked to control the pest population of *H. armigera* (Hubner) with an ecosafe entomopathogen *B. bassiana* (Balsamo). Four different concentrations (0.1, 0.125, 0.2 and 0.25 ml $\times 10^8$ spores/ml) were sprayed topically against most damaging 4th instar larvae of *H. armigera* (Hubner) and a dose dependent mortality was observed that went up to 76.7 percent with highest dose of 0.25 ml $\times 10^8$ spores/ml. Mortality started after two to three days of treatment. Senthamizhlselvan, *et al.* (2010) collected fungal isolates from Karaikal and tested against seven insect pests using different inoculation methods *viz*., spraying, crawling and dipping. Dipping method was found to be highly effective (82.50 per cent) to coccinellids followed by spraying and crawling methods (48.75 and 13.75 per cent). Larval mortality of spotted pod borer in pulses was more with spraying method (85.00 per cent). Among the three methods, spraying method was considered to be superior.

Similarly, Kumar and Chowdhry (2004) also reported that 18 *B. bassiana* isolates were found pathogenic to *H. armigera*. The larval mortality ranged from 40.0 to 90.0%. The maximum larval mortality was recorded in isolate HBB-2 (90.0%), followed by DBB-1 (87.5%) and HBB-1 (75.0%). The mean LC50 values for *B. bassiana* HBB-2 and *M. anisopliae* HMA-2 against the second instar larvae of *H. armigera* were $0.955x10^3$ and $1.243x10^3$ spores/ml, respectively. Hatting, (2012) bioassayed three entomopathogenic fungi, *B. bassiana* (Balsamo-Crivelli) Vuillemin, *N. rileyi* (Farlow) Samson and *Isaria fumosorosea* Wize in laboratory employing topical versus *per os* inoculation techniques. At a dose of 3.75×10^5 conidia per larva, *N. rileyi* out-performed both *I. fumosorosea* and *B. bassiana*, causing a mean of 87±1.4% mortality. Notably, no difference was detected between the two inoculations techniques employed with any of the three fungal species assayed. The incubation periods for topical applications ranged from 4 days (PPRI 7201 and 8072) to 7 days (PPRI 7758) while *per os* treatments responded on Day 4 (PPRI 8072) and Day 5 (PPRI 7758 and 7201) post-inoculation.

Table 1. "*In vitro* bio-efficacy of *Beauveria bassiana* against *H. armigera* Hubner on Chickpea year 2015-16

T. N.	Treatments	Doses kg/ha	Percent mortality of *H. armigera* up to 8 days							
			1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day
T_1	*B. bassiana* 1.15% WP ($1x10^8$ cfu/g)	@2000g/ha	0.00 [c] (0.544)	0.00 [d] (0.544)	0.00 [d] (0.544)	10.00 [c] (18.439)	10.00 [c] (18.439)	15.00 [d] (22.500)	15.00 [d] (22.500)	15.00 (22.500)
T_2	*B. bassiana* 1.15% WP ($1x10^8$ cfu/g)	@2500g/ha	0.00 [c] (0.544)	0.00 [d] (0.544)	0.00 [d] (0.544)	15.00 [c] (22.500)	15.00 [c] (22.500)	20.00 [d] (26.565)	30.00 [cd] (33.055)	32.50 (34.557)
T_3	*B.bassiana* 1.15% WP ($1x10^8$ cfu/g)	@3000g/ha	0.00 [c] (0.544)	0.00 [d] (0.544)	5.00 [d] (9.481)	15.00 [c] (20.051)	15.00 [c] (20.051)	30.00 [c] (32.898)	40.00 [c] (39.164)	45.00 (42.057)
T_4	*B. bassiana* 1.15% WP ($1x10^8$ cfu/g)	@5000g/ha	0.00 [c] (0.544)	5.00 [c] (9.481)	15.00 [c] (22.500)	25.00 [c] (29.889)	30.00 [b] (32.832)	60.00 [b] (50.895)	75.00 [b] (63.453)	75.00 (63.453)
T_5	Neem oil Azadirechtin 0.03% EC	@5000ml/ha	60.00 [b] (50.835)	72.50 [b] (58.608)	85.00 [b] (69.948)	90.00 [b] (76.447)	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 (89.455)
T_6	Profenophos 50% EC	@1000ml/ha	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 [a] (89.455)	100.00 (89.455)
T_7	Control	-	0.00 [c] (0.544)	0.00 [d] (0.544)	0.00 [d] (0.544)	0.00 [d] (0.544)	0.00 [d] (0.544)	0.00 [e] (0.544)	0.00 [e] (0.544)	0.00 [e] (0.544)
	CD at (0.05%)		2.677	6.663	9.594	11.556	9.082	6.136	10.847	11.449
	SE(m)		0.909	2.266	3.263	3.927	3.088	2.086	3.687	3.892
	C.V.		8.908	19.865	23.668	21.361	15.819	9.351	15.284	15.933

*Figures in parentheses are arc sine transformed values.

Table 2. "*In vitro* bio-efficacy of *B. Bassiana* against *H. armigera* Hubner on Chickpea year 2016-17

T. N.	Treatments	Doses kg/ha	Percent mortality of *H. armigera* up to 8 days							
			1 day	2 day	3 day	4 day	5 day	6 day	7 day	8 day
T_1	*B. bassiana* 1.15% WP ($1x10^8$ cfu/g)	@2000g/ha	0.00 [c] (0.544)	0.00 (0.544)	0.00 (0.544)	10.00 (15.990)	12.50 (20.464)	12.50 (20.467)	12.50 (20.464)	12.50 (20.464)
T_2	*B.bassiana* 1.15% WP ($1x10^8$ cfu/g)	@2500g/ha	0.00 [c] (0.544)	0.00 (0.544)	5.00 (9.481)	15.00 (22.500)	15.00 (22.500)	22.50 (28.227)	32.50 (34.557)	32.50 (34.557)
T_3	*B. bassiana* 1.15% WP ($1x10^8$ cfu/g)	@3000g/ha	0.00 [c] (0.544)	0.00 (0.544)	5.00 (7.043)	17.50 (24.535)	17.50 (24.535)	30.00 (32.898)	40.00 (39.104)	45.00 (42.057)
T_4	*B. bassiana* 1.15%	@5000g/ha	0.00 [c]	5.00	15.00	25.00	30.00	55.00	67.50	72.50

	WP ($1x10^8$ cfu/g)		(0.544)	(9.481)	(19.680)	(29.736)	(32.832)	(47.947)	(58.998)	(61.941)
T_5	Neem oil Azadirechtin 0.03% EC	@5000ml/ha	57.50 [b] (49.383)	77.50 (65.117)	90.00 (76.447)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)
T_6	Profenophos 50% EC	@1000ml/ha	100.00 [a] (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)	100.00 (89.455)
T_7	Control	-	0.00 [c] (0.544)	0.00 [c] (0.544)	0.00 [d] (0.544)	0.00 [d] (0.544)	0.00 [f] (0.544)	0.00 [e] (0.544)	0.00 [e] (0.544)	0.00 [e] (0.544)
	CD at (0.05%)		3.133	11.071	14.726	7.854	6.355	6.530	12.939	11.848
	SE(m)		1.065	3.765	5.004	2.669	2.160	2.220	4.397	4.028
	CV		10.523	31.714	34.478	13.723	10.801	10.058	18.500	16.668

*Figures in parentheses are arc sine transformed values.

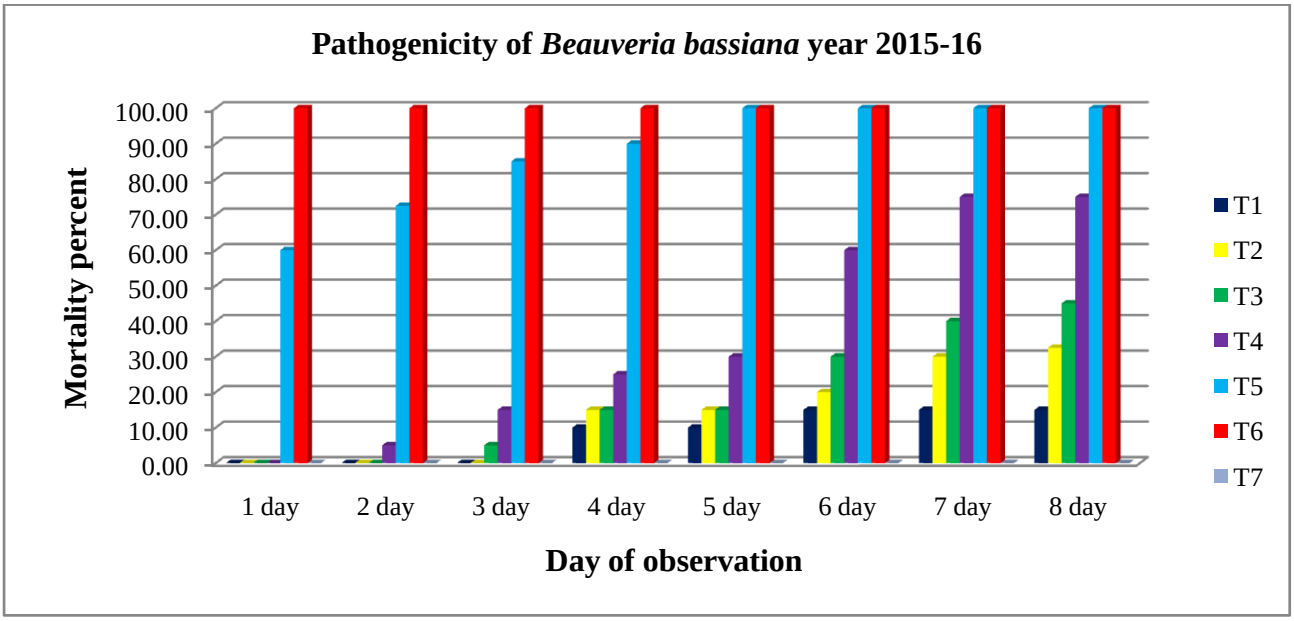


Fig 1: *In vitro* pathogenicity of *Beauveria bassiana* against *Helicoverpa armigera* Hubner

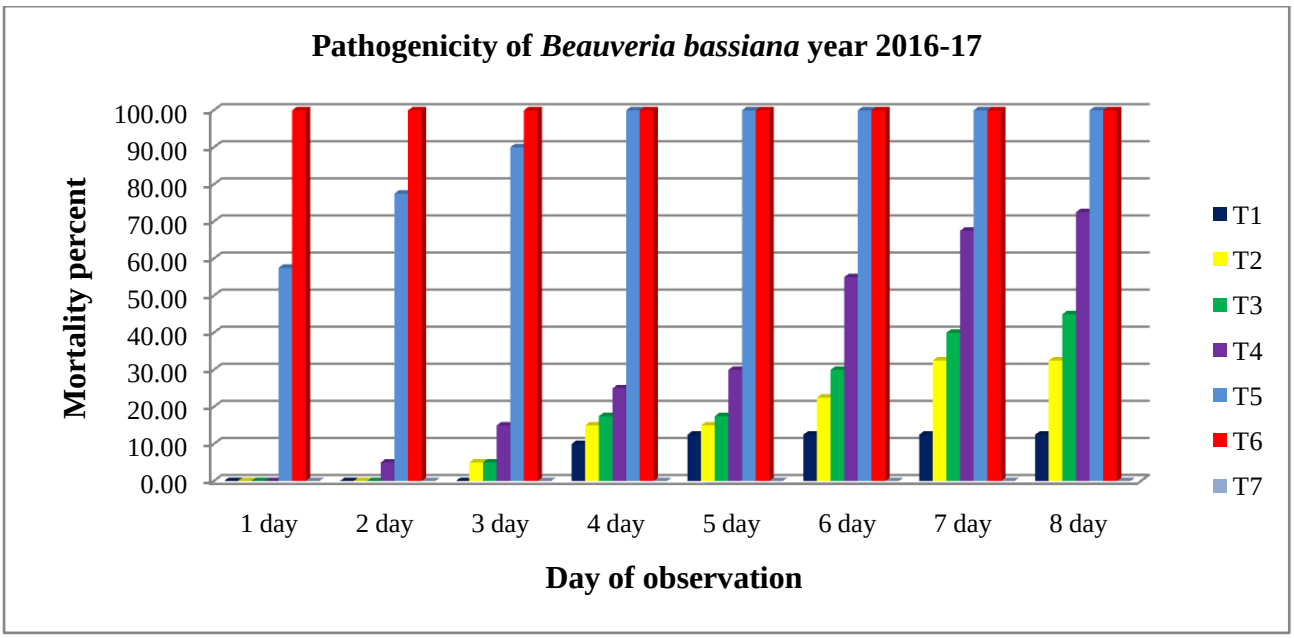


Fig 2: *In vitro* pathogenicity of *Beauveria bassiana* against *Helicoverpa armigera* Hubner

CONCLUSION

In both the year among the doses of *B. bassiana* T_4 (*B. bassiana* @5000g/ha) show the maximum mortality followed by T_3 (*B. bassiana* @3000g/ha), T_2 (*B. bassiana* @2500g/ha). Lower percent mortality recorded in T_1 (*B. bassiana* @2000g/ha) but superior than control T_7. In this experiment we can conclude that *B. bassiana* can useful bio-pesticide in integrated insect pest management for eco-friendly management of insect pests.

REFERENCES

Ahmed, K., Khalique, F. and Malik, B. A. (1998). Modified artificial diet for mass rearing of chickpea pod borer, *Helicoverpa armigera* (Hub.). *Pakistan Journal of Biological Sciences*, **1**(3):183-187.

Annonymous (2016). India Stat, 2016, Ministry of Agriculture and Farmers Welfare, Govt. of India. pp.1-2.

Elizabeth, R., H., Brown, P. M. J., Rothery, P., Ware, R. L. and Majerus, M. E. N. (2008). Interaction between the fungal pathogen *Beauveria bassiana* and three species of coccinellid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata. Biocontrol*, **53**: 265-276.

Fields, P. G. (1998). Diatomaceous earth: advantages and limitations. Proceedings of the 7th International Working Conference on Stored product Protection, Beijing China, **1**: 781-784.

Hatting, J. L. (2012). Comparison of three entomopathogenic fungi against the bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), employing topical vs per os inoculation techniques. African Entomology, **20** (1):91-100.

Hong, W. (2003). Molecular biology of the entomopathogenic fungus *Beauveria bassiana*: Insectcuticle Degrading enzymes and development of a new selection marker for fungal transformation. PhD. Thesis, University of Heidelberg, Germany, pp. 147.

Kontsedalov, S., Abu-Moch, F., Lebedev, G., Czosnek, H., Horowitz, R., Ghanim, M. (2012). *Helicoverpa armigera* biotype dynamics and resistance to insecticides in Israel during the years 2008-2010. *J Integrative Agric.*, **11**:312-320.

Krishnareddy, B. and Hanur, S. V. (2015). Enhanced synthetic diet for rearing of *Helicoverpa armigera* under laboratory conditions. *Journal of Entomological and Zoology studies*, **3**(1):165-167.

Kumar, V. and Chowdhry, P. N. (2004). Virulence of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* against tomato fruit borer, *Helicoverpa armigera*. *Indian Phytopathology*, **57**(2): 208-212.

Langle, T. (2006). *Beauveria bassiana* (Bals.Criv.) Vuill.: A biocontrol agent with more than 100 years of history of safe use. REBECA, pp. 37.

Li, Y. W. and J. H. Yang (1988). Prospects for the use of entomogenous fungus against forests pests, In: Study and application of entomogenous fungus in China, (Y. W. Li, Z. Z. Li, Z. Q. Liang, J. W. Wu, Z. K. Wu and Q. F. Xu ed.). Periodical Press, Beijing, pp. 10-14.

Lingappa, S., Saxena, H. and Devi Vimala, P. S. (2005). Role of biocontrol agents in management of Helicoverpa armigera (Hubner). In: Recent advances in Helicoverpa armigera Management (Hem Saxena, A. B. Rai, R. Ahmad and Sanjeev Gupta eds.). Indian Society of Pulses Research and development, IIPR, Kanpur, pp 159-184.

Mccaffery A. R., Head, D. J., Jianguo, T., Dubbeldam, A. A., Subramaniam, V. R. and Callaghan, A. (1998). Nerve insensitivity resistance to pyrethroids in Heliothine lepidoptera. *Pestic Sci*, **51**: 315-320.

Namasivayam, S. K. R., Aarthi, R. and Anbazhahan, P. (2015). Studies on factors influencing the viability of entomopathogenic fungi *Metarhizium anisopliae* in soil adapting culture dependent method. *J. Biopest*., **8**(1):23-27.

Nauen R, and Bretschneider T. (2002). New modes of action of insecticides. *Pesticide outlook*, **13**:241-245.

Neoliya, N. K., Singh, D. and Sangwan, R. S. (2007). Azadirachtin-based insecticides induce alternation in *Helicoverpa armigera* Hub. head polypeptides. *J. Current Science*, **92** (1):94-99.

Prakash, M. R., Ram, U. and Tariq, A. (2007). Evaluation of chickpea (*Cicer arietinum* L.) germplasm for the resistance to gram pod borer, *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae). *J. entomol. Res*., **31**: 215-218.

Prasad, A., Syed, N. and Purohit, S. (2010). *Beauveria bassiana* (Balsamo) vuillemin: a successful biopesticide against key pest *Helicoverpa armigera* (Hubner). *Internat. J. of Pharma and Bio Sciences*, **1** (2):30.

Prasad, V. D., Jayaraj, S. and Rabindra, R. J. (1990). Susceptibility of gram caterpillar, *Heliothis armigera* Hbn. (Noctuidae: Lepidoptera) to certain entomogenous fungi. *J. of Biological Control*, **4**(1): 44-47.

Senthamizhlselvan, P., Alice J., Sujeetha, R. P. and Jeyalakshmi, C. (2010). Efficacy of the native entomopathogenic fungal isolates with different inoculation methods. *J. of Biopesticides*, **3**(3): 550 – 552.

Senthamizhlselvan, P., Alice J., Sujeetha, R. P. and Jeyalakshmi, C. (2010). Efficacy of the native entomopathogenic fungal isolates with different inoculation methods. *J. of Biopesticides*, **3**(3): 550 – 552.

Sigsgaard, L., Greenstone, M. H. and Duffield, S. J. (2002). Egg cannibalism in *Helicoverpa armigra* on sorghum and *pigeonpea*. *Biological control*, **47**: 151-165.

Tefera, T. and Pringle, K. L. (2003). Effect of exposure method to *Beauveria bassiana* and conidia concentration on mortality, mycosis, and sporulation in cadavers of *Chilo partellus* (Lepidoptera: Pyralidae). *Journal of Invertebrate Pathology*, **84**:90–95.

RESEARCH ARTICLE

FEEDING POTENTIAL OF LADY BIRD BEETLE *CHEILOMENES SEXMACULATA*, FABRICIUS (COLEOPTERA: COCCINELLIDAE) ON COTTON MEALY BUG *PHENACOCCUS SOLENOPSIS* (TINSLEY) UNDER CHOICE AND NO CHOICE CONDITION OF LABORATORY

Nirali J. Sanghani, G.R. Bhanderi* and R.D. Patel

Cotton Entomology, Main Cotton Research Station,
Navsari Agricultural University, Surat- 395007, Gujarat
Email: grbhanderi@yahoo.co.in

Received-05.06.2017, Revised-22.06.2017

Abstract: An experiment was conducted to determine the feeding potential of lady bird beetle, *cheilomenes sexmaculata* (Fab.) on cotton Mealy bug, *Phenacoccus solenopsis* under choice and no choice condition in the laboratory at Department of Agricultural Entomology, Navsari Agricultural University, Navsari during July–August, 2016. The results revealed that *C. sexmaculata* was mostly preferred eggs of *P.solenopsis* as compared to nymph and adult of mealybug. The per day prey consumption rate of larval and adult stage of *C. sexmaculata* were varied from 35.00 to 44.00 (Av. 38.80±2.18) and 25.58 to 27.03 (Av. 26.38±0.35) eggs, 13.00 to 18.33 (Av. 14.97±1.47) and 15.52 (Av. 14.83±0.45) nymphs, and 9.67 to 14.00 (Av. 11.58±1.14) and 10.95 to 12.79 (Av. 12.02±0.40) adults of mealybug, respectively in no choice condition. In free choice feeding, grub of *C. sexmaculata* preferred eggs of mealybug more as compared to adult and nymph stage of mealybug. Which indicated by consumption of 65.55 ± 16.63 eggs, 10.75 ± 3.78 nymphs and 5.70±1.75 adults out of 82.00±21.04 (mixed stage) by larvae and 490.55±53.39 eggs, 139.35±15.56 nymphs and 93.25±7.72 adults out of 723.15±3.15 (mixed stage) by adult of *C. sexmaculata* on mealybug.

Keywords: *Cheilomenes sexmaculata*, *Phenacoccus solenopsis*, Feeding potential

INTRODUCTION

The cotton crop is attacked by both sucking and chewing type insects. The sucking type including, whitefly, aphid, thrips, mealy bugs and mites while chewing type including, grasshopper, termite, weevils and lepidopteron insects. Among these mealy bug is the serious pest of cotton and has resulted in severe damage during the last few years (Solangi *et al.*, 2008; Lohar and Khuhro, 2005). Mealybug, (Hemiptera: Pseudococcidae) are small, soft-bodied, sap sucking insect that cause severe damage to various field crops, fruits and vegetables (Arif *et al.*, 2009). Cotton mealybug, *Phenacoccus solenopsis* Tinsley was described originally from USA in 1898 (Tinsley, 1898) and regarded as an exotic pest of Asia. Mealybug which was not hitherto familiar earlier started destroying cotton crops caused economic damage, reducing yields up to 40-50% in affected 6 fields since 2006. Several parts of Gujarat which are located on the border of Pakistan which had recent history of mealybug infestation were severely affected. Mealybug infestation were recorded in 2006 on *G. hirsutum* in all the nine cotton-growing states of India, Punjab, Haryana, Rajasthan, Gujarat, Madhya Pradesh, Maharashtra, Tamil (Rao and David, 1958; Dean *et al.*, 1971).

The ladybird beetle belongs to the family coccinellidae of order Coleoptera. The members of the family are exclusively predator on aphids, mealybugs, scales, whiteflies, thrips, leafhoppers, mites and other small soft bodied insect pests (Omkar and Pervez, 2000). The predatory coccinellids occupy all the habitats and niches of their prey and distributed worldwide. Nine different species of ladybird beetle have been recorded in middle Gujarat. Of these, *Cheilomenes sexmaculata* (Fabricius.) found to be predominant species in middle Gujarat region. The ladybird beetles are predaceous on nymphs of cowpea aphids (*Aphis craccivora*), nymphs of cotton mealybug (*P. solenopsis*), nymphs of sorghum aphids (*Rhophalosiphum maydis*), nymphs of cotton aphids (*A. gossypii*) (Shepard, 1998). Keeping in view the above facts and importance of biological control of cotton mealy bug, the study on feeding potential of *Cheilomenes sexmaculata* against cotton mealy bug in laboratory was undertaken

MATERIAL AND METHOD

Maintenance of *C. sexmaculata* culture: Laboratory experiments were conducted under ambient and protected conditions at Bio-control Laboratory, Department of Agricultural Entomology, N. M. College of Agriculture, Navsari Agricultural University, Navsari during 2016-2017. Grubs, eggs and adults of predatory coccinellids were collected from the cotton fields of Research farm, Main Cotton Research Station, Navsari Agricultural University, Surat during 2016-17 and brought to the laboratory.

Maintenance of prey insects (aphid and mealybug) culture: The initial culture of mealybug collected from the Research farm, Main Cotton Research Station, Navsari Agricultural University, Surat during 2016-17. For the purpose, mealybug

*Corresponding Author

infested central twig with leaves were plucked and collected in perforated plastic bags, separately. The initial culture was released on 45 days old cotton plants raised in pots of dimension 45 × 18.5 cm in the wire netting inventory of Bio-control Laboratory, Department of Agricultural Entomology, Navsari Agricultural University, Navsari for establishment. The culture was established within one and half month which utilized as prey hosts for studying feeding potential of *C. sexmaculata*.

Assessment of feeding potential of *C. sexmaculata* on *P. solenopsis:* The predatory potential of different stages of *C. sexmaculata* against eggs (ovisac), nymphs and adults (freshly formed) stage of mealybug studied under no choice and free choice feeding trials separately under laboratory condition. In no choice feeding experiment, 20 larva of *C. sexmaculata*were kept individually in the plastic vial (5.0 × 2.5 cm). Three such sets of 20 larvae under plastic vials were prepared. One set was utilized for studying predatory potential against eggs (ovisac) of mealy bug; second set for nymphs and third for adults were provided as prey insect stages throughout the total larval and adult development of *C. sexmaculata*. Number of prey consumed by the predatory larvae in each instars and adult stage of *C. sexmaculata* calculated for each individual and the total consumption during total larval and adult stage of *C. sexmaculata* worked out. In free choice feeding, 20 larvae of *C. sexmaculata* kept individually in the plastic vial (5.0 × 2.5 cm). The set of 20 larvae under plastic vials were prepared. The set utilized for studying predatory potential against mixed stages of mealybug (ovisac, nymph and adult) as prey. In the set, known numbers of mixed stages offered as food and the record maintained separately on rate of consumption daily. On next day, again counted numbers of mixed stages offered as food and the consumption of prey insect calculated daily. Number of prey consumed by the predatory larvae and adult of *C. sexmaculata* in each prey stage and in each instars and adult of *C. sexmaculata*was calculated for each individual and the total consumption during total larval and adult stage of *C. sexmaculata* worked out. The total larval and adult duration of *C. sexmaculata*was also estimated and per day consumption of prey stages calculated.

RESULTS AND DISCUSSION

No choice condition

The data on feeding potential of all four larval instars and adult of *C. sexmaculata* on eggs (ovisac), nymphs and adults of *P. solenopsis* under no choice feeding conditions presented in Table 1 and Fig. 4, fig. 5 and fig. 6. Grubs and adult of *C. sexmaculata* consumed 8.35±2.11, 11.95±1.85, 34.50±9.10, 43.70±7.03 and 764.00±94.36 eggs(ovisac) of mealybug in developmental durations of 2.15±0.49, 2.30±0.47, 2.50±0.51, 2.75±0.44 and 28.95±3.41 days of first, second, third and fourth instar of grub, and adult respectively. The total grub period consumed 71.00 to 118.00 (Av. 98.50±16.05) eggs of mealybug during developmental durations of 9.70±1.56 days. The prey consumption rate varied from 2.33 to 6.00 (Av. 3.93±0.86), 4.33 to 6.50 (Av. 5.27±0.62) and 11.00 to 16.00 (Av. 13.68±1.45), 13.67 to 18.00 (Av. 15.93±1.05) and 25.58 to 27.03 (Av. 26.38±0.35) eggs of mealybug per day during first, second, third, fourth instar larvae, and adult of *C. sexmaculata* respectively. During entire larval period, per day prey consumption rate was varied from 35.00 to 44.00 (Av. 38.80 ± 2.18) eggs. The grubs and adult of *C. sexmaculata* consumed 1.20±0.52, 1.90±0.91, 13.10±1.92, 22.50 ± 6.94 and 400.45±35.34 nymphs of mealybug in developmental durations of 1.85±0.37, 2.10±0.31, 2.50±0.51, 2.75±0.44 and 27.00±2.22 days of first, second, third and fourth larval instars, and adult respectively. The *C. sexmaculata* grub consumed 13.00 to 18.33 (Av. 14.97±1.47) nymphs of mealybug during developmental durations of 5.00 to 7.00 (Av. 9.20±0.69) days. The prey consumption rate varied from 0.00 to 2.00 (Av. 0.73±0.50), 0.50 to 1.50 (Av. 0.89±0.37), 4.33 to 6.50 (Av. 5.33±0.59), 5.50 to 11.33 (Av. 8.03±1.62) and 13.88 to 15.52 (Av. 14.83±0.45) nymphs of mealybug per day during first, second, third and fourth instar, and adult of *C. sexmaculata*, respectively. During entire larval period, prey consumption rate varied from 13.00 to 18.33 (Av. 14.97±1.47) nymphs per day. When grubs and adult of *C. sexmaculata* feed on adults of mealybug, consumed 1.25±0.79, 1.80±0.62, 10.65±2.39, 15.70±3.20 and 342.90±49.59 adults of mealybug in developmental durations of 1.45±0.51, 1.80±0.62, 2.75±0.44, 2.75±0.44 and 28.50±3.87 days of first, second, third and fourth larval instars, and adult respectively. The *C. sexmaculata* grub consumed 20.00 to 38.00 (Av. 29.40±4.73) adults of mealybug during developmental durations of 6.00 to 10.00 (Av. 8.75±1.25) days. The prey consumption rate varied from 0.00 to 2.00 (Av. 0.85±0.56), 1.00 to 2.00 (Av. 1.10±0.31), 2.50 to 5.67 (Av. 3.91±0.81), 4.33 to 7.00 (Av. 5.73±0.79) and 10.95 to 12.79 (Av. 12.02±0.40) adults of mealybug per day during first, second, third and fourth instar grubs, and adult of *C. sexmaculata* respectively. During entire larval period, prey consumption rate varied from 9.67 to 14.00 (Av. 11.58±1.14) adults.

No choice condition

The data (Table 2 and Fig. 3) showed that grubs of *C. sexmaculata* consumed 6.90±2.43, 10.40±3.22, 27.50±9.58, 37.20±12.15 and 723.15±73.15 mixed stages of mealybug (eggs, nymphs and adult) in developmental durations of 1.65±0.49, 1.75±0.44, 1.90±0.64, 2.15±0.81 and 28.60±2.96 days of first, second, third and fourth larval instars, and adults respectively. The *C. sexmaculata* grubs consumed 42.00 to 113.00 (Av. 82.00±21.04) numbers of

mealybug (mixed stages) during developmental durations of 4.00 to 10.00 (Av. 7.45±1.85) days. In free choice feeding, grub of *C. sexmaculata* preferred eggs of mealybug as compared to adult and nymph stage of mealybug. It indicated by consumption of 65.55±16.63 eggs, 10.75±3.78 nymphs and 5.70±1.75 adults out of 82.00 ± 21.04 (mixed stage) by larvae and 490.55±53.39 eggs, 139.35±15.56 nymphs and 93.25±7.72 adults out of 723.15 ±73.15 (mixed stage) by adult of *C. sexmaculata* on mealybug. This might be due to soft body, small size of younger stages of mealybug favoring the easy capture and disliking of waxy coating on body of adult stage of mealybug. The prey consumption rate varied from 2.00 to 6.00 (Av. 4.15±0.88), 4.00 to 7.50 (Av. 5.88±0.92) and 12.50 to 17.00 (Av. 14.39±1.24), 14.33 to 22.00, (Av. 17.81±2.12), and 24.27 to 26.00 (Av. 25.30±0.43) number of mealybug (mixed stages) per day during first, second third and fourth instar, and adults of *C. sexmaculata* respectively. During entire larval period, prey consumption rate varied from 36.83 to 48.50 (Av. 42.53±3.19) numbers of mealybug (mixed stages) per day.

In present study, observed the larvae and adult of *C. sexmaculata* preferred eggs (ovisac) of mealybug and nymph as compared to freshly formed adult mealybug as prey in free choice feeding condition. Which was confirmed with different workers *viz.*, Kaur and virk (2012) reported that fourth instar grubs and adults of *C. montrouzieri* were the most voracious feeders on first instars of mealybug.. Lad *et al.* (2012) reported the consumption of total 306.6 ± 10.79 and 345.60 ± 3.56 nymphs of mealy bug during total larval and adult period. Asifa *et al.* (2013) found that first, second, third, fourth instar grubs of beetle consumed 44.00 and 5.44, 45.00 and 7.11, 54.33 and 8.21, 33.00 and 7.33 medium sized nymph and adult of cotton mealybug. Aggarwal and Neetan (2014) found that the. *C. sexmaculata* beetle consumed first, second, third instar nymphs and adult of mealybug were 1371.10, 361.40, 225.40, and 27.90, respectively during its life cycle. Dumaniya *et al.* (2015) observed that *C. montrouzieri* grub and adult consumed186.52 ± 9.23 and 944.12 ± 31.02 nymphs and 117.24 ± 3.73 and 93.64 ± 3.86 adults of *P. solenopsis.*

CONCLUSION

The larvae and adult of *C. sexmaculata* preferred eggs (ovisac) of mealybug and nymph as compared to freshly formed adult mealybug as prey in free choice feeding condition. There was not much variation in development duration of larvae of *C. sexmaculata* when fed on mixed stages (eggs, nymphs and adults) in no choice feeding then fed on eggs, nymphs and adults of mealybug fed in free choice feeding conditions. However, larvae of *C. sexmaculata* developed little bit slower when fed exceptionally on eggs of mealybug in no choice feeding conditions.

