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**Cover photos: Left: Adult mud wasp *(Sceliphron madraspatanum* F.) and Right: Nest of mud wasp with emerging holes (See the Article by Jaydeep Halder *et al.,* on page no. 373)**


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**Review Article**

# The ladybird predator *Serangium parcesetosum* Sicard (Coleoptera: Coccinellidae): current status and future perspectives

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**ABSTRACT**: Whiteflies have been causing extensive damage for almost a century and continue to be destructive pests of several agricultural crops in much of the world. Biological control is recognized as the best alternative to the use of insecticides for controlling insect pests. *Serangium parcesetosum* Sicard (Col., Coccinellidae) is a specialist, oligophagous and efficient predator that has demonstrated a potential for biological control of many whitefly species. Concomitantly, this paper reviews the morphology, phenology and biology of *S. parcesetosum*. In addition, studies conducted during the last decade on the predator's predation potential and preferences are summarized. Furthermore, *S. parcesetosum* releases against some whitefly species are herein presented. Finally, this paper presents the current efforts in biological control of whiteflies using *S. parcesetosum* in greenhouses and open fields, and highlights research gaps and directions deserving further development to create a better understanding of *S. parcesetosum* on different agricultural crops to control whiteflies. The available data indicate that long survival of *S. parcesetosum* adults accomplished by their voracious feeding is a great feature that resulted in successful control of whiteflies. An additional positive feature of *S. parcesetosum* is that it could establish and disperse throughout citrus fields. In conclusion, *S. parcesetosum* could develop, survive, reproduce and prey upon whiteflies, and build up its population successfully. Consequently, it is likely that *S. parcesetosum* could effectively function as a sole biological control agent or in conjunction with other natural enemies to develop new management strategies to provide a great level of suppression of whiteflies worldwide.

**KEY WORDS**: *Serangium parcesetosum*, predator, whiteflies, biology, predation, preference, population dynamics, biological control

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## INTRODUCTION

Coccinellidae (Coleoptera) is a wellknown beetle family, worldwide distributed (Almeida *et al*., 2011), and divided into six subfamilies: Coccidulinae, Coccinellinae, Scymninae, Chilocorinae, Sticholotidinae and Epilachninae (Vandenberg, 2002). The predaceous insects of family Coccinellidae are commonly known variously as ladybirds, ladybugs, lady beetles or coccinellid beetles (Sharma and Joshi, 2010). Predaceous ladybird beetles occur within the first five subfamilies whereas the Epilachninae are phytophagous (Hodek and Honek, 1996; Dixon, 2000). The coccinellids are of extremely diverse habits, predators of a variety of pests such as aphids, leafhoppers, scale insects, mealybugs, whiteflies, thrips and mites in all parts of the world (Majerus, 1994; Omkar and Bind, 1996; Al-Zyoud, 2007, 2008, 2012). The introduction of the Vedalia ladybird, *Rodolia cardinalis* Mulsant from Australia into California in 1888 to control the cottony cushion scale, *Icerya purchasi*, which threatened the citrus industry, is widely regarded as the most successful instance of biological pest control by coccinellids (Majerus, 1994). About 6000 species of Coccinellidae are known Worldwide (Vandenberg, 2000) with over 300 species known from the Indo-Pakistan subcontinent (Rahatullah *et al.,* 2010). Poorani (2002) has given an annotated checklist of the family Coccinellidae for the Indian sub-region, which lists 400 species, under 79 genera, 22 tribes and five subfamilies. Thirty one species were identified, of which 19 species were recorded for the first time within the Haridwar district of India (Joshi and Sharma, 2008). Fourteen species from 12 genera belonging to 4 subfamilies were listed in Pakistan (Rahatullah *et al.,* 2011). Also, Khan *et al*. (2007) have recorded 12 species of coccinellid beetles from Chitral district of Pakistan. Shah (1985) studied the coccinellids of Peshawar valley and recorded 16 species along with geographical distribution and host plants. Singh and Singh (1990) have reported 16 species of aphidophagous coccinellids from Mizoram state, a north eastern state of India. In addition, Omkar and Pervez (2000, 2002) have reported 17 more species

of coccinellids from the same region. Also, in India, 8 species of ladybird beetles belonging to 6 genera were recorded (Sharma *et al.,* 2011). Similarly, Usman and Puttarudriah (1955) recorded 48 species of predaceous coccinellids from the Mysore state, to which Kapur (1972) further added 17 species. Furthermore, 30 coccinellid species belonging to 18 genera (Pajni and Singh, 1982) and 25 coccinellid species from 15 genera from the Chandigarh region (Pajni and Varma, 1985) were recorded.

Biological control of whiteflies through the release of natural enemies has been attempted for at least 30 years (Goolsby *et al.,* 2000), and many attempts have ended in complete success. Some species of fungi such as *Aschersonia aleyrodis*, *Verticillium lecanii*, *Paecilomyces fumosoroseus* and *Beauveria bassiana* have been developed as microbial agents against whiteflies (Mor *et al.,* 1996; Chen and Feng, 1999; James and Jaronski, 2000), but, the development of fungi as control agents of whiteflies is still at a fairly early stage. However, extensive listed fauna of parasitoids were used to control whiteflies of various species of the genera *Eretmocerus* and *Encarsia* (DeBarro *et al.,* 2000; Hu *et al.,* 2003; Urbaneja and Stansly, 2004). Nevertheless, the required releases of *Er. eremicus* (Rose and Zolnerowich) were 27-fold more expensive than the use of insecticides (Driesche *et al.,* 1999). Moreover, *En. formosa* Gahan was unable to build-up its populations on *B. tabaci* and its activity is reduced during winter (Arno and Gabarra, 1996). Nevertheless, in spite of whiteflies being hosts of many parasitoids, it seems that the control of these pests by parasitoids is not achieved due to the extensive host range and mobility of the pests (Gerling and Steinberg, 2003). Thus, biological control strategies should include the release of additional natural enemies.

Predators play a key role in regulating pest populations (Jazzar and Hammad, 2004). Predators range from generalists that require additional food and specialists whose metabolism is adjusted to a specific biochemical composition of food. Hundreds of predators have been reported to prey upon whiteflies including arthropods belonging to 9 orders and 31 families. Heteropteran predators are usually polyphagous and prey specificity is rare (Fauvel, 1999). The predatory mirid bug, *Dicyphus tamaninii* Wagner (Lucas and Alomar, 2002) and *Nesidiocoris tenuis* Reuter (Calvo *et al.,* 2009), and the anthocorid bugs, *Orius laevigatus* (Fieber) and *O. majusculus* (Reuter) (Montserrat *et al.,* 2000) were used to control whiteflies. However, plant feeding by some species of predatory Heteroptera may cause economic injury (Sanchez and Lacasa, 2008) making their use controversial. The green lacewing, *Chrysoperla carnea* (Stephens) (Abd-Rabou and El-Naggar, 2003), and the phytoseiid predatory mites, *Euseius scutalis* (Athias-Henriot) and *Typhlodromips swirskii* (Athias-Henriot) appear to be promising biological control agents of whiteflies (Nomikou *et al.,* 2003). Many species of Coccinellidae are considered important natural enemies of whiteflies and may exhibit various degrees of oligophagy (Obrycki and Kring, 1998). *Delphastus catalinae* (Horn) feeds on immature whitefly but there are conflicting reports on prey consumption rates (Heinz and Parrella, 1994a).

The genus *Serangium* (Coleoptera: Coccinellidae) was erected by Blackburn (1889) with *Serangium mysticum* Blackburn from Australia as the type species. The name Serangiini was introduced by Blackwelder (1945) in his checklist and was validated by Pope (1962). *Serangium* is the largest genus of Serangiini with 45 described species, mostly occurring in the Oriental Region (Slipinski and Burckhardt, 2006). Wang *et al.* (2011) reviewed and described 12 species of *Serangium* from China. *Serangium* spp. are widely distributed in the World and known to be useful predators of many whitefly species. *Serangium parcesetosum* Sicard is a specialist, oligophagous and efficient predator that has demonstrated a potential for the biological control of many whitefly species. *S. parcesetosum* was firstly found in India and described by Sicard (1929) and reported there to be a very host specific on the cotton whitefly, *Bemisia tabaci* (Genn.) (Kapadia and Puri, 1992) and on the sugarcane whitefly, *Aleurolobus barodensis* Mask. (Shah *et al.,* 1986; Patel *et al.,* 1996). This predatory species was originally collected from India in 1929 for the release as a bio-agent of the citrus whitefly, *Dialeurodes citri* (Ashmead) in the Union of Soviet Socialist Republic (Timofeyeva and Nhuan, 1979). According to field studies carried out in Turkey, *S. parcesetosum* has successfully adapted in citrus growing areas to control *D. citri* (Ulusoy *et al.,* 1996). *S. parcesetosum* was introduced into Georgia in 1974 and into France from Georgia in 1985 for the biological control of *D. citri* (Malausa *et al.,* 1988). This ladybird was released to control the wooly whitefly, *Aleurothrixus floccosus* Maskell in Israel (Argov, 1994), and *B. tabaci* and *D. citri* in Syria (Abboud and Ahmad, 1998 Ahmad and Abboud, 2001). In addition, *S. parcesetosum* was investigated as a predator of the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (*B. tabaci* biotype B) in the USA (Ellis *et al.,* 2001; Legaspi *et al.,* 2001). *S. parcesetosum* release was evaluated in grapefruit orchard to control the citrus blackfly, *Aleurocanthus woglumi* Ashby (Legaspi *et al.,* 2001). The biological and ecological parameters of

*S. parcesetosum* have thoroughly been investigated on *B. tabaci* (Al-Zyoud and Sengonca, 2004; Al-Zyoud *et al.,* 2004, 2005b, 2006; Sengonca *et al.,* 2004, 2005; Al-Zyoud, 2007, 2008). According to Al-Zyoud *et al.* (2005a), *S. parcesetosum* was found to be a promising predator of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood. *S. parcesetosum* releases were evaluated to control *B. tabaci* on cotton and cucumber (Al-Zyoud *et al.,* 2007; Al-Zyoud, 2012), and *B. argentifolii* on poinsettias (Ellis *et al.,* 2001) under greenhouse conditions.

Concomitantly, this paper reviews the morphology, phenology and biology of *S. parcesetosum*. In addition, the studies conducted during the last decades on the predator's predation potential and preferences are summarized. Finally, *S. parcesetosum* releases against some whitefly species are herein presented. Also, this paper presents the current efforts in biological control of some whiteflies using *S. parcesetosum* in greenhouses and open fields, and highlights research gaps and directions deserving further development to create a better understanding of this predator on different agricultural crops to control whiteflies.

## MORPHOLOGY AND PHENOLOGY OF *SERANGIUM PARCESETOSUM*

The adult of *S. parcesetosum* is small, hemispherical, shiny, and yellow-brown. The fronts' mouthparts and legs are usually slightly lighter, and the eyes are black. The head is sub-merged into pronotum, and directed downward. Antennae have 9 segments, where the $4^{th}$-$8^{th}$ segments are equal. Legs are covered with hairs and the femur strongly broadened and tarsi conceitedly 4 segmented. The abdomen is semicircular. Adult's body length is 2.0–2.1 mm, width of 1.7–1.8 mm and depth of 1.1 mm (Timofeyeva and Nhuan, 1979; Poorani, 1999). The last larval instar is 4.0–5.3 mm in length, fusiform in shape, and widest on the metathorax. Larval head has indistinct brownish spots, and has 3 black ocelli on each side, and the antenna is short with 3 segments. The larval $1^{st}$ – $8^{th}$ abdominal segments are almost of identical length, following segments gradually narrowing to the end. Larval body is densely covered with setae surrounded by pigmented areas, and legs are long and slender with sparse hairs, and brown claw. The pupa is 2.3–2.4 mm long, 1.8 mm wide, white yellowish, covered with dense long gray hairs on prothoracic segment (Timofeyeva and Nhuan, 1979).

The predator, *S. parcesetosum* overwinters as adult in dry rolled up leaves and underneath bark, congregating in large groups. *S. parcesetosum* emerges from its winter hibernation at early April. Thereafter, *S. parcesetosum* feeds actively for 40–50 days on its prey eggs and larvae, after which it started egg laying, and continues to lay eggs until end of June. *S. parcesetosum* has 4–5 generations/year (Timofeyeva and Nhuan, 1979). The predator lays its eggs singly or in groups on the under surface of the leaf among the whitefly eggs (Ahmad and Abboud, 2001; Al-Zyoud *et al.,* 2005b). The first larval instar ($L_1$) to hatch frequently consumes the eggs on their own egg batch. Newly hatched $L_1$ are relatively immobile and feed on whitefly eggs and larvae over a limited leaf surface. The $L_2$ and $L_3$ move rapidly over the leaves, like the adults, they feed on all development stages of the host (Patel *et al.,* 1996; Ellis *et al.,* 2001; Al-Zyoud and Sengonca, 2004). Males of *S. parcesetosum* follow females, feeding on the remains of the prey of the females and rarely feeding independently. Having attached themselves, pupae frequently become the victims of cannibalism by older larvae (Timofeyeva and Nhuan, 1979).

### Prey species of *Serangium parcesetosum*

The predator, *S. parcesetosum* feeds successfully upon many whiteflies in the family Aleyrodidae (Hom.), including *B. tabaci* (Al-Zyoud *et al.,* 2006, 2007; Al-Zyoud, 2008), *A. barodensis* (Kapadia and Butani, 1997; Patel *et al.,* 1996), *D. citri* (Yigit, 1992b; Uygun *et al.,* 1997; Yigit *et al.,* 2003), *B. argentifolii* (Ellis *et al.,* 2001; Legaspi *et al.,* 2001), *A. floccosus* (Argov, 1994), *T. vaporariorum* (Al-Zyoud *et al.,* 2005a), *A. woglumi* (Kalidas, 1995), the castor bean whitefly, *Trialeurodes ricini* (Misra) (Al-Zyoud, 2007), the spiraling whitefly, *Aleurodicus dispersus* (Russell) and the arecanut whitefly, *Aleurocanthus arecae* David (Legaspi *et al.,* 1996). From the family Coccidae (Homoptera), *S. parcesetosum* has been reported to feed on the brown soft scale, *Coccus hesperidum* L. (Yigit *et al.,* 2003), the citrus soft scale, *C. pseudo-magnoliarum* (Kuwana) (Abboud *et al.,* 2009), and the striped mealybug, *Ferrisia virgata* (Cockerell) (Legaspi *et al.,* 1996).

### Biology of *Serangium parcesetosum*

In order to use a predator in biological control programs against a pest species, it is important to investigate its biology, which is considered one of the most important features that should be taken into account. However, the biology of *S. parcesetosum* has been affected by temperature, prey's host plant species or cultivar, and prey species or even strain (Abboud and Ahmad, 1998, 2006; Al-Zyoud *et al.,* 2004, 2005a, b; Al-Zyoud, 2008). Summarizing the data available on

biology of *S. parcesetosum* may enhance the options for using this specialized predator in pest management programs to control whiteflies in both greenhouses and open fields.