Table 1. Feeding potential of *C. sexmaculata* on different stages of *P. solenopsis* (no choice feeding)

Larval stages of *C. sexmaculata*	No. of larvae exposed	No. of mealy bug consumed			Rate of consumption/day			Developmental duration in days		
		Min.	Max.	Av. ± S. D.	Min.	Max.	Av. ± S. D.	Min.	Max.	Av. ± S. D.
Eggs of Mealy bug as prey										
I instar	20	3.00	12.00	8.35 ± 2.11	2.33	6.00	3.93 ± 0.86	1.00	3.00	2.15 ± 0.49
II instar	20	9.00	16.00	11.95 ± 1.85	4.33	6.50	5.27 ± 0.62	2.00	3.00	2.30 ± 0.47
III instar	20	22.00	48.00	34.50 ± 9.10	11.00	16.00	13.68 ± 1.45	2.00	3.00	2.50 ± 0.51
IV instar	20	29.00	51.00	43.70 ± 7.03	13.67	18.00	15.93 ± 1.05	2.00	3.00	2.75 ± 0.44
Total	80	71.00	118.00	98.50 ± 16.05	35.00	44.00	38.80 ± 2.18	7.00	12.00	9.70 ± 1.56
Adult	20	597.00	931.00	764.00 ± 94.36	25.58	27.03	26.38 ± 0.35	23.00	35.00	28.95 ± 3.41
Nymphs of Mealy bug as prey										
I instar	20	0.00	2.00	1.20 ± 0.52	0.00	2.00	0.73 ± 0.50	1.00	2.00	1.85 ± 0.37
II instar	20	1.00	4.00	1.90 ± 0.91	0.50	1.50	0.89 ± 0.37	2.00	3.00	2.10 ± 0.31
III instar	20	10.00	16.00	13.10 ± 1.92	4.33	6.50	5.33 ± 0.59	2.00	3.00	2.50 ± 0.51
IV instar	20	11.00	34.00	22.50 ± 6.94	5.50	11.33	8.03 ± 1.62	2.00	3.00	2.75 ± 0.44
Total	80	26.00	52.00	38.70 ± 8.35	13.00	18.33	14.97 ± 1.47	5.00	7.00	9.20 ± 0.69
Adult	20	326.00	459.00	400.45 ± 35.34	13.88	15.52	14.83 ± 0.45	22.00	30.00	27.00 ± 2.22
adults of Mealy bug as prey										

I instar	20	0.00	2.00	1.25 ± 0.79	0.00	2.00	0.85 ± 0.56	1.00	2.00	1.45 ± 0.51
II instar	20	1.00	3.00	1.80 ± 0.62	1.00	2.00	1.10 ± 0.31	1.00	3.00	1.80 ± 0.62
III instar	20	5.00	17.00	10.65 ± 2.39	2.50	5.67	3.91 ± 0.81	2.00	3.00	2.75 ± 0.44
IV instar	20	10.00	21.00	15.70 ± 3.20	4.33	7.00	5.73 ± 0.79	2.00	3.00	2.75 ± 0.44
Total	80	20.00	38.00	29.40 ± 4.73	9.67	14.00	11.58 ± 1.14	6.00	10.00	8.75 ± 1.25
adult	20	230.00	401.00	342.90 ± 49.59	10.95	12.79	12.02 ± 0.40	21.00	33.00	28.50 ± 3.87

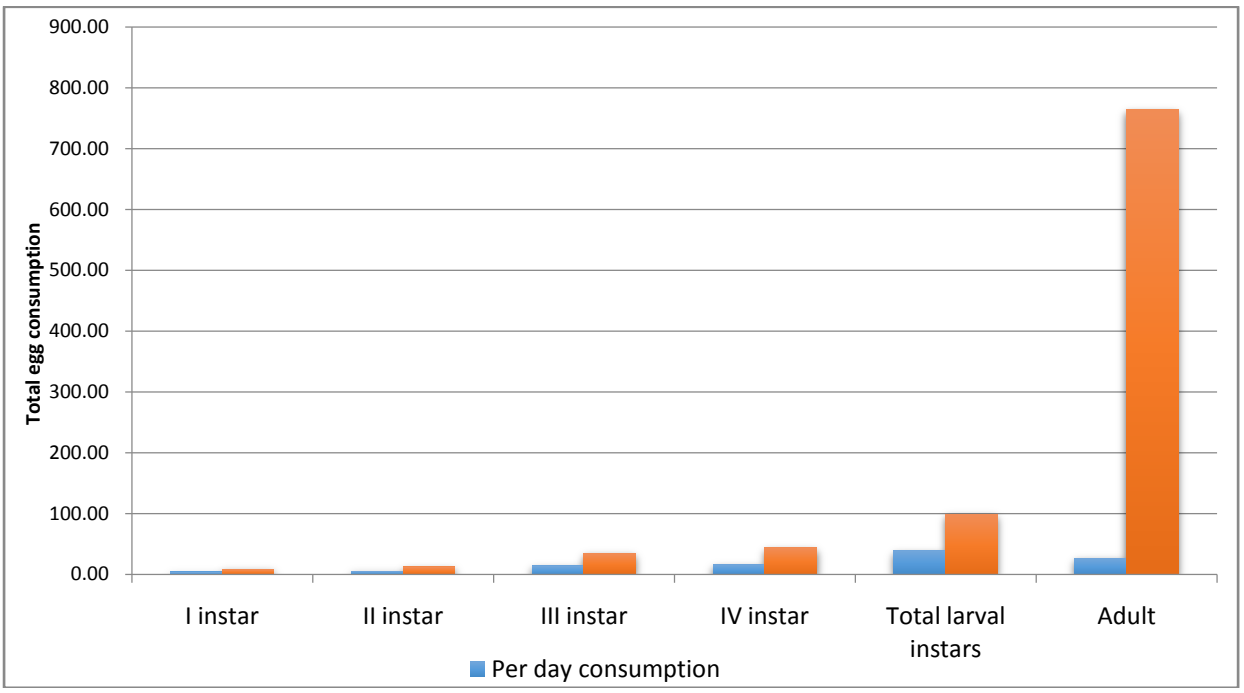


Fig. 1: Feeding potential of larva and adult of *C. sexmaculata* on eggs of *P. solenopsis* Tinsely (No choice condition)

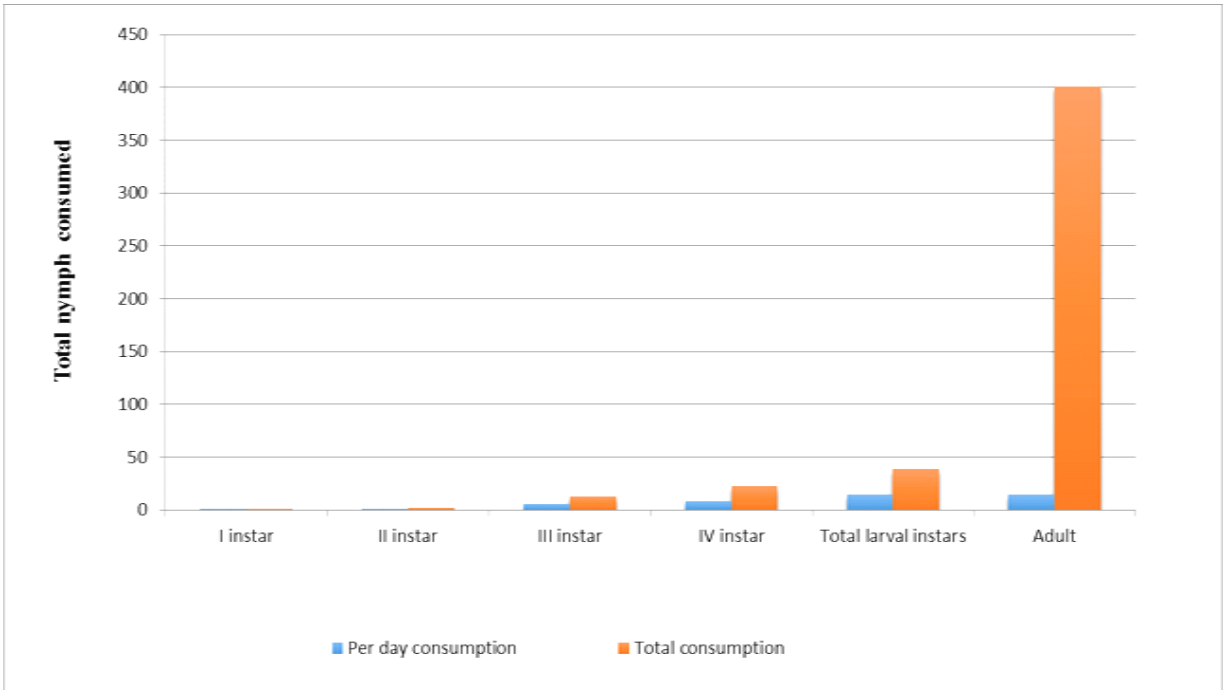


Fig. 2: Feeding potential of larva and adult of *C. sexmaculata* on nymph of *P. solenopsis* Tinsely (No choice condition)

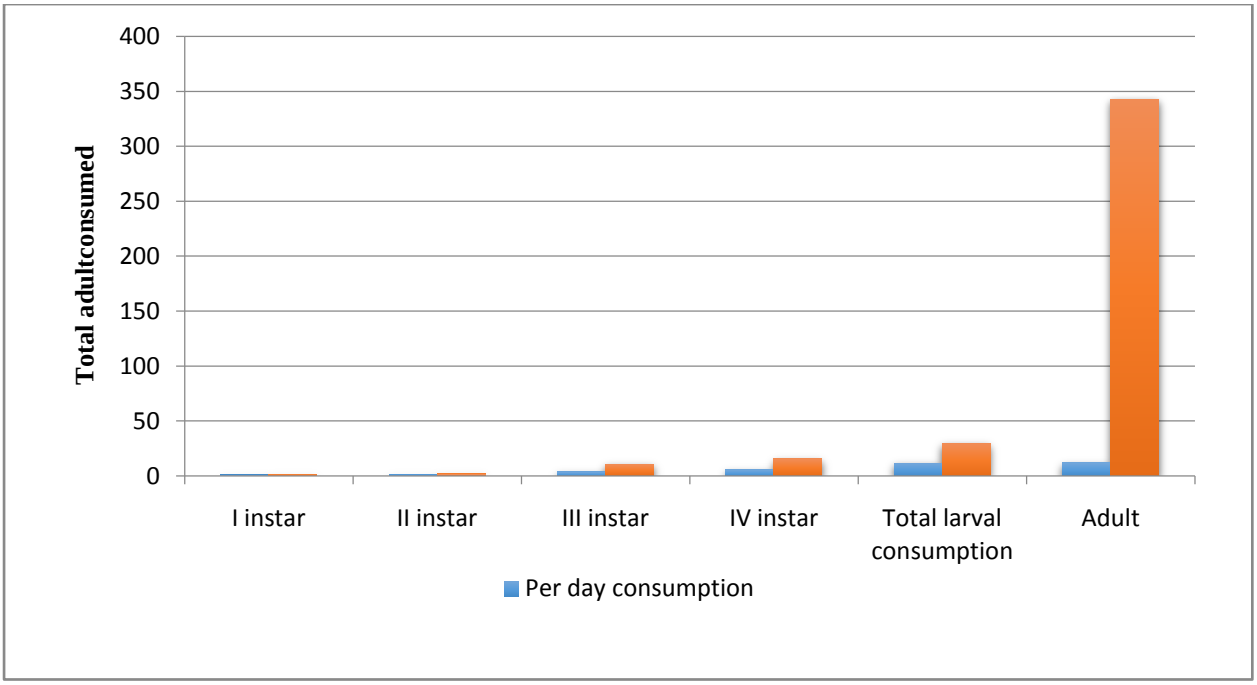


Fig. 3: Feeding potential of larva and adult of *C. sexmaculata* on adult of *P. solenopsis* Tinsely (No choice condition)

Table 2. Feeding potential of larva and adult of *C. sexmaculata on P. solenopsis* (free choice method)

Larval stages of *C. sexmaculata*	Mathematical functions	No. of mixed stages of mealy bug consumed				Rate of consumption (No./day)				Duration in days
		Eggs	Nymphs	Adults	Total	Eggs	Nymphs	Adults	Total	
I instar	Min.	2.00	0.00	0.00	2.00	2.00	0.00	0.00	2.00	1.00
	Max.	9.00	2.00	1.00	10.00	4.50	1.00	1.00	6.00	2.00
	Av ± S. D.	5.10 ± 2.02	1.20 ± 0.62	0.60 ± 0.50	6.90 ± 2.43	3.05 ± 0.72	0.73 ± 0.34	0.38 ± 0.36	4.15 ± 0.88	1.65 ± 0.49
II instar	Min.	4.00	0.00	0.00	4.00	3.50	0.00	0.00	4.00	1.00
	Max.	11.00	3.00	2.00	15.00	5.50	1.50	1.00	7.50	2.00
	Av ± S. D.	8.25 ± 2.45	1.30 ± 0.80	0.85 ± 0.59	10.40 ± 3.22	4.70 ± 0.62	0.73 ± 0.41	0.45 ± 0.32	5.88 ± 0.92	1.75 ± 0.44
III instar	Min.	10.00	1.00	0.00	13.00	10.00	0.50	0.00	12.50	1.00
	Max.	37.00	6.00	3.00	44.00	14.50	2.00	1.50	17.00	3.00
	Av ± S. D.	23.25 ± 8.02	2.75 ± 1.52	1.50 ± 0.83	27.50 ± 9.58	12.23 ± 1.23	1.41 ± 0.45	0.75 ± 0.40	14.39 ± 1.24	1.90 ± 0.64
IV instar	Min.	15.00	1.00	1.00	17.00	11.67	1.00	0.50	14.33	1.00
	Max.	42.00	9.00	5.00	55.00	17.00	4.00	2.50	22.00	3.00
	Av ± S. D.	28.95 ± 9.09	5.50 ± 2.44	2.75 ± 1.37	37.20 ± 12.15	13.94 ± 1.70	2.51 ± 0.64	1.36 ± 0.57	17.81 ± 2.12	2.15 ± 0.81
Total	Min.	35.00	4.00	3.00	42.00	28.83	2.50	1.67	36.83	4.00
	Max.	87.00	18.00	8.00	113.00	38.50	6.67	4.50	48.50	10.00
	Av ± S. D.	65.55 ± 16.63	10.75 ±3.78	5.70 ± 1.75	82.00 ± 21.04	33.93 ± 2.76	5.37 ± 1.02	2.93 ± 0.81	42.23 ± 3.11	7.45 ± 1.85
Adult	Min.	387.00	114.00	77.00	582.00	16.00	4.38	2.83	24.27	23.00
	Max.	574.00	170.00	108.00	840.00	17.60	5.43	3.78	26.00	33.00
	Av ± S. D.	490.55 ± 53.39	139.35±5.65	93.25±7.72	723.15 ± 73.15	17.14±0.25	4.88 ±0.28	3.28±0.25	25.30 ±0.43	28.60 ±2.96

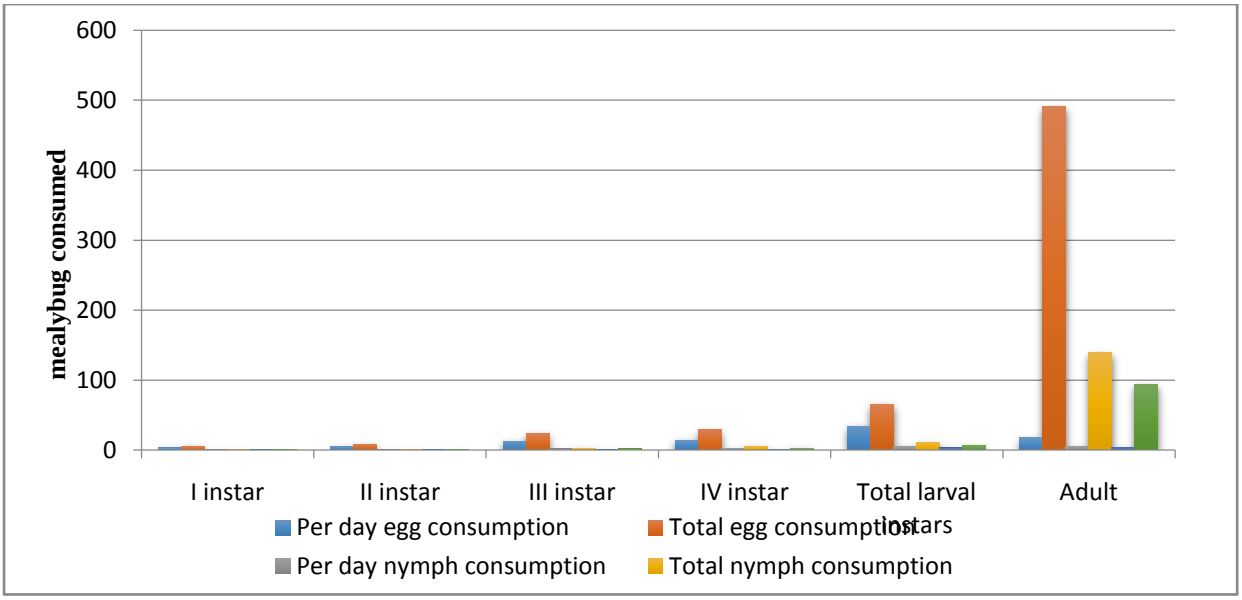


Fig. 4: Feeding potential of larva and adult of *C. sexmaculata* on eggs, nymph and adult of *P. solenopsis* Tinsely (Free choice condition).

REFERENCES

Aggarwal, N. and Neetan (2014). Predatory efficiency of *Cheilomenes sexmaculata* (Fab.) and *Chrysoperla zastrowi sillemi* (Esben-Peterson) on cotton mealybug, *Phenacoccus solenopsis* Tinsley under laboratory conditions.*Acta Phytopathologica et Entomological Hungerica.*,**49**(1): 73-81.

Arif, M. I., Rafiq, M. and Ghaffar, A. (2009). Host plants of cotton mealybug (*Phenacoccus solenopsis*): a new menace to cotton agro ecosystem of Punjab, *Pakistan. Int. J. Agric. Biol.*, **11**: 163–167.

Asifa, H., Saleem, M., Haider, K., Saghir, A., Mussarat, H., Wajid, N. Akram, M., Hammad, H., and Shuaib, F. (2013).Life history and predatory potential of eleven spotted beetle (*Coccinella undecimpunctata* Linnaeus) on cotton mealybug (*Phenacoccus solenopsis* Tinsley).*Afr. J. Agric Res.*, **8**(48): 6142-6148.

Dean, H. A., Hart, W. G. and Ingle, S. J. (1971). Citrus mealybug, a potential problem on Texas grapefruit.*J. Rio Grande Val. Hortic. Soc.*, **15**: 46-53.

Dumaniya, S. G., Patel, M. B., Siddhapara, M. R. And Kapadiya, I. B. (2015).Feeding potential of lady bird beetle, *Cryptolaemus montrouzieri* (Mulsant) on cotton mealybug, *Phenacoccus solenopsis* (Tinsley).*J. Cotton Res. Dev.*, **29**(2):311-314.

Kaur, H., Virk, J. S. (2012). Feeding potential of *Cryptolaemus montrouzieri* against the mealybug *P. solenopsis.Phytoparasitica.*, **40**(2): 131–136.

Lad, S. K., Patil, P. D., and Narangalkar, A. L. (2012).Feeding potential of *cryptolaemus montrouzieri* (mulsant) on mealy bug *maconellicoccus hirsutus* (green) under climatic conditions of Konkan, Maharashtra (India).*J. Appl. Zool. Res.*,**23**(2): 112- 114.

Lohar, M.K. and Khuhro, R.D. (2005). First Annual Report on Mass rearing of Coccinellid Predators on different insect pests, Submitted to Higher Education Commission, Islamabad and Sindh Agric. Univ. Tandojam, Pakistan. 211p.

Omkar, S. and Pervez, A. (2000).Biodiversity in predaceous coccinellids in India.*A review. J. Aphido*. **14**(1&2): 41-66.

Rao, T. V. and David, L. A. (1958). The biological control of coccid pests in South India by use of beetle, *Cryptolaemus montrouzieri*Muslant.*Indian J. Agric. Sci.*, **28**: 545-552.

Shepard, B. M. (1998). Insects and their natural enemies association with vegetables and soybean in Southeast Asia.Quality Printing Co. Orangeburg, South Carolina, U.S.A. pp. 22-24.

Solangi, G.S., Mahar, G.M. and Oad, F.C. (2008). Presence and abundance of different insect predators against suckinginsect pest of cotton. J. Entomol. 5(1): 31-37.

Tinsley, J. D. (1898). Notes on Coccidae, with descriptions of new species.*Canadian Ento.*, **30**: 317-320.

RESEARCH ARTICLE

IMPACT OF FLD CONDUCTED ON PLANT PROTECTION SCHEDULE AND USE OF CERTIFIED SEED IN THE YIELD OF POTATO

Rajni Agashe[1]*, Dharmpal Kerketta[2] and R.K. Mishra[3]

[1]SMS, Extension, KVK Surguja I.G.K.V.V. Raipur (C.G.)
[2]Programme Assistant, KVK, Surguja I.G.K.V.V. Raipur (C.G.)
[3]Programme coordinator, KVK, Surguja I.G.K.V.V. Raipur (C.G.)

Received-07.06.2017, Revised-21.06.2017

Abstract: In Surguja district of Northern Hilly zone of Chhattisgarh, potato is grown as *rabi* and *kharif* also, The use of seed treatment and recommended plant protection schedule like 3 sprays of recommended pesticides for management of major diseases (i.e. –blight Phytopthora wilt etc) and insect pest, (i.e. Potato tuber moth aphids & sucking pest) will achieve the expected yield of potato. The present study was carried out during the year 2012-15 in Surguja district of Chhattisgarh state. Findings of the study showed the data significantly indicated that before Front line demonstration (FLD), the majority of respondents were (57.14 to 74.28 per cent) in the category of low level of knowledge for various aspects of study. With respect to knowledge level in medium category in each aspect of plant protection, remaining respondents were included and the share of respondents ranged between 25.72 per cent to 42.86 per cent. There was a rise in the number of respondents in middle level of knowledge from low level and the respondents belonging to this category after FLDs ranged between 49.00 to 51.42 per cent as against 25.71 to 42.86 per cent before FLDs. 22.85 to 43.29 per cent respondents become successful in acquiring high level of knowledge pertaining to the various aspects of plant protection in potato production. There was increase in the number of respondents in middle level of adoption and the respondents belonging to this category after FLDs ranged between 37.14 to 54.28 per cent as against 22.86 to 37.14 per cent before FLDs.22.86 to 37.14 per cent respondents become successful in acquiring high level of adoption pertaining to the various aspects of plant protection in potato production after FLDs. With respect to various aspects of certified seed of potato, the FLDs helped the respondents to improve their knowledge. There was increase in no of respondents in high knowledge level and medium knowledge level category and reduction of respondents in low knowledge level category. With respect to adoption level in medium category in each aspect of use of certified seed of potato remaining respondents were included and the share of respondents ranged between 17.14 to 54.28 per cent. Data further revealed that average yield before FLD were 70q/ha^{-1}. However it increased to 110q/ha^{-1} and increase in yield was 57% after FLDs. Problems faced by respondents regarding use of plant protection schedule indicated that maximum number of respondents had problems about uncertain weather condition like frost, rainfall and hailstorm (97.14%) followed by Disease infestation especially early and late blight of potato (88.57%) respectively. Problems faced by respondents regarding use of certified seed maximum respondents having problems of more demand of local red variety of potato by consumer (100.00%) and more cost of seed potato(100.00%) both followed by non availability of certified seed of potato in market and lack of facility of cold storage(94.28%) both.

Keyword: FLDs, Potato, Surguja districts (C.G.)

INTRODUCTION

Potato (*Solanum tuberosum* L.), family Solanaceae, is one of the most popular vegetables grown in India because of its higher nutritive and higher production. It is the cheapest source of dietary carbohydrates (20.6%), protein (2.1%), fat (0.3%), crude fiber (1.1%), ash (0.9%), starch and vitamins especially C and B_1 and minerals. Potato is a good source of energy as they are rich in carbohydrates and therefore, is the fourth most important food crop after rice, wheat and maize. India is the second largest producer of potato in the world. Uttar Pradesh is the largest potato producing state in the country and accounts for 32% of total production. Potato is a very popular and important cash crop in district Surguja of C.G. but due to improper adoption of improved technology, its productivity is far below the average productivity of the state.

There is inadequate supply of certified seeds to the extent that farmers almost solely depend on informal seed sources (farm-saved, local markets or neighbors). Self-supply is the major source of seed for most farmers. The high altitude farms being the sole source of clean basic seed in India, its physical and human capacity is limited, as it struggles with the double mandate of research and commercial basic seed production. It is handicapped by institutional arrangement in which there are very little incentives for increased productivity and efficiency it produces between 25 and 55 tonnes of basic seeds per year. Only about 1% of potato farmers can access quality planting material, since the collapse of the national seed distribution network in the quantities of certified seed potato which is not only inadequate but also highly priced with the implication that majority of farmers resort to using used seed of doubtful quality from various sources. This exacerbates the spread of seed-borne diseases especially bacterial wilt. From one season to the next, farmers select seed at harvest from their own farm but periodically go outside their farms to bring in "new" or "fresh" seed (seed renewal) from the other sources. Farmers renew seed for various reasons including acquiring a new variety, seed degeneration, disease and weather

*Corresponding Author

calamities (floods, drought etc). The concept of certified seed is not clear to most farmers and most people believe that when productivity of a variety decreases, then the variety is "used to" or "too familiar" to the soil. They, then usually buy seed from another area, exchange their seed with their neighbours or try and change the location of their potato plot. The farmers frequently plant the smallest tubers (chatts) as seeds and either eat or sell the bigger ones .These small seed tubers produce single stems, produce few tubers and are susceptible to diseases such as bacterial wilt and other environmental stresses (Lung'aho pers. Comm.). An economic analysis comparing use of farmers' seeds with use of certified seeds showed that, under the agronomic practices currently practiced by farmers and current prices of the certified seed and ware potatoes, farmers will incur loss, if they use certified seeds as opposed to their own seeds

Improper plant protection schedule leads to increased infestation of many insect pests as well as attack of diseases in unfavorable condition. Likewise local variety doesn't perform better for higher yield. The present system such as seed/soil treatment and recommended plant protection schedule, use of certified seed of suitable variety, 3 sprays of recommended plant protection chemical for control of major insect- pests and diseases enhanced the yield of potato. The proposed study will work out the extent of knowledge and adoption of plant protection practices demonstrated and advocated schedule to farmers under FLD conducted during the year 2012-15 with objectives to study the level of knowledge regarding use of plant protection schedule certified seed, to measure the extent of adoption regarding use of plant protection schedule and certified seed and to find out constraint in adoption of plant protection schedule and use of certified seed.

MATERIAL AND METHOD

The study was carried out in Surguja district of C.G. The FLDs on assessment of use of plant protection schedule and certified seed were conducted in the Village-Pando nagar, block Surajpur, District – Surguja (prior) during the year in *rabi* season 2012-15. Listed beneficiaries of FLDs were selected as respondents for study. For collecting in formations semi structure interview schedule designed on the basis of available literature was used and the data have been collected by personal interview or discussion with all respondents. The data were analyzed by using appropriate statistical framework such as frequency, mean and percentage, the level of knowledge and adoption measured by knowledge and adoption index.

The thirty five front line demonstrations were conducted at the farmers' field. These FLDs on potato were purposively conducted in tribal belt village Pandonagar. The soil of the district is mostly sandy loam in texture and suitable for the major crop of the district i.e. potato. Although maximum area of *rabi* and *kharif* both season covered by potato crop, still the average yield of the district is very low i.e. 15t/ha^{-1}. Improper plant protection schedule and lack of use of improved or certified seed of potato account low yield. To assess the performance of seed of improved variety / certified and use of recommended plant protection schedule at proper stage of crop, the front line demonstration were conducted during *rabi* seasons 2012-15. The area under each demonstration was 0.25 hectare. The major problems diagnosed were the early and late blight of potato. Use of certified seed of suitable variety and 3 sprays of recommended plant protection chemicals for control of major diseases i.e. blight, phytopthora wilt etc. and insect pests i.e. borer, potato tuber moth, aphids and sucking pests were demonstrated in FLDs.

To measure the extent of adoption of plant protection schedule and certified seed of potato, questions related with the various aspects of FLDs were identified / prepared in consultation with the experts in this field. The responses obtained from the respondents to these questions were rated on the three point continuum i.e. complete adoption, partial adoption and no adoption with the numerical score of 2, 1 and 0, respectively, before and after the training. The maximum score of an individual could be two and minimum zero.

The knowledge test composed of items called questions for constructing the knowledge test of all the conducted FLDs. The questions developed were discussed with the subject matter specialist, in the disciplines and were finalized. Answers to these questions were non-structured. The correctness of the answer was judged against the predetermined answers and was categorized as complete, partial and no knowledge and scored as 2, 1 and 0. The sum of scores of all the items of test for a particular individual was taken as the knowledge of that individual before and after the training. The knowledge index and adoption index were worked out with the help of following formula:

$$\textbf{Knowledge index} = \frac{\text{Knowledge score actually obtained by the respondents}}{\text{Maximum obtainable knowledge score by the respondents}} \times 100$$

The respondents were grouped into three categories on the basis of knowledge level by using following formula:

KI = Mean ($\overline{X}$) ± S.D. (Standard Deviation)

Categories	**Computed by**
Low level of knowledge	(< $\overline{X}$ - SD)

Medium level of knowledge (in between $\overline{X} \pm$ SD)

High level of knowledge (> $\overline{X}$ + SD)

$$\textbf{Adoption index} = \frac{\text{Adoption score actually obtained by the respondents}}{\text{Maximum obtainable adoption score by the respondents}} \times 100$$

The extent of adoption was as certained in terms of selected aspects of the use of plant protection schedule home science practices adopted by the trainees. The respondents were grouped into the following categories on the basis of using following formula:

AI = Mean ($\overline{X}$) ± S.D. (Standard Deviation)

Categories	**Computed by**
Low level of adoption	(< $\overline{X}$ - SD)
Medium level of adoption	(in between $\overline{X} \pm$ SD)
High level of adoption	(> $\overline{X}$ + SD)

RESULT AND DISCUSSION

Level of knowledge regarding various aspects of plant protection in potato production.

The data contained in table 1 clearly revealed the positive impact of Front line demonstration (FLD) pertaining to the various aspects of plant protection in potato right from the seed treatment.

The data significantly indicated that before FLDs, the majority of respondents were in the category of low level of knowledge. In this low level of category, the highest per cent of respondents (74.25 %) were found with regards to time or schedule of use of insecticide/ pesticide for storage pest, followed by seed treatment (71.43%), use of insecticide/ pesticide for storage pests (86.75%) ,time or schedule of use of insecticide / pesticide (65.72) and time or schedule of use of fungicide or other chemicals for disease control (62.86) . In other aspects of plant protection, the low level of knowledge was possessed by less than 60% respondents but not below 57 per cent.

With respect to knowledge level in medium category in each aspect of plant protection, remaining respondents were included and the share of respondents ranged between 25.72 per cent to 42.86 per cent as indicated by the data in table 1.

It was surprising that nome of the respondents belonged is high level of knowledge before FLDs.

The data given in table 1 regarding the knowledge level of FLDs clearly revealed the positive impact of FLDs and there was substantial increase in the knowledge of the respondents. This resulted in drastic reduction of respondents in low knowledge level category, increase in medium knowledge level category and shifting of some farmers into high level of knowledge category. 20.00 to 28.57 per cent respondents belonged to low level of knowledge after FLDs as against 57.14 to 74.28 per cent before FLDs with regards to various aspects of plant protection in potato crop.

There was a rise in the no. of respondents in middle level of knowledge and the respondents belonging to this category after FLDs ranged between 49.00 and 51.42 per cent as against 25.71 to 42.86 per cent before FLDs. 22.85 to 43.29 per cent respondents become successful in acquiring high level of knowledge pertaining to the various aspects of plant protection in potato production.

The data further indicated that there is a need to have successful FLDs in potato production so that none of the respondents will be found Saikh *et.al*.(1993),Sarda and Khurana (1994) , reported that farmers did not apply chemicals at proper time ,because they had information about time of application of chemicals low level of knowledge category.

Level of adoption regarding various aspects of plant protection in potato production.

The data (Table 2) revealed the significant impact of front line demonstration; data positively showed that before FLDs, the majority of respondents were in the category of low level of adoption. In this low level of category, the highest per cent of respondents (77.14%) were found with regards to seed treatment and time or schedule of use of insecticide/pesticide for storage pests both , followed by time or schedule of use of insecticide/pesticide and use of insecticide/pesticide for storage pests (71.43 %) both, use of insecticide / pesticide (65.71%), use of fungicide or other chemical for diseases control (63.86%) and time or schedule of use of fungicide or other chemical for diseases control (60.00%). In other aspects of plant protection, the low level of adoption was possessed by 60 % respondents.

With respect to adoption level in medium category in each aspect of plant protection, remaining respondents were included and the share of respondents ranged, between 22.86 per cent and 37.14 per cent as showed in table 2.

With regards to the adoption level in high category in various aspects of plant protection respondents were

included in range between 0.00 per cent and 28.75 per cent. Quadri et al. (2013) reported that high cost as a reason for not using plant protection measures by about 47.88 per cent respondents.

The data given in table 2 regarding the adoption level after FLDs revealed the positive impact of FLDs and there was substantial increase in the adoption of the respondents. This resulted into drastic reduction of respondents in low adoption level category, increase in medium adoption level category and shifting of some farmers into high level adoption category. 14.20 to 77.14 per cent respondents belonged to low level of adoption after FLDs as compared to 60.00 to 77.14 per cent before FLDs with regards to various aspects of plant protection in potato crop.

There was increase in the no. of respondents in middle level of adoption and the respondents belonging to this category after FLDs ranged between 37.14 to 54.28 per cent as against 22.86 to 37.14 per cent before FLDs.22.86 to 37.14 per cent respondents become successful in acquiring high level of adoption pertaining to the various aspects of plant protection in potato production.

The result further showed that there is a need to have successful FLDs in potato production so that none of the respondents will be found in low level of adoption category. Ibrahim, et.al. (2014), observed that in Munshiganj Sadar, highest proportion of the respondents was observed in high adoption categories in case of recommended potato variety (72.6%) after FLDs.

Level of knowledge regarding use of certified seed of potato

Data presented in table 3 revealed the level of knowledge regarding use of certified seed of potato before FLDs. Majority of the respondents belonged medium level of knowledge with regarding time of sowing (54.28%) followed by earthing up (48.57%), time of harvesting (45.71 %), seed rate (37.14), application of fertilizer(34.28%) respectively .However low level of knowledge were possessed by majority of the respondents in respect of source of availability of certified seed of potato and variety (82.86%), spacing(65.71%), seed rate (60.00 %), application of fertilizer(57.14 %) respectively. While high level of knowledge was about time of harvesting (25.71%), earthing up (22.86%) and time of sowing (14.28 %) . A very few respondents possessed high level of knowledge and maximum respondents (25.7 %) had high level of knowledge for time of harvesting followed by earthing up.

With respect to various aspects of certified seed of potato, the FLDs helped the respondents to improve their knowledge. There was increase in number of respondents in high knowledge level and medium knowledge level category and reduction of respondents in low knowledge level category.

Table 3 clearly revealed the positive impact of Front line demonstrations (FLDs) pertaining the knowledge regarding use of certified seed of potato right from the Source of availability of certified seed of potato.

Data significantly indicated that after FLDs the majority of respondents were included medium category. The share of respondents declined between 0.00 to 20.00 per cent as compared to 28.57 to 82.86 per cent in knowledge level before FLDs in low level of category. In medium category, share of respondents ranged between 37.14 to 54.28 per cent as compared to 17.1 to 54.28 per cent before FLDs. In high level category, after FLDs there was rise in respondents to 31.42 to 57.14 per cent as compared to 0.00 to 25.71 per cent before FLDs.

Level of Adoption regarding use of certified seed of potato

The data significantly indicated (Table 2) that before FLDs, the majority of respondents were in the category of low level of adoption. In this low level of category, the highest per cent of respondents (85.71%) were found with regards to seed rate followed by source of availability of certified seed of potato and use of variety (82.86%) both, spacing (54.29 %), use of time of sowing (48.5 7%) and application of fertilizer, earthing up time of harvesting (28.57%) respectively.

With respect to adoption level in medium category in each aspect of use of certified seed of potato remaining respondents were included and the share of respondents ranged between 17.14 to 54.28 per cent as indicated by the data in table 4. None of the respondents belonged is high level of adoption before FLDs.

The data given in table 1 regarding level of adoption regarding use of certified seed of potato revealed the positive impact of FLDs and there was substantial increase in adoption of the respondents. This resulted in to drastic reduction of respondents in low adoption level category, increase in medium adoption level category and shifting of some farmers into high level of adoption category. 0.00 to 20.00 per cent respondents belonged to low level of adoption after FLDs as against 28.57 to 85.71 per cent before FLDs with regards to various aspect of use of certified seed of potato.

There was a rise in the no of respondents in middle level of adoption and the respondents belonging to this category after FLDs ranged between 31.43 to 60.00 per cent as against 14.29 to 65.71 per cent before FLDs.

The data further indicated that there is a need to have successful FLDs in potato production so that none of the respondents will be found in low level of adoption category. Huque *et.al*. 1996 Findings revealed that majority of the potato growers (63 per cent) had moderate level of adoption, 32 per cent low and only 5 per cent had high adoption. Singh *et.al* 2010 concluded that about 82 per cent of the vegetable growers had low or medium adoption of Commercial potato cultivation practices

Impact of FLD on plant protection schedule and certified seed of potato on yield of potato

Data showed in table 5 revealed that average yield before FLD were 70q/ha^{-1} however it increased to 110q/ha^{-1} with increased in yield by 57%.

Problems faced by respondents regarding use of plant protection schedule

Data presented in table 6 revealed problems faced by respondents regarding use of plant protection schedule. Maximum number of respondents had problems about uncertain weather condition like frost, rainfall and hailstorm (97.14%) followed by disease infestation especially early and late blight of potato (88.57%) respectively.

Problems faced by respondents regarding use of certified seed

In case of problems faced by respondents (table 7) regarding use of certified seed, maximum respondents faced problems of more demand of local red variety of potato by consumer (100.00%) and more cost of seed potato(100.00%) both followed by non availability of certified seed of potato in market and lack of facility of cold storage(94.28%) both.

Table 1. Level of Knowledge regarding plant protection schedule of potato n=35

SN	Particular	Knowledge level Before FLD						Knowledge level After FLD					
		Low		Medium		High		Low		Medium		High	
		F	%	F	%	F	%	F	%	F	%	F	%
1	Seed Treatment	25	71.43	10	28.57	0	0.00	7	20.00	18	51.42	10	28.57
2	Use of insecticide / pesticide	21	60.00	11	31.42	3	8.58	10	28.57	17	48.58	8	22.85
3	Time or schedule of use of insecticide/ pesticide	23	65.72	8	22.85	4	11.42	10	28.57	16	45.71	9	25.71
4	Use of fungicide or other chemicals for diseases control	20	57.14	15	42.86	0	0.00	9	25.71	14	40.00	12	34.29
5	Time or schedule of use of fungicide or other chemical for diseases control	22	62.86	11	31.42	2	5.71	7	20.00	18	51.42	10	28.57
6	Use of insecticide /pesticide for storage pests	24	68.57	10	28.57	1	2.86	9	25.72	18	51.42	8	22.85
7	Time or schedule of use of insecticide /pesticide for storage pest	26	74.28	9	25.71	0	0.00	7	20.00	16	45.71	12	34.29

Table 2. Level of Adoption regarding plant protection schedule of potato n=35

SN	Particular	Adoption level Before FLD						Adoption level After FLD					
		Low		Medium		High		Low		Medium		High	
		F	%	F	%	F	%	F	%	F	%	F	%
1	Seed Treatment	27	77.14	8	22.86	0	0.00	5	14.28	18	51.43	12	34.29
2	Use of insecticide / pesticide	23	65.71	11	31.43	1	2.86	9	25.71	18	51.43	8	22.86
3	Time or schedule of use of insecticide/ pesticide	25	71.43	8	22.86	2	5.71	10	28.57	13	37.14	12	34.29
4	Use of fungicide or other chemical for diseases control	22	62.86	13	37.14	0	0.0	7	20.00	15	42.86	13	37.14
5	Time or schedule of use of fungicide or other chemical for diseases control	21	60.00	10	28.57	4	11.43	5	14.28	18	51.43	12	34.29
6	Use of insecticide/ pesticide for storage pests	25	71.43	10	28.57	0	0.00	5	14.28	19	54.28	11	31.43
7	Time or schedule of use of insecticide/pesticide for storage pests	27	77.14	8	22.86	0	0.00	6	17.14	17	48.57	12	34.29

Table 3. Level of Knowledge regarding use of certified seed of potato n=35

SN	Particular	Knowledge level before FLD						Knowledge level after FLD					
		Low		Medium		High		Low		Medium		High	
		F	%	F	%	F	%	F	%	F	%	F	%
1	Source of availability of certified seed of potato	29	82.86	6	17.14	0	0.00	3	8.57	17	48.58	12	34.28
2	Variety	29	82.86	6	17.14	0	0.0	2	5.71	19	54.28	14	40.00
3	Seed rate	21	60.00	13	37.14	1	2.85	5	14.28	15	42.86	15	42.86
4	Spacing	23	65.71	11	31.43	1	2.85	3	8.57	14	40.00	18	51.43
5	Time of sowing	11	31.43	19	54.28	5	14.28	2	5.71	22	62.85	11	31.42
6	Application of fertilizer	20	57.14	12	34.28	3	8.57	7	20.00	11	31.42	17	48.57
7	Earthing up	10	28.57	17	48.57	8	22.86	0	0.00	21	60.00	14	40.00
8	Time of harvesting	10	28.57	16	45.71	9	25.71	1	2.86	14	40.00	20	57.14

Table 4. Level of Adoption regarding use of certified seed of potato n=35

S N	Particular	Adoption Before FLD						Adoption After FLD					
		Low		Medium		High		Low		Medium		High	
		F	%	F	%	F	%	F	%	F	%	F	%
1	Source of availability of certified seed of potato	29	82.86	6	17.14	0	0.00	7	20.00	19	54.28	9	25.71
2	Variety	29	82.86	6	17.14	0	0.00	7	20.00	18	51.42	10	28.57
3	Seed rate	30	85.71	5	14.29	0	0.00	5	14.28	17	48.57	13	37.14
4	Spacing	19	54.29	11	31.43	5	14.28	1	2.86	20	57.14	14	40.00
5	Time of sowing	17	48.57	13	37.14	5	14.28	3	8.57	11	31.43	21	60.00
6	Application of fertilizer	10	28.57	23	65.71	2	5.71	2	5.71	15	42.85	18	51.42
7	Earthing up	10	28.57	17	48.57	8	22.86	0	0.00	21	60.00	14	40.00
8	Time of harvesting	10	28.57	19	54.28	6	17.14	0	0.00	19	54.28	16	45.71

Table 5. Impact of FLD on plant protection schedule & certified seed of potato on yield of potato

Crop	Yield before FLD	Yield after FLD	% Increase in yield
Potato	70q/ha	110q/ha	57

Table 6. Problems faced by respondents regarding use of plant protection schedule

S.No.	Problems	F	Yes	F	No
1	Uncertain weather condition like frost, rainfall and hailstorm.	34	97.14	1	2.86
2	Uncertain disease infestation specially early and late blight of potato.	31	88.57	4	11.43

Table 7. Problems faced by respondents regarding use of certified seed

Sr. No	Problems	Yes		No	
		F	%	F	%
1	Non availability of certified seed of potato in market	33	94.28	2	5.72
2	More demand of local red variety of potato by consumer (due to taste).	35	100.00	-	-
3	Lack of facility of cold storage	35	94.28	2	5.72
4	More cost of seed potato	35	100.00	-	-

REFERENCES

Huque, M.M., Rashid, M.H., Rahman, M.L. (1996). Adoption of improved practices by potato farmers. *Bangladesh J Agric Econs* **XIX** (1 & 2): 59-69.

Ibrahim, M.D, Khalil, Enamul Haque, M.D and Ziaul, Haque (2014). Adoption of recommended potato (*solanum tuberosum*) production technologies by the potato growers of some selected areas of Bangladesh. J. agril. Res. **39(1)**: 79-92.

Quadri Javeed Ahmad Peer, S.K. Kher, Nafees Ahmad, M.H., Chesti, J.S. Manhas, Kumar, Satesh and Gupta, Bhavana (2013). An Analysis of Reasons of Non- adoption of Selecting different crop production practices by potato growers in Sub-Tropics of Jammu Division. International j. of scie. **2(6)**:1-3.

Sarda, M.K. and Khurana, G.S. (1994). Adoption gap in the package of practices for rice crop in Ludhiana district Punjab. J.of Res. PAU, **31**: 22-26.

Singh, B.K., Singh, Dhiraj, Kumar, Yadav, V.P.S. and Singh, Lotan (2010). Adoption behaviour of commercial potato growers in district ghaziabad (up). *Indian Res J Ext Edu* **10** (3):5-9.

RESEARCH ARTICLE

IMPACT OF SPENT WASH IRRIGATION ON DIFFERENT SOIL CHARACTERISTICS

Sarika Goel[1]*, Sarika Maheshwari[2] and Kamakshi Saxena

[1]Dept of Botany, S.D. (P.G.) College, Muzaffarnagar
[2]Dept of Life Sciences, SRM University, Modinagar
[3]Dept of Applied Sciences, SRM University, Modinagar

Received-23.05.2017, Revised-15.06.2017

Abstract: Soil is the upper most surface of earth on which plants can be grown. Soil is a system, made up of small particles of different size. This system consists of water and minerals, different microorganisms, organic and inorganic content. Contamination of soil by any mean results in soil pollution that may cause harmful effect. One of such contamination is industrial waste which is discharge improperly into land and water bodies without any treatments. One of such industrial waste is distillery spent wash. India is a major producer and consumer of sugar in the world. A huge quantity of spent wash has been generated by these distillery whose disposable in to water bodies and land causes a number of environmental problems. To overcome this, the spent wash can be utilized in agricultural for irrigation purpose, as fertilizer and as manure. Application of spent wash in agriculture gives better crop productivity if used after proper dilution. The present investigation has been conducted to observe irrigation effect on different soil characteristics.

Keywords: Soil properties, Distillery spent wash, Irrigation, Fertilizer

INTRODUCTION

Soil is the loose and unconsolidated outer layer of earth's crust that is powdery in nature and made up of small particles of different sizes. Soil ecosystem includes inorganic and organic constituents, and the microbial groups.

India being a developing country, had establishing a large number of industries such as sugar, distillery, steel, paper, textile that play important role in progress of nation. These industries along with their product produce waste, causes various environmental problems. One of such industry is distillery, which produce spent wash as waste material. About 40 billion litre of spent wash is generated from distilleries in India (Chindankumar et al., 2009). The spent wash is acidic and loaded with organic and inorganic salts. Being plant origin, the spent wash contains considerable amount of plant nutrients and organic matter (Sindhu et al., 2007). Spent wash is an acidic effluent rich in organic carbon, K, Ca, Mg and S, considerable amount of N, P, traces of micronutrients viz Fe, Mn, Zn, and Cu, traces of sugar are also observed (Saliha et al., 2005). The distillery waste water is non-toxic, biodegrable, purely of plant origin and contains large quantities of soluable organic matter and plant nutrients but the problem with is high BOD and COD content. This waste water is used for irrigation purpose in many countries including India. In most of the areas, water scarcity has forced the farmers to use this spent wash as a substitute of irrigation water (Mittal and Tawari, 2008).

Different soil properties directly influence growth and productivity of plants and hence effects on economy and prosperity of human being. The present investigation deals with impact of distillery spent wash irrigation on different soil properties.

*Corresponding Author

MATERIAL AND METHOD

Different physiochemical characteristics of residual soil treated with different concentration of distillery spent wash have been analyzed. Different concentration of spent wash used were 5.0%, 10.0%, 15.8%, 25.1%, 36.6% and 39.8% (Anonymous, 1975) , collected from M/S Sir Shadi Lal Distillery and Chemical Works, Mansurpur, Muzaffarnagar. The residual soil treated with ordinary water served as control. Different parameters observed were soil pH, water holding capacity, wilting coefficient and total N, P, K content. The soil samples are taken initially and at different time interval after taking the plant samples at 60, 75, 90, 105, 120 and 135 days treated with different concentration of spent wash.

Preliminary examination of soil sample was done by squeezing a small amount of soil between thumb and fingers. For the determination of soil pH, 10 g of each soil sample was taken in 50 ml beaker and 25 ml of distilled water was added in each case. The beakers were kept for 6 hrs at room temperature. The pH readings were taken with the help of pH paper. Water holding capacity was determined by method of Pandeya *et al*., 1968. Wilting Coefficient is the percentage of water in the soil when the plant growing in it reached just a condition of permanent wilting was defined as wilting coefficient (Krammer, 1969). For the estimation of NPK content, method of Misra, 1968 and Jackson, 1973 were used.

The different aspect of physico-chemical characteristics of spent wash have been analyzed i.e. pH, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total nitrogen, phosphate and potassium. pH of the soil sample was determined by pH paper. DO and BOD was determined as per Manivaskam (1987). BOD was estimated after 5 days incubation in dark at 20^0C. COD was determined according to the method of Anonymous (1975). Estimation for nitrogen, phosphate and potassium was done as per procedure described by Manivaskam, 1987.

RESULT

To study distillery spent wash irrigation effect on different soil properties, it is necessary to observe some characteristics of waste water. All concentration of spent wash shows that waste water is acidic. Dissolved oxygen is undectable in all concentration of spent wash. BOD and COD values are very high, however good amount of NPK was observed.

Table 1. Physico-chemical characteristics of different concentration of spent wash

Character of spent Wash	**Concentration of spent wash**					
	5.0%	**10.0%**	**15.8%**	**25.1%**	**36.6%**	**39.8%**
pH	6.6	6.3	6.2	5.8	5.5	5.4
DO	Nil	Nil	Nil	Nil	Nil	Nil
BOD	2550 mg/l	5239 mg/l	8058 mg/l	12801 mg/l	18666 mg/l	20298 mg/l
COD	4429 mg/l	8358 mg/l	13047 mg/l	20727 mg/l	30224 mg/l	32867 mg/l
Total N	46.64 mg/l	93.73 mg/l	146 mg/l	232 mg/l	339 mg/l	369 mg/l
Total P	4.30 mg/l	6.40 mg/l	7.78 mg/l	11.45 mg/l	16.69 mg/l	19.25 mg/l
Total K	4.73 mg/l	7.30 mg/l	9.64 mg/l	15.31 mg/l	22.23 mg/l	24.28 mg/l

The soil of experimental plots was blackish gray with sandy loam texture. The soil comprised clay, slit, fine sand and coarse sand particles besides the humus, the organic fraction. The topsoil contained sand particles and humus in greater proportion than the other components. Humus contents were at the optimum level in the soil. On the whole sand particles constitute more than half of the total soil components. Soil moisture was in a highly dynamic condition and varied with seasonal fluctuation and irrigation regimes. Wilting point was 9.8%. Water holding capacity of the soil remained almost unaltered during the entire growth span of the plants. Analysis of soil for pH depicted the soil to be neutral. Mineral estimation of soil showed presence of potassium, phosphorus and nitrogen content. Potassium content was higher than the nitrogen contents.

Table 2. Physico-chemical properties of the soil of study site.

Soil texture	
Coarse sand (%)	1.5
Fine sand (%)	55.6
Slit (%)	21.5
Clay (%)	21.0
Wilting coefficient (%)	9.8
pH	7.20
Minerals	
Nitrogen (gm/m^2)	0.30
Phosphorus (gm/m^2)	0.75
Potassium (gm/m^2)	7.52

Soil pH remained almost unchanged from 60 to 135 days of crop growth in control as well as in various concentration of spent wash. Soil pH under the treatment of 5.0%, 10.0% and 15.8% spent wash was almost similar to the control. However, under the treatment of 25.1%, 36.6% and 39.8% spent wash, the soil pH was lowered. Maximum reduction in soil pH was observed in the soil irrigated with 39.8% spent wash. Water holding capacity of the soil remained almost unchanged from 60 to 135 days of plant growth in all spent wash treatments as well as in the control. The values were almost similar in various treatments of spent wash as compared to control. Nitrogen percentage in the soil increases from 60 to 105 days of growth and thereafter reduced from 120 to 135 days age of the plant in all treatments as well as in the control. Whereas phosphorus and potassium percentage in the soil increases from 60 to 90 days and thereafter reduced from 105 to 135 days of plant growth.

Table 3. Physical characteristics at different day interval of the soil treated with different concentration of distillery spent wash.

PHYSICAL CHARACTERISTICS OF TREATED SOIL						
Concentration of spent wash	**Age in days**					
	60	**75**	**90**	**105**	**120**	**135**
pH						
Control	7.58±0.04	7.63±0.02	7.50±0.01	7.65±0.01	7.70±0.02	7.73±0.04
5.0%	7.61±0.01	7.56±0.02	7.86±0.01	7.64±0.01	7.70±0.02	7.75±0.02
10.0%	7.60±0.03	7.59±0.02	7.80±0.16	7.61±0.02	7.64±0.01	7.61±0.02
15.8%	7.57±0.03	7.54±0.01	7.58±0.02	7.70±0.02	7.59±0.02	7.52±0.01
25.1%	7.50±0.02	7.49±0.02	7.50±0.02	7.51±0.01	7.42±0.03	7.40±0.02
36.6%	7.41±0.02	7.44±0.01	7.41±0.01	7.40±0.02	7.39±0.03	7.35±0.02
39.8%	7.01±0.10	7.11±0.03	7.02±0.10	7.12±0.02	7.00±0.10	6.99±0.09
Water Holding Capacity (%)						
Control	28.25±0.06	28.29±0.04	28.33±0.02	28.35±0.02	28.36±0.01	28.38±0.02
5.0%	28.15±0.02	28.17±0.03	28.19±0.03	28.21±0.03	28.33±0.03	28.25±0.02
10.0%	28.13±0.02	28.15±0.02	28.16±0.03	28.18±0.02	28.21±0.03	28.22±0.01
15.8%	28.10±0.03	28.11±0.02	28.12±0.03	28.15±0.02	28.17±0.03	28.19±0.03
25.1%	28.15±0.02	28.17±0.02	28.19±0.03	28.21±0.03	28.23±0.04	28.82±0.11
36.6%	28.22±0.03	28.24±0.02	28.28±0.04	28.29±0.03	28.31±0.03	28.54±0.10
39.8%	28.25±0.02	28.28±0.03	28.32±0.04	28.30±0.03	28.33±0.04	28.36±0.03
Wiliting Cofficient (%)						
Control	23.12±0.02	31.44±0.04	21.35±0.02	14.60±0.10	19.62±0.02	18.63±0.02
5.0%	24.21±0.05	30.40±0.18	22.53±0.08	16.40±0.14	20.19±0.14	19.19±0.05
10.0%	23.22±0.02	32.42±0.34	24.35±0.05	17.48±0.02	21.10±0.03	20.21±0.02
15.8%	21.77±0.04	30.04±0.04	19.53±0.05	13.30±0.02	18.26±0.04	17.63±0.03
25.1%	20.87±0.19	28.32±0.03	18.19±0.04	13.45±0.02	18.62±0.03	17.92±0.04
36.6%	18.83±0.05	25.52±0.09	17.19±0.02	13.02±0.01	17.26±0.03	17.02±0.04
39.8%	17.20±0.02	22.29±0.04	16.91±0.03	12.98±0.01	16.19±0.02	16.98±0.02
Values are mean ± S.E.						

When the values of nitrogen, phosphorus and potassium percentage of the soil under spent wash treatments were compared with the values in control, it was observed that the mineral percentage of the soil under the treatment of 5.0% and 10.0% spent wash was less than that of control while in the presence of 15.8%, 25.1%, 36.6% and 39.8% spent wash the mineral percentage of the soil was more than that of control. However the mineral percentage in the presence of 5.0% and 10.0% spent wash was less than that of control, in case of 10.0% spent wash the mineral percentage was less than 5.0% spent wash treatment.

Table 4. Available mineral (NPK) status (gm/m^2) of the soil at different days interval treated with different concentrations of spent wash.

MINERAL CONTENT OF SOIL						
Concentration of spent wash	**Age in Days**					
	60	**75**	**90**	**105**	**120**	**135**
Nitrogen content (gm/m^2)						
Control	0.33±0.02	0.19±0.06	0.28±0.36	0.43±0.65	0.34±0.03	0.32±0.02
5.0%	0.27±0.06	0.16±0.05	0.20±0.31	0.39±0.99	0.24±0.01	0.22±0.03
10.0%	0.31±0.04	0.22±0.09	0.22±0.20	0.40±0.90	0.31±0.06	0.25±0.04
15.8%	0.40±0.03	0.29±0.07	0.35±0.21	0.42±0.09	0.34±0.09	0.31±0.09
25.1%	0.43±0.08	0.39±0.12	0.40±0.12	0.43±0.08	0.36±0.08	0.33±0.07
36.6%	0.45±0.12	0.41±0.23	0.48±0.13	0.53±0.06	0.40±0.12	0.35±0.05
39.8%	0.48±0.13	0.45±0.025	0.52±0.17	0.56±0.02	0.46±0.12	0.37±0.01
Phosphorus content (gm/m^2)						
Control	0.90±0.03	1.35±0.12	1.75±0.12	1.35±0.03	1.05±0.03	0.90±0.06
5.0%	0.45±0.04	0.75±0.23	1.20±0.13	0.90±0.06	0.75±0.06	0.39±0.05
10.0%	0.75±0.06	0.90±0.34	1.35±0.15	1.08±0.05	0.90±0.02	0.45±0.07
15.8%	1.35±0.01	1.79±0.45	1.99±0.01	1.38±0.02	1.15±0.04	1.01±0.08
25.1%	1.75±0.02	1.80±0.21	2.05±0.09	1.45±0.01	1.35±0.08	1.20±0.09
36.6%	1.85±0.78	1.90±0.01	2.50±0.98	1.53±0.05	1.45±0.09	1.32±0.12
39.8%	2.00±0.12	2.03±0.02	2.80±0.87	1.59±0.08	1.48±0.99	1.37±0.14
Potassium content (gm/m^2)						

Control	13.50±0.32	13.90±0.09	16.60±0.03	7.20±0.21	6.30±0.02	4.50±0.02
5.0%	8.10±0.21	7.20±0.08	9.90±0.02	6.10±0.20	5.08±0.03	4.31±0.03
10.0%	11.70±0.12	9.00±0.05	13.90±0.04	6.98±0.12	5.58±0.02	4.38±0.02
15.8%	13.90±0.05	15.20±0.06	17.20±0.02	7.60±0.13	6.90±0.01	5.45±0.05
25.1%	13.50±0.06	16.40±0.07	18.20±0.01	8.50±0.14	7.40±0.01	6.35±0.01
36.6%	16.60±0.02	17.30±0.02	20.10±0.06	9.70±0.02	8.30±0.09	7.43±0.08
39.8%	19.80±0.01	20.20±0.03	21.30±0.02	10.10±0.09	9.00±0.08	8.16±0.09
Values are mean ± S.E.						

DISCUSSION

In an ecosystem, energy fixation and dry matter production of green plants depends greatly upon the supply of biogenic salts (Odum, 1970). The soil is the main source of the necessary elements from where they are absorbed and accumulated in the bodies of the vascular plants to make them available to the other living organisms of the ecosystem. In croplands nitrogen, phosphorus and potassium are added in the form of fertilizers since paucity of these elements between natural and man-made ecosystem is that, in the former, nutrient status of the soil is maintained through natural process of biogeochemical cycling, whereas in the latter a significant proportion of the soil nutrients removed by the plants do not reach to the crop fields but used by herbivores. Thus for a good crop, nutrients status of the soil is maintained at a sufficiently high level by using chemical fertilizers, organic manure and spent wash irrigation.

Soil pH decreased when irrigated with 25.1% to 39.8% spent wash. However irrigation with low concentration of spent wash does not affected much the pH of the soil. Hati *et al.,* 2005 also observed no appreciable changes in soil pH with the application of diluted spent wash. Other physico-chemical characteristics of soil such as water holding capacity and wilting coefficient were not much affected under all the treatments of spent wash. Zalawadia *et al.,* 1997 observe betterment in some physical characteristics of soil as application of spent wash in sugarcane field. More percentage of nitrogen, phosphorus and potassium was observed under the treatment of high concentration of spent wash (15.8% to 39.8%). Chindankumar and Chandraju, 2008, Kaushik and Khan, 2008 and Sindhu *et al.,* 2007 have presented similar reports. At the time of irrigation with higher concentration of spent wash water is absorbed by the plants and the salts carried in it, left behind in the soil. In the event of limited or no rainfall and inadequate applications of irrigation water, leaching of accumulated salts slows. Continuous irrigation with water containing effluents having high salt concentration causes soil to become more saline with time (Boralkar *et al.,* 1982).

Chemical analysis of spent wash depicts a regular increase in the amount of minerals in soil with increase in concentration of spent wash, which consequently increase the salinity of soil solution. Watering with the higher concentrations of spent wash having high BOD and COD results in the depletion of soil oxygen. This creates anaerobic conditions in the soil, which has been reported to affect adversely mineral composition of the soil (Kumar *et al.,* 1994). Application of spent wash improves the physical characteristics of soil as proved by Singhandhupe *et al*., 2009. Irrigation with distillery wastewater seems to be an attractive agriculture practice, which not only augment crops yield but also provides a plausible solution for the land disposable of the spent wash. The spent wash contained N, P, K, Ca, Mg and S and thus valued as a fertilizer when applied to soil through irrigation with water (Samuel, 1986). Application of distillery spent wash should be done after proper dilution (1:10 to 1:50) with irrigation water or by pre-plant application (40-60 days before planting) (Baskar *et al*., 2003). Kaushik and Khan, (2008) called spent wash as liquid gold for agriculture because of its valuable macro and micronutrients. The spent wash contains an excess of various form of cations and anions, which are injurious to plant growth and these constituents should be reduced to beneficial level by diluting spent wash, which can be used as a substitute for chemical fertilizer (Sahai *et al*., 1983). The diluted spent wash irrigation improved the physical and chemical properties of the soil and further increased soil microflora (Kaushik *et al*., 2005). The distillery spent wash contained all necessary elements and bio fertilizer microbes (*Rhizobia, Azospirilla, Azotobacter and Phosphobacteria*) to support the growth of plants (Babu *et al*., 1996).

CONCLUSION

It may be concluded form the present study that 5.0% and 10.0% concentration of spent wash may be used as a fertilizer. Up to 10 to 20 times dilution, the distillery spent wash may be used to improve the physicochemical characteristics of soil which are affected adversely due to irrigation with higher concentration of spent wash. Besides conventional, biological and chemical treatments by the distillreies, the spent wash should be properly diluted with water before discharge on land and use for irrigation purposes as fertilizer. The utilization of distillery spent wash in agriculture would save cost on fertilizer, better crop productivity and facilitate reduction in pollution load on aquatic system.

REFERENCES

Annonymous (1975). In: " Standard methods for the examination of water and waste water" 13th ed. (M. J. Taras, Arnold E. Greenberg, R. D. Hoak and M. C. Rand eds.) American Public Health Association, Washington.

Babu, R. S., Saralabai, V. C., Muralidharan, K. S. and Vivekanandam, M. (1996). Foliar application of distillery-spent wash as a liquid fertilizer for betterment of growth of *Sorghum vulgare* and *Cajanus cajan. Journal Applied Biochemistry and Biotechnology***. 59**(1):p.87-91

Baskar, M., Kayalvizhi, C., Bose, M.S.C. (2003). Eco-friendly utilization of distillery effluent in agriculture-a review. *Agricultural Reviews*. **24**(1):p.16-30

Boralkar, D. S., Trivedy, R. K., Pathak, S. C., Deshmukh, A. M. and Patil, M. A. (1982). Studies on soil pollution along the Krishna river in state of Maharashtra. *J. Env. Biol*. **3**:p.113

Chidankumar, C. S. and Chandraju, S. (2008). Impact of irrigation of distillery spent wash on the nutrients of pulses in untreated and treated soil. Journal Sugar Tech. **10(4):** p.314-318

Chidankumar, C. S., Chandraju, S. and Nagendraswamy, R. (2009). Impact of distillery spent wash irrigation on the yield of top vegetables (Creepers*). J. World Applied Science*. **6**(9): p.1270-1273

Hati, K. M., Biswas, A. K., Bandyopadhay, K. K., Mandal, K. G. and Misra, A. K. (2005). Influence of added spent wash on soil physical properties and yield of wheat under soyabean-wheat system in a vertisol of central India. *Env and Eco.* **23**(3):p.631-634

Jackson, M. L. (1973). Soil chemical analysis. Prantice Hall of India Pvt. Ltd., New Delhi.