**Development**

The predator, *S. parcesetosum* is able to complete its development on many whitefly species i.e. *B. tabaci* (Al-Zyoud, 2008), *A. barodensis* (Patel *et al.,* 1996), *D. citri* (Yigit *et al.,* 2003), *B. argentifolii* (Ellis *et al.,* 2001), *A. floccosus* (Argov, 1994), *T. vaporariorum* (Al-Zyoud *et al.,* 2005a), *A. woglumi* (Legaspi *et al.,* 2001), *T. ricini* (Al-Zyoud, 2007), *A. dispersus* and *A. arecae* (Legaspi *et al.,* 1996). The development of *S. parcesetosum* consists of an egg stage, four larval instars and a pupal stage (Patel *et al.,* 1996; Abboud and Ahmad, 1998; Sengonca *et al.,* 2004; Al-Zyoud *et al.,* 2005a; Al-Zyoud, 2008). Klausnitzer and Klausnitzer (1997) mentioned that most of the well-known coccinellids have four larval instars during development. However, prey species, temperature, prey's host plant species and predatory sex influence the developmental duration of *S. parcesetosum* as shown in Table 1. Data indicate that males develop faster than females do in all studies undertaken (Sengonca *et al.,* 2004; Al-Zyoud *et al.,* 2005a; Al-Zyoud, 2008). At the same temperature and prey species, *S. parcesetosum* develops faster on cotton than cucumber (Sengonca *et al.,* 2004), and on cabbage than eggplant. This indicates that plant species plays a key role in the development of the predator. The shortest developmental duration (13.2 days) was recorded when the predator reared at 27°C on sugarcane infested with *A. barodensis* (Patel *et al.,* 1996), while the longest development was reported at 18°C on cucumber infested with *B. tabaci* (45.2 days). Besides, this also indicates that temperature plays a vital role in the development of *S. parcesetosum* since the developmental duration is 3-fold at 18°C than at 27°C.

**Table 1. Mean developmental duration from egg to adult emergence of *Serangium parcesetosum* fed on different whitefly species reared on different plants and temperatures**

| Temp (°C) | Prey species | Plant species | Predator Sex | Developmental duration (days) | Reference |
|---|---|---|---|---|---|
| 18 | *Bemisia tabaci* | Cotton | Male | 43.4 | Sengonca*et al.* (2004) |
| 18 | *B. tabaci* | Cotton | Female | 42.4 | Sengonca *et al.* (2004) |
| 18 | *B. tabaci* | Cucumber | Male | 45.2 | Sengonca *et al.* (2004) |
| 18 | *B. tabaci* | Cucumber | Female | 43.4 | Sengonca *et al.* (2004) |
| 21 | *B. tabaci* | Cabbage | – | 23.8 | Ahmad and Abboud (2001) |
| 25 | *B. tabaci* | Cabbage | – | 15.8 | Abboud and Ahmad (1998) |
| 25 | *Aleurothrixus floccosus* | Cabbage | – | 17.3 | Abboud and Ahmad (1998) |
| 25 | *Dialeurodes citri* | Cabbage | – | 17.9 | Abboud and Ahmad (1998) |
| 25 | *Bemisia tabaci* | Cucumber | Male | 20.0 | Al-Zyoud (2008) |
| 25 | *B. tabaci* | Cassava | – | 21.0 | Asiimwe *et al.* (2007) |
| 25 | *B. tabaci* | Cotton | – | 22.9 | Vatanesever *et al.* (2003) |
| 25 | *B. tabaci* | Egg plant | – | 28.0 | Vatanesever *et al.* (2003) |
| 23–33 | *B. tabaci* | Cucumber | Male | 17.4 | Al-Zyoud (2008) |
| 27 | *Aleurolobus barodensis* | Sugarcane | – | 13.2 | Patel *et al.* (1996) |
| 27 | *Bemisia tabaci* | Cabbage | – | 15.7 | Ahmad and Abboud (2001) |
| 27–32 | *B. tabaci* | Cabbage | – | 12.9 | Ahmad and Abboud (2001) |
| 30 | *B. tabaci* | Cotton | Male | 17.2 | Sengonca*et al.* (2004) |
| 30 | *B. tabaci* | Cotton | Female | 16.2 | Sengonca *et al*. (2004) |
| 30 | *B. tabaci* | Cucumber | Male | 15.9 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cucumber | Female | 15.1 | Sengonca *et al.* (2004) |
| 30 | *Trialcurodes vaporariorum* | Cucumber | Male | 17.4 | Al-Zyoud *et al.* (2005a) |
| 30 | *T. vaporariorum* | Cucumber | Female | 16.9 | Al-Zyoud *et al.* (2005a) |
| 32 | *Bemisia tabaci* | Cabbage | – | 14.3 | Ahmad and Abboud (2001) |

## Mortality

Mortality occurs during all developmental stages of *S. parcesetosum*. Mortality in $L_1$ instar was the highest as compared to other larval instars, and the mortality in the pupal stage was the highest compared to other immature stages (Sengonca *et al.,* 2004; Al-Zyoud *et al.,* 2005a; Al-Zyoud, 2008). Temperature influences the mortality of *S. parcesetosum*, of which it was higher at 18°C (33 and 31%) than at 30°C (24 and 21%) on cotton and cucumber infested by *B. tabaci*, respectively (Sengonca *et al.,* 2004). Abboud and Ahmad (1998) stated that mortality of *S. parcesetosum* fed on *B. tabaci* was 40, 22, 20% and 10% at 32, 27, 21°C and 27-32°C, respectively. In addition, plant species influenced the predator's mortality, that is, it was higher on cotton than cucumber (Sengonca *et al.,* 2004). According to Ahmad and Abboud (2001), the mortality was 100, 30, 18% and 5%, when *S. parcesetosum* fed on *B. tabaci* on bean, cabbage, eggplant and okra at 27°C, respectively. In addition, mortality was the lowest on cotton (21%) and the highest on eggplant (49%) at 25°C (Vatanesever *et al.,* 2003). It was suggested that hair density on plant leaves helps positively in reducing the predator's mortality (Ahmad and Abboud, 2001; Sengonca *et al.,* 2004). Furthermore, mortality is affected by prey species, given that it was higher when *S. parcesetosum* fed on *T. vaporariorum* (26%) (Al-Zyoud *et al.,* 2005a) than on *B. tabaci* (21%) (Sengonca *et al.,* 2004) reared on cucumber at 30°C. Furthermore, mortality might be affected by the combination of plant-whitefly-predator (tritrophic) interaction.

## Sex ratio

Sex ratio (female : male) of *S. parcesetosum* fed on *B. tabaci* is affected by temperature and plant species. It was 1:0.9 and 1:0.8 at 18°C, and 1:1.1 at 30°C on cotton and cucumber under laboratory conditions, respectively (Sengonca *et al.,* 2004). Kapadia and Puri (1992) reported a sex ratio of 1:0.8 and 1:1 under field and laboratory conditions, respectively.

## Longevity

One of the most important features for a successful predator is to survive for a long period and feed continuously on the prey species. Long survival of *S. parcesetosum* adults (Sengonca *et al.,* 2004) accomplished by voracious feeding (Sengonca *et al.,* 2005) is a great feature that results in a successful control of *B. argentifolii* (Ellis *et al.,* 2001) and *B. tabaci* (Sengonca *et al.,* 2004; Al-Zyoud, 2008). However, *S. parcesetosum* longevity varies according to temperature, prey's host plant species or cultivars, prey species or even strains and predatory sex as shown in Table 2. The longest longevity (6 months)