Kaushik, K., Nisha, R., Jaggeeta, K. and Kaushik, C. P. (2005). Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with bio-amendments. *Bioresource Technol*. **96**(17)**:**p.1860-1866

Kaushik, P. and Khan, M. A. A. (2008). Ferti-irrigation of treated spent wash and its effect on sugarcane (*Saccharum officinarum* L.) and soil fertility: Sustainable technology option for reuse of waste water. *Crop Res*. **35(**3): p.218-225

Krammer, P. J. (1969). Plant and soil water relationship, a modern analysis. Mc Graw Hill Book Company, New York

Kumar, K. T. R. (1994). Effect of waste pollution on some crop plants. *Guj. Univ. Bull.* **25**:p.220-221

Manivaskam, N. (1987). Industrial effluent origin, characteristics, effects analysis and treatment. Sakthi Publications, Coimbatore, India.

Misra, R. (1968). Ecology work book. Oxford and IBH Publications, Calcutta, India.

Mittal, R. and Tiwari, A. (2008). Irrigation effect of Rairu distillery effluent on phosphorous content of wheat crop. *Natural Product Radiance*. **7**(4):p.344-346

Odum, H. T. (1970). Rain forest structure and mineral cycling homeostasis. A tropical rain forest. (ed. H.T.Odum) Division of technical information. US atomic energy commission

Pandeya, S. C., Puri, G. S. and Singh, J. S. (1968). Research methods in plant ecology. Asia Publishing House, Bombay

Sahai, R., Jabeen, S. and Saxena, P. K. (1983). Effect of distillery waste on seed germination, seedling growth and pigment content of rice. *Indian J. Eco*. **10:**p.7-10

Saliha, B. B., Krishnakumar, S., Saravaran, S. and Natarajan, S. K. (2005). Microbial and enzyme dynamics in distillery spent wash treated soil. *Research Journal of Agriculture and Biological Sciences*. **1**(2): 166-169

Samuel, G. (1986). The use of alcohol distillery waste as a fertilizer. *Proceeding of International American sugarcane seminar*.p.245-252

Sindhu, S. K., Sharma, A. and Ikram, S. (2007). Analysis and recommendation of agricultural use of distillery spent wash in Rampur district, India. *Journal of Chemistry***. 4(**3):p.390-396

Singhandhupe, R. B., Muduli, S. D., Chakrabarty, H. and Kumar, A. (2009). Evaluation of distillery effluent for crop irrigation. *JSIR*. **68**(4):p.332-337

Zalawadia, N. M., Raman, S. and Patil, R. G. (1997). Influence of diluted spent wash of sugar industries application on yield of and nutrient uptake by sugarcane and changes in soil properties. *J. of Indian Society of Soil Science.* **45**(4):p.767-769.

RESEARCH ARTICLE

EFFECT OF SULPHUR AND BORON ON GROWTH, YIELD AND ECONOMICS OF SOYBAEN (*GLYSINE MAX*L.)

Lomendra Kulhary,* T.D. Sahu, V.N. Mishra, Pradeep Kumar Dewangan and Yugal Kishor Sahu

Department of Soil Science and Agricultural Chemistry
Indira Gandhi Krishi Viswavidyalaya, Krishak Nagar Raipur-492012 (Chhattisgarh)
Email: lomendrakulhary01@gmail.com

Received-12.06.2017, Revised-24.06.2017

Abstract: An experiment was conducted at Indira Gandhi Krishi Viswavidyalaya, Krishak Nagar Raipur (Chhattisgarh) during *kharif* season 2015 in vertisol with objective to determine the effect of sulphur and boron application on yield and economics of soybean.The experiment was laid out in a RCBD with 16 treatments comprised four levels of sulphur *viz* 0, 15, 30 and 45 kg ha^{-1} and four levels of boron *viz* 0, 0.5, 1.0 and 1.5 kg ha^{-1}. Result revealed that yield of soybean was significantly influenced by different sulphur levels and maximum yield (20.04 kg ha^{-1} Seed yield and 22.55 kg ha^{-1}stover yield) was observed with 30 kg sulphur per hectare. Among boron levels, 1.0 kg boron per hectare was superior to others for getting maximum soybean yield (18.82 Seed yield and 21.05 kg ha^{-1}stover yield). Interaction of sulphur and boron levels had no significant different parameters under study Gross return (68388 `$ha^{-1}$) and net return (42286 `ha^{-1}) was significantly higher with the application of T_{11} ($S_{30}B_{1.0}$) Statistically highest Benefit cost ratio (2.64) was observed also with T_{11} ($S_{30}B_{1.0}$).

Keywords: Boron, Economics, Soybean, Sulphur, Yield

INTRODUCTION

Soybean designated as "miracle bean" has established its potential as an industrially vital and viable oilseed crop in many areas of India. It is a cheapest source of vegetable oil and protein. It contains about 40% protein, well balanced in essential amino acids, 20% oil rich with poly unsaturated fatty acids specially Omega 6 and Omega 3 fatty acids, 6-7% total mineral, 5-6% - area of 12.2 m ha, with production potential of 11.95 million tonnes and average productivity of 979.3 kg ha^{-1} (Anon., 2013a). The productivity of soybean is less in India as compared to world average (2484.1 kg ha^{-1}). Global area and production of soybean is 111.27 m ha and 276.4 million tonnes respectively (Anon., 2013b). The imbalanced and inadequate nutrition is found to be one of the major limiting factors for its poor yield. Among the major nutrients, sulphur is found to be quite important now a day in many soybean-growing areas. It is the 13th most abundant element in the earth crust with an average concentration of 0.06%. It is now considered as the 4th major plant nutrient after nitrogen (N), phosphorous (P) and potassium (K)for oilseeds. Sulphur is an important part of every living cell, required for the formation of chlorophyll and for the activity of ATP-sulphurylase (the enzyme involved in sulphur metabolism). It is involved in several important physiological functions in soybean including oil synthesis and acts as precursor for many amino acids, namely cysteine (26%S), cystine (27%S) and methionine (21%S) which act as building blocks for the synthesis of protein. As soybean is rich in both oil and protein, the requirement of sulphur is quite high. Over the years due to intensive cultivation and use of sulphur free fertilizers, the deficiency of sulphur has begun to appear and it is slowly becoming a major constraint for realizing higher yield in soybean. Sulphur deficiencies are now widespread in Indian soil and reports of more areas found deficient in S are coming in regularly. Recently, soil fertility survey by the Indian Council of Agricultural Research based on the analysis of 47,000 soil samples has shown S deficiencies to be a widespread problem. Besides sulphur, boron is another element, which is highly important in the physiological functions in soybean. Boron's widespread role within the plant includes cell wall synthesis, sugar transport, cell division, differentiation, membrane functioning, root elongation, and regulation of plant hormone levels. Boron has particularly attended an important position in intensive agriculture. Boron is required for the proper development of growing tips, phloem and xylem. Boron helps in germination and growth of pollen grains and also development of pollen-tube thus facilitating fertilization in plant and grain yield.

In Chhattisgarh, agriculture is mainly based on rainwater; therefore most of the crops are grown as rainfed in *kharif* season. Soybean occupies 1.52 lac hectares in Chhattisgarh with a productivity of 11.54 q ha^{-1}. More acreage of soybean in Chhattisgarh state is in plain area namely Durg, Bemetara, Rajnandgaon, Mungeli and Kabirdham districts. In Kabirdham district soybean is grown in 44.25 thousand hectare area with a productivity of 12.10 q ha^{-1}. Among the fertilizer elements, sulphur requirement of oilseed crops is quite high as compared to other crops. Oil seed crops respond to liberal application of sulphur and it is involved in the synthesis of fatty acids and also increases protein

*Corresponding Author

quality through the synthesis of certain amino acids such as cystine, cystein and methionine. Boron is associated with calcium utilization, cell division, flowering and fruiting, water relations, and protein synthesis. The fertility status of soils has been declining continuously due to non-judicious use of chemical fertilizers and intensive cropping without proper replenishment of nutrients and organic matter. Consequently, in addition to N, P and K deficiencies, deficiencies of some other nutrients such as S, Zn and B are being observed in many parts of the country. Many research works have been done on the effect of N, P and K fertilizers on the yield of soybean crop. But, a few works have been carried out on the effect of sulphur and boron on yield of soybean.

MATERIAL AND METHOD

The experiment was carried out during *kharif*, 2015at Indira Gandhi Krishi Viswavidyalaya, Krishak Nagar Raipur (Chhattisgarh) in vertisol with objective to determine the effect of sulphur and boron application on yield and economics of soybean.The experiment was laid out in a RCBD with 16 treatments comprised four levels of sulphur*viz* 0, 15, 30 and 45 kg ha^{-1} and four levels of boron *viz* 0, 0.5, 1.0 and 1.5 $kgha^{-1}$, replicated thrice.Soybean*var*.JS-335 was sown with spacing of 70 x 20 cm, seed rate 20 kg ha^{-1} and RDF 120:60:40 kg ha^{-1}.Height was measured in cm from ground surface to the tip of main stem. Height of five tagged plants in each plot was recorded in cm at harvest and then average was worked out and used for statistical analysis. Total number of pods was recorded from five randomly tagged plants and mean was worked out by dividing the total number of pods by five and used for statistical analysis. Randomly seed samples were taken from each net plot. 100 healthy seeds from the produce of each plot were counted and same were oven dried till constant weight and then weight was recorded in gram accurately by using an electronic digital balance. Seed yield of the net plot was noted down, after threshing, winnowing and drying then calculated in q ha^{-1} with appropriate multiplication factor. The harvested produce from each net plot was tied in bundles separately. Stover yield of plot was calculated after subtraction of seed yield from bundle weight. Bundle weight was recorded with the help of spring balance and converted into qha^{-1}.The harvest index was determined by using the formula given by Donald (1962).Gross return and cost of cultivation was calculated for each treatment, using current purchase price of inputs and the selling price of outputs prevailing in local market. Net profit was calculated as gross income subtracted by cost of cultivation. Benefit cost ratio was computed as the ratio of net return and cost of cultivation in the following formula:

Gross return (`$ha^{-1}$) = Income received from sale of grain, stover and stone (`ha^{-1})

Net return (`$ha^{-1}$) = Gross return (`ha^{-1}) - cost of cultivation (`ha^{-1})

$$\text{B:C ratio} = \frac{\text{Net return (`ha}^{-1}\text{)}}{\text{Cost of cultivation (`ha}^{-1}\text{)}}$$

Analysis of variance method (Gomez and Gomez, 2003) was followed for statistical analysis of various data. Significance of different sources of variations was tested by "error mean square method" of Fisher Snedecor's 'F' test at probability level 5%. In the tables of result the standard error of mean (SEm±) and the value of least significant difference (critical difference) at 5% between mean have been provided. Data on weed count and weed biomass were subjected to square root transformation $\sqrt{X+0.5}$ to make the analysis of variance valid (Gomez and Gomez, 2003).

RESULT AND DISCUSSION

Effect on growth

Plant height of soybean (Table - 1) ranged from 35.43 cm to 43.90 cm. Irrespective of the boron level, application of sulphur significantly affected plant height and maximum height (42.89 cm) was observed with application of 45 kg S ha^{-1} followed by 30 kg S ha^{-1} (41.63 cm) and 15 kg S ha^{-1} (39.18 cm). Plant height with application of 0, 0.5, 1.0 and 1.5 kg B ha^{-1} were 38.03, 40.99, 40.75 and 40.98 cm respectively and were statistically *at par*. Maximum plant height (43.90cm) was observed in the treatment T16 ($S_{45}B_{1.5}$) and minimum (35.43 cm) in control where no sulphur and boron were applied i.e. T1 (S_0B_0). Interaction effect between sulphur and boron level was found to be non-significant. Similar findings were reported by Chaubey*et al.* (2000)

Effect on yield attributes

Number of pods plant-1(Table - 1) significantly varied due to application of different sulphur levels. Number of $podsplant^{-1}$ ranged from 42.00 to 50.04. Minimum number was associated 0 kg S ha^{-1}. Which was increased with increasing level of sulphur and maximum number was observed with 45 kg S ha^{-1}. Maximum number of pods $plant^{-1}$ (47.17) was recorded due to application of 1.5 kg B ha^{-1} followed by 1.0 kg B ha^{-1} (46.92), 0.5 kg B ha^{-1} (46.75) and minimum number with 0 kg B ha^{-1} (44.33). However, number of pods $plant^{-1}$ under different boron levels did not differ significantly. Interaction of sulphur and boron level did not have any significant effect on the number of pods $plant^{-1}$ and maximum (50.04) and minimum (42.00) value were associated with T_{16} ($S_{45}B_{1.5}$) and T_1 (S_0B_0) respectively.

Table 1. Effect of sulphur and boron on growth, yield attributes and yield of soybean

Treatment	Plant height (cm)	Pods plant^{-1} (No.)	100 seed weight (g)	Seed yield (q ha^{-1})	Stover yield (q ha^{-1})	Harvest Index (%)
T_1 - S_0B_0	35.43	42.00	10.31	13.72	15.57	46.51
T_2 - $S_0B_{0.5}$	38.57	44.67	10.34	13.77	15.98	46.30
T_3 - $S_0B_{1.0}$	37.13	43.00	10.73	14.52	16.96	46.16
T_4- $S_0B_{1.5}$	36.03	45.00	10.80	14.60	16.64	46.73
T_5 - $S_{15}B_0$	36.47	44.33	10.45	13.90	16.56	46.03
T_6 – $S_{15}B_{0.5}$	38.90	44.00	10.57	16.66	18.49	47.39
T_7 – $S_{15}B_{1.0}$	41.97	45.67	12.46	18.12	20.02	47.47
T_8 – $S_{15}B_{1.5}$	40.43	44.67	12.39	18.96	20.48	48.08
T_9 – $S_{30}B_0$	39.43	45.33	10.34	19.78	21.04	48.47
T_{10} – $S_{30}B_{0.5}$	43.13	48.33	13.66	21.75	22.66	48.94
T_{11} – $S_{30}B_{1.0}$	40.40	49.33	12.92	21.83	23.26	48.32
T_{12} – $S_{30}B_{1.5}$	43.57	49.00	12.36	20.81	23.25	47.25
T_{13} – $S_{45}B_0$	40.80	45.67	10.54	16.68	18.59	47.27
T_{14} – $S_{45}B_{0.5}$	43.37	50.00	11.56	20.73	23.10	47.33
T_{15} – $S_{45}B_{1.0}$	43.50	49.67	12.87	20.82	23.96	46.38
T_{16} – $S_{45}B_{1.5}$	43.90	50.04	12.20	20.47	23.15	46.89
S levels (kg ha^{-1})						
0	37.05	43.67	10.55	14.15	16.29	46.42
15	39.18	44.67	11.47	16.91	18.89	47.24
30	41.63	48.00	12.32	21.04	22.55	48.25
45	42.89	48.83	11.79	19.67	22.20	46.97
B levels (kg ha^{-1})						
0	38.03	44.33	10.41	16.02	17.94	47.07
0.5	40.99	46.75	11.53	18.23	20.06	47.49
1.0	40.75	46.92	12.25	18.82	21.05	47.08
1.5	40.98	47.17	11.94	18.71	20.88	47.24
SEm±						
S Level	0.90	0.93	0.43	0.74	0.78	0.58
B Level	0.90	0.93	0.43	0.74	0.78	0.58
(SXB) Interaction	1.80	1.86	0.86	1.47	1.55	1.17
CD (P=0.05)						
S Level	2.59	2.69	1.24	2.13	2.24	NS
B Level	NS	NS	1.24	2.13	2.24	NS
(SXB) Interaction	NS	NS	NS	NS	NS	NS

Different level of sulphur had significant effect on 100 seed weight (Table-1). The highest weight of 100 seed (12.32 g) was found with 30 kg S ha^{-1} and the lowest 100 seed weight (10.55 g) was found with 0 kg S ha^{-1}. 100 seed weight with application of 30 kg S ha^{-1} and 45 kg S ha^{-1} (11.79 g) were found to be statistically *at par* with each other but significantly higher than that of 15 kg S ha^{-1} (11.47 g) and 0 kg S ha^{-1} (10.55 g) Irrespective of the sulphur level, boron level had significant effect on 100 seed weight. 100 seed weight of soybean was increased with increase in boron level up to 1.0 kg B ha^{-1} and beyond this level 100 seed weight was decreased. 100 seed weight with 0 kg B ha^{-1}, 0.5 kg B ha^{-1}, 1.0 kg B ha^{-1} and 1.5 kg B ha^{-1} were 10.41, 11.53, 12.25 and 11.94 respectively. Interaction of sulphur and boron level did not have any significant effect on 100 seed weight. Highest 100 seed weight (12.92 g) was observed with T_{11} ($S_{30}B_{1.0}$) and lowest 100 seed weight (10.31 g) was recorded in T_1 (S_0B_0).Highest harvest index was associated with 30 kg S ha^{-1} (48.25 %), 0.5 kg B ha^{-1} (47.49 %) and T_{10} - $S_{30}B_{0.5}$ (48.94 %).Result confirmed by Chaubey *et al.* (2000) and also supported by Halepyati (2001) and Singaravel *et al.* (2006).

Effect on yield

Seed yield of soybean ranged from 13.72 to 21.83 q ha^{-1}(Table 1). The highest seed yield (21.04 q ha^{-1}) was obtained with 30 kg S ha^{-1} followed by (19.67 q ha^{-1}) was obtained from 45 kg S ha^{-1}. The lowest one 14.15 q ha^{-1} was associated with 0 kg S ha^{-1}Seed yield of soybean was significantly influenced by boron level. Significantly higher seed yield was (18.82 q ha^{-1}) with 1.0 kg Bha^{-1} followed by 1.5 kg B ha^{-1} (18.71 q ha^{-1}) and 0.5 kg B ha^{-1} (18.23 q ha^{-1}) whereas the lowest seed yield (16.02 q ha^{-1}) was obtained from 0 kg B ha^{-1}.

The highest seed yield (21.83 q ha^{-1}) was recorded with T_{11} ($S_{30}B_{1.0}$) and the lowest one (13.72 q ha^{-1}) with T_1 (0 kg B ha^{-1} and 0 kg S ha^{-1}). But interaction effect between sulphur and boron level was found to be non-significant. Stover yield of soybean significant influence with different sulphur level. The highest stover yield of 22.55 q ha^{-1} was recorded with 30 kg S ha^{-1} and the lowest one (16.29 q ha^{-1}) was found with 0 kg S ha^{-1}. Boron level showed

significant influence on stover yield of soybean. The stover yield was more (21 .05q ha^{-1}) with 1.0 kg B ha^{-1} as compared to the stover yield of 20.88 and 20.06 q ha^{-1} which was associated with 1.5 and 0.5 kg B ha^{-1} respectively and lowest stover yield (17.94 q ha^{-1}) was obtained from 0 kg B ha^{-1}. The stover yield of soybean did not vary significantly by the interaction effect of sulphur and boron level. The sum total effect will be higher seed yield. The results confirm the findings of Kumar *et al*. (1992) and Sarkar*et al*. (2002).These finding were also supported by Halepyati (2001) and Singaravel *et al.* (2006).Results are in accordance with that of Singh *et al*. (2003), who documented that crop yields, in general, have been promoted by regular application of boron. Chowdhury *et al*. (2000) also reported that seed yield of cowpea increased significantly with the increase in boron application.

Effect on economics

Cost of cultivation of soybean was significantly varied due to application of different sulphur levels. Cost of cultivation(`$ha^{-1}$)of soybean ranged from 22557 to 27286. Significantly lowest cost of cultivation ` 22557 $ha^{-1}$was applied in T_3 ($S_0B_{1.0}$) and maximum cultivation ` 27286 $ha^{-1}$was applied in $T_{13}$($S_{45}B_0$).Gross return (68388 `ha^{-1}) and net return (42286 `$ha^{-1}$) was significantly higher with the application of $T_{11}$ ($S_{30}B_{1.0}$) which was *at par with* $T_{10}$ ($S_{30}B_{0.5}$) as compare to rest of treatments and significantly lowest gross (43547`ha^{-1}) and net return (17543 `ha^{-1}) found in control. Statistically highest Benefit cost ratio (2.64) was observed also with T_{11} ($S_{30}B_{1.0}$) which was *at par* being with T_{10} ($S_{30}B_{0.5}$) and T_{15} ($S_{45}B_{1.0}$). Similar findings were observed by Singaravel *et al.* (2006) and Singh *et al*. (2003).

Table 2. Effect of sulphur and boron on economics of soybean

Treatment	Cost of Cultivation (`ha^{-1})	Gross Return (` ha^{-1})	Net Return (` ha^{-1})	B:C Ratio
T_1 - S_0B_0	23914	43457	17543	1.61
T_2 - $S_0B_{0.5}$	22926	43792	20866	1.93
T_3 - $S_0B_{1.0}$	22557	46232	23675	2.05
T_4- $S_0B_{1.5}$	24270	46280	22010	1.91
T_5 - $S_{15}B_0$	24956	44420	19464	1.78
T_6 – $S_{15}B_{0.5}$	26093	52668	26789	2.03
T_7 – $S_{15}B_{1.0}$	26323	57122	30799	2.17
T_8 – $S_{15}B_{1.5}$	27061	59536	32475	2.20
T_9 – $S_{30}B_0$	24680	61948	37268	2.51
T_{10} – $S_{30}B_{0.5}$	25830	67880	42050	2.62
T_{11} – $S_{30}B_{1.0}$	26102	68388	42286	2.64
T_{12} – $S_{30}B_{1.5}$	25938	65731	39793	2.53
T_{13} – $S_{45}B_0$	27286	52663	25377	1.93
T_{14} – $S_{45}B_{0.5}$	27156	65448	38292	2.41
T_{15} – $S_{45}B_{1.0}$	25834	66112	40278	2.55
T_{16} – $S_{45}B_{1.5}$	26521	64797	38276	2.51
S levels (kg ha^{-1})				
0	23916	44940	21023	1.87
15	26108	53436	27381	2.05
30	25637	65986	40349	2.58
45	26699	62255	35555	2.35
B levels (kg ha^{-1})				
0	25709	50622	24913	1.96
0.5	25501	57447	31999	2.25

1.0	25204	59463	34259	2.35
1.5	25947	59086	33138	2.29
SEm±				
S Level	413.24	206.62	208.41	0.03
B Level	413.24	206.62	208.41	0.03
(SXB) Interaction	826.48	413.24	416.82	0.06
CD (P=0.05)				
S Level	1193.53	596.77	601.94	0.09
B Level	NS	596.77	601.94	0.09
(SXB) Interaction	NS	S	S	S

REFRENCES

Anon (2013a.) **and Anon.** (2013b). Food and Agriculture Organization (FAO) of theUnited Nations.*www.faostat.org*.

Chowdhury, M.M.U., Ullah, M.H., Rahman, M.A. and Islam, M.S. (2000). Effect ofboron and nitrogen fertilization on cowpea growth, nodulation and grain yield in Rangamati, Bangladesh. *Legume Research* **23**: 9-14.

Das, K.N. and Das, K. (1994). Effect of sulphur and nitrogen fertilization on yield andN uptake by rapeseed. *Journal of Indian Society of Soil Science* **42**: 476-478.

Donald (1962). In search of yield. *Journal of Aus. Agri. Science*. 28: 171-178.

Gomez, K.A. and Gomez, A.A. (2003). *Statistical Procedure for Agricultural Research*. John Wiley and Sons, London, U.K. *pp*. 139-167 and 204-207.

Halepyati, A.S. (2001). Conjunctive use of organic and inorganic fertilizers in rabi/summer groundnut in UKP area.*Karnataka Journal of Agricultural Science* **14 (2)**: 454-455.

Kumar, A., Singh, K.P., Singh, R.P. and Sarkar, A.K. (1996). Response of groundnut to boron application in acid sedimentary soil. *Journal of Indian Society of Soil Science* **44**:178-179.

Sarkar, S.K., Chowdhury, M.A.H. and Zakir, H.M. (2002). Sulphur and boronfertilization on yield quality and nutrient uptake by Bangladesh soybean. *Journal of Biological Science* **2**:729-733.

Singaravel, R., Parasath, V. and Elayaraja, D. (2006). Effect of organics and micro nutrients on the growth, yield of groundnut in coastal soil. *International Journal of agricultural Science* **2 (2)**: 401-402.

Singh, M.V., Patel, K.P. and Ramani, V.P. (2003). Crop responses to secondary and micronutrients in swell-shrink soils. *Fertilizer News* **48(4)**:63-66.

RESEARCH ARTICLE

EFFECT OF DATE OF SOWING ON GROWTH AND DEVELOPMENT OF COTTON

V.K. Vekariya*, H.R. Ramani, G.O. Faldu, K.B. Sankat, B.G. Solanki

Main Cotton Research Station, Navsari Agricultural University, Athwa Farm, Surat-395007
Email: vvekaria@nau.in

Received-24.05.2017, Revised-11.06.2017

Abstract: A field experiment was conducted during *kharif* seasons of 2013-14 at Main Cotton Research Station, Navasari Agricultural University, Surat to assess the effect of environment on cotton growth and development. The experiment was laid out in split plot Design comprising three dates of sowing as main plot and six genotypes as sub plot treatments replicated thrice. The result was indicated that no of days and GDD required to attain different phenological stages are significantly higher in normal sown condition. *Bt* hybrids required less no. of days and GDD to attain all phenological stage as compare to Non *Bt* Hybrids. G.Cot. Hy-8 BG-II was required lower GDD and days to attain all phenological stages. The Plant height, no. of sympodia, no. of bolls per plant and seed cotton yield was significantly decreased in delayed sown condition. ANKUR-3028 BG-II has significantly higher plant height, no. of sympodia, no. of bolls per plant and seed cotton yield as compare to other genotypes.

Keyworlds: Cotton, Climate change, Date of sowing, GDD, Growth

INTRODUCTION

Climate change is extremely affecting agriculture production. Deviation in temperatures will eventually reduce yields and increase the incidence of pests and diseases. Changes in precipitation are likely to lead to crop failures and production declines. There will be some gains depending on crops grown and regions. This evaluation applies largely to the regional impacts of cotton production (Cotton and Climate Change, 2011). In India cotton is the crop which affects the GDP of India. Cotton is stand for long time in the field and encounter different environments during their life cycle of 150 days to 210 days. The temperature and Growing Degree Days (GDD) represent two important spatially-dynamic climatic variables, as both play vital roles in influencing forest development by directly affecting plant functions such as evaporation, photosynthesis, plant respiration, plant water and nutrient movement. Crop growth and development refers to the increase in crop height, volume or area and weight in a certain time scale (Gudadhe *et al.*, 2013). The seed cotton yield per unit area is affected by a number of factors including land selection, sowing time, weeding, irrigation, chemical fertilizers etc. Of these, sowing period plays significant role in crop production process (Varlev *et al*., 2000 and Mohammad *et al*., 2015). Ariyo (1987) evaluated sowing 15 okra genotypes in 5 different environments and the results showed a significant environmental effect for all studied characters. Olasantan and Olowe (2006) reported that sowing dates significantly affected on vegetative growth, flowering, fruiting and harvesting stages (El-Waraky, Y. B., 2014). Climate change will impact on many facets of cotton physiology.

An integrative research process will be needed to assess the exact effect of climate change will have on cotton production (Michael Bange, 2007). Thereof, in this study effect of date of sowing on cotton growth and development has been carried out.

MATERIAL AND METHOD

A field experiment was conducted during *Kharif*-2013 at Main Cotton Research Station, Navasari Agricultural University, Surat to assess the effect of environment on cotton growth and development. The soils of the experimental field was clayey in texture having pH 7.3, medium in organic carbon (0.42 %) and available phosphorus and high in available potash (565 kg/ha).The experiment was laid out in split plot Design comprising three dates of sowing viz., D-1= early sowing (20 June 2013), D-2= normal sowing (11 July 2013), iii) D-3= Delayed sowing (6 August 2013) as main plot and six genotypes namely NCS-145 BG-II, RAH-100, G.Coy. Hy.-8 BG-II, G.Cot. Hy. 12, ANKUR-3028 BG-II, FHH-141as sub plot treatments replicated thrice. 1.20 m x 0.45 m plant spacing was kept and fertilized with 240:40:00 N:P:K. In each plot recommended agronomic practices were followed and plant stand was uniformly maintained. The observation were recorded on five randomly selected plants form each plot at harvest for all the traits viz., plant height, No. of sympodia, and yield components *viz.*, number of bolls per plant, average boll weight (g), and seed cotton yield per plant (g). The weather conditions *viz.*, rainfall, temperature and relative humidity were obtained from the main cotton Research Station, Surat. Heat units accumulated in each stage were calculated as per the equation (Growing degree days = $\sum[(T_{max} + T_{min}) /2 - T_b]$, T_b: base temperature. Statistical analysis was carried out at 5% level for

*Corresponding Author

significance, with date of sowing as main plot and genotypes as sub plot.

RESULT AND DISCUSSION

Phenology

Result showed that days required to attain different phonological stages differ significantly due to different dates of sowing and genotypes (Table-1). There was significant difference between dates of sowing for all phenological stages. The days to 50 % squaring, days to 50 % flowering, days to 50 % boll opening and days to maturity was significantly reduced as observed in delayed sowing (D-2) and early sowing. The genotype, G. Cot Hybrid-8 BG-II was earliest considering all phenological stages followed by Ankur-3028 BG-II while RAH-100 was required more days for all phonological stage. The interaction of sowing dates and genotype for all phonological stage except maturity days showed significant. G. Cot Hybrid-8 BG-II sown in early condition required less days for 50% squaring, 50% flowering and 50% boll opening followed by G. Cot Hybrid-8 BG-II sown in delayed condition and NCS-145 BG-II and ANKUR-3028 BG-II sown in early condition. while RAH-100 sown in normal condition was required more days for 50% squaring, 50% flowering and 50% boll opening followed by RAH-100 sown in delayed condition and LHH-144 sown in normal condition. Similar result was observed by Ghulam *et al.* (2014) and Mohammad *et al*. (2015), The result was also in accordance with result found by Sikder (2009) and Taher *et al.* (2015) for wheat.

Growing Degree Days (GDD)

The growing degree days to attain different physiological stage for cotton genotypes in different climate are presented in table -2. No. of GDD required was significantly lower in early sown condition for 50 % squaring, 50 % flowering, 50 % boll opening, while significant reduction was observed in number of GDD for maturity in late sown condition. The Genotype RAH-100 required significantly higher no of GDD for 50 % squaring, 50 % flowering, 50 % boll opening and maturity. The trend also showed that non Bt hybrids required higher no of GDD as compared to Bt hybrids for all phonological stage. The similar results were reported by Hebbar *et al.* (2002), Gudadhe *et al.* (2013) and Bandhopadhyay *et al.* (2008).

Plant height and sympodia per plant

The plant height and number of sympodia at harvest showed significant difference due to date of sowing and genotypes (Table-3). The plant height and number of sympodia were significantly reduced under delayed sowing whereas significant higher in early sowing and it was at par with normal sowing. Mohammad *et al.* (2015) who was reported that plant height and number sympodia per plant was significantly higher in early sown date. Plant height was significantly higher in ANKUR-3028 BG-II (114 cm) while G.Cot. Hy-8 BG-II showed significantly lower plant height. No of sympodia was observed significantly higher in ANKUR-3028 BG-II and it was at par with G.Cot. Hy-8 BG-II and NCS-145 BG-II. The significant interaction was observed for plant height. ANKUR-3028 BG-II sown in early condition attended significant higher plant height which was at par with G.Cot. Hy-12 sown in early condition and ANKUR-3028 BG-II sown in Normal condition. Plant height was observed significantly lower for G.Cot. Hy-8 BG-II sown in delayed condition which was at par with G.Cot. Hy-12 and RAH-100 sown in delayed condition. The plant height was significantly affected due to environment and genotypes (Hussain *et al.* 2007).

Yield and yield component

Table 4 showed that the significant reduction was observed in mean number of bolls in delayed sowing. Significantly higher no. of ball was observed in genotype ANKUR-3028 BG-II which was at par with G.Cot. Hy-8 BG-II. The average boll weight was significantly reduced for delayed sowing. Significantly higher boll weight was observed in ANKUR-3028 BG-II which was at par with NCS-145 BG-II. Plant seed cotton yield attain significantly higher in early sowing and it was at par with normal sowing. The genotype Ankur-3028 BG-II recorded significantly highest seed cotton yield per plant (118 g) over rest of the genotypes. Interaction for sowing date and genotype was significant. Ankur-3028 BG-II recorded significantly highest seed cotton yield per plant sown in early condition while LHH-144 showed lowest seed cotton yield per plant in delayed sawn condition. The seed cotton yield was significantly lower in late sown condition was also reported by Mohammad *et al.* (2015), Khalid *et al*. (2016).

Table 1. Phenological parameters in cotton genotypes under early, normal and delayed sown condition during *kharif 2013.*

Genotypes	Phenological Stages															
	Days to 50 % squaring				Days to 50 % flowering				Days to 50 % boll opening				Days to maturity			
	D-1	**D-2**	**D-3**	**mean**	**D-1**	**D-2**	**D-3**	**mean**	**D-1**	**D-2**	**D-3**	**mean**	**D-1**	**D-2**	**D-3**	**mean**
NCS-145 BG-II	65	70	67.3	**67.4**	83.7	90.3	84.3	**86.1**	128.7	133.3	132.3	**131.4**	149.3	167.3	151.7	**156.1**
RAH-100	67	77.3	75	**73.1**	85.3	99	95	**93.1**	129.7	141.3	142.7	**137.9**	154.3	172.3	161.3	**162.7**
G.Cot. Hy-8 BG-II	60	65	63.3	**62.8**	77.3	85.7	81.3	**81.4**	120.3	130.3	129.7	**126.8**	141.0	162.7	147.3	**150.3**

G.Cot. Hy-12	71	71	69.7	**70.6**	90.3	94	86.7	**90.3**	133.3	137.7	136.7	**135.9**	153.0	171.0	155.3	**159.8**
ANKUR-3028 BG-II	65	67.3	65.3	**65.9**	82.3	88	84.7	**85.0**	123.3	135.3	130.3	**129.6**	144.3	162.7	149.3	**152.1**
LHH-144	71	74.7	69.3	**71.7**	89.3	95.7	89.3	**91.4**	131	140.7	137.7	**136.5**	151.7	174.3	156.7	**160.9**
Mean	**66.5**	**70.9**	**68.3**		**84.7**	**92.1**	**86.9**		**127.7**	**136.4**	**134.9**		**127.7**	**136.4**	**134.9**	
LSD (p≤0.05)																
Sowing Dates (D)	1				1.8				1.2				0.8			
Genotype (V)	1				1				1.6				2			
D x V	1.8				1.8				2.7				NS			

Table 2. Growing degree days required by cotton genotypes under early, normal and delay sown condition during *kharif 2013*

Genotype	Growing Degree Days															
	Days to 50 % squaring				Days to 50 % flowering				Days to 50 % boll opening				Days to maturity			
	D-1	**D-2**	**D-3**	**mean**	**D-1**	**D-2**	**D-3**	**mean**	**D-1**	**D-2**	**D-3**	**mean**	**D-1**	**D-2**	**D-3**	**mean**
NCS-145 BG-II	1204	1307	1292	**1268**	1564	1699	1636	**1633**	2451	2515	2473	**2480**	2837	3064	2745	**2882**
RAH-100	1240	1447	1450	**1379**	1598	1872	1836	**1769**	2472	2661	2619	**2584**	2922	3136	2875	**2978**
G.Cot. Hy-8 BG-II	1107	1206	1215	**1176**	1439	1607	1574	**1540**	2285	2466	2435	**2395**	2688	2996	2686	**2790**
G.Cot. Hy-12	1314	1327	1338	**1327**	1700	1769	1680	**1716**	2544	2590	2532	**2555**	2900	3117	2795	**2937**
ANKUR-3028 BG-II	1204	1254	1254	**1237**	1537	1654	1643	**1611**	2346	2549	2444	**2447**	2748	2997	2712	**2819**
LHH-144	1314	1403	1332	**1350**	1680	1802	1729	**1737**	2499	2648	2546	**2564**	2877	3162	2813	**2951**
Mean	**1231**	**1324**	**1313**		**1586**	**1734**	**1683**		**2433**	**2571**	**2508**		**2829**	**3079**	**2771**	
LSD (p≤0.05)																
Sowing Dates (D)	20.7				36.8				20.8				13.2			
Genotype (V)	20.6				20				27.6				31.6			
D x V	38.2				47.8				48.1				NS			

Table 3. Growth characters in cotton genotypes under early, normal and delay sown condition during *kharif 2013*

Genotype	Plant Height (Cm)				Number of sympodia per plant			
	D-1	D-2	D-3	mean	D-1	D-2	D-3	mean
NCS-145 BG-II	116.0	113.7	88.3	**106.0**	23.7	23.4	18.8	**22.0**
RAH-100	106.0	102.5	85.6	**98.0**	20.7	19.7	14.8	**18.4**
G.Cot. Hy-8 BG-II	97.7	89.7	74.6	**87.3**	23.3	23.8	19.7	**22.3**
G.Cot. Hy-12	126.0	108.7	81.4	**105.4**	21.7	21.1	16.2	**19.7**
ANKUR-3028 BG-II	126.3	125.1	90.7	**114.0**	24.3	24.0	18.8	**22.4**
LHH-144	111.0	111.0	87.9	**103.3**	21.7	21.0	15.9	**19.5**
Mean	**113.8**	**108.5**	**84.8**		**22.6**	**22.2**	**17.4**	
LSD (p≤0.05)								
Sowing Dates (D)	7.74				2.69			
Genotype (V)	5.8				2.78			
D x V	10.04				NS			

Table 4. Yield contributing characters in cotton genotypes under early, normal and delay sown condition during *kharif 2013*

Genotype	No. of Bolls per plant				Avg. boll wieght (g)				Seed cotton yield per plant (g)			
	D-1	D-2	D-3	mean	D-1	D-2	D-3	mean	D-1	D-2	D-3	mean
NCS-145 BG-II	39.8	38.3	28.7	**35.6**	3.1	3.7	3.3	**3.4**	124.7	124.9	65.9	**105.2**

RAH-100	32.0	31.7	24.0	**29.2**	2.5	3.0	2.6	**2.7**	80.7	83.3	51.5	**71.8**
G.Cot. Hy-8 BG-II	43.4	38.0	29.0	**36.8**	2.7	3.4	3.0	**3.0**	115.7	107.1	67.6	**96.8**
G.Cot. Hy-12	34.3	34.0	28.3	**32.2**	2.6	3.1	2.7	**2.8**	102.3	101.4	64.9	**89.5**
ANKUR-3028 BG-II	45.7	40.3	31.3	**39.1**	3.2	3.8	3.4	**3.5**	144.3	136.6	73.0	**118.0**
LHH-144	28.2	28.3	23.7	**26.7**	2.5	3.1	2.6	**2.7**	79.0	76.2	50.2	**68.5**
Mean	**37.2**	**35.1**	**27.5**		**2.8**	**3.4**	**2.9**		**107.8**	**104.9**	**62.2**	
LSD (p≤0.05)												
Sowing Dates (D)	4.03				0.08				12.59			
Genotype (V)	3.16				0.16				8			
D x V	NS				NS				13.85			

REFERENCES

Ariyo, O.J. (1987). Stability of performance of okra as influenced by planting date. *Theor. Appl. Genet*, 74: 83- 86.

Bandhopadhyay *et al.* (2008). Predicting cotton production using infocrop-cotton simulation model, remote sensing and spatial agro-climatic data. *Current Science*, 95(11): 1570-1579.

Cotton and Climate Change: (2011). Impacts and Options to Mitigate and Adapt. International Trade Centre (ITC). Geneva: ITC, xii, 32

El-Waraky, Y. B. (2014). Effect of Sowing Date, Plant Density and Phosphorus Fertilization on Seed Yield of Okra. *Alex. J. Agric. Res.*, 59(1): 27-41.

Ghulam, M., Ehsanullah, Saif-ul-Malook, Muhammad, S., Muhammad, K. Shahbaz, U. C. and Qurban, A. (2014). A review of production for various Bt and non Bt cotton varieties in Pakistan. *Nature and Science*, 12(11): 81-91.

Gudadhe, N. N., Neeraj, K., Pisal, R. R., Mote, B. M. And Dhonde, M. B. (2013). Evaluation of Agrometeorological Indices in Relation to Crop Phenology of Cotton (Gossipium spp.) and Chickpea (Cicer aritinum L.) at Rahuri Region of Maharashtra. *Trends in Biosciences*,6 (3): 246-250.

Hebbar, K. B., Venugopalan, M. V., Rao, M. R. K., Gadade, G. D., Chatterji, S., and Mayee, C. D. (2002). Effect of sowing dates and fertilizer levels on phenology, growth and yield of cotton. *Indian Journal of Plant Physiology*, 7(4): 380–383.

Hussain, M., Ahmad, A. and Zamir, S.I. (2007). Evaluation of agro-qualitative characters of five cotton cultivars (*Gossypium hirsutum* L.) grown under Toba Tek Singh conditions. *Pak. J. Agri. Sci*., 44(4): 575-580.

Khalid, U., Ayatullah, Niamatullah, K. And Sohrab, K. (2016). Genotype-by-sowing date interaction effects on cotton yield and quality in irrigated condition of dera ismail khan, pakistan. *Pak. J. Bot*., 48(5): 1933-1944.

Michael, Bange (2007). Effects of climate change on cotton growth and development. *The Australian Cottongrower*, June-July: 41-45.

Mohammad, S., Akram, S., Abdul, R., Jamil, K., Muhammad, Y., Adnan, N. S., Nadil, S., Allah, B., Muhammad, Y. B. (2015). Influence of Different Planting Scheduling and Cultivar on the Growth and Yield of Cotton Crop. *Journal of Biology, Agriculture and Healthcare*, 5 (1) : 192-203.

Olasantan, F. O. and Olowe, V.I. (2006). Effect of sowing dates on response of okra (Abelmoshus esculentus) to intercropping with contrasting cassava cultivars. *International J. Tropical Agric*, 24: 1 – 2.

S. Sikder. (2009). Accumulated Heat Unit and Phenology of Wheat Cultivars as Influenced by Late Sowing Heat Stress Condition. Journal of Agriculture & Rural Development, **7**(1&2): 57-64.

Taher, B. F., Eman, I. El., Mosaad, K. H., and Ahmed, M. (2015). Evaluation and prediction of some wheat cultivars productivity in relation to different sowing dates under North Sinai region conditions. *Annals of Agricultural Science*, 60(1): 11–20.

Varlev, I., Popova, Z. and Gospodinov, I. (2000). Cotton sowing technologies. Productivity enhancement in cash crops. *Proc. 1st Int. Conf. Lisbon, Portugal, September*, 311-319.

RESEARCH ARTICLE

BIOLOGY OF *BRACON HEBETOR* SAY (BRACONIDAE: HYMENOPTERA) A LARVAL ECTO- PARASITOID ON RICE MEAL MOTH, *CORCYRA CEPHALONICA* STAINTON (LEPIDOPTERA : PYRALIDAE)

Sanjay Kumar,* Ghirtlahre, Rajeev Gupta and Jayalaxmi Ganguli

Department of Entomology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur-492012, Chhattisgarh, India.
Email: sanjayentomology@gmail.com

Received-08.06.2017, Revised-20.06.2017

Abstract: Biology study was made on reproductive parameters of *B. hebetor* reared on Rice meal moth**,***Corcyra cephalonica* Stainton at ordinary room temperature under laboratory conditions. The mean incubation period was 1.5 day and larval period 0.37 days. The pupal period lasted for 0.37 days with a range from 5-4 days. The mean development period of the parasitoid was 18.78 days. The females lived longer period (10.20 days) than males (6.40 days).

Keywords: Biology, *Bracon hebetor*, Rice meal moth, *Corcyra cephalonica*

INTRODUCTION

Biological control has been a valuable tactic in pest management programs around the world for many years. Biological control is a natural phenomenon of plant and animal regulation by their natural enemies. Biological control is a tool used in Integrated Pest Management (IPM) for several field agricultural systems and in protected crops systems. This technology is economically viable, of low environmental impact, and does not present risks of environmental contamination, human health nor for domestic animals (Orr, 2009). In the case of pest management, the major natural enemies are other insects, known as entomophagous, or microorganisms as entomopathogens. The entomophagous group is represented by predators and parasitoids. Most of the natural enemies belong to the order Hymenoptera.

Hymenopterans are one of the four megadiverse orders at world level. Females typically have a special ovipositor for inserting eggs into hosts or otherwise accessible places. The ovipositor is often modified into a stinger. Braconidae is the second largest family of Hymenoptera, comprising about 4,000 species. The braconid (Hymenoptera:Braconidae) is a cosmopolitan, gregarious, idiobiont arrhenotokous, ecto-parasitoid of Lepidoptera, Coleoptera and Diptera. Braconid is an important biological control agent for several insect pests (Heimpel *et al*., 1997; Darwish *et al*., 2003). Braconids have been widely used in various studies related to host-parasitoid interactions due to its high reproductive rate, short generation time, and considerable range of host species (Yu *et al*., 2002). Life cycle (biology) information on *B. hebetor* is not available. Such information is necessary for projecting population growth and designing insect mass rearing programs. Considering the above facts the present study was undertaken with the following objectives to measure the duration of different developmental stages and other related parameters of *B. hebetor* reared on the larvae of *Corcyra cephalonica.*

MATERIAL AND METHOD

2.5 kg of grains (Jwar+Maize+Bajra) were kept in wooden cages (45cm× 30cm×15cm). The grains were sterilized in hot air oven for one hour at 100^0C. After cooling the grains were grinded coarsely. 5 ml of 10% honey solution along with 5g of yeast and a pinch of Streptomycin were mixed in each container. Finally the containers were charged with 0.25cc (about 4750 eggs) of *C. cephalonica.* A study was made on reproductive parameters of *B. hebetor* on *C.cephalonica* at ordinary room temperature under laboratory conditions. The mouth of glass jar (15 cm x 9 cm) containing newly emerged one males and one females of *B. hebetor* was covered with a piece of white muslin cloth over which one full grown larvae of *C. cephalonica* were placed. After placing the larvae on the mouth of glass jar again another piece of white muslin cloth of same size was placed over the host larvae and kept in position with the help of rubber bands. Five replicates were used for each host species. After 24 h the parasitized larvae of each host species were removed gently without damage and were kept individually in plastic bowls (4.50cm x 3.50cm) for further study on various biological parameters of *B. hebetor*.

*Corresponding Author

RESULT AND DISCUSSION

Bracon hebetor Say mated frequently during day and night. Its females preferred to attack and oviposit on the fourth instar larval host. Ovipositing females were found to locate their hosts probably via trails containing semiochemicals produced in the mandibular gland of the host larvae (Shonouda and Nasr, 1998). Once the host was located, the female *B. hebetor* injected venom, which resulted complete paralysis of host within 15 min. It was also reported by Hagstrum and Smittle (1978). After the host was paralyzed, the female oviposited, usually placing a clutch of several eggs on the ventral surface of the host or on the side that was in contact with the substrate. Similar behavior of oviposition was also reported by Benson (1973) and Strand and Godfray (1989). The developmental periods of each life stages of *B. hebetor* observed in the present studies are shown in Table 1 and 2.

Egg: Freshly laid eggs were creamy white in colour and become translucent later. The deposited eggs were spindle shaped slightly curved, hyaline colourless and loosely attached to the surface of the host body. The mean incubation period of egg was 1.50 days which varied from 1 to 2 days (Table 1).

Larvae: The mature larvae were creamy white in colour and apodous. The mean larval duration was 3.60 days which varied from 3 to 4 days (Table 1).

Pupae: There was a pre- pupal stage, which lasts from 0.63 to 0.83 days. Pupation was found to take place outside the host body within a white coloured cocoon. Pupa was attached with each other by silken threads. The mature pupae were dark brown and exarate type. The mean pupal duration was 4.6 days with a range of 4.0 to 5.0 days.

Adult: The colour variation of adult parasitoid of *B. hebetor* was common with variable pterostigma having dark brown, sometimes with a large pale basal spot; body is nearly completely yellowish brown to largely dark brown or black. The last abdominal segment of the adult female was acute and possessed very short ovipositor. The last abdominal segment of male parasitoid being almost round. The average adult longevity was 6.40 and 10.20 days for male and female, respectively. In case of male, the minimum adult longevity was 5.0 days and maximum was 8.0 days. In this study the longevity of female is longer than male because female are larger and heavier than males due to slower rate of weight loss than that of males (Griggs, 1959).

Dabhi *et al.* (2011) studied the biology of *B. hebetor* on *G. mellonella* and found that the egg, larva and pupal period were 1.12, 3.42 and 4.64 days respectively. Longevity of male and female adult of *B. hebetor* was 8.27 and 24.12 days, respectively. Landge *et al.* (2009) studied the comparative biology of *Bracon hebetor* on *C. cephalonica* and *Opisina arenosella* Walker. In their study they found the incubation period, larval period and pupal period lasted for 23.32 hr and 24.26 hr, 64.8 hr and 72.48 hr, and 4.37 and 5.3 days on *C. cephalonica* and *O. arenosella*, respectively. Larval stage completed within five instars, pupation took place in white silken cocoon close to the host. Male and female adults from *C. cephalonica* and *O. arenosella* survived for 14.2 and 37.9, 12.05 and 20.85 days, respectively. Life-cycle of *B. hebetor* on *C. cephalonica* and *O. arenosella* was completed within 8.25 and 10.56 days, respectively. Farag *et al*., (2012) recorded adult longevity of *Habrobracon hebetor* reared on *Cadra* (*Ephestia*) *cautella* was 7.9 and 6.6 days for female and male, respectively. Pre-ovopositional and ovipositional periods lasted <12 h and 7.4 days, respectively. Total number of eggs/female was 69.3 eggs, with a mean of 9.45 eggs/day. Mean durations of immature stages reached 14.99 hrs, 2.48 and 5.65 days for egg, larval and pupal stages, respectively. These three observations were in agreement with the findings of the present study.

CONCLUSION

The biological study is important for mass rearing program in inundative release to ensure successful biological control of insect pests. So there is a great opportunity to use *Bracon hebetor* as a component of Integrated Pest Management programme for the suppression of lepidopteran pests.

Table 1. Development time (days) of different life-stages of *B. hebetor* reared on *Corcyra cephalonica* Stainton

Egg	Larva	Pre-pupa	Pupa	Adult		Female			Total life cycle	
				Male	Female	Pre-oviposition	Oviposition	Post - oviposition	Male	Female
1.50	3.60	0.78	4.60	6.40	10.20	0.62	8.20	2.00	16.88	20.68

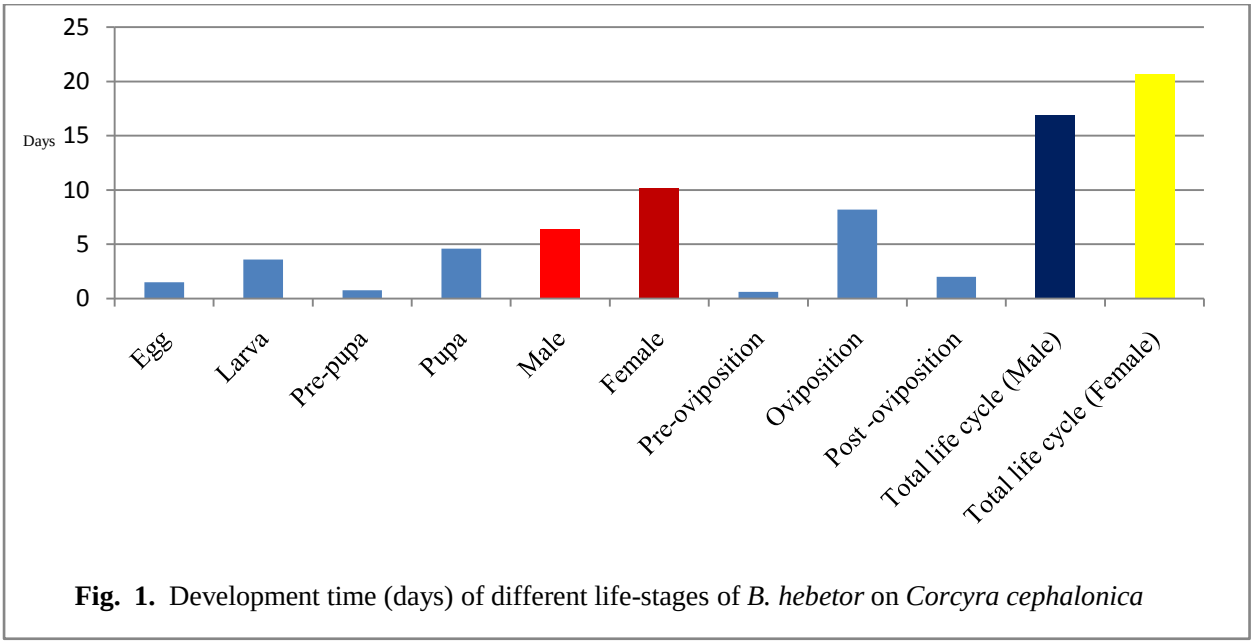


Fig. 1. Development time (days) of different life-stages of *B. hebetor* on *Corcyra cephalonica*

Table 2. Influence of *Corcyra cephalonica* larvae on fecundity, egg hatching (%), eclosion (%), viability (%) and sex ratio of *B. hebetor.*

Fecundity	Egg hatching (%)	Eclosion (%)	Viability (%)	Sex ratio (F : M)
112.80	70.79	91.25	44.53	1.67

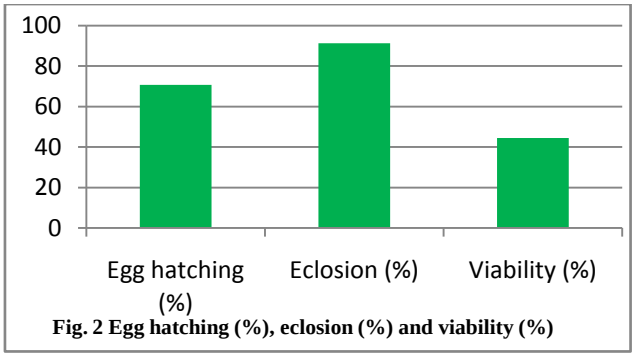


Fig. 2 Egg hatching (%), eclosion (%) and viability (%)

Table 3. Percentage of survival of immature stages of *Bracon hebetor* reared on six lepidopteran host species.

Survivorship (%)			
Egg	**Larva**	**Pupa**	**Egg-adult**
70.79	68.92	91.25	44.53

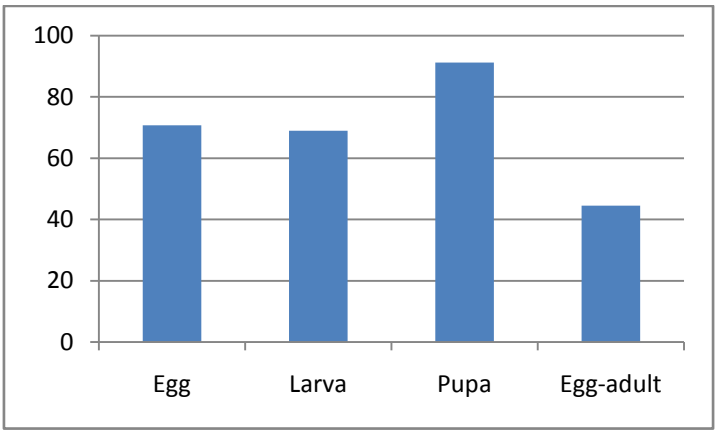


Fig. 3. Survivorship (%) of *Bracon hebetor*

REFERENCES

Benson, J. F. (1973). Intraspecific competition in the population dynamics of *Bracon hebetor* Say. Journal of Animal Ecology, 42: 193-197.

Dabhi, M. R., Korat, D.M. and Vaishnav, P. R. (2013). Reproductive parameters of *Bracon hebetor* Say on seven different hosts. African Journal of Agricultural Research. 8(25), pp. 3251-3254.

Dabhi, M. R., Korat, D. M. and Vaishnav, P. R. (2011). Comparative biology of *Bracon hebetor* Say on seven lepidopteran hosts. Karnataka J. Agric. Sci., 24 (4) : (549-550).

Darwish,-E; El-Shazly,-M; El-Sherif,-H. (2003). The choice of probing sites by Bracon hebetor Say (Hymenoptera: Braconidae) foraging for Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). Journal-of-Stored-Products-Research. 39(3): 265-276.

Farag,-M-M-A; Sayeda,-S-A; El-Husseini,-M-M. (2012). Life history of Habrobracon hebetor Say (Hymenoptera: Braconidae) parasitizing Cadra (Ephestia) cautella (Walker) (Lepidoptera: Pyralidae) on dried date fruits. Egyptian-Journal-of-Biological-Pest-Control. 22(1): 73-77.

Grisggs, R. C. (1959). A study of succinic dehydrogenase activity in the wasp *Habrobracon juglandis* (Ashmead). Bios, 202-207.

Hagstrum, D. W. and Smittle, B. J. (1978). Host utilization by *Bracon hebetor*. Environmental Entomology, 7: 596-600.

Heimpel, G.E., Antolin M.F., Franqui R.A., Strand M.R. (1997). Reproductive isolation and genetic variation between two "strains" of *Bracon hebetor* (Hymenoptera: Braconidae). Biological Control. 9**,** 149–156.

Landge, S. A., Wankhede, S. M and Gangurde, S. M. (2009). Comparative Biology of *Bracon hebetor* Say on *Corcyra cephalonica* Stainton and *Opisina arenosella* Walker. International Journal of Plant Protection, 2 (2): 278-280.

Orr, D. (2009). Biological Control and Integrated Pest Management. Department of Entomology, North Carolina State University, Raleigh, North Carolina, 27695- 7613, USA.

Shonouda, M. L. and Nasr, F. N. (1998). Impact of larval-extract (kairomone) of *Ephesiia kuehnie//a* Zell. (Lep., Pyralidae), on the behaviour of the parasitoid *Bracon hebetor* Say. (Hym., Braconidae). Journal of Applied Entomology, 122: 33-35.

Strand, M. R. and Godfray, H. C. J. (1989). Superparasitism and ovicide in parasitic Hymenoptera: theory and a case study of the ectoparasitoid *Bracon hebetor*. Behaviour Ecology and Sociobiology, 24: 421-432.

Yu S.H., Ryoo M.I., Na J.H., Choi W.I. (2002). Effect of host density on egg dispersion and the sex ratio of progeny of *Bracon hebetor* (Hymenoptera: Braconidae). J. Stored Prod. Res. 39 (4): 385–393.

RESEARCH ARTICLE

ADOPTION OF INTEGRATED PEST MANAGEMENT PRACTICES AMONG SOYBEAN GROWERS IN REWA DISTRICT (M.P.)

Anil Mandloi*, A.S. Chouhan, Sanjay Singh and Dharmendra

Department of Extension Education,
College of Agriculture Rewa, JNKVV Jabalpur M.P.

Received-10.06.2017, Revised-22.06.2017

Abstract: Keeping in mind the importance of Integrated Pest Management (IPM) in soybean crop, the present study was conducted in Rewa District with the objective to study the extent of adoption of Integrated Pest Management practices among the by soybean growers. Primary data were conducted from 120 farmers from Rewa blocks of Rewa district usingproportionate random sampling method. Pre- structured interview schedule was used for data collection by using personal interview method. The findings of study indicated that higher percentage 44.17 percent respondents had low adoption of Integrated Pest Management practices.On the basis of mean adoption score regarding different management practices it may be concluded that the adoption of thecultural practices was found to be highest followed by chemical, mechanical and biological practices on the basis of farmers feedback obtained in the study it suggests .it is an urgent need of trials and demonstration on IPM practices and skill oriented trainingprogramme for soybean growers in Rewa districtfor higher and safer soybean production.

Keywords: Integrated Pest Management, Adoption, Mean adoption score, Soybean

INTRODUCTION

Soybean (*Glycine max* L. merill) became the miracle crop of the 21st century. Soybean is the single largest oilseed produced in the world of the total 310-320 million tones oilseeds produced annually. On the global scale, it tops on the list of oilseed crops. Amongst soybean producing countries USA enjoys first rank while India is placed in fifth position. In India in the year 2014-15 area under soybean crop was 116.28 lakh hectare with the production of 86.42 lakh ton (SOPA 2014-15). Madhya Pradesh is known as "Golden State or Soya State" because of highest area sown in soybean as compared to other states in India. The total area under soybean in M.P. was recorded as 6164.40 thousand hectares and production of 4517.30 thousand ton with the productivity 733 kg/ha (M.P. Govt. in 2013-14). The productivity of soybean is affected by many factors viz. crop genetics, resource managements and climatic factors. Yield losses due to individual diseases/ insect/ weed species ranges from 20 to 100 percentage(Anonymous 2014).Integrated Pest Management (IPM) approaches have been globally accepted for achieving sustainability in agriculture and maintaining the agro-eco-system. It has more relevant due to a number of advantages like safely to environment, pesticide-free food commodities, low input based crop production. Keeping this in view the present study was under taken to assess the extent of adoption of Integrated Pest Management practices among the soybean growers.

METHODOLOGY

The study was purposively conducted in Rewa district of Madhya Pradesh due to larger area undersoybean crop. The district comprises 9 blocks out of which Rewa block was selected purposively because this block is occupying the largest area under soybean crop presently. From this block, five villages and soybean growers were selected by using proportionate random sampling method, to make a sample of 120 respondents. The selected respondents were interviewed with the help of a pre- structured interview schedule. The collected data were analyzed in the light of the objective. To study the extent of adoption of Integrated Pest Management practices by soybean growers.An index was developed in consultation with the experts and scientists of College of Agriculture Rewa, JNKVV (M.P.). All the recommended practices of IPM namely cultural, mechanical, biological and chemical practices were incorporated in the index. The responses of the respondents were recorded on 3 points scale as complete, partial and incomplete with the scores 2,1 and 0 respectively.

*Corresponding Author

RESULT

Table 1. Practice wise adoption of Integrated Pest Management practices (N=120)

S. No.	Statement	Extent of Adoption			Total score	Mean adoption score	Rank
		complete	partial	Incomplete			
A	**Cultural practices**						
1	Deep summer ploughing	85 (70.83)	24 (20.00)	11 (9.16)	194	1.61	I
2	Use of Recommended dose of chemical fertilizer i.e. NPK	44 (36.67)	52 (43.33)	24 (20.00)	140	1.16	VII
3	Use of resistant varieties.	73 (60.83)	30 (25.00)	17 (14.17)	176	1.46	V
4	Hand weeding or hoeing.	79 (65.83)	27 (22.50)	14 (11.67)	185	1.54	III
5	Timely sowing	82 (68.33)	28 (23.33)	10 (8.33)	192	1.60	II
6	Seed rate and proper spacing	57 (47.50)	51 (42.50)	12 (10.00)	165	1.37	VI
7.	Removal and destruction of infected and infested stubbles	77 (64.17)	26 (21.67)	17 (14.16)	180	1.50	IV
8.	Crop rotation	43 (35.83)	48 (40.00)	29 (24.17)	134	1.11	VIII
9	Inter-cropping	49 (40.83)	29 (24.17)	42 (35.00)	127	1.05	IX
	Over all mean Score					1.37	
B	**Mechanical practices**						
10	Hand picking and destruction of larvae/eggs	38 (31.66)	22 (18.33)	60 (50.00)	98	0.81	III
11	Use of yellow trap	20 (16.66)	44 (36.66)	56 (46.66)	84	0.70	IV
12	Use of Pheromone trap	10 (8.33)	24 (20.00)	86 (71.66)	44	0.36	V
13	Use of light trap	30 (25.00)	40 (33.33)	50 (41.66)	100	0.88	II
14	Collection and destruction of infested plant parts	64 (53.33)	36 (30.00)	20 (16.66)	120	1.36	I
	Over all mean Score					0.82	
C.	**Biological control**						
15	Seed treatment with *Trichodermaviride*(@ 4-5 g/kg seed).	40 (33.33)	45 (37.50)	35 (29.16)	120	1.04	III
16	Use of bio fertilizer	49 (40.83)	46 (38.33)	25 (20.84)	144	1.20	I
17	Spraying of NPV.	5 (4.16)	17 (14.16)	98 (81.66)	27	0.22	V
18	Installing perchers for birds	17 (14.16)	23 (19.16)	80 (66.66)	57	0.47	IV
19	Use of NeemKarnel Extract 4% or Nemecticide	47 (39.16)	33 (27.50)	40 (33.33)	127	1.05	II
	Over all mean Score					0.79	
D	**Chemical practices**						
20	Soil application of Phorate 10 G @ 15 kg/ha at sowing time	24 (20.00)	20 (16.66)	76 (63.33)	68	0.56	IV
21	Seed treatment with Thiram+Carbendazim.	66	28	26	160	1.33	II

		(55.00)	(23.33)	(21.66)			
22	Spraying of Trizophos 40 EC @ 800ml/ha or chloropayriphos 20EC @ 1500ml/ha insecticide	74 (61.66)	26 (21.66)	20 (16.66)	174	1.45	I
23	Seed treatment with Thiamethoxam 30FS @10ml/kg seed.	56 (46.66)	35 (29.16)	29 (24.16)	147	1.22	III
	Over all mean Score					1.44	
	Mean adoption score of all the practices					1.10	

The practices wise distribution of the respondents according to adoption about IPM practices is presented in under Table 1. It is clear from Table 1 that in care of cultural practices the mean adoption score was highest in summer deep ploughing (1.61) followed by timely sowing (1.60), hand weeding (1.54), removal and destruction of infected and infested stubbles (1.50), use of resistant varieties (1.46), seed rate (1.37), use of recommended dose of chemical fertilizer (1.16), crop rotation (1.11) and intercropping (1.05).