**Table 2. Mean longevity of *Serangium parcesetosum* fed on different whitefly species reared on different plants and temperatures**

| Temp (°C) | Prey species | Plant species | Predator Sex | Longevity period (days) | Reference |
|---|---|---|---|---|---|
| 18 | *Bemisia tabaci* | Cotton | Male | 175.4 | Sengonca *et al.* (2004) |
| 18 | *B. tabaci* | Cotton | Female | 144.5 | Sengonca *et al.* (2004) |
| 18 | *B. tabaci* | Cucumber | Male | 122.2 | Sengonca *et al.* (2004) |
| 18 | *B. tabaci* | Cucumber | Female | 94.3 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cotton | Male | 92.4 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cotton | Female | 52.5 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cucumber | Male | 63.4 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cucumber | Female | 50.3 | Sengonca *et al.* (2004) |
| 30 | *Trialcurodes vaporariorum* | Cucumber | Male | 70.8 | Al-Zyoud *et al.* (2005a) |
| 30 | *T. vaporariorum* | Cucumber | Female | 59.9 | Al-Zyoud *et al.* (2005a) |
| 25 | *Bemisia tabaci* | Cucumber | Male | 79.9 | Al-Zyoud (2008) |
| 23–33 | *B. tabaci* | Cucumber | Male | 95.1 | Al-Zyoud (2008) |
| 25 | *B. tabaci* | Cucumber | Female | 65.2 | Al-Zyoud (2008) |
| 23–33 | *B. tabaci* | Cucumber | Female | 71.5 | Al-Zyoud (2008) |
| 23.7 | *B. tabaci* | Egg plant | Male | 50.5 | Kapadia and Puri (1992) |
| 23.7 | *B. tabaci* | Egg plant | Female | 22.6 | Kapadia and Puri (1992) |
| 27 | *Aleurolobus barodensis* | Sugarcane | – | 29.8 | Patel *et al.* (1996) |
| 20–23 | *Bemisia argentifolii* | Hibiscus | – | 44.2 | Legaspi *et al.* (1996) |
| 20–23 | *B. argentifolii* | Cucumber | – | 24.5 | Legaspi *et al.* (1996) |
| 20–23 | *B. argentifolii* | Cantaloupe | – | 27.6 | Legaspi *et al.* (1996) |
| 20–23 | *B. argentifolii* | Tomato | – | 27.8 | Legaspi *et al.* (1996) |
| 20 | *B. argentifolii* | Cantaloupe | – | 79.2 | Legaspi *et al.* (1996) |
| 30 | *B. argentifolii* | Cantaloupe | – | 26.9 | Legaspi *et al.* (1996) |
| 40 | *B. argentifolii* | Cantaloupe | – | 1.40 | Legaspi *et al.* (1996) |

was reported on cotton infested by *B. tabaci* at 18°C (Sengonca *et al.,* 2004), while the shortest (1.4 days) was recorded on cantaloupe infested by *B. argentifolii* at 40°C (Legaspi *et al.,* 1996). Moreover, within the same temperature *S. parcesetosum* survives longer on cotton than cucumber (Sengonca *et al.,* 2004), and on hibiscus than cucumber, cantaloupe and tomato (Legaspi *et al.,* 1996). In all studies, females survive longer than males do. It is worth mentioning that the extremely high longevity on cotton may partly be explained by that *S. parcesetosum* was reared on *B. tabaci* using cotton as a host plant. Therefore, it might be that the predator has adapted itself on cotton plants and lived much more on it (Legaspi *et al.,* 1996; Al-Zyoud *et al.,* 2004). Additionally, the morphological characteristics of the host plant and the interaction of the plant-whitefly-predator (tritrophic) may have a major effect on the longevity of the predator, suggesting a high degree of specialization of *S. parcesetosum* on a plant species. *S. parcesetosum* lived longer when the predator fed on *T. vaporariorum* than on *B. tabaci* (Al-Zyoud *et al.,* 2005a), *B. argentifolii* (Legaspi *et al.,* 1996), and *A. barodensis* (Patel *et al.,* 1996).

The periods of pre-oviposition, oviposition and post-oviposition are affected by temperature, prey and plant species as shown in Table 3. Range of periods of pre-oviposition, oviposition and post-oviposition were 6.8-26.3, 24.3-121.1 and 8.6-59.7 days, respectively.

The ability of a natural enemy to survive on alternative nutritional sources may have an advantage in stabilizing its population dynamics (Lalonde *et al.,* 1999). However, a major stumbling block to use biological control on a large scale is that it has been difficult to produce adequate numbers of predatory insects to effect reduction of large outbreaks of pest populations. For example, predators have been used successfully for decades to control insect pests however, the scale of their use has been limited because of inadequate methods to artificially produce these predatory insects. In case of *S. parcesetosum,* a growth medium composed of a mixture of an adherent and fibrous retention substrate, a protein-lipid paste, and a liquid was successfully used to rear the predator for three generations (Hodek and Honek, 2009). In addition, it was found that *S. parcesetosum* adults survive on honey emulsion for 27 days at 18°C, and for 14 days at 30°C (Al-Zyoud *et al.,* 2006).

**Fecundity**

The ability of a predator to oviposit successfully on the host plant on which its prey lives is one of the major factors in determining its ability to successfully control the pest. Prey's host plant species or even cultivar temperature and prey species influence the daily and total fecundity of *S. parcesetosum*. The maximum number of eggs laid per day by *S. parcesetosum* was 4.7 eggs/female (fed on *B. tabaci* Sengonca *et al.,* 2004) and 1.5 eggs/female (fed on *T. vaporariorum* Al-Zyoud *et al.,* 2005a) on cucumber at 30°C. A maximum daily laid eggs of 8.7 and 6.6 eggs/female was reported when *S. parcesetosum* fed on *B. tabaci* at 25°C and 23-33°C, respectively (Al-Zyoud, 2008). The highest total fecundity of *S. parcesetosum* (443.9 eggs/female) was recorded on cabbage when the predator fed on *B. tabaci* at 27°C (Ahmad and Abboud, 2001) as shown in Table 4. This was followed by 354.7 eggs/female when the predator fed on *B. tabaci* on cotton at 25°C (Vatanesever *et al.,* 2003). It seems that temperature ranges from 25°C to 27°C is the most preferred for the predator. Within the same temperature and plant species, the fecundity is 3-fold higher when *S. parcesetosum* fed on *B. tabaci* (98 eggs/female) (Sengonca *et al.,* 2004) than on *T. vaporariorum* (28 eggs/female) (Al-Zyoud *et al.,* 2005a). In addition, when *S. parcesetosum* kept together with 5 different plant species infested with *B. tabaci*, the predator laid more eggs on cucumber (115 eggs) than on tobacco (42 eggs), cotton (33 eggs), tomato (30 eggs),

**Table 3. Mean pre-oviposition, oviposition and post-oviposition periods of *Serangium parcesetosum* fed on different whitefly species reared on different plants and temperatures**

| Temp (°C) | Prey species | Plant species | Pre-ovip. | Ovip. | Post-ovip | Reference |
|---|---|---|---|---|---|---|
| 18 | *Bemisia tabaci* | Cotton | 18.8 | 121.1 | 35.5 | Sengonca *et al.* (2004) |
| 18 | *B. tabaci* | Cucumber | 26.3 | 36.2 | 59.7 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cotton | 07.7 | 28.0 | 56.7 | Al-Zyoud *et al.* (2004) |
| 30 | *B. tabaci* | Cucumber | 12.1 | 40.6 | 10.7 | Al-Zyoud *et al.* (2004 |
| 30 | *Trialcurodes vaporariorum* | Cucumber | 08.8 | 46.0 | 16.0 | Al-Zyoud *et al.* (2005a) |
| 23.7 | *Bemisia tabaci* | Eggplant | 16.3 | 24.3 | 08.6 | Kapadia and Puri (1992) |
| 23–33 | *B. tabaci* | Cucumber | 06.8 | 52.5 | 35.8 | Al-Zyoud (2008) |
| 25 | *B. tabaci* | Cucumber | 08.2 | 42.3 | 29.5 | Al-Zyoud (2008) |