As far as mechanical management practices was concerned mean adoption score was arranged in descending order as collection and destruction of infected plant part (1.36) followed by use of light trap (0.88), hand picking and destruction (0.81), use of light trap (0.70), use of pheromone trap (0.36).

As regarding the biological management practices mean adoption score was highest in use of bio fertilizer (1.20) followed by use of nemecticide (1.05), Seed treatment with *Trichodermaviride*, installing perchers for birds (0.47) and spray of NPV (0.22).

Among the chemical management practices it was observed that the mean adoption score was highest in spray of trizophos (1.45) followed by seed treatment with thiram + carbendazim (1.33), seed treatment with thiamethoxam (1.22) and application of phorate (0.56).

On the basis of mean adoption score it may be concluded that the adoption of thecultural practices was found to be highest followed by chemical, mechanical and biological. This finding is supported byVenkatesh Gandhi *et al* (2008).

Table 2. Distribution of respondents according to their adoption level regarding integrated pest management practices

S. No.	Extent of Adoption	No. of respondents	Percentage
1.	Low	53	44.17
2.	Medium	38	31.67
3.	High	29	24.16
Total		**120**	**100.00**

The Table 2 reveals that out of total selected soybean growers, 44.17 per cent had low adoption level, followed by 31.67 percent had medium and only 24.16 per cent had high adoption level of integrated pest management practices.This finding is supported byMahoviya (2006) and Raghuwansi (2010).

CONCLUSION

The result of the studyrevealed that adoption of IPM amongsoybean growersthere was considerably low. Generally, soybean growers hadhigher adoptionregardly cultural practices followed by chemical,mechanical and biological practices.Therefore, it is an urgent need of conducting trials and demonstration and skill oriented trainingprogrammeon IPM forsoybean growers regarding to enhance the adoption of IPM among the soybean growers.

REFERENCES

Anonymous (2014). Report of National Centre for Integrated Pest Management.

Mahoviya, R. (2006). A study to investigate the extent of adoption of recommended mustard cultivation technology in different farm size of farmers of Morena block of Morena district (M.P.). M.Sc. (Ag.) Thesis (unpublished) JNKVV, Jabalpur

Raghuwansi, S. (2010). A study on adoption of improved production technology by the soybean growers in Hoshangabad of M.P. M.Sc. (Ag) Thesis (Unpublished) J.V.K.V.V., Jabalpur: 63-65

Soybean Production Association area and production of soybean in India kharif 2015 survey conducted by indore with effect from September 15-27

Venkatesh Gandhi, R., Hanchinal, S.N., Shivamurthy, M. and Hittalmani, S. (2008). Adoption of integrated pest management practices among the tomato growers. Karnataka J. Agric. Sci. Vol. 21(1): 17-19.

RESEARCH ARTICLE

IN-VITRO EVALUATION OF VARIOUS FUNGICIDES, PLANT EXTRACTS AND BIO CONTROL AGENTS AGAINST ROOT ROT OF AJWAIN

Babu Lal Fagodia*, B.L. Mali and R.K. Fagodiya

Department of Plant Pathology, Rajasthan College of Agriculture (MPUAT), Udaipur, Rajasthan 313 001
Email: blfagodia25@gmail.com

Received-07.06.2017, Revised-25.06.2017

Abstract: An incubation study was conducted at Department of Plant pathology, Rajasthan College of Agriculture, Udaipur to evaluation of different fungicides, plant extracts and bio-agents against ajwain (*Trachyspermum ammi* L.) root rot caused by *Rhizoctonia solani* and results revealed that treatment comprising of fungicide Bavistin, Plant extracts Neem oil and bio control agent *Trichoderma viride* at 7 days of incubation at 28±1^{0}C was found significantly superior over control and gave maximum percent growth inhibition of *R. solani.*

Keywords: *Ajwain, R. solani fungicide, Plant extracts, Bio-agents*

INTRODUCTION

Ajwain (*Trachyspermum ammi* L.) also known as Bishop's weed and Carom, is one of the most important seed spice crop it belongs to family *Apiaceae* and is believed to have originated from India and Egypt. In India it is widely distributed and its production is concentrated mainly in Rajasthan followed by Gujarat, Madhya Pradesh, Bihar, Utter Pradesh, Punjab, Tamil Nadu, Andhra Pradesh and West Bengal, respectively. Since ancient time the state of Rajasthan and Gujarat has emerged as "Seed spices bowl". Whose dried fruit of seeds are used as spices.

In Rajasthan, it is cultivated in the districts of Chittorgarh, Udaipur, Jhalawar, Baran, Rajsamand, Bhilwara and Kota covering an area of 11658 hectares with the production and productivity is 4672 tonnes per annum and 401 kg/ha, respectively (Anonymous, 2015-16). In India, Rajasthan contributes 73 per cent of total production of ajwain. The healthy seeds are having economic values in the market but the large number of diseases affects the ajwain crop in the field and a huge damage is caused by the pathogens carried to the harvested seeds during transit and storage. Root rot (*Rhizoctonia solani* Kuhn) and Powdery mildew (*Erysiphe polygoni* D.C.) are two major diseases of ajwain (Dhanbir, 2000 and Meena *et al.* 2009). Among these, the root rot disease is most and destructive common disease of ajwain, caused by *R. solani*, resulted losses in yield as well as quality of the crop. Madhusudhan *et al*. (2010) tested six fungicides viz., Carbendazim (50%WP), Propiconazole (25%EC), and Hexaconazole (5%EC) by poisoned food technique for their efficacy on *R. solani* compatibility different concentrations viz., 50, 100,250, 500 and 1000 ppm. *T. viride* and *T. harzianum* were reported by several workers as the best antagonists for growth inhibition of several soil and seed borne plant pathogens (Dubey 2002, 2003; Poddar *et al*. 2004).

MATERIAL AND METHOD

***In vitro* efficacy of fungicides (Poison food technique):** The relative efficacy of different systemic and non-systemic fungicides evaluated against *R. solani* by using poisoned food technique (Schmitzs, 1930). Five fungicides *viz.*, Bavistin 50% WP [Carbendazim, Methyl-2-benzimidazole carbamate (MBC)] BASF India Ltd., Mumbai, Hexaconazole 5% EC [2-(2,4-dichlorophenyl) -1-(1H-1,2,4-triazol-1-yl) hexan-2-ol], Crop Life Science Ltd., Gujarat, Tebuconazole 25.9 w/w [1-(4-chlorophenyl) -4,4-dimethyl-3-(1,2,4-triazol-1-methyl) peptan-3-ol (Follicular 250 EC)] Bayer Crop Science, India Ltd., Mumbai, Dithane M-45 75% WP [mancozeb, Manganese ethylene bis-dithiocarbamate+zinc ions 2%] Indofil Chemicals Ltd., Mumbai and Saaf [(Carbendazim+Mancozeb) 75 WP (Methyl-2-benzimidazole carbamate (MBC +Manganese ethylene bis-dithiocarbamate + zinc ions 2%] United Phosphorus Limited, Mumbai were tested at two concentrations i.e. 0.1 and 0.2 per cent against *R. solani.* Desired quantity of each fungicide was added separately to sterilized medium, mixed thoroughly and poured in sterilized Petri plates and then allowed to solidify. For each treatment, three replications were taken and each plate was inoculated with 3 mm disc of *R. solani* and incubated at 28±1°C. The linear growth was measured after seven days and control treatment was also maintained (without fungicide). Per cent inhibition of radial growth of mycelium was calculated using formula as given below.

$$I = \frac{C - T}{C} X\ 100$$

Where,
I= Per cent inhibition
C= Colony diameter in control

*Corresponding Author

T= Colony diameter in treatment

***In vitro* evaluations of plant extracts (Poison food technique)**: Efficacy of Neem oil, Karanj oil, Garlic oil and Neem formulation were evaluated at 1 and 2 per cent concentration against *R. solani* by using poison food technique. One and two ml of individual plant extracts was added to 100 ml sterilized PDA in the conical flasks so as to obtain the final concentration in the medium. The plant extracts amended medium was poured aseptically in 90 mm sterilized Petri plates @ 20 ml per plate and allowed to solidify. Three mm bits of *R. solani* removed from the periphery of seven days old cultures and aseptically inoculated at the centre of each plate. For comparison, plates having PDA without plant extracts were kept as control. For each treatment, three replications were maintained. The plates were incubated at 28±1°C for seven days and then colony diameters were measured along with control plates. The per cent inhibition zone was calculated using formula given as above.

***In vitro* efficacy of fungal biocontrol agents (dual culture technique**): The efficacy of biocontrol agents *i.e. Trichoderma viride and T. harzianum* were tested by using dual culture plate method on PDA medium (Johnson *et al*., 1959). The antagonistic effect of *T. viride, T. harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested against *R. Solani.* Three mm diameter mycelium bit of seven days old culture of *Rhizoctonia* was inoculated in center of 1st half of Petri plate and *Trichoderma* spp. in center of 2nd half of Petri plate containing sterilized PDA medium. For each treatment three replications were taken. Inoculated plates were incubated at 28±1°C temperature in incubator. Observations on colony diameter were recorded up to the complete coverage of control plates, which was inoculated with only pathogen. The linear growth after seven days of incubation was recorded and per cent inhibition zone was calculated.

***In vitro* efficacy of bacterial biocontrol agents (dual culture technique):** Dual culture method was used for assessing inhibition of radial growth of the pathogen by bacteria (biocontrol agents) inoculated on King's B agar medium in sterilized Petri dishes. A loopful of bacterial suspension from the 24 hours old cultures was streaked on two sides of each plate and then placed 3 mm disc of *R. solani* in the centre. Control plates were inoculated by pathogens individually. Three replications were maintained for each treatment and were inoculated at 28±1°C. The measurement of radial growth of the test pathogens was recorded after five days and compared with respective controls.

RESULT AND DISCUSSION

***In vitro* evaluation of fungicides (poisoned food technique):** Five fungicides Bavistin, Hexaconazole, Tebuconazole, Dithane M-45 and SAAF were evaluated at two concentrations *viz.*, 1000 and 2000 ppm with poisoned food technique against *R. solani*. All the test fungicides significantly inhibited the mycelial growth of *R. solani* at both concentrations.

The effect was more on *R. solani* where two fungicides Bavistin and Tebuconazole completely inhibited the growth both at 1000 and 2000 ppm concentrations. Further, the fungicides varied in their efficacy on the particular pathogen. Here Bavistin and Tebuconazole at 1000 and 2000 ppm exhibited 100 per cent growth inhibition of *R. solani*. This was followed by Hexaconazole which exhibited 98.1 per cent and 98.9 per cent growth inhibition at 1000 and 2000 ppm, respectively. The other test fungicides Dithane M-45 and SAAF were found to show very weak efficacy to control of *R. solani* at both 1000 and 2000 ppm (Table 1). The per cent growth inhibition for SAAF was 55.5 per cent each at 1000 ppm and 64.4 per cent growth inhibition at 2000 ppm and Dithane M-45 the per cent growth inhibition was 44.4 per cent each at 1000 ppm and 53.3 per cent as well as 2000 ppm as compared to control (Table 1).

Table 1: *In vitro* evaluation of different fungicides (systemic and non-systemic) against of *R.solani* at 1000 and 2000 ppm concentrations after 7 days of incubation at 28 ± 1°C (Poison food technique)

S.No.	Fungicides	Colony diameter (mm)*			Per cent growth inhibition*		
		1000 ppm	2000 ppm	Mean	1000 ppm	2000 ppm	Mean
1.	Bavistin	0.0	0.0	0.0	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
2.	Hexaconzole	1.7	1.0	1.35	98.1 (82.10)	98.9 (83.9)	98.5 (83.0)
3.	Tebuconazole	0.0	0.0	0.0	100.0 (90.00)	100.0 (90.0)	100.0 (90.0)
4.	Dithane M-45	50.0	42.0	46.0	44.4 (41.81)	53.3 (46.9)	48.9 (44.3)
5.	SAAF	40.0	32.0	36.0	55.5 (48.19)	64.4 (53.4)	59.9 (50.7)
6.	Control	90.0	90.0	90.0	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
		SEm ±	CD at 5 %		SEm±	CD at 5%	
Fungicides		0.366	1.076		0.237	0.695	

concentration		0.211	0.621		0.136	0.401	
F X C		0.518	1.52		0.335	0.983	

* Mean of three replications; Figures in parentheses are arcsine √ per cent angular transformed values

Table 2. *In vitro* evaluation of various plant extracts against of *R.solani* at 1% and 2% concentration after 7 days of incubation at 28 ± 1°C (Poison food technique)

S.No.	Plant extracts	Colony diameter (mm)*			Per cent growth inhibition*		
		1%	2%	Mean	1%	2%	Mean
1.	Neem oil	68.0	65.0	66.5	24.4 (29.5)	27.8 (31.7)	26.1 (30.6)
2.	Karanj oil	87.0	85.0	86.0	3.33 (10.2)	5.60 (13.5)	4.46 (11.9)
3.	Garlic oil	82.0	79.0	80.5	8.90 (17.0)	12.2 (20.2)	10.5 (18.7)
4.	Neem formulation	75.0	72.0	73.5	16.7 (24.0)	22.2 (26.5)	19.4 (25.3)
5.	Control	90.0	90.0	90.0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm ±		1.956	1.879	1.917	1.970	1.654	1.787
CD at 5 %		6.379	6.127	6.253	6.425	5.396	5.829

* Mean of three replications; Figures in parentheses are arcsine √ per cent angular transformed values

Table 3. *In vitro* evaluation of different biocontrol agents (fungal and bacterial) against on growth of *R.solani* after 7 days of incubation at 28 ± 1°C (Dual culture technique)

S. No.	Biocontrol agents	Colony diameter of *R. solani* (mm)*	Per cent growth inhibition* of *R. solani*
1.	*Trichoderma viride*	16.2	82.0 (64.9)
2.	*T. harzianum*	35.0	61.1 (51.4)
3.	*Bacillus subtilis*	20.0	77.8 (61.8)
4.	*Pseudomonas fluorescence*	42.0	53.3 (46.9)
5.	Control	90.0	0.00 (0.00)
SEm ±		0.571	0.422
CD at 5%		1.863	1.376

*Mean of three replications; Figures in parentheses are arcsine √ per cent angular transformed values

On the other hand two fungicides bavistin and tebuconazole could inhibit the growth (100%) of *R. solani* both at 500 and 1000 ppm concentrations. These were followed by hexaconazole where per cent growth inhibition was 98.1 per cent and 99.9 per cent at 1000 and 2000 ppm, respectively. SAAF fungicide was found at par in inhibiting of *R. solani* at 1000 ppm, where per cent growth inhibition was 55.5 per cent and 2000 ppm 64.4 per cent, respectively. Dithane M-45 was found to be the weakest fungicide both at 1000 (44.4 per cent) and 2000 ppm (53.3 per cent) as compared to the control (Table 1). Various fungicides were reported effective against root rot pathogen in different crops, but larger of such information on these diseases in ajwain is inadequate. Similar observations were made by Nikam *et al.* (2007), Mukhtar (2007), Christian *et al.* (2007), Mddhusudhan *et al.* (2010), Subhani *et al.* (2011), Andrabi *et al.* (2011), Tetarwal *et al*. (2013) and Padamini (2014).

***In vitro* evaluation of botanicals (poisoned food technique):** Four phyto-extracts viz., Neem oil, Karanj oil, Garlic oil and Neem formulation were evaluated at 1.0 and 2.0 per cent concentration with poisoned food technique against *R. solani.* Neem oil best effective caused 24.4 per cent inhibition at 1.0% concentration and 27.8 per cent inhibition at 2.0% growth of *R. solani* followed by Neem formulation caused 16.7 per cent growth inhibition at 1.0% concentration and 22.2 per cent inhibition at 2.0% concentration while, Karanj oil was less effective, and caused 3.33 per cent inhibition at 1.0% concentration and 5.60 per cent inhibition at 2.0% concentration of linear growth of *R. solani*. In general, both the botanical were not much effective in this study and therefore these were not taken forward for further pot culture experiments (Table 2). Fungicides can effectively control the disease but the residual problems are increasing and this is causing health hazards in human beings and animals. Similar results were obtained by Nwachukewe and

Umechuruba (2001), Singh and Chand (2004), Sitara *et al*. (2008), Tetarwal *et al.* (2013) and Padamini (2014).

***In vitro* evaluation of biocontrol agents (Dual culture technique)**: Efficacy of biocontrol agents the local isolates of *T. viride*, *T. harzianum* and bacteria *Bacillus subtilis*, *Pseudomonas fluorescence* as studied *in vitro* as described in Materials and Methods, using dual culture technique. Data revealed that *T. viride* and bacteria (*Bacillus* spp.) were potential antagonists of *R. solani*.

Maximum and significant high per cent inhibition of growth (82.0%) by *T. viride* was observed in dual culture method for *R. solani*, followed by *Bacillus subtilis* (77.8%), respectively. Efficacy of *T. viride* was found comparatively lower than bacteria *Bacillus subtilis*. The minimum per cent inhibition of growth of *Pseudomonas fluorescence* (53.3%) followed by *T. harzianum* (61.1%), respectively (Table 3). This result was similar with the results obtained by Meki *et al*. (2011) and Subhani *et al*. (2013).

REFFERENCES

Andrabi, M., Vaid, A. and Razdan, V.K. (2011). Evaluation of different measures to control wilt causing pathogens in chickpea. J. Pl. Protec. Res. **51**(1): 55-59.

Anonymous. (2015-16). Rajasthan Agriculture Statistics. Directorate of Agriculture, Pant Krishi Bhawan, Jaipur, *Rabi* crops pp. 2-6.

Christian, L., Morel, N. and Luiz, R. (2007). *In-vitro* susceptibility to fungicides by invertebrate pathogenic and saprobic fungi. *Mycopathologica*. **164** (9): 39-47.

Dhanbir, S. (2000). Diseases of spices crop and their control. Kalyani publishers, New Delhi, pp. 148-158.

Dubey, S.C. (2002). Bio-agent based integrated management of collar rot of French bean. Indian Phytopath., **55**: 230-231.

Dubey, S.C. (2003). Integrated management of web blight of urd/mung bean by bio-seed treatment. Indian Phytopath, **56**: 34-38.

Johnson, L.F., Curl, E.A., Bond, J.H. and Fribourg, H.A. (1959). Methods for studying soil micro-flora plant disease relationships. Burgess Publ. Co., Minneapolis, Minn. **66**: 111-119.

Madhusudhan, P., Gopal, K., Haritha, V., Sangale, U.R. and Rao, S.V.R.K. (2010). Compatibility of Trichoderma viride with fungicides and efficacy against Fusarium solani. J. Pl. Dis. Sci. **5**(1): 23-26.

Meena, S. S., Mehta, R .S., Anwar, M. M., Lal, G., Sharma, Y.K., Kakani, R. K. and Saxena, S. N. (2009). Advance production technology of ajwain, ICAR-NRCSS, Ajmer Technical Bulletin, pp. 1-2.

Meki, S., Ahmedb, S. and Sakhujac, P.K. (2011). Control of chickpea wilt (Fusarium oxysporum f.sp. ciceris) using Trichoderma spp. in Ethiopia. Archiv. Phytopath. Pl. Protect. **44** (5): 432- 440.

Mukhtar, I. (2007). Comparison of phyto-chemical and chemical control of Fusarium oxysporium f sp. ciceri. Mycopathology, **5**(2): 107-110.

Nikam, P.S., Jagtap, G.P. and Sontakke, P.L. (2007). Management of chickpea wilt caused by Fusarium oxysporum f. sp. ciceri. African J. Agric. Res. **2**(2): 692-697.

Nwachukwu, E.O and Umechuruba, C.I. (2001). Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. *J. App. Sci. Environ. Managmnt*. **5**(1): 29-32.

Padamini (2014). Studies on integrated management of wilt and root rot complex of chickpea (*Cicer arietinum* L.) caused by *Fusarium* spp. and *Rhizoctonia solani***.** Ph.D. Thesis, MPUAT, Udaipur (Raj.) pp. 130.

Poddar, R.K., Singh, D.V. and Dubey, S.C. (2004). Integrated application of Trichoderma harzianum mutants and Carbendazim to manage chickpea wilt (Fusarium oxysporum f. sp. ciceri). Indian J. Agri. Sci. **74**: 346-348.

Schmitzs, H. (1930). A suggested toximetric method for wood preservation. Indus. Engia. Chem. Analyt. Ed. **2**:361-363.

Singh, S. and Chand, H. (2004). Control of chickpea wilt (Fusarium oxysporum f. sp. ciceri) using bio-agents and plant extracts. Indian J. Pl. Protec. **32**(1):162-163.

Sitara, U. I., Naseem, J.N. and Sultana, N. (2008). Antifungal effect of essential oils on in vitro growth of pathogenic fungi. Pak. J. Botany, **40**(1): 409-414.

Subhani, M.N., Sahi, S.T., Hussain, S., Ali, A., Iqbal, J. and Hameed, K. (2011). Evaluation of various fungicides for the control of gram wilt caused by *Fusarium oxysporium* f.sp.*ciceri. African J. Agri. Res*. **6**(19): 4555-4559.

Subhani, M.N., Sahi, S.T., Hussain, S., Ali, A., Iqbal, J. and Hussain, N. (2013). Management of Chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceris* through antagonistic microorganisms. *Can. J. Pl. Protect*. **1**(1): 1-6.

Tetarwal J.P., Trivedi, A. and Mathur, K. (2013). Sustainable and eco-friendly management of root rot of soybean caused by Rhizoctonia solani and Fusarium solani. Indian Phytopath. **66** (2) : 182-185.

RESEARCH ARTICLE

STUDY OF PATTERNS OF SENESCENCE IN LEAFLETS OF *TECOMA STANS* (LINN.) H.B. & K.

Amita Sharma*

Department of Botany,
R.G. (P.G.) College, Meerut

Received-29.03.2017, Revised-15.06.2017

Abstract: Senescence is the last stage in the development of leaf, it involves both leaf decay and a removal of the nutrients that are stored in the leaves to other parts of the plant. *Tecoma stans* has compound leaves which are oppositely arranged. Biochemical analysis was carried out for Total N, Total P, Total Chl. and some enzymes i.e. Protease, Amylase, IAA oxidase and RNase. Before biochemical analysis visual observations were carried out in different excised leaves of *Tecoma stans*, petioles were dipped in distilled water and dark incubated to study the pattern of senescence. According to visual observations leaflets of young leaf senescence a bit faster than leaflets of mature leaves. Pattern of changes of levels of constituents indicates that Total Chl., Total N, and Total P increases up to mature stage and then declines. Amylase, IAA oxidase, Protease and RNase increases up to presenescent stage in the leaflets.

Keywords: Senescence, Leaflets, Biochemical changes, Visual observations, *Tecoma stans*

INTRODUCTION

Senescence is the last phase of development of an organism. Leaves have been extensively used to understand the process of senescence. Besides, regulation by phytohormones etc., senescence is established to be a genetically programmed phenomenon. It is of interest to distinguish between the terms ageing and senescence. All organisms from the beginning of their life cycle undergo ageing which culminates in the final phase of senescence leading to death. The process leading to onset of senescence and accompanying it and modification of senescence have been of major interest. It was, therefore, of interest to extend such studies and in this paper results of certain visual observations and biochemical analysis of the leaves of *Tecoma stans* are presented. The system *Tecoma stans* have compound leaves which are oppositely arranged. The interesting feature of this system is that it shows polarity which can be visually observed. The leaves which are towards the earth are somewhat larger than their opposite leaves. Further this distinction is also clear in the opposite leaflets.

In this communication those leaflets were selected from a single leaf and visual observations and biochemical studies were carried out. The terminal leaflets were also taken for comparison.

MATERIAL AND METHOD

Leaves of *Tecoma stans* were collected from C.C.S. University, Meerut Campus. (Plate-1). Leaflets were selected from single leaf and sets were prepared. Visual observations were carried out. The terminal leaflets were also taken into comparison. Young growing leaves and fully expanded mature leaves were excised. They were surfaced sterilized in mercuric chloride solution. After washing in distilled water, excised leaves were kept with petiole dipping in distilled water in vials. Incubation was done in dark and visual changes were recorded till completion of senescence. Experiment was repeated at least thrice in triplicate.

Biochemical analysis were carried out for total nitrogen, total phosphate, chlorophylls and some enzymes i.e. protease, amylase, IAA Oxidase & RNase in *Tecoma stans*. All the data are averages of at least four experiments, each done in triplicate.

Total Nitrogen : For estimation of nitrogen, digestion was done according to Snell and Snell (1954) and the digest was estimated by colorimetric method.

Total Phosphate : Total phosphate was estimated after Allen (1940) using metol reagent.

Total Chlorophyll : For the estimation of total chlorophyll leaf sample of known weight was homogenized with 80% acetone with a pinch of sodium bicarbonate. The amount of chlorophyll a and Chlorophyll b were calculated according to the following formulae (Arnon, 1949).

Chl. a (mg/l) = $12.72\ A_{665} - 2.28\ A_{645}$

Chl. b (mg/l) = $22.87\ A_{648} - 4.67\ A_{663}$

Enzymes : A common Tris – maleate-NaOH buffer pH 6.8 (Vimala, Y, 1983) was used as the extraction cum assays medium for amylase, protease, IAA oxidase and RNase activity.

Amylase : It was estimated by the method given by Filner and Varner, (1967) with iodine reagent.

Protease : It was estimated with sulphate reagent and Pholin phenol reagent (Yamo & Varner, 1973).

IAA oxidase : Gordon & Weber (1951) with Salkowaski reagent.

RNase : Citrate phosphate buffer pH 5.0 used as a extraction medium. Method of Anfinsen et al. (1954) was used for enzyme estimation.

OBSERVATIONS AND CONCLUSIONS

Table 1. shows visual changes accompanying the senescence of young and mature excised leaves of *Tecoma stans* incubated in dark.

*Corresponding Author

The young as well as mature leaves were green initially. No change was observed in any of the stage till 4th day. By the 6th days tips of young leaves were started curling. Browning of the young leaves started from the margin on the 8th day and by 14th day whole leaf turned brown showing complete senescence. In mature leaves browning started as brown spots at the base of leaf on 10th day and complete senescence was observed by the 18th day. As far as different positions of leaflets in young and mature leaves were concerned there was no significant difference observed. The observations, thus, showed that the young leaves senescence a bit faster than the mature leaves. The colour changes may be due to involvement of phenol oxidases besides chlorophyllases.

Table 2. shows changes in fresh weight, dry weight, pigment levels, some chemical constituents and activities of some enzymes accompanying compound leaf development and senescence in *Tecoma stans* on per leaf basis.

According to table 2 fresh weight increased upto mature stage in terminal leaflets and then declined. The right and left leaflets showed increase upto presenescent stages. Same case was noted in case of dry weight. Total chlorophyll rise upto the mature stage and then decline. Total Chlorophyll was maximum in terminal leaflets in young presenescent leaves. Total chlorophyll was showing no significant differences in left and right leaflets of young, mature and presenescent leaves. While total nitrogen and total phosphate increased upto mature stage followed by decline. Total N and Total P was more in terminal leaflets and showed no significant difference in left and right leaflets.

Amylase activity increased continuously upto presenescent stage. Activity was more in left & right leaflets in case of mature and presenescent leaves.

IAA-oxidase activity increased continuously upto presenescent stage. Activity was more in terminal leaflets in case of mature and presenescent leaves than the sides leaflets.

Protease activity increased upto presenescent stage in all the three types of leaflets.

RNase activity showed rise upto presenescent stage. The level was more in side leaflets (left & right) upto presenescent stage.

In this system leaf as a whole shows normal pattern of senescence (e.g. chlorophyll wise). There are, however, difference in levels of chemical constituents and enzyme activities in different leaflets of the same leaf. Thus, the position of leaflet in a compound leaf is important as the position of leaf on a node.

The value of the present work is that, it indicates the importance of studying various patterns of senescence which further shows that generalizations with only standardized model systems may not necessarily lead to a unified concept. These studies form the basis for future indepth studies.

The author acknowledge the valuable suggestions and help given by Prof. D. Banerji and Prof. C.M. Govil.

Table 1. Visual changes accompanying senescence of different excised leaf lets of young and mature compound leaves of *Tecoma stans* during incubation in dark.

Position of Leaflet	Days of Incubation	Colour etc. changes in leaf stages	
		Young	Mature
Terminal	0	Light Green	Green
	2	Light Green	Green
	4	Light Green	Green
	6	Leaf Tip Curl	Leaf Tip Curl
	8	Brown Spots on Margin	Leaf Tip Curl
	10	Half Leaf Brown	Brown Spot at base
	12	More than Half Leaf Brown	Brown Spot at base
	14	Whole Leaf Brown	Half Leaf Brown
	16	-	Half Leaf Brown
	18	-	Whole leaf Brown

Position of Leaflet	Days of Incubation	Colour etc. changes in leaf stages	
		Young	Mature
Left/Right	0	Light Green	Green
	2	Light Green	Green
	4	Leaf Tip Curl	Leaf Tip Curl
	6	Leaf Wrinkled	Margin Brown
	8	Margins Brown	Margin Brown
	10	Half Leaf Brown	Margin Brown
	12	Whole Leaf Brown	Half Leaf Brown
	14	Whole Leaf Brown	Half Leaf Brown
	16	Whole Leaf Brown	More than Half Leaf Brown
	18	Whole Leaf Brown	Whole leaf Brown

Table 2. Changes in Pigment level, some biochemical components and enzyme activities in different leaflets Terminal (T), Left (L) and Right (R) of Young, mature and presenescent intact compound leaves of *Tecoma stans* (per organ basis)

Parameter	Position of Leaflet	Stages of Leaf		
		Young	Mature	Presenescent
Fresh weight (mg) ±SD	T	44.66 ± 2.06	209.16 ± 6.04	152.5 ± 1.80
	L	29.5 ± 1.04	106.66 ± 3.55	11.75 ± 1.60
	R	30.5 ± 1.87	116.83 ± 4.87	122.5 ± 1.62
Dry weight (mg) ±SD	T	6.60 ± 0.63	74.16 ± 2.13	60.5 ± 0.92
	L	4.82 ± 0.16	26.2 ± 1.13	50.5 ± 1.20
	R	5.57 ± 1.78	33.96 ± 1.09	52.9 ± 1.80
Chlorophyll a (mg/leaf) ±SD	T	0.028 ± 0.004	0.076 ± 0.007	0.054 ± 0.002
	L	0.009 ± 0.000	0.088 ± 0.023	0.055 ± 0.001
	R	0.014 ± 0.005	0.120 ± 0.001	0.064 ± 0.005
Chlorophyll b (mg/leaf) ±SD	T	0.020 ± 0.001	0.064 ± 0.001	0.051 ± 0.007
	L	0.003 ± 0.002	0.074 ± 0.001	0.036 ± 0.002
	R	0.027 ± 0.006	0.082 ± 0.001	0.036 ± 0.002
Total Chlorophyll (mg/leaf) ±SD	T	0.049 ± 0.001	0.136 ± 0.002	0.113 ± 0.004
	L	0.014 ± 0.002	0.162 ± 0.056	0.102 ± 0.003
	R	0.027 ± 0.006	0.194 ± 0.001	0.100 ± 0.001
Total Nitrogen (mg/leaf) ±SD	T	0.035 ± 0.005	0.094 ± 0.003	0.031 ± 0.001
	L	0.021 ± 0.001	0.082 ± 0.007	0.032 ± 0.001
	R	0.022 ± 0.001	0.093 ± 0.001	0.021 ± 0.003
Total Phosphate (mg/leaf) ±SD	T	0.039 ± 0.008	0.051 ± 0.001	0.043 ± 0.004
	L	0.009 ± 0.004	0.015 ± 0.002	0.025 ± 0.002
	R	0.088 ± 0.003	0.044 ± 0.001	0.029 ± 0.002
Amylase (µg starch degraded min^{-1} $leaf^{1}$±SD	T	0.077 ± 0.007	0.155 ± 0.008	0.196 ± 0.004
	L	0.053 ± 0.001	0.183 ± 0.001	0.232 ± 0.003
	R	0.091 ± 0.002	0.230 ± 0.001	0.250 ± 0.004
IAA Oxidase (µg IAA degraded min^{-1} $leaf^{1}$±SD	T	0.015 ± 0.003	0.056 ± 0.001	0.072 ± 0.003
	L	0.045 ± 0.001	0.013 ± 0.000	0.069 ± 0.009
	R	0.050 ± 0.001	0.019 ± 0.004	0.067 ± 0.012
Protease (µg aa released min^{-1} $leaf^{1}$)±SD	T	0.851 ± 0.004	2.657 ± 0.001	3.899 ± 0.083
	L	1.046 ± 0.016	2.254 ± 0.010	3.815 ± 0.002
	R	1.098 ± 0.019	2.259 ± 0.040	3.195 ± 0.012
RNase (µg RNA degraded hr^{-1} $leaf^{1}$)±SD	T	0.427 ± 0.003	0.953 ± 0.002	1.242 ± 0.004
	L	0.685 ± 0.004	0.898 ± 0.001	1.192 ± 0.007
	R	0.536 ± 0.001	1.012 ± 0.001	1.145 ± 0.008

Shrub of *Tecoma stans Stans* having terminal leaflet & right & left leaflets

Compound Leaf of *Tecoma*

Plate -1

REFERENCES

Adam, W.W. III Klaus Winter, Ulrich Schrebeiber and Sxhramel, P. (1990). Photosynthesis and chlorophyll fluorescence characteristics in relation to changes in pigment element composition of leaves of Platanus occidentalis L., during autumnal leaf senescence. Plant Physiol (Bethesda).92(4) : 1184-1190

Allen, R.J.L. (1940). The estimation of phosphorus. Biochem J **34** 858-865.

Anfinsen, C.B., Redfield, R.R., Choate, W.I. Page, J. and Carroll, W.R. (1954). Studied on the gross structure, cross linkages & terminal sequences in Ribonucleases. J Bio Chem **207** 201-210.

Arnon, D.I. (1949). Copper enzymes in isolated choloroplases, polyphenotoxidase in Beta vulgaris. Plant Physiol **24** 1-15

Cuello, J., Josequiles, M., Garcia, C. and Sabater, Bartolome (1990). Effect of light and growth substances on senescence of barley leaf segments at different development stages. Bot. Bull. Acad. Scin. (Taipei). 41(2) : 107-112

Davies, T.G. Emyr, Horward Thomas, Thomas, B.J. and Rojers, L.J. (1990). Leaf senescence in a non – yellowing mutants of Festuca paratensis. Plant Cell Physiol. 32(4):555-561.

Filner, P. and Varner, J.E. (1967). A test for de novo synthesis of enzymes. Density labeling with H_20 of Barley alphamylase induced by GA Proc Natl Acad Sci **58** 1520-1526

Gordon, S.A. and Weber, R.P. (1951). IAA-oxidase. Plant Physiol **26** 162. In : Modern Methods of Plant Analysis Springer Verlag Berlin.

Nicholas, N. Boersma, Frank, G. Dohleman, Fernando E. Miguez, Emily A. Heaton (2015). Autumn leaf Senescence in Miscanthus giganteus and leaf (N) differ by stand age. Journal of Experimental Botany. 66. 14, 4395

Snell, F.D. and Snell, C.T. *et. al* (1954). Colorimetric methods of Analysis Volume 3rd D-Von Nostrand Company Inc New York.

Thomas, H. (2003). Do Plants age and if so how? Topics in current genetics. 3:145 (Discuss relationships between the senescence and life span of plants and their parts).

Thomas, H. (2010). Leaf senescence and autumn coloration. McGraw Hill. 2010 Year Book of Science and Technology .pp 211-214 (Consider how and why leaves change their color when they senesce.)

Thomas, H. (2012). Plant senescence. In : Menellim Contrafatto G, etd. Biological Science Fundamentals and Systematics Emcyclopedia of Life Support System (EOLSS), Developed under the Auspices of UNESCO .Oxford, UK : EOLSS Publishers.

Thomas, H. (2013). Senescence, ageing and death of the whole plant. New Phytologist. Vol. 197, Issue 3 pages 697-711

Thomas, H. and Oughan, Helen (2015). Senescence and Crop Performance. Chapter 10 Crop Physiology (2nd Edn.) Academic Press .ISBN 978-0-12-4171046 page 223-249.

Thiman, K.V. (1987). Plant Senescence : A proposed Integration of constituent process. American Society of Plant Physiologists 1-19 ISBN 0-943088-10-0

Yomo, H. and Varner, J.E. (1973). Control of the formation of amylase and protease in the cotyledons of germinating peas. Plant Physiol **51** 708-713.

RESEARCH ARTICLE

DOCUMENTATION OF WEED FLORA IN KARNATAKA COLLEGE CAMPUS AT DHARWAD IN SOUTH INDIA

S.N. Agadi*, D. Thangadurai, P.L. Talawar and S.N. Emmi

Department of Botany, Karnataka University, Dharwad, Karnataka 580003, India
Email: shekaragadi@rocketmail.com

Received-03.06.2017, Revised-18.06.2017

Abstract: Plants which are grown in unwanted places are considered as weeds. A small attempt has been made to document the weed flora of Karnataka College Campus at Dharwad in Karnataka. The present work was undertaken during 2011 to 2015 and nearly 73 species of weeds belonging to 26 families have been documented. Some weeds are effectively preadapted to grow and proliferate in human-disturbed areas such as agricultural fields, lawns, roadsides, and construction sites. In locations where predation and mutually competitive relationships are absent, weeds have increased resources available for growth and reproduction. College campus and Botanical garden weeds have large ecological amplitude so they multiply and flourish well even in changed conditions. Since they have unique potentialities for adaptation, they survive almost in any environment and adjust themselves to changed conditions, which are also supported by the outcome of the present study.

Keywords: Botanic Garden, College Campus, Crops, Lawns, Soil, Weeds

INTRODUCTION

Plants are found everywhere in all kinds of soils, unless it happen to be unable to support plant life. Man tries to grow only a fraction of plants that he needs and the original inhabitants of soil become useless to him referring as weeds. The term weed has been defined in various ways by different authors and most of them convey only a partial meaning. Perhaps the most comprehensive one means that "weed is a plant out of a place". Some people define it as "a plant growing in a place where something else is expected to grow". This definition does not therefore recognize weeds of waste land; because man does not cultivate any plant in waste places and if he does they are no more such. Usually weeds are useless or relatively useless plants causing sometimes great damage to crops or live-stock or making a place ugly. They grow in the fields where they compete with crops for water, soil nutrients, light and space and thus reduce crop yields. They also harbor insects, pests and microorganism. Certain weeds release into the soil, the inhibitors or poisonous substances which are harmful to the plants, human beings and live stocks. They increase the expenditure on labor and equipment, render harvesting difficult, and reduce the quality and marketability of agricultural produce. They block the drainage and impede the flow of water in canals and water-transport channels and their growth in the rivers renders navigation very difficult. The dense growth of weeds in water pollutes the water because they deoxygenate the water and kill the fish.

When land is cultivated to raise crops, weeds spring up naturally along with the crop plants. Ordinarily under any sort of cultivation the natives of the soil try to assert themselves and no land is free from them, and as such they become "necessary corollaries of agriculture". Hence the continual struggle between the ryot and weeds. Being well adapted to the conditions of soil and environment the weeds are not easily destroyed. Therefore it becomes necessary that they should be studied in various aspects so that, ways and means may be devised to control or utilize them.

Despite the use of disease free and healthy seeds, ploughing, cultivation, hand pulling and crop rotation, weeds persist because of our inability to cope up with their great reproductive capacity and mass recycling potential. In contrast to cultivated plants, the weed is an invader, an uninvited guest in any cultivated field or garden or campus of various places. Weeds are excellent example of the struggle for existence. Out of more than 3,00,000 plant species known in the world about 3,000 species are the weeds.

College campus and Botanical garden weeds have large ecological amplitude so they multiply and flourish well even in changed conditions. Since they have unique potentialities for adaptation, they survive almost in any environment and adjust themselves to changed conditions. Man has been mostly different towards weeds and has allowed them to create havoc by growing, spreading and disseminating their seeds. Fortunately a large majority of the weeds are not harmful to cultivated with which they are associated.

Dharwad is one of the 29 districts of Karnataka in southern India. Karnataka College is situated in western part of Dharwad (14°, 78′ to 15, 5′N longitude and 74°, 48′ 76°, 00′ E longitude) elevation of 678 MSL above. The vegetation is dry deciduous type in the west to scrubby jungles in the eastern dryer parts. Eastern plains of the district have black cotton soil and western parts are with literate soil. College campus is with abundant red soil, in which

*Corresponding Author

large number of phanerogamic plants grows. This leads to grow many weeds in the campus. The present work is designed mainly to document, evaluate the richness, distribution, effect and medicinal uses of the weed flora of Karnataka College Campus at Dharwad

MATERIAL AND METHOD

Plants were collected during 2011 to 2015 and identified with the help of flora up to species level [1-10]. Standard herbarium techniques are followed and specimens are preserved in the Departmental Herbarium for further reference.

RESULT AND DISCUSSION

Karnataka College Campus at Dharwad in Karnataka is rich in weed plants, as over 73 plant species of 26 families are observed and identified at species level (Table 1). Some of them are medicinally useful and among surveyed, plants belonging to Asteraceae > Poaceae > Malvaceae > Acanthaceae and > Euphorbiaceae are large in numbers, respectively. These weeds have useful aspects such that (1) it minimizes the force of falling rain drops, (2) it checks soil erosion and sloping land mainly in belly terrains, (3) many weeds have medicinal importance, and, (4) some weeds fix atmospheric nitrogen in soil. Hence, sustainable utilization of these weeds resources warrants further study.

Table 1. List of weeds collected and documented from Karnataka College Campus at Dharwad in South India.

Family	Species
Acanthaceae	*Blepharis maderaspatensis* Juss.
	Crossandra infundibuliformis (L.) Needs.
	Justicia betonica Linn.
	Peristrophe bicalyculata (Retz) Nees.
	Thunbergia alata Boj. ex Sims
	Strobilanthes ciliatus Wall.ex Nees
Amaranthaceae	*Achyranthes aspera* Linn.
	Alternanthera triandra Lam.
Asclepiadaceae	*Gymnema sylvestre* (Retz) R. Br.
Asteraceae	*Ageratum conyzoides* Linn.
	Artemisia annua Linn.
	Blainvillea rhomboidea Cass.
	Blumea oxyodonta DC.
	Blumea eriantha DC.
	Conyza stricta Willd.
	Eclipta alba Haask.
	Lagasca mollis Cav.
	Parthenium hysterophorus Linn.
	Poidea biferata Linn.
	Sonchus aspera Hill.
	Spilanthes acmella Murr.
	Vernonia cinerea Less.
	Vicoa auriculata Cass.
	Tridax procumbens Linn.
Basellaceae	*Basella alba* Linn.
Ceasalpinaceae	*Cassia tora* Linn.
Chenopodiaceae	*Chenopodium murale* Linn.
Commelinaceae	*Commelina benghalensis* Linn.
	Cyanotis cristata Schult.
Convolvulaceae	*Ipomea biloba* Forsk.
Cuscutaceae	*Cuscuta reflexa* Roxb.
Cyperaceae	*Cyperus rotundus* Linn.
Euphorbiaceae	*Acalypha indica* Linn.
	Euphorbia corrigioloides Boiss.
	Euphorbia hirsute Linn.
	Euphorbia geniculata Ort.
	Euphorbia pulcharrima Willd.
Lamiaceae	*Coleus forskohlii* (Wild.) Briq.
	Leucas aspera R. Br.
	Leucas martinicensis (Jacq.) R. Br.
Malvaceae	*Abutilon indicum* G. Don.
	Malvestrum coromandalianum Gorcke.
	Sida acuta Burm.
	Sida cardifolia Linn.
	Sida rombifolia Linn.

	Sida mysorensis Wight & Arn.
Minispermaceae	*Cocculus hirsutus* (L.) Diels
Mimosaceae	*Mimosa pudica* Linn.
Nyctaginaceae	*Boerhaavia diffusa* Linn.
Oxalidaceae	*Oxalis corymbosa* (DC.) Lourteig
	Oxalis carniculata Linn.
Papaveraceae	*Argemone mexicana* Linn.
Papilioniaceae	*Desmodium triflorum* DC.
	Crotolaria albida Heyne.
	Indigofera hirsute Linn.
	Indigofera parviflora Heyne.
Poaceae	*Cenchrus ciliaris* Linn.
	Cynodon dactylon Pers.
	Digitaria sanguinalis (L.) Scop.
	Digitaria ciliaris (Retz.) Koeler.
	Eleusine indica Gaern.
	Eragrostis aspera Nees.
	Eragrostis bifaris (Vahl) Wight
	Panicum dichotomiflorum Michx.
	Setaria glauca (L.) Beauv.
	Themeda triandra Forssk.
Plumbagenaceae	*Plumbago zeylanica* Linn.
Rubiaceae	*Heliotropium indicum* Linn.
Solanaceae	*Datura innoxia* Linn.
	Datura metel Linn.
	Solanum nigrum Linn.
Umbelliferae	*Centella asiatica* Linn.
Verbenaceae	*Lantana camera* Linn.

REFERENCES

Arora, R.K., Khanna, P.P. and Singh, R. (1976). Weeds of North India, Ministry of Agriculture and Education, Government of India, New Delhi, pp 1-93.

Caton, B.P., Mortimer, M. and Hill, J.E. (2004). A practical field guide to weeds of rice in Asia, International Rice Research Institute, Los Baños, Philippines, pp 1-116.

Cooke, T. (1905). Flora of the Presidency of Bombay, Botanical Survey of India, Calcutta.

Quammen, D. (2012). Planet of Weeds, Harper's Magazine, November 15, 2012.

Elmore, C.D. (1990). Weed Identification Guide. Southern Weed Science Society, Champaign, IL, USA.

Gamble, J.S. (2012) Flora of the Presidency of Madras, Botanical Survey of India, Calcutta.

Sastry, K.S.K., Boraih, G., Govindu, H.C. and Khaleel, T.F. (1980). Weeds of Karnataka, University of Agricultural Sciences, Bangalore, India.

Parimala, R. (2011). Flora of Dharwad district, PhD Thesis, Karnatak University, Dharwad, Karnataka.

Stubbendieck, J., Friisoe, G.Y. and Bolick, M.R. (1994). Weeds of Nebraska and the great plains, Nebrasks Department of Agriculture, USA.

Whitson, T.D. and Burrill, L.C. (1991). Weeds of West, Western Society of Weed Science and Western United States Land Grant Universities Cooperative Extension Services, USA.

RESEARCH ARTICLE

CHARACTERIZATION AND PRELIMINARY EVALUATION OF DIFFERENT GENOTYPES OF LEAFY VEGETABLE CHENCH (*CORCHOROUS ACUTANGULUS* LAM.)

Bhupendra Singh Rana*, Pravin Kumar Sharma, Amit Dixit and Kamal Kumar

Department of Vegetable science, College of Agriculture,
Indira Gandhi Krishi Vishwavidyalya Raipur -492006, India
Email: bsrana305@gmail.com

Received-12.06.2017, Revised-24.06.2017

Abstract: The study was carried out to characterization and evaluation of Forty-six indigenous genotypes of Chench (Corchorus acutangulus Lam.) where collected from different place of Raigarh, Kanker, Bastar, Narayanpur and kondagaon district of Chhattisgarh during 2015 at Indira Gandhi Krishi Vishwavidyalaya Raipur, which was planted with three replication in RCBD design for qualitative and quantitative characters. Among forty-six genotypes IGCB-2013-23 found higher yield (55.42q/ha) followed by IGCB-2015-9 (53.58q/ha). IGCB-2013-23 recorded highest leaf weight (5.13g) while maximum stem weight was observed in IGCB-2015-8 (6.60 g) were maximum leaf width and leaf length was recorded in IGCB-2013-23 (5.47cm) IGCB-2013-23 (8.39cm) respectively. Among the qualitative characters, all the genotypes were erect, had a tap root. Other morphological characters exhibited large variability.

Keyword: Chench, Genotypes, Characterization, Preliminary evaluation, Qualitative, Quantitative characters

INTRODUCTION

Chench (*Corchorus acutangulus* Lam.) is one of the unexploited and underutilized leafy vegetable and also know as vegetable jute in India. Chenh is one of the main species of taxonomically diverse group of leaf vegetables. The nutritional value of chench is excellent because of its high content of essential minerals (iron, calcium) and good source of vitamins (vitamin C and folic acid). Chench belongs to the genus Corchorous of the family Tiliaceae. *Corchorous* has many species which are used as leafy vegetables, Many wild species occurs out of which, only seven species are cultivated *C. fascicularis, C. trilocularis, C. acutangulus, C. tridens, C. capsularis, C. olitorius, C. depresses* (Choudhary *et al*., 2013). It is widely cultivated throughout India especially during the summer and rainy seasons. There was little information on the extent and kind of diversity present in the collection maintained in Chhattisgarh, hence characterization and preliminary evaluation of these genotypes was considered an important area of study.

MATERIAL AND METHOD

Forty-six genotypes were collected at Agriculture Research Station, IGKV, Krishak Nagar, Raipur were characterized and evaluated during the *rabi* season of 2015-16 at Research and Instructional Farm, Department of Horticulture of, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). Each genotype was grown in 2 m long rows with a spacing of 50 cm between rows and 30 cm within rows, under recommended growing conditions. For characterization, IBPGR descriptor (7 qualitative and 12 quantitative traits) were considered. Qualitative traits studied includes growth habit, branching index, stem pigmentation, leaf pigmentation, leaf shape, Leaf vein pigmentation, prominence of leaf veins, petiole pigmentation, while quantitative traits recorded were plant height, number of branches, leaf length, leaf breadth, leaf weight, stem weight and leaf yield. The scoring for these characters was done as per the IBPGR Amaranth Descriptor List (Grubben and van Sloten 1981). Data were collected from five randomly selected plants on various quantitative characters. Mean data were subjected to statistical analysis to calculate range, standard deviation and coefficient of variability which were used to group the genotypes into different categories (Panse and Sukhatme, 1978).

RESULT AND DISCUSSION

With respect to the quantitative traits, the genotypes showed a wide range of variability in plant height (21.23–42.82cm), number of branches(2.57-14.90), Number of leaves per plant (10.67-18.23), Internodal length (1.07-1.67cm), Petiole length (1.33-5.70cm), leaf length (3.72-8.39cm), leaf width (1.38–5.47cm), leaf weight per plant (0.77- 5.13g), stem weight per plant (1.80-6.60g), Days to 50% flowering (43.33 to 67.00), Dry matter percentage of plant (11.48-40.73 %) and leaf yield q/ha (27.08-55.42q/ha) indicating the possibility of exploiting this variation for varietal improvement in chench. Similarly, Wu *et al*. (2000) reported the presence of wide diversity in agronomic traits among amaranth genotypes and also identified several genotypes having the required agronomic traits for cultivar development.

Among the qualitative characters, all the genotypes were erect, had a tap root. Other morphological

*Corresponding Author

characters exhibited large variability (Table 1). Branches were found all along the stem in almost all the genotypes (28) while some branches were confined to the top (18). Stem pigmentation ranged from dark red (27) to medium red (1) to light red (10) to light green (4) to greenish red (1) and dark green (3). Leaf pigmentation ranged from dark

Table 1. Grouping of chench genotypes for qualitative parameters

Character	Category
Branching Index	(a) Branches all over the stem (28), (b)Only at top(18)
Stem pigmentation	(a) Dark red(27), (b) Medium red(1), (c) Light red(10), (d) Light green(4), (e) Greenish red(1), (d) Dark green(3)
Leaf pigmentation	(a) dark green (20), (b) Medium green (8), (c) Red green (1) (d) Light reddish green (14), (e) Light green (3)
Leaf shape	(a) Lanceolate (22), (b) Ovate lanceolate (24)
Leaf vein pigmentation	(a) Green(7), (b) Pink (39)
Prominence of leaf veins	(a) Smooth (46)
Petiole pigmentation	(a) Light reddish green(2), (b) Light red(29), (c) Medium red(7), (d) Light green(7) (e) Dark red(1)

Table 2. Promising chench entries identified for different biometric traits

Character	Range	Genotypes
Plant height	>35cm	IGCB-2015-2, IGCB-2015-7, IGCB-2015-8, IGCB-2015-10, IGCB-2015-12, IGCB-2015-13, IGCB-2015-14, IGCB-2015-15, IGCB-2015-16, IGCB-2015-17, IGCB-2015-18
Number of Branches	>10	IGCB-2013-11, IGCB-2015-1, IGCB-2015-2, IGCB-2015-3, IGCB-2015-4, IGCB-2015-5, IGCB-2015-6, IGCB-2015-7, IGCB-2015-8, IGCB-2015-9, IGCB-2015-11, IGCB-2015-12, IGCB-2015-13, IGCB-2015-14, IGCB-2015-15, IGCB-2015-18, IGCB-2015-19, IGCB-2015-20, IGCB-2015-21
Number of leaves per plant	>17	IGCB-2015-13, IGCB-2015-15, IGCB-2015-14, IGCB-2015-9, IGCB-2015-8, IGCB-2015-5, IGCB-2013-19, IGCB-2015-11, IGCB-2015-12
Internodal length (cm)	>1.5cm	IGCB-2015-1, IGCB-2015-12, IGCB-2015-10, IGCB-2015-8, IGCB-2015-15, IGCB-2015-14, IGCB-2015-7
Petiole length (cm)	>5cm	IGCB-2015-8
Leaf length	>7cm	IGCB-2015-8, IGCB-2015-10, IGCB-2015-12, IGCB-2015-14
Leaf breadth	>3cm	IGCB-2015-8, IGCB-2015-10, IGCB-2015-11, IGCB-2015-12, IGCB-2015-14
Leaf weight per plant	>4g	IGCB-2015-8, IGCB-2015-10, IGCB-2015-14
Stem weight per plant	>5g	IGCB-2013-21, IGCB-2013-22, IGCB-2015-2, IGCB-2015-3, IGCB-2015-4, IGCB-2015-5, IGCB-2015-8, IGCB-2015-9, IGCB-2015-10, IGCB-2015-12, IGCB-2015-15, IGCB-2015-16
Days to 50% flowering	<50 days	IGCB-2013-23, IGCB-2013-25, ICGB-2015-10, IGCB-2015-11
Dry matter percentage of plant	>25 %	IGCB-2013-9, IGCB-2015-6, IGCB-2015-12, IGCB-2013-6, IGCB-2013-14, IGCB-2013-19, IGCB-2013-7, IGCB-2013-28, IGCB-2013-20, IGCB-2013-16, IGCB-2013-15, IGCB-2015-1, IGCB-2013-11, IGCB-2015-13, IGCB-2013-18 and IGCB-2013-21
Leaf yield q/ha	>50q/ha	IGCB-2013-15, IGCB-2015-2, IGCB-2015-8, IGCB-2015-14, IGCB-2015-9, IGCB-2015-10

Table 3. Mean performance of Genotypes

Genotype	Plant height	Number of Branches	Number of leaves per plant	Internodal length (cm)	Petiole length (cm)	Leaf length	Leaf breadth	Leaf weight per plant	Stem weight per plant	Days to 50% flowering	Dry matter percentage of plant	Leaf yield q/ha
IGCB-2013-1	29.69	3.73	12.27	1.08	1.65	3.82	1.65	2.96	1.87	57.33	16.00	43.75
IGCB-2013-2	31.16	2.57	10.67	1.07	1.57	4.34	1.69	2.61	1.80	62.67	12.58	27.58
IGCB-2013-3	30.75	2.93	12.20	1.16	1.33	3.72	1.38	3.29	2.64	55.33	16.86	35.92
IGCB-2013-4	29.92	3.37	11.60	1.24	1.64	4.03	1.64	1.96	1.89	62.33	15.77	40.92
IGCB-2013-5	21.23	3.27	14.20	1.26	2.14	4.93	2.08	3.74	2.70	60.00	21.60	27.08
IGCB-2013-6	24.02	3.70	12.33	1.21	2.45	5.76	2.22	3.27	1.81	63.67	32.59	29.25
IGCB-2013-7	26.94	5.27	14.70	1.13	1.93	4.62	1.95	2.90	2.49	59.67	28.23	39.75
IGCB-2013-8	27.98	2.67	12.93	1.33	2.31	4.62	2.31	4.78	3.41	56.67	24.63	34.42
IGCB-	31.93	3.67	13.43	1.27	2.43	5.36	2.20	3.72	2.82	61.67	40.73	29.25

2013-9												
IGCB-2013-10	37.75	8.33	15.50	1.33	2.10	5.26	2.12	6.02	4.30	62.33	21.54	32.25
IGCB-2013-11	30.76	12.90	15.93	1.41	2.26	5.83	2.30	6.00	3.51	67.00	25.55	31.25
IGCB-2013-12	30.93	6.23	13.97	1.27	2.19	5.32	2.11	4.06	2.21	56.67	23.50	33.25
IGCB-2013-13	30.36	3.90	13.43	1.24	2.06	5.01	1.94	4.00	2.75	65.00	23.96	28.25
IGCB-2013-14	28.32	3.20	12.13	1.30	1.72	4.91	1.64	5.23	3.17	66.67	28.39	37.75
IGCB-2013-15	28.60	5.40	13.27	1.36	2.27	5.42	2.24	8.26	4.35	64.33	26.18	52.08
IGCB-2013-16	31.00	5.23	14.47	1.18	1.90	5.11	1.87	4.23	3.14	63.33	26.56	42.08
IGCB-2013-17	31.73	9.77	15.03	1.29	2.20	5.78	2.15	6.31	4.59	63.33	23.39	35.25
IGCB-2013-18	30.53	8.37	16.67	1.18	3.13	6.37	2.73	8.23	4.85	63.33	25.24	40.58
IGCB-2013-19	31.20	9.93	17.40	1.43	2.35	5.60	2.24	6.36	4.44	55.67	28.37	41.08
IGCB-2013-20	32.49	8.00	15.83	1.32	2.49	4.89	2.47	6.20	4.66	62.67	26.63	34.42
IGCB-2013-21	30.17	8.07	14.27	1.40	2.46	5.71	2.52	8.62	5.71	57.67	25.11	45.75
IGCB-2013-22	30.15	6.77	13.40	1.33	2.40	5.54	2.40	7.76	5.57	60.00	17.18	42.42
IGCB-2013-23	40.59	6.30	15.70	1.31	5.70	8.39	5.47	7.07	4.28	48.67	14.38	55.42
IGCB-2013-24	29.77	5.97	15.80	1.24	2.55	5.77	2.30	7.93	4.16	62.67	11.84	32.75
IGCB-2013-25	29.12	6.10	14.57	1.27	2.53	5.43	2.53	5.79	4.24	43.33	15.72	32.75
IGCB-2015-1	27.96	11.27	15.63	1.67	2.20	5.73	2.18	7.01	4.90	57.33	26.07	35.58
IGCB-2015-2	39.85	10.37	16.07	1.41	2.83	6.03	2.73	8.34	5.26	63.67	17.12	50.33
IGCB-2015-3	33.98	11.37	15.30	1.24	2.51	5.02	2.51	6.00	5.24	57.67	28.16	34.08
IGCB-2015-4	24.48	10.63	16.07	1.24	2.68	4.51	2.74	5.95	5.08	60.00	19.93	42.08
IGCB-2015-5	26.35	11.60	17.53	1.32	2.44	4.58	2.39	6.18	5.03	54.67	17.19	44.42
IGCB-2015-6	32.88	11.23	15.50	1.13	1.74	5.11	1.82	4.32	4.01	65.67	33.18	29.58
IGCB-2015-7	35.25	11.33	15.63	1.51	2.05	5.66	2.06	5.98	4.27	58.67	24.56	32.08
IGCB-2015-8	42.82	13.00	17.67	1.56	2.40	5.13	3.28	10.96	6.60	57.67	18.46	51.58
IGCB-2015-9	34.60	14.90	17.70	1.45	2.82	6.84	2.84	11.61	6.52	62.33	18.94	53.58
IGCB-2015-10	35.65	9.80	15.93	1.61	3.12	7.11	3.06	12.37	6.29	44.00	19.38	44.75
IGCB-2015-11	30.07	12.57	17.03	1.44	3.13	6.05	3.22	6.78	4.91	45.33	21.14	42.58
IGCB-2015-12	37.41	12.97	17.13	1.65	3.17	7.06	3.26	7.49	5.54	56.67	32.80	34.75
IGCB-2015-13	35.45	13.47	18.23	1.44	2.99	6.31	2.99	6.12	2.96	63.67	25.54	32.75
IGCB-2015-14	41.59	14.00	17.80	1.54	3.15	7.31	3.24	10.86	4.41	64.33	19.54	52.58
IGCB-2015-15	37.46	13.07	18.03	1.56	2.45	6.51	2.46	7.68	5.53	62.67	14.72	36.25
IGCB-2015-16	37.86	8.47	15.87	1.31	2.47	5.55	2.47	8.14	5.21	59.00	16.58	39.25
IGCB-2015-17	36.52	9.43	15.87	1.35	2.36	5.34	2.32	7.80	3.74	62.33	19.51	37.25
IGCB-2015-18	36.22	11.73	16.57	1.42	2.58	5.86	2.65	10.87	3.78	61.67	16.47	41.92
IGCB-2015-19	34.33	10.57	16.10	1.24	2.58	4.91	2.42	7.45	3.78	57.00	15.26	41.08
IGCB-2015-20	33.07	10.93	15.63	1.33	2.54	5.65	2.53	8.08	3.86	62.00	19.01	34.75
IGCB-2015-21	31.85	10.97	16.30	1.34	2.85	5.14	2.72	7.14	4.70	59.33	19.36	39.42

green (20), light reddish green (14) to medium green (8) to light green (3) and reddish green (1). Leaf shape ranged from lanceolate (22) to ovate lanceolate (24). Wu-Huai Xiang *et al*. (2000) observed wide diversity for stem and leaf colour while evaluating the genetic resource collection from China. Xiao *et al*. (2000) classified 31 vegetable amaranth varieties based on 17 biological characters, of which leaf shape and colour were considered more practical for classifying amaranth varieties. Leaf veins pigmentation were found green (7), to pink (39). Leaf vein prominent in all the genotypes it was smooth (46) and the petiole pigmentation ranged from light red (29), medium red (7) to light green (7)

and dark red (1). Kurrey (2015) observed wide range of variability in 25 genotypes of chench for plant height, leaf length, leaf width, number of branches per plant, leaf yield kg per plot, leaf yield q per ha.

CONCLUSION

Promising entries identified for different important biometric traits are given in Table 2. These include lines with maximum plant height, leaf length, breadth, leaf and stem weight. IGCB-2015-10 recorded highest leaf weight (5.13g) followed by IGCB-2015-14 (4.59 g) while maximum stem weight was observed in IGCB-2015-8 (6.60 g) followed by IGCB-2015-9 (6.52g), were maximum leaf width and leaf length was recorded in IGCB-2015-8 (5.47cm) followed by IGCB-2015-12 (3.26cm) and IGCB-2015-8 (8.39cm) followed by IGCB-2015-14 (7.31cm) respectively which is a desirable character in leafy vegetables. The variability present in the stem, leaf and branch characters and in some quantitative characters such as plant height, leaf number and days to flowering can be successfully utilized for commercial exploitation of chench. These lines can either be directly used for commercial cultivation or can be utilized for multi locational trials in different location or also can utilized in intervarietal hybridization to obtain segregating population.

ACKNOWLEDGEMENT

I am heartily thankful to Dr. Pravin Kumar Sharma and Deepika Chandravanshi my family and friends for their suggestion & support in search of literature and in compiling this paper. The authors are also grateful to publishers/ authors/editors of all those books, articles and journals from where the literature for this article has been reviewed and discussed.

REFERENCES

Choudhary, S.B., Sharma, H.K., Karmakar, P.G., Kumar, A.A., Saha, A.R., Hazra, P. and Mahapatra, B.S. (2013). Nutritional profile of cultivated and wild jute (Corchorus species) AJCS **7**(13):1973-1982.

Grubben GJH, van Sloten DH. (1981). Genetic resources of amaranths, IBPGR, Rome, Italy.

Joshi, V., Vijaya, M., Sireesha, K. and Latha, P. M. (2011). Characterization and preliminary evaluation of Vegetable amaranth (Amaranthus spp.). Veg. Sci., **38**(2): 239-240.

Kurrey, V. K. (2015). Collection, evaluation and identification of suitable genotypes of chench (*Corchorus acutangulus* Lam.) for Chhattisgarh plain condition. M. Sc.(Ag) Thesis IGKV, Raipur

Panse, V.G. and Sukhatme, V.G. (1989). Statistical methods for agricultural workers. ICAR, New Delhi.