**Table 4. Mean total fecundity of *Serangium parcesetosum* fed on different whitefly species reared on different plants and temperatures**

| Temp (°C) | Prey species | Plant species | Fecundity | Reference |
|---|---|---|---|---|
| 18 | *Bemisia tabaci* | Cotton | 52 | Sengonca *et al.* (2004) |
| 18 | *B. tabaci* | Cucumber | 25 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cucumber | 98 | Sengonca *et al.* (2004) |
| 30 | *B. tabaci* | Cotton | 31 | Sengonca *et al.* (2004) |
| 30 | *Trialeurodes vaporariorum* | Cucumber | 28 | Al-Zyoud *et al.* (2005a) |
| 25 | *Bemisia tabaci* | Cucumber | 228 | Al-Zyoud (2008) |
| 23–33 | *B. tabaci* | Cucumber | 143 | Al-Zyoud (2008) |
| 23.7 | *B. tabaci* | Eggplant | 22.7 | Kapadia and Puri (1992) |
| 27 | *B. tabaci* | Cabbage | 443.9 | Ahmad and Abboud (2001) |
| 20–23 | *Dialeurodes citri* | Citrus | 135–185 | Timofeyeva and Nhuan (1979) |
| 25 | *B. tabaci* | Eggplant | 135.2 | Vatanesever *et al.* (2003) |
| 25 | *B. tabaci* | Cotton | 354.7 | Vatanesever *et al.* (2003) |

and sweet pepper (only 3 eggs) (Al-Zyoud *et al.,* 2004). Host plant and prey species have a major impact on natural enemies by influencing their searching success and the quality of their dietary resources, and consequently their biology (Coll and Ridgway, 1995). Several researchers have stated that plant architecture and surface texture influence the search behavior of coccinellid predators (Kareiva and Sahakian, 1990). Sweet pepper has overly smooth leaf surfaces which may have a negative effect on the oviposition of the predator (Carter *et al.,* 1984). The other four plants are characterized by hairiness leaf surfaces therefore, it might be that leaf pubescence helps positively increase the probability of more eggs being laid enhacing the protection of eggs by the pubescence of the host plant of *S. parcesetosum*. Vatanesever *et al.* (2003) reported that cotton infested with *B. tabaci* constitutes more suitable plant species for mass rearing due to short development time, low mortality rate and high fecundity of *S. parcesetosum*. In addition, *B. tabaci* is an insect that is easily reared under laboratory conditions and suitable for rearing *S. parcesetosum* (Yigit, 1992a). The data also show that the interaction between *S. parcesetosum* and its prey influences not only by prey species but also by the suitability of the food plants used by the prey that serves as food for the predator. In conclusion, *S. parcesetosum* seems to prefer a number of whiteflies host plant species for oviposition and can complete its full development successfully on them.

## PREDATION POTENTIAL OF *SERANGIUM PARCESETOSUM*

A successful biological control of a pest species depends on the fact that the predator destroys, kills or consumes a sufficient number of the pest to keep its population bellow the economic threshold level (Sengonca *et al.,* 2005). *Serangium parcesetosum* are common in the Mediterranean region and both adults and larvae are predaceous stages (Santos *et al.,* 2009). Despite their polyphagy, coccinellid adults tend to feed more on certain types of food (Iperti, 1999) and the beneficial effect that food has on individual predators leads to increased rates of growth, development and fertility, and decreased rates of mortality (Begon *et al.,* 1996). Moreover, larvae are the most voracious stages of coccinellids requiring great amount of food to grow up rapidly (Stathas, 2000). However, predation potential data reveal that *S. parcesetosum* larvae and adults exhibit the ability to prey voraciously upon many whitefly species.

### Predation potential of larval instars

The larvae of *S. parcesetosum* are able to prey successfully upon different whitefly species reared on different plants at different temperatures. The larvae consume 310 and 261 of *B. tabaci* immatures/day at 25°C and 23–33°C, respectively on cucumber (Al-Zyoud, 2008). Sengonca *et al.* (2005) mentioned a maximum daily predation of 161 nymphs and 27 puparia at 18°C, and 235 nymphs and 36 puparia of *B. tabaci* at 30°C on cotton. Predation potential of the separate larval instars of *S. parcesetosum* at 18°C indicated that $L_1$ instar consumes a total of 115 nymphs or 27 puparia of *B. tabaci*. The mean total predation increased with the progress of development until it was the highest by the $L_4$ instar with 964 nymphs or 152 puparia. At 30°C, $L_1$ instar fed on 79 nymphs or 18 puparia, while $L_4$ instar consumed 676 nymphs or 102 puparia of *B. tabaci* (Sengonca *et al.,* 2005). Asiimwe *et al.* (2007) reported that $L_1$ instar

consumed only 51 nymphs, while $L_4$ feeds on 551 nymphs of *B. tabaci* on cassava, indicating that $L_4$ consumes 10-fold higher than $L_1$. The $L_1$ consumed a total of 44 nymphs or 18 puparia of *T. vaporariorum*, while $L_4$ instar consumed 722 nymphs or 110 puparia *B. tabaci* (Al-Zyoud *et al.,* 2005b). Means total of 122 and 75 ($L_1$), and 924 and 733 ($L_4$) *B. tabaci* immatures were consumed at 25°C and 23-33°C, respectively (Al-Zyoud, 2008).

However, *S. parcesetosum* during its entire larval development consumed more prey at 18°C (1566 nymphs or 280 puparia) than at 30°C (1119 nymphs or 188 puparia) (Sengonca *et al.,* 2005). This may be explained by that the larval developmental period at 30°C was only a half of that one at 18°C (Sengonca *et al.,* 2004). *S. parcesetosum* consumed 1012 nymphs or 184 puparia of *T. vaporariorum* during its development (Al-Zyoud *et al.,* 2005b). The predatory larvae consumed more *B. tabaci* at 25°C (1542) than at 23–33°C (1095 immatures) (Al-Zyoud, 2008). Timofeyeva and Nhuan (1979) reported that *S. parcesetosum* larval instars consumed a total of 900–1000 eggs of *D. citri* at 20–23°C. In addition, *S. parcesetosum* consumed during its larval duration 1678 eggs or 195 puparia of *B. tabaci* on cabbage at 27°C (Ahmad and Abboud, 2001), 1055 nymphs of *B. tabaci* on cassava (Asiimwe *et al.,* 2007), and 671 nymphs and puparia of *A. barodensis* on sugarcane at 27°C (Patel *et al.,* 1996) respectively. Differences in the results might be due to the fact that different prey stages or species, host plants and temperatures used in the different studies.

**Predation potential of adults**

The available data from prior studies on the predation potential of *S. parcesetosum* adults indicated that predatory females and males feed on 15 and 13 nymphs or 10 and 9 puparia of *B. tabaci* on the 1st day after adult emergence, and reach a peak of 49 and 44 nymphs or 22 and 18 puparia/day at 18°C, respectively. While at 30°C, 41 and 23 nymphs or 24 and 23 puparia were consumed on the 1st day, and consumption reached a peak of 74 and 71 nymphs or 40 and 33 puparia/day by females and males, respectively (Sengonca *et al.,* 2005). *S. parcesetosum* females and males fed on 31 and 30 nymphs or 20 and 18 puparia of *T. vaporariorum* on the 1st day after adult emergence, and reached a peak of 84 and 71 nymphs or 34 and 29 puparia/day, respectively (Al-Zyoud *et al.,* 2005a). At 25 and 23-33°C, adults consumed 84 and 92 immatures of *B. tabaci* on the 1st day after adult emergence and reached a peak of 144 and 130 immatures/day, respectively (Al-Zyoud, 2008). *S. parcesetosum* adults consumed daily 99 nymphs and puparia of *A. barodensis* and 170-200 eggs and immature stages of *B. argentifolii* at 27°C (Patel *et al.,* 1996), and 271 eggs or 23 puparia of *B. tabaci* (Ahmad and Abboud, 2001).

Within 60 days of longevity, *S. parcesetosum* adults consumed 2188 (males) and 1994 (females) nymphs or 727 (males) and 625 (females) puparia at 18°C, and 3948 (males) and 3577 (females) nymphs or 1601 (males) and 1449 (females) puparia of *B. tabaci* at 30°C (Sengonca *et al.,* 2005), as well as 3842 (males) and 3507 (females) nymphs or 1482 (males) and 1368 (females) puparia of *T. vaporariorum* (Al-Zyoud *et al.,* 2005a). While over 80 days of longevity, the predator consumed 7805 and 7502 of *B. tabaci* immatures at 25°C and 23–33°C, respectively (Al-Zyoud, 2008). The maximum cumulative lifetime predation was measured at >10,000 of *B. argentifolii* consumed in the most long-lived individuals (Legaspi *et al.,* 1996). The daily predation rate of adults increased with increasing temperature, where it was 139, 181, and 187 of *B. argentifolii* immatures at 20°C, 30°C and 40°C on cantaloupe, respectively (Legaspi *et al.,* 1996). In all the studies, females consumed more prey than males, which justifies a stronger need for nutrients for egg laying by females. Differences in predation rate among the different studies could be attributed to different prey species, prey stages, plant species, temperatures and feeding periods used in the different studies. It can be concluded that *S. parcesetosum* successfully developed, survived, reproduced and fed upon many whitefly species. Consequently, this ladybird seems to have a potential to be a bio-agent of whiteflies, which could be employed in biological control programs against these pests under greenhouses and open field conditions.

**Predation potential by changing prey number**

The prey's population available in the agro-ecosystem for a natural enemy will never be constant and fluctuates in relation to many factors. To be considered as an efficient natural enemy, a predator is expected to be able to adapt itself to such a fluctuation in prey availability. However, *S. parcesetosum* was smoothly able to adapt itself to prey availability fluctuation. A range of 3–5, 6–9, 14–17 and 25–30 of *B. tabaci* puparia/day was consumed by *S. parcesetosum* when 5, 10, 20 and 50 puparia were offered/day, respectively (Sengonca *et al.,* 2005). Thus, daily predation rate became higher when more prey was offered, in contrast, most of prey individuals offered were consumed when the daily prey offer was only 5 puparia. These results are going along with a conclusion made by Alvarado *et al.* (1997) who reported a considerable increase in the daily predation rate in relation to prey density.

**Density-dependent response of *Serangium parcesetosum***

It is of vital importance in biological control to find the predator response to prey because it may contribute to stability of predator-prey system (Taylor, 1984). The predator, *S. parcesetosum* imposes positive density dependent with *B. tabaci* (type III functional response). The functional response of *S. parcesetosum* can be simulated by Hollings disc equation and expressed as $Ne=0.82N/1+0.0016N$, and by the reciprocal linear transformation of Hollings equation as y=1.2218x–0.0019. The estimated search rate is 5.74 cm and the handling time is 3 min (Araj *et al.,* 2012). Predators having such a type of response allow long-term population persistence (Pech *et al.,* 1992), and in turn will effectively stabilize their prey population. *S. parcesetosum* causes higher mortality levels at moderate whitefly densities. So, it is recommended to use the predator at a moderate infestation of whiteflies'.

## PREFERENCES OF *SERANGIUM PARCESETOSUM*

Before considering a predator in biological control, it is important to investigate its affinity toward a certain developmental stage of the target pest or even the pest species to be controlled and a possible interaction with other natural enemies. This is true especially when it is taken into account that under greenhouses and open field conditions there are naturally several pest species that might serve as potential prey for the predator, in addition, there are several natural enemies that could interact with it.

**Prey stage preferences**

Investigating the preferred prey stage would be useful in determining which developmental stage of the prey is the most predated, and this will facilitate further laboratory rearing of the predator, which is a prime objective in biological control (Sahayaraj and Paulraj, 2001). However, *S. parcesetosum* $L_2$, $L_4$ instars and adults prefer puparia and nymphs to the eggs of *B. tabaci* on cotton (Al-Zyoud and Sengonca, 2004). Patel *et al.* (1996) reported that the predator to be highly specific and feeds voraciously on eggs, nymphs and puparia of *A. barodensis*. *S. parcesetosum* predates eggs and puparia of *A. barodensis* (Shah *et al.,* 1986). According to Ahmad and Abboud (2001), *S. parcesetosum* could feed on all *B. tabaci* developmental stages. In general, predation and preference depend mostly on the characteristics of the prey's tegument (Honda and Luck, 1995), relation between size of predator and prey, and prey's nutritional quality (Roger *et al.,* 2000). However, regardless of the whitefly species used in the different studies, *S. parcesetosum* has the ability to feed on all developmental stages of whiteflies offered.

**Prey species preferences**

Al-Zyoud and Sengonca (2004) offered five different prey species to *Serangium parcesetosum* Sicard separately on cotton, and it is found that predatory larvae and adults have prey preference toward the whitefly species used (*B. tabaci* and *T. vaporariorum*) consuming very few individuals from the non-whitefly species *Aphis gossypii*, *Frankliniella occidentalis* and *Tetranychus urticae*. In addition, the predator had more preference for *B. tabaci* rather than *T. vaporariorum*. In addition, when *S. parcesetosum* offered five different prey species together or separately on cucumber, the predator also preferred the whitefly species tested *B. tabaci* and *T. ricini* rather than *T. urticae*, *A. gossypii* and *Liriomyza huidobrensis* (Al-Zyoud, 2007). Legaspi *et al.* (1996) mentioned that when *S. parcesetosum* was simultaneously offered the eggs of *Helicoverpa zea* and *Manduca sexta*, and *B. argentifolii* reared on poinsettia, cantaloupe and cucumber respectively, the predatory adults did not feed on *H. zea* and *M. sexta*, indicating a preference for *B. argentifolii.* Abboud and Ahmad (1998) in a study conducted on the preference of *S. parcesetosum* for different whitefly species observed that the whitefly, *Paraleyrodes minei* Laccarino is not suitable prey for *S. parcesetosum*, while *B. tabaci*, *D. citri* and *A. floccosus* were found to be suitable for the predator. In addition, they found that *S. parcesetosum* prefers *B. tabaci* more than *D. citri* and *A. floccosus*. Legaspi *et al.* (2001) noted that *S. parcesetosum* is not as voracious on *A. woglumi* eggs as on *B. argentifolii* nymphs. However, the degree of preference of *S. parcesetosum* for one whitefly species upon another might be due to size of the whitefly, thickness and hardness of the cuticle, and many other physical and chemical factors. Moreover, it might be that nutrient differences among prey species have a substantial impact on predator choice. Concomitantly, *S. parcesetosum* is a specialist predator of whiteflies.

**Interaction and combined use of natural enemies**

The predator, *S. parcesetosum* $L_2$, $L_4$, adult females and males tend to avoid parasitized *B. tabaci* puparia by *En. formosa* and feed instead on unparasitized ones. The predator consumed daily 8.7 and 0.2 ($L_2$), 11.1 and 0.6 ($L_4$), 12.1 and 1.0 (male), and 10.5 and 0.2 (female) unparasitized and parasitized *B. tabaci* puparia, respectively (Al-Zyoud and Sengonca, 2004). In addition, larvae and adults of *S. parcesetosum* significantly tend to avoid parasitized puparia and feed instead on unparasitized

puparia of *B. tabaci* by *Er. mundus*, i.e. 8.3 and 1.3 ($L_4$) and 8.5 and 1.3 (adult) unparasitized and parasitized puparia, respectively (Al-Zyoud, 2007). Furthermore, *S. parcesetosum* survivorship has not affected by the rates of the entomopathogenic fungi, *B. bassiana* and *P. fumosoroseus*, and cumulative predation showed that *S. parcesetosum* sprayed with *P. fumosoroseus* consumes prey at a rate similar to that in the control (Poprawski *et al.,* 1998). Overall, these results enhance the options for the use of *S. parcesetosum* in pest management programs in conjunction with parasitoids and pathogens. The results suggest that because the parasitized whiteflies by *En. formosa* and *Er. mundus* are currently in use worldwide to control whiteflies (Abd-Rabou, 1999) from one hand and on the other hand these parasitoids are avoided by *S. parcesetosum*. There is a feasible potential for integration of these natural enemies into whiteflies management programs in order to provide a great level of the pest suppression. In this regard, Zapata *et al.* (2003) mentioned that release of *Er. mundus* in combination with *Macrolophus caliginosus* provides a great level of whitefly suppression.

## EGG LAYING BEHAVIOUR OF *SERANGIUM PARCESETOSUM*

Studying of egg-laying behaviour and oviposition strategy of a natural enemy is of a great value that leads to a better understanding of its ecological characteristics and helps positively in using it in a biological control program against a pest species. A female insect must take at least two decisions to oviposit on a host where to lay its eggs and how many *eggs* to lay in each site. The answers to these questions could explain the oviposition strategy, which determines the insect fitness of offspring and growth rate in the population (Danho and Haubruge, 2003). However, Al-Zyoud *et al.* (2005b) investigated the egg-laying behaviour of *S. parcesetosum* in the absence and presence of *C. carnea*, one of the main predators associated with *B. tabaci* population, on cucumber and cotton infested with *B. tabaci*. They found that *S. parcesetosum* prefers to lay its eggs between the veins and close to the veins in the absence of *C. carnea*, while in its presence more eggs were deposited close to veins and petiole on cucumber leaves. In contract, on cotton leaves S. *parcesetosum* prefers to deposit its eggs close to the veins and petiole in the absence and presence of *C. carnea*. Timofeyeva and Nhuan (1979) stated that *S. parcesetosum* fed on *D. citri* lays its eggs on the under surface of citrus leaves. *S. parcesetosum* deposits its eggs singly on the under surface of eggplant leaves infested with *B. tabaci* (Kapadia and Puri, 1992). According to Patel *et al.* (1996), *S. parcesetosum* fed on *A. barodensis* lays its eggs singly. While, Ahmad and Abboud (2001) mentioned that *S. parcesetosum* fed on *B. tabaci* deposits its eggs singly or in irregular groups on the plant leaves near the prey stages. It appears that *S. parcesetosum* could lay its eggs singly or in groups. Also, the results indicate that the presence of *C. carnea* and plant species influence the distribution of eggs on the leaves.

## RELEASES OF *SERANGIUM PARCESETOSUM*

The predator, *S. parcesetosum* is a promising bio-agent against many whitefly species because of its voracity and preference. Both larvae and adults of *S. parcesetosum* could feed on all developmental stages of whiteflies (Kapadia and Puri, 1992; Ahmad and Abboud, 2001; Al-Zyoud *et al.,* 2005a.). However, because of the success of *S. parcesetosum* in the laboratory and in order to be considered as an efficient predator for a biological control program and to be successfully used to control whiteflies, it has been evaluated to check its effectiveness in reducing the population of some whitefly species under more natural conditions such as greenhouses and open fields. However, when *S. parcesetosum* introduced 1 and 2 weeks after infestation with *B. tabaci* as well as a control treatment on cotton plants under glasshouse conditions, the number of *B. tabaci* was 75, 123 and 685 (1 predator: 25 whiteflies) in the last experimental week ($7^{th}$ week), respectively (A-Zyoud *et al.,* 2007). On cucumber plants, the number of *B. tabaci* was significantly higher in the control treatment compared with 1- and 2-week treatments when *S. parcesetosum* was introduced at densities of 1:30 and 1:20. Initial whitefly release rates (1:30 or 1:20) greatly affected the final population density of the whitefly. This effect was most evident when whitefly populations were left uncontrolled, in which *B. tabaci* numbers in the last experimental week were 955, 336 and 364 (1:30) as well as 670, 253 and 267 (1:20) in control, 1 and 2 weeks after *S. parcesetosum* introduction, respectively (Al-Zyoud, 2012). It could be concluded that release rate of 1 predator: 20 whiteflies would be more efficient in suppressing the pest than 1:30. A single release of one adult *S. parcesetosum* beetle was effective at stopping the growth of *B. tabaci* populations on cucumber and cotton for 7 weeks. In general, *S. parcesetosum* was able to successfully feed, reproduce and consume *B. tabaci* infested cotton and cucumber under greenhouse conditions. In addition, the number of whitefly was lower when the predator introduced one week rather than two weeks after the whitefly infestation (A-Zyoud *et al.,* 2007; Al-Zyoud, 2012). In similar fashion, Ellis *et al.* (2001) found that introduction of *S. parcesetosum* adults was

extremely effective at stopping the growth of *B. argentifolii* population on poinsettias under greenhouse conditions for 10 weeks. They further mentioned that after six weeks of introducing *S. parcesetosum*, *B. argentifolii* population densities were dramatically lower in the cages with *S. parcesetosum* than in the control cages. An early introduction of *S. parcesetosum*, while the density of *B. tabaci* population is still low, would be more effective in its control. On eggplants infested by *B. tabaci*, followed by the introduction of *S. parcesetosum* within three weeks at weekly intervals at rates of 0, 3, 6 adults/plant, the number of whiteflies increased in treated cages until the 3rd week, and then began to decrease 7 weeks later. Whereas, the density of whitefly population in the control treatment increased 3-fold during the same period (Abboud *et al.,* 2006).

Reductions in *B. tabaci* population of 65 and 62% (1:30) as well as 62 and 60% (1:20) on cucumber plants, and 89 and 82% (1:25) on cotton plants were reported in the last experimental week (7th week) when the predator was introduced 1 and 2 weeks, respectively (A-Zyoud *et al.,* 2007; Al-Zyoud, 2012). *B. tabaci* population in cages receiving 2 and 4 *S. parcesetosum* adults/plant showed 56 and 53% reductions on eggplants, respectively (Kutuk *et al.,* 2008). In addition, when 1 *S. parcesetosum* was released in cages filled with *A. woglumi* eggs on grapefruit, it was found that predation by *S. parcesetosum* for 12 days reduced egg hatch by 12.5% (Legaspi *et al.,* 2001). Variation among the different studies might be due to differences in prey species or strain, temperature, host plant and release rate used in the different studies.

However, it is to be mentioned that even without a reproductive success, introducing *S. parcesetosum* prevents *B. tabaci* population from increasing over a 7-week-period (Al-Zyoud *et al.,* 2007 Al-Zyoud, 2012) and *B. argentifolii* population over a 10-week-period (Ellis *et al.,* 2001). This can be explained by the fact that laboratory studies up to date show that the ladybird's adults could survive for 2-6 months (Sengonca *et al.,* 2004) and 3 months (Legaspi *et al.,* 1996 Al-Zyoud *et al.,* 2007). In addition, the predator's adults are voracious feeders capable for consuming large numbers of whiteflies, where they reached just over 80 days of longevity to 7805 whiteflies (Al-Zyoud, 2008), and >10,000 *B. argentifolii*/ lifetime (Legaspi *et al.,* 1996). Therefore, depending on these results, it appears that this success in controlling whiteflies was primarily, in addition to the feeding of the larvae, due to the prolonged survival and continuous feeding of *S. parcesetosum* adults.

Furthermore, *En. formosa* and *S. parcesetosum* were released at a rate of 1 adult/plant to control *B. argentifolii* infecting a greenhouse crop of poinsettias. Whitefly densities within the control treatments were considerably greater than those of each of the two natural enemy treatments. At the end of the study (week 13), the whitefly population was less than 1/100 and 1/150 in the greenhouse area receiving both natural enemies and *S. parcesetosum* alone, respectively from those in the control (Weaver and Ciomperlik, 2000a). Furthermore, releases of *S. parcesetosum* were evaluated for their ability to disperse throughout a greenhouse crop of poinsettias infested with *B. argentifolii*. Whiteflies were introduced at a rate of 1.25 adult/plant in week 0 into two separate greenhouses and releases of *S. parcesetosum* were made on weeks 5, 7, and 9. However, results indicated that if whitefly densities were high, the beetles did not disperse as readily as when whitefly densities were low (Weaver and Ciomperlik, 2000b). Heinz and Parrella (1994b) recovered several adult *D. catalinae* three weeks after the last release, but no evidence of successful predator reproduction was reported. In contrast, S. *parcesetosum* larvae were first observed 1 week after adults have been released (A-Zyoud *et al.,* 2007 Al-Zyoud, 2012). However, *S. parcesetosum* would be useful especially for suppressing localized pest population in the greenhouse. An additional positive feature of *S. parcesetosum* that its ability to distinguish between parasitized and unparasitized *B. tabaci* by *En. formosa* (Al-Zyoud and Sengonca, 2004) and *Er. mundus* (Al-Zyoud, 2007) and feed on more unparasitized whiteflies. In Jordan, Sharaf and Hassan (2003) mentioned a high parasitization rate when either *Er. mundus* (72.2%) or *En. formosa* (75.8%) were released against *B. tabaci* at a ratio of 1 parasitoid: 2 whiteflies. As an obligate whitefly predator with a voracious feeding potential, *S. parcesetosum* is capable for checking rapid increases in whitefly populations, thus potentially enabling whitefly parasitoid species such as *Eretmocerus* or *Encarsia* to suppress whiteflies to acceptable thresholds. Thus, there is a feasible potential for integration of the predator and the two parasitoids into a biological control program to suppress *B. tabaci*. This conclusion is supported by Heinz and Nelson (1996) who found that the specific whitefly predator, *D. catalinae* provided the greatest suppression of the silverleaf whitefly when used in conjunction with *Encarsia*. Also, Zapata *et al.* (2003) showed that releases of *Er. mundus* alone or in combination with *M. caliginosus* provided a great level of whitefly suppression. Based on these data it appears that *S. parcesetosum* might be best suited for inclusion in a multiple species biological control approach for management of whiteflies. Another positive feature which makes the predator, *S. parcesetosum* more distinguished and effective

compared to other predators is that the predator is specific for whiteflies (Legaspi *et al.,* 1996 Abboud and Ahmad, 1998; Al-Zyoud and Sengonca, 2004; Al-Zyoud, 2007).

Releases of *S. parcesetosum* in citrus orchards infested with *Diaphernia citri* resulted in its establishment on citrus and dispersal throughout the citrus-growing regions in Turkey. Also, *S. parcesetosum* could tolerate large temperature intervals in the region of Turkey. The success in colonization of *S. parcesetosum* within a certain period shows its high potential of searching capacity in addition to prey suitability (Yigit and Canhalal, 2005). Antadze and Timofeyeva (1975) indicated that *S. parcesetosum* could overwinter in Georgia where the temperature was –2°C. While, Yasnosh and Chaidze (1986) mentioned that the predator overwintered as adults and could resist –6°C to –8°C. On cotton, the predator population increased in the 1st generation to 9-fold, and in cages into which 16-18 adult coccinellids were introduced, produced an average of 157 adults (Yigit, 1992a).

## CONCLUSION

The ladybird, *S. parcesetosum* is a specialist, oligophagous and efficient predator that has demonstrated a potential for biological control of many whiteflies. *S. parcesetosum* is able to develop successfully on many whitefly species and it could survive for up to 6 months. *S. parcesetosum* adults survived for a period of time on artificial nutritional sources, which may have an advantage in stabilizing its population dynamics. An artificial growth medium was successfully used to rear *S. parcesetosum* for 3 generations.

Cotton infested with *B. tabaci* constituted more suitable plant species for mass rearing of *S. parcesetosum*. *S. parcesetosum* seems to occupy a number of host plant species for oviposition and can complete its development successfully on them. Data presented herein provide opportunities to better understand interactions of the plant-whitefly-predator and demonstrated that successful biological control of pests should integrate the environmental aspects of each trophic level.

The predator exhibited the ability to prey successfully upon many whitefly species. The predatory larvae could consume up to 1566 whitefly immatures/day during its entire larval development and adults feed on >10,000 *B. argentifolii* in the most long-lived individuals. In addition, *S. parcesetosum* imposes positive density dependent with *B. tabaci*, which allows long-term population persistence, and in turn will effectively stabilize its prey population. In addition, *S. parcesetosum* could feed on all developmental stages of whiteflies offered, and has a prey preference toward whitefly species used rather than the non-whitefly species. Thus, *S. parcesetosum* is a specialist predator of whiteflies. Furthermore, *S. parcesetosum* tended to avoid parasitized puparia of *B. tabaci* by *En. formosa* and *Er. mundus* and feed instead on unparasitized puparia. Moreover, *S. parcesetosum* sprayed with *P. fumosoroseus* consumed prey at a rate similar to that of the control. Thus, there is a feasible potential for integration of these natural enemies into management programs for whiteflies in order to provide a great level of suppression.

Under greenhouse conditions, *B. tabaci* population was significantly lower when *S. parcesetosum* was introduced after 1 or 2 weeks than control treatment. Also, the number of *B. tabaci* was lower when the predator was introduced after 1 week rather than 2 weeks. A single release of one *S. parcesetosum*/plant was effectively checked further increases in prey population on cotton and cucumber for up to 7 weeks, and on poinsettias for 10 weeks. It is speculated that early release of *S. parcesetosum* would be more effective in biological control of whiteflies. *S. parcesetosum* could spread out throughout cotton orchards with heavily infested by *D. citri* by forming a colony, and tolerate large temperature intervals. It is concluded that releases of the predatory beetle should preferably be done in central point in an orchard, heavily infested by the prey to spread the predator to other orchards.

Long survival of *S. parcesetosum* adults accomplished by their voracious feeding is a great feature that resulted in a successful control of whiteflies. These information will lead to enhance the options for using this specialized whitefly predator in pest management programs to control many whitefly species in greenhouses and open fields. Finally, the ladybird predator, *S. parcesetosum* showed the ability to develop, survive, reproduce and prey successfully upon and build up its population as well as cause a high reduction in whiteflies population. Consequently, it is likely that *S. parcesetosum* could function effectively as the sole biological control agent or in conjunction with other natural enemies to provide a great level of whiteflies suppression, as well as to develop new managing strategies to successfully suppress these worldwide pests. However, additional studies mentioned below are worth consideration: (1) searching behavior of *S. parcesetosum* that permits subsistence at low whitefly densities; (2) optimal exploitation of *S. parcesetosum* must consider the fact that several species may be present

contemporaneously and act in a complementary way; (3) the effect of insecticides on *S. parcesetosum*; (4) the discontinuous nature of annual crops which do not provide a stable environment for the predator establishment and finally, (5) the presence of other pests that may require additional management considerations.

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**Research Article**

# Fitness cost associated with resistance to *Bacillus thuringiensis* Cry1Ac toxin in *Helicoverpa armigera* (Hübner)

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**ABSTRACT**: Transgenic cotton producing a *Bacillus thuringiensis* (*Bt*) Cry1Ac toxin is widely used for controlling the cotton bollworm, *Helicoverpa armigera*. The lessons learnt from the usage of insecticides suggest deployment of effective resistance management strategies to preserve the long-term utility of Bt-cotton. Consequently, it is important to understand the interaction of Cry1Ac toxin with distinct populations of the resistant alleles (homozygote resistant RR, susceptible SS and heterozygote RS or SR) keeping in mind the fitness cost associated with resistance. The present studies were under taken to understand the *in vitro* response of all such allelic populations. A critical analysis on the effects of Bt-toxin on different development stages shows that irrespective of the allelic genotype, the toxin exerts inhibitory influence on all the developmental stages. This effect is visualized as an enormous decrease in larval, pupal and adult weight, wing expanse of adults, adult life span and sex-ratio that was coupled with increase in time taken to pupate, pupal duration and total developmental period. Majority of the emerged adults possessed different types of abnormalities (wingless, deformed wings). They did not mate to lay eggs and if eggs were laid, they normally did not hatch, thus resulting in total loss of population(s). Cry1Ac toxin exerts a high cost of fitness on *H. armigera* and in this context susceptible and heterozygous genotypes were the most affected.

**KEY WORDS**: American bollworm, *Bacillus thuringiensis*, Bt toxin, fitness cost, resistance, transgenic cotton.

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## INTRODUCTION

Transgenic cotton incorporating Cry1Ac gene derived from *Bacillus thuringiensis* Berliner