Wu HX, Sun M, Yue SX, Sun HL, Cai YZ, Huang RH, Brenner D, Corke H. (2000). Field evaluation of an *Amaranthus* genetic resource collection in China. Genetic Resources and Crop Evolution **47**(1):43–53.

Xiao SG, Liu ZM, Song Y, Yang G; Xiao SG, Liu ZM, Song, Yang G. (2000). Classification of vegetable amaranth variety resources. Journal of Hunan Agricultural University. **26**(4):274–277.

RESEARCH ARTICLE

ASSESSMENT OF LOSSES DUE TO PULSE BEETLE IN CHICKPEA UNDER LABORATORY CONDITION

Rahul Singh*, Gaje Singh, S.K. Sachan, D.V. Singh, Rajendra Singh, and Prashant Mishra

**Department of Entomology, Department of Plant Pathology,*
Sardar Vallabhbhai Patel University of Agriculture and Technology,
Modipuram Meerut 250110, (U.P.) India
Email: dodwalrahul@gmail.com

Received-10.06.2017, Revised-26.06.2017

Abstract: A laboratory studies on assessment of losses due to pulse beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) in stored chickpea under laboratory condition during 2016. The losses caused by pulse beetle were estimated by releasing 1, 2, 4, 8 and 16 pairs of adults in jars each containing 100g chickpea grains. The lowest mean grain damage, weight loss and germination loss were recorded in case of 1 pair of adult pulse beetle *i.e.,* 6.25, 1.25 and 4.00 per cent. While, highest losses were recorded in case of release of 16 pair *i.e.,* 60.25, 9.00 and 43.5 per cent after 30 days of storage, respectively. The losses followed the same trend after 90 days of storage and reached to highest *i.e.,* 40.75, 18.75 and 28.5 per cent in case of release of 1 pair of adult, While, 98, 45.75 and 99 per cent, respectively, in case of release of 16 pair of adult pulse beetle. The losses were increased with increase in storage period.

Keywords: Pulse beetle, Chickpea grains, Abiotic factors

INTRODUCTION

Pulses are important food crops due to their high protein and essential amino acid content. Apart from being an important source of dietary protein for human consumption, the pulse crops are also important for the management of soil fertility due to nitrogen fixing ability (Kantar *et al.,* 2007). Chickpea (*Cicer arietinum* L.) is a highly nutritious pulse cultivated throughout the world and is placed third in the importance list of the food legumes. India is the largest producer of this pulse contributing to around 63% of the world's total production (ICRISAT, 2007). Chickpea is used in arrange of different preparation in our cuisine and has a good source of energy i.e. 416 calories/100gm chickpea (Shrestha, 2001). It contains protein (18.22%) carbohydrates (52-70%) fat (4-10%), minerals (calcium, phosphorous and iron) and vitamins. It is already a traditional component of the Indian diet but is becoming increasingly scarce. Likewise, chickpea can be an important contributor to soil fertility and organic matter to soil (Kumar Rao *et al*. 1998). It is recorded that 55- 60 per cent loss in seed weight and 45.50 to 66.30 per cent loss in protein content of pulses is due to infestation caused by pulse beetle (Faruk *et al*. 2011). Plant-derived materials are more readily biodegradable, relatively specific in the mode of action and easy to use. They are environmentally safe, less hazardous, less expensive and readily available (Das, 1986). There is a steady increase in the use of medicinal plant products and edible oils as a cheaper and ecologically safer means of protecting stored products against infestation by insects. The above studies emphasize the need in controlling the pulse beetle *Callosobruchus chinensis* through plant derived oil extract and edible oils.

*Corresponding Author

MATERIAL AND METHOD

To estimate the losses at different population levels of the pulse beetle 1 pair, 2 pairs, 4 pairs, 8 pairs and 16 pairs of adults (both male and female) were released in separate jars containing 100g chickpea seeds. The experiment was replicated four times. The observations given below was recorded at 30, 60, 90 days after release of adults of beetles.

Mean grain damage (%)

A sample of 100g of chickpea grains were take from the jars of each replicate of every set after 30 days. The damaged grains were separated out from the total number of grains taken for observation in each replication. Care was taken to avoid recount of damage grain. The data taken was used for calculating the mean per cent damaged grains. The same procedure was adopted for recording observations at 30, 60 and 90 days after release of pulse beetle. The following formula was used for determination of mean damage percent as described.

$$\text{Grain damage (\%)} = \frac{\text{Total number of damaged grains}}{\text{Total number of grains}} \times 100$$

Mean germination loss (%)

To investigate the effect of plant leaf extracts oil and edible oils on seed viability, 100 seeds were taken from each treatment and were placed in petridish separately having water soaked blotting paper at its bottom. The petridishes was placed in B.O.D. at 18 ± 25^{0}C temperature and $75\pm$ 5% relative humidity. After incubation, the germinated seeds was counted and worked out the percent seed germination. The mean per cent germination loss was calculated by following formula:

Per cent germination

$$= \frac{\text{Number of germinated seeds}}{\text{Total number of selected seeds}} \times 100$$

Mean weight loss

After removing the beetles from each jar the weight of grains were taken separately on an electric balance from each replicate after 60 and 90 days of release. The mean per cent loss in weight was calculated by the following formula:

$$\textbf{Mean weight loss} = \frac{I-F}{I} \times 100$$

Where,

I=initial weight of grains

F=final weight of grains

RESULT AND DISCUSSION

Grain damage %

(Table 1)The minimum grain damage after 30 days of storage was recorded in case of release of 1 pair adult with 6.25 and was maximum in case of release of 16 pair of adult with 60.25 per cent. The grain damage was further increased up to 19.75 and 93.75 per cent after 60 days of storage and ultimately reached 40.75 and 98 per cent after 90 days of storage, respectively. Similar results were also reported by Doharey *et al.* (1987) who observed that the grain damage by *C. chinensis* increased from1.35 per cent to 99.91 per cent after 120 days of storage. Patil *et al.* (2003) tested the chickpea seeds cv. PG-12 were stored in jars, each containing 0, 1, 2, 4 or 8 pairs of newly emerged adults of *C. maculatus* and they reported that population count and seed infestation were directly proportional to the number of pairs of adult beetles released.

Weight loss %

The minimum weight loss (Table 2) after 30 days of storage was recorded with 1.25 per cent in case of release of 1 pair of adult and maximum in case of release of 16 pair of adult with13.25 per cent. It was further increased up to 6.75 and 32.50 per cent after 60 days of storage and ultimately reached 18.75 and 45.75 per cent after 90 days of storage, respectively. Anandhi *et al.* (2008) revealed that the release of five pairs of *C. chinensis* about in 250g of pulse increased to a mean population of 648.3 after 180 days of storage. The loss in weight increased up to 17.3 during the period. Venkatesham *et al.* (2015) evaluated the losses caused by pulse beetle, *Callsobruchus chinensis* L. were determined by releasing five pair of adults in a glass jar each containing 500g chickpea grains. The mean seed damage, Weight loss was 7.87 per cent, 4.19 percent, respectively after 30 days of release which increased with the storage duration resulting in 99.33 and 48.73 per cent, respectively after 120 days.

Germination loss %

The minimum germination loss (Table 3) after 30 days of storage was recorded with 4 per cent in case of release of 1 pair of adult and maximum in case of release of 16 pair of adult with 43.5 per cent. It was further increased up to 12 and 81 per cent after 60 days of storage and ultimately reached 28.5 and 99 per cent after 90 days of storage, respectively. Patil *et al. (*2003) reported that 100 g seeds of chickpea cv. PG-12 were stored in plastic jar, each containing 0, 1, 2, 4 or 8 pairs of newly emerged adults of *C. maculates* A significant reduction in germination was recorded when more than 2 pairs of adult beetles were released in a jar. A germination level of 61.0% was recorded for seeds stored with 8 pairs of adult beetles. Similar results were also reported by Jat *et al*. (2013) conducted an experiment by releasing 1, 2, 4, 8 and 16 pairs of adults in jars each containing 100g chickpea grains and recorded the losses caused by pulse beetle. The lowest mean grain damage, weight loss and germination loss were recorded in case of 1 pair of adult pulse beetle *i.e.*, 7.79, 1.81 and 4.55 per cent. While, highest losses were recorded in case of release of 16 pair *i.e.*, 60.93, 13.99 and 44.57 per cent after 30 days of storage, respectively.

Table 1. Effect of pulse beetle on percent grain damage at different population density level

No of pairs released	Grain damage (%)		
	30DAR	60DAR	90DAR
1	6.25 (14.42)	19.75 (26.36)	40.75 (39.65)
2	16.25 (23.74)	35.75 (36.70)	53.5 (46.99)
4	25.5 (30.30)	48.75 (44.26)	74 (59.32)
8	42.75 (40.81)	67.5 (55.22)	93.75 (75.59)
16	60.25 (50.89)	93.75 (75.65)	98 (81.97)
CD at 5% level	**1.813**	**2.417**	**2.370**
SE(m) ±	**0.596**	**0.795**	**0.779**

Table 2. Effect of pulse beetle on percent weight loss at different population density level

No of pairs released	Weight loss (%)		
	30DAR	60DAR	90DAR
1	1.25 (6.33)	6.75 (14.96)	18.75 (25.80)
2	3.75 (11.09)	12 (20.22)	24.5 (29.65)
4	5.75 (13.83)	15.5 (23.31)	33 (35.03)
8	9 (17.43)	21.75 (27.76)	41.5 (40.08)
16	13.25 (21.32)	32.5 (34.73)	45.75 (42.54)
CD at 5% level	**1.68**	**2.62**	**2.36**
SE(m) ±	**0.553**	**0.862**	**0.778**

Table 3. Effect of pulse beetle on percent germination loss at different population density level

No of pairs released	Germination loss (%)		
	30DAR	60DAR	90DAR
1	4 (11.48)	12 (20.22)	28.5 (32.24)
2	11 (19.31)	26.75 (31.12)	55.75 (48.28)
4	20 (26.36)	46 (42.68)	80.75 (63.99)
8	31.75 (34.27)	61.75 (51.78)	94 (75.81)
16	43.5 (41.24)	81 (63.95)	99 (83.62)
CD at 5% level	**1.851**	**1.912**	**1.968**
SE(m) ±	**0.609**	**0.629**	**0.647**

REFERENCES

Anandhi, P., Varma, S and Sarvanan, L. (2008). Estimation of losses and evaluation of different storage containers against pulse beetle, *Callosobruchus chinensis* (Linnaeuss) in bengal gram. *Jounal of Insect science*. 21 (1): 40-43

Dhoarey, R.S., katiyar, R.N. and Singh, K.M. (1987). Toxiecological studies on pulse beetle infesting green gram. *Bulletin of grain technology* **25**(2): 152-156

Jat, N. R, RANA B. S. and. Jat S. K. (2013). Estimation of losses due to pulse beetle in chickpea. *The Boicsan An International Quarterly Journal of Life Science*. **8**(3): 861-863

Patil, S.K. Tanpure, S.V. and Mate, S.N. (2003). Effect of different levels of pulse beetle (*Callasobruchus maculates*F.)Infestation on chickpea during storage.Seed Res. **31** (1): 119- 120.

Venkatesham, V., Meena, R.S. and Laichattiwar, M.A. (2015). Assessment of losses due to pulse beet*le Callosobruchus chinensis* l. in chickpea.Ecology, Environment and Conservation, **21**(2) :759-761.

Das, G.P. (1986). Pesticidal efficacy of some indigenous plant oils against the pulse beetle, *Callosobruchus chinensis* Linn. (Coleoptera: Bruchidae). *Bangladesh Journal Zoololgy*, **14** (1): 15-18.

Faruk, K.I., Varol and Bayram, M. (2011). The effect of carbon dioxide at high pressure under different developmental stages of *Callosobruchus maculates* (F) hosting on chickpea. *African J. Biotechnol.*, **10** (11): 2053-2057.

ICRISAT (2007). Chickpea (Internet). International Crop Research Institute for the Semi-Arid Tropics. Available from*www.icrisat.org*, Accessed 2007 Feb. 17.

Kantar, F., Hafeez, F.Y., Shivkumar, B.G., Sundaram, S.P., Tejera, N.A., Aslam, A., Bano, A. and Raja, P. (2007). Chickpea:Rhizobium Management and nitrogen fixation. *In Chickpea Breeding and Management* Yadav S, Redden R, Chen W, Sharma, B.(Eds.) pp. 179-192.

Kumar Rao, J.V.D.K., Johansen, C. and Rego, T.J. (1998). Chickpea can be an important contributor to soil fertility and organic matter to soil. *Indian journal of Lep*, **16** (13) : 23-29.

Shrestha, U.K. (2001). Need for chickpea cultination in nepalin :Pande, C. johansen, P. C. Stevensum and D. Grzwack (eds). Proceeding of the international workshop on planting and implementation of on farm chickpea in nepal, 6-7 september 2001. Kathmandu, Pp 33-47.

RESEARCH ARTICLE

GROWTH, YIELD AND QUALITY OF SUGARCANE (*SACCHARUM OFFICINARUM* L.) AS INFLUENCED BY DIFFERENT VARIETIES AND NUTRIENT MANAGEMENT PLANTED IN SPRING SEASON OF CHHATTISGARH PLAINS

Hemant Kumar Jangde*, N. Tiwari, Dinesh Kumar Marapi, Birendra Tigga and Bhujendra Kumar

**Department of Agronomy, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) 492012*

Received-03.06.2017, Revised-19.06.2017

Abstract: A field investigation was carried out at Research cum Instructional Farm of IGKV, Raipur, Chhattisgarh during spring season of 2014-15 to evaluate the effect of different varieties of sugarcane and nutrient management on growth, yield and quality of sugarcane. Experiment was laid out in split plot design (SPD) with three replications. The treatments consisted of five varieties *viz.*, SG-Co-86032, Local Rasgulla, MSG-CoM-0265, SG-CoC-671 and EG-VSI-08121 in main plots and four nutrient management treatment of N: P_2O_5:K_2O kg ha^{-1} (200:70:70, 250:80:80, 300:90:90 and 350:100:100) in sub-plots. Among the varieties tested, Local Rasgulla was recorded significantly improvement of higher growth, yield attributes and yield in terms of tillers (124.99 $\times 10^3$ ha^{-1}), plant height (319.20 cm), single cane weight (1374 g), average cane diameter (3.15 cm) and cane yield (97.51 t ha^{-1}) were recorded under the variety Local Rasgulla. Among the nutrient management treatments higher no of tillers (117.42 $\times 10^3$ ha^{-1}), plant height (311.50 cm), Single cane weight (1387 g), average cane diameter (3.11 cm) and cane yield (103.45 t ha^{-1}) was recorded with application of 350: 100:100 kg N: P_2O_5:K_2O kg ha^{-1}.

Keywords: *Varieties, Nutrient management, Sugarcane, Cane yield*

INTRODUCTION

Sugarcane (*Saccharum spp*. hybrid complex) is an important sugar and commercial crop in India and plays a pivotal role in agricultural and industrial economy of our country. It provides rich source of sucrose, alcohol and organic matter waste which is utilized as fertilizer. There are many reasons for low yields of sugarcane. Among the multiplicity of factors responsible for low yield, adoption of faulty nutrient management practice and cultivation of obsolete cane varieties are the main reasons for poor productivity of sugarcane crop. Sugarcane variety shows a tendency to decline in yield and vigor which needs replacement of existing varieties with the new one and yield potentiality of a crop would not reach maximum unless proper nutrient management practices is used. This situation may be overcome using newly introduced varieties of sugarcane having better production potential and also adopting improved nutrient management practices. Sugarcane is a long duration exhaustive crop and it depletes the nutrients from soil heavily. On an average 1 kg N, 0.6 kg P_2O_5 and 2.25 kg K_2O are removed by one tonne of sugarcane. Thus a 100 t crop removes 100, 60 and 225 kg N, P_2O_5 and K_2O ha^{-1} from soil, respectively (Lakshmi *et al.*, 2010). Earlier studies showed positive response of sugarcane genotypes to fertility level under diverse planting season (Shukla, 2007). Application of major plant nutrients in right proportion and in optimum quantity through correct method for specific soil- climatic condition is the key input for sustained crop production. There is differential response of the genotypes to the higher level of nutrients due to differential genetic potentiality of the particular genotypes (Sinha *et al*., 2005).

An increase in cane productivity is the interaction of varieties and amount of nutrients applied to the crop. Thus it is important to select varieties along with its appropriate fertilizer doses for sustainable sugarcane production. In view of above, the present study was undertaken to find out the effect of nutrient management on growth, nutrient uptake and productivity of different varieties of sugarcane planted in spring season.

MATERIAL AND METHOD

A field experiment was conducted during spring season of 2014-15 at Research cum Instructional Farm of IGKV, Raipur, Chhattisgarh. Geographically, Raipur is situated in south eastern part of Chhattisgarh at 21˚4'N latitude, 81˚35' longitude with an altitude of 290.20 m above the mean sea level. The experimental soil of field was clay in texture with approximately neutral in reaction pH (7.62), EC (0.16dsm^{-1}), low, medium and high in available nitrogen (245.73 kg ha^{-1}), phosphorus (23.35 kg ha^{-1}) and potassium (385.02 kg ha^{-1}). Experiment was laid out in split plot design (SPD) with three replications. The treatments consisted of five varieties *viz.*, SG-Co-86032 (V_1), Local Rasgulla (V_2), MSG-CoM-0265 (V_3), SG-CoC-671 (V_4) and EG-VSI-08121 (V_5) in main plots and four nutrient management viz. 200:70:70 kg N: P_2O_5:K_2O ha^{-1} (F_1), 250:80:80 kg N: P_2O_5: K_2O ha^{-1} (F_2), 300:90:90 kg N:P_2O_5:K_2O ha^{-1} (F_3) and 350:100:100 kg N:P_2O_5:K_2O ha^{-1} (F_4) in sub plots. Fertilizer nitrogen, phosphorous, potassium was applied in the

*Corresponding Author

form of urea, single super phosphate and muriate of potash respectively and applied as per treatments in each plot. The full dose of single super phosphate and 1/3rd dose of muriate of potash is applied as basal dose and urea was applied in 3 split 30, 90, 120 DAP and rest of the muriate of potash was applied in 2 split at tillering and final earthing up. The sugarcane was planted in second week of February, 2014 and harvested on January-February; 2015.The mean rainfall received during the cropping season was 1257.80 mm. Growth and yield attributing parameters like number of tillers , plant height, single cane weight, average cane diameter were recorded at the time of harvest. Cane yield was recorded after stripping the leaves and detopping. Juice quality parameters *viz.* Brix, sucrose%, purity% and CCS% were recorded at harvest by following standard procedures (Spencer and Meade, 1963). Brix was recorded by using hydrometer and sucrose was estimated by recording pol % using polarimeter. Purity % was calculated by using the formula: (Sucrose% x 100) / Brix. CCS % was calculated by using the formula: (1.022 S) - (0.292 B). Observations were recorded and analyzed as per standard statistical procedure (split plot design) suggested by Gomez and Gomez (1984).

RESULT AND DISCUSSION

Growth and yield attributes

The data pertaining to growth and yield attributes have been summarized and presented in Table 1. A perusal of mean data reveals that different varieties of sugarcane had significant effect on germination percentage. Variety Local Rasgulla had recorded the highest germination (42.27%) which was significantly superior to varieties SG-Co-86032 (31.22%) and SG-CoC-671 (32.94%) but was statistically at par with varieties MSG- CoM-0265 (39.50%) and EG-VSI-08121 (38.89%). The variation in germination percentage was owing to chemical composition of soluble solids in juice as well as enzymes and hormones present in cell sap, which varies from genotype to genotype. Germination percentage of sugarcane unaffected due to nutrient management.

Plant height differed significantly among the different varieties at 270 days after planting. Variety Local Rasgulla recorded the significantly highest plant height (319.20 cm) but it was statistically similar to varieties MSG-CoM-0265 (310.95 cm) and EG-VSI-08121 (299.67 cm). The result in the variation of plant height among the different varieties might be due to different growth rates which were manifested in the form of varied plant height at different stages of plant growth. This was probably due to the genetically characteristic of the variety in term of variation in assimilating capacity of photosynthetic apparatus such as leaf size, orientation and chlorophyll content of leaves. The varietal difference in plant height of sugarcane confirms with the findings of Shukla and Singh (2011). From perusals of the data, it has been observed that different nutrient management treatments had significant influence on plant height at 270 DAP and the tallest plant (311.50 cm) was observed under the treatment 350:100:100 kg N, P_2O_5, K_2O ha^{-1} While, it remained at par with the treatment 300:90:90 kg N, P_2O_5, K_2O ha^{-1}(306.45 cm) at 270 DAP. Such higher plant height might be due to assured supply of nutrients during grand growth stage, improved nutrient availability in root zone to support the cell elongation and their proper root development, which resulted vigorous growth. The results are in accordance with Naga Madhurai *et al.* (2011) for promising early maturing sugarcane varieties.

Variety Local Rasgulla produced significantly highest number of tillers (124.99×10^3 ha^{-1}) at 120 DAP but it was comparable to varieties MSG-CoM-0265 (115.50×10^3 ha^{-1}) and EG-VSI-08121(113.41×10^3 ha^{-1}). The variation in number of tillers among different variety might be due to genetic characters of varieties. Sinare *et al.* (2006), Munir *et al.* (2009) and Aravinth and Wahab (2011) also concluded that tillering behaviors was significantly affected by different varieties. Different nutrient management treatments had significant effect on number of tillers. Significantly higher numbers of tillers (117.42×10^3 ha^{-1}) at 120 DAP was recorded under treatment 350:100:100 kg N, P_2O_5, K_2O ha^{-1}, however it was at par to treatment 300:90:90 kg N, P_2O_5, K_2O ha^{-1}(115.78×10^3 ha^{-1}) at 120 DAP. This might be due to higher doses of chemical fertilizers which increased the population of tillers due to immediate and quick supply of plant nutrients to tillers at the tillering stage. Furthur, higher doses of NPK also reduced the mortality of tillers. The results are in agreement with the findings of Virida and Patel (2010). Lal *et al.* (2008) also observed that significant increase in production of tillers due to increasing nitrogen doses from 75% to 100% recommendation.

Citation of the data regarding average cane diameter at harvest reveals that different varieties of sugarcane show significant effect on average diameter of cane while highest average diameter of cane was recorded in variety Local Rasgulla which was found significantly superior than SG-Co-86032, SG-CoC-671 and EG-VSI-08121.Whereas; it was statistically similar to variety MSG-CoM-0265. Improvement in average diameter of cane was due to increased metabolic processes in plant, resulting in greater metabolic activity thereby improving the sink size which manifested in to thicker canes. These results confirm the findings of Pandey and Shukla (2003).

A critical examination of data indicates that different nutrient management differs significantly among them with regards to average diameter of cane at harvest. The highest average diameter of cane was

recorded under treatment 350:100:100 kg N, P_2O_5, K_2O ha^{-1} which was found significantly superior over other treatments. These findings are in accordance with Ahmad (2002) who obtained maximum cane diameter at higher doses of NPK.

Different varieties of sugarcane had significant difference in single cane weight and highest single cane weight was obtained under variety Local Rasgulla but it was comparable to varieties MSG-CoM-0265 and EG-VSI-08121.This is might be due to fact that growth of varieties is the outcome of genomic, environmental and agronomic interactions. Since all the varieties of sugarcane were grown under identical agronomic environment, the observed variation in overall growth of varieties could be ascribed to their biochemical activities and external environmental factors to which these were exposed during the course of development. Variations in the varietal response were reported by Srinivas *et al*. (2003).

Application of different nutrient management treatments exerted significant influence on single cane weight. Maximum single cane weight is recorded under the treatment 350:100:100 kg N, P_2O_5, K_2O ha^{-1}but it remained at par with treatment 300:90:90 kg N, P_2O_5, K_2O ha^{-1}. This is might be due to higher level of NPK, assured supply of nutrients to sugarcane for growth and development. This result is agreement with the finding of Manickam *et al.* (2008) was recorded highest single cane weight under 125% of the recommended NPK rate.

Yield and quality

The data pertaining to cane yield and quality have been summarized and presented in (Table 1). The highest cane yield was recorded by Local Rasgulla (97.51 t ha^{-1}) followed by MSG-CoM-0265 (95.28 t ha^{-1}). Enhanced yield with suitable varieties was due to the fact that production of significantly highest growth and yield attributes *viz.* plant height, tillers and millable canes. Some varieties have ability to absorb and utilize more nutrients from a soil under the same climatic condition and produce more cane yield. Performance of different varieties with variation in the yield was reported by Kadam *et al.* (2008), Munir *et al.* (2009) and Charumathi *et al.* (2012).

Different varieties of sugarcane influences significantly variation in juice quality with respect of brix percentage, pol percentage and CCS percentage but purity percentage are influences non significantly. Among the varieties SG-Co-86032 showed higher brix (19.95%), pol (17.25%) and CCS (11.81%) in juice followed V_4-SG-CoC-671, EG-VSI-08121, MSG-CoM-0265 and Local Rasgulla. This might be due to genetic ability of this variety to accumulate more sucrose in juice.

Among the different nutrient management treatments the highest cane yield (103.45 t ha^{-1}) was noted under the treatment 350:100:100 kg N: P_2O_5: K_2O ha^{-1} which was significantly superior over other treatments, while it remained at par with treatment 300:90:90 kg N: P_2O_5: K_2O ha^{-1} (100.71 t ha^{-1}). The interaction between varieties and nutrient management was found significant with respect to cane yield. Highest shoot population coupled with efficient conversion of tillers in to millable canes at harvest contributed to higher cane yield. Significant response up to 375 N kg ha^{-1} for variety 83R23 has been reported by Srinivas *et al*., 2003 and for variety 2003V46 by Naga Madhuri *et al.,* 2011. Cane juice quality parameters including brix percentage, pol percentage, purity percetage and CCS percentage did not show significantly among the nutrient management.

Table 1. Growth, yield attributes and quality of sugarcane as influenced by different varieties and nutrient management

Varieties	Germination (%) at 45 DAP	Plant height (cm) at 270 DAP	No. of tillers ($\times 10^3$) at 120 DAP	Average cane diameter (cm)	Single cane weight (g)	Cane yield (t ha^{-1})	Brix (%)	Pol (%)	Purity (%)	CCS (%)
V_1-SG-Co-86032	31.22	281.71	97.42	2.86	1191	80.85	19.95	17.25	86.50	11.81
V_2-Local Rasgulla	42.27	319.20	124.99	3.15	1374	97.51	19.22	16.07	84.87	10.81
V_3-MSG-CoM-0265	39.50	310.95	115.50	3.12	1334	95.28	19.26	16.16	84.94	10.89
V_4-SG-CoC-671	32.94	291.48	105.19	2.92	1225	83.69	19.80	16.92	86.25	11.51
V_5-EG-VSI-08121	38.89	299.67	113.41	3.06	1319	89.50	19.51	16.54	86.12	11.20
SEm±	**1.17**	**6.01**	**3.68**	**0.02**	**34**	**2.60**	**0.15**	**0.13**	**0.91**	**0.14**
CD (P=0.05)	**3.82**	**19.61**	**12.01**	**0.08**	**111**	**8.62**	**0.50**	**0.41**	**NS**	**0.46**
Nutrient Management (N:P_2O_5:K_2O kg ha^{-1})										
F_1-200:70:70	34.62	289.65	104.37	2.95	1187	70.13	19.74	16.79	86.06	11.40
F_2-250:80:80	35.82	294.79	107.63	2.99	1252	83.13	19.64	16.75	85.73	11.38
F_3-300:90:90	38.35	306.45	115.78	3.04	1329	100.71	19.50	16.49	85.62	11.16
F_4-350:100:100	39.05	311.50	117.42	3.11	1387	103.45	19.30	16.33	85.53	11.05
SEm±	**1.29**	**2.95**	**2.35**	**0.02**	**30.85**	**1.13**	**0.13**	**0.13**	**0.87**	**0.15**
CD(P=0.05)	**NS**	**8.52**	**6.79**	**0.06**	**89.11**	**3.28**	**NS**	**NS**	**NS**	**NS**

CONCLUSION

The results of experiment have clearly showed increased growth, yield attributes and cane yield indicating that all the sugarcane varieties tested under the different nutrient management practices are responding to increased level of nutrients. Among the varieties Local Rasgulla was found significantly

superior over others varieties in terms of growth (plant height, no. of tillers), yield attributes (single cane weight, average cane diameter) and cane yield but it was at par with MSG-CoM-0265 and EG-VSI-08121. As regard to nutrient management, application of 350:100:100 kg N, P2O5, K2O ha^{-1} was significantly superior than other nutrient management in terms of growth, yield attributes and cane yield which was at par with application of 300:90:90 kg N,P_2O_5, K_2O ha^{-1}.

REFERENCES

Ahmad, I. (2002). Bioeconomic efficiency of spring planted sugarcane as influenced by spatial arrangement and nutrient management. Ph.D. Thesis, Deptt. Agron., Univ. of Agric., Faisalabad.

Aravinth, V. and Wahab, K. (2011). Studies on the evaluation of varieties for growth and yield of sugarcane. *Plant Archives*, 11(1): 89-90.

Charumathi, M., Rao, K.P., Naidu, N.V. and Adilakshmi, D. (2012). Performance of promising early and midlate sugarcane clones suitable for Andhra Pradesh. Cooperative Sugar, 44(2): 31-34.

Gomez, K.A. and Gomez, A.A. (1984). Statistical procedure for Agricultural Research. John Wiley and Sons Publication 2nd edition.

Kadam, B.S., More, S.M., Veer, D.M. and Nale, V.N. (2008). Response of promising sugarcane genotypes with different levels of nutrients under vertisol for south Maharashtra. Cooperative Sugar, 40(2): 51-54.

Lakshmi, C.S.R., Sreelatha, T., Rani, T.U. and Naidu, N.V. (2010). Organic manures- key inputs for sustaining soil fertility in sugarcane. Indian Sugar, LX(6):31-34.

Lal, R., Chand, M., Khippal, A., Singh, S. and Singh, R. (2008). Response of newly developed sugarcane varieties of north- west zone of India to fertility level. In: Proceedings of National Seminar on varietal planning for improving productivity and sugar recovery in Sugarcane held at G.B.P.U.A. & T. Pantnagar, 14-15 Feb. 2008, p. 202-206.

Manickam, G., Panneerselvam, R., Jayachandran, M., Karunanidhi, K. and Rajendran, B. (2008). Effect of varied levels of NPK fertilization on growth and yield of sugarcane (*Saccharum officinarum*) genotypes. Cooperative Sugar, 39(9): 35-37.

Munir, M.A., Sarwar, M.A., Hussain, F. and Chattha, A.A. (2009). Yield and quality comparison of promising varieties of autumn sown sugarcane. Pakistan Sugar J., XXIV(2): 5-8.

Naga Madhuri, K. V. N, Hemanth, Kumar M and Sarala, N. V. (2011). Influnce of higher doses of nitrogen on yield and quality of early maturing sugarcane varieties. Sugar Tech 13(1):96-98.

Pandey, M.B. and Shukla, S.K. (2003). Growth-cum-tillering pattern and its effect on productivity of sugarcane (*Saccharum spp.*Hybrid complex) genotypes under different planting seasons and nitrogen levels in subtropical India. Indian J. of Agricultural Sciences, 73(1): 23–28.

Shukla, S. K. (2007). Growth, yield and quality of high sugar cane (*Saccharum officinarum*) genotypes as influenced due to planting seasons and fertility levels. Indian J. of Agricultural Sciences, **77**(9): 569–573.

Shukla, S.K. and Singh, I. (2011).Tillering pattern, growth and sugarcane yield of promising genotypes under different planting seasons and fertility levels in subtropical India. Indian J. of Sugarcane Technology, 26(2): 10-13.

Sinare, B. T., Bhoite, D. S., Dixit, R. M. and Bhoi, P. G. (2006). Response of promising new sugarcane genotypes to fertilizer levels in autumn planting. Indian Sugar, 56(4): 29-32.

Sinha, U. P., Singh, H. and Singh, B. K. (2005). Effect of genotypes and fertility levels on growth, yield and quality of sugarcane under rainfed condition. Indian Sugar, 55(7): 23-26.

Spencer, G.L. and Meade, G.P. (1964). Cane sugar handbook, Edition 2nd, p. 433-437. John Wiley and Sons.

Srinivas, D., Rao, B.R.B., Suresh, M., Vijay Kumar, M. and Reddy, L. K. (2003). Influence of level of nitrogen on the yield and quality of early-maturing sugarcane varieties. Cooperative Sugar, 34(6): 479-482.

Virida, H.M. and Patel, C.L. (2010). Integrated nutrient management for sugarcane (*Saccharum* spp. hybrid complex) plant-ratoon system. Indian J. of Agronomy, 55(2): 147–51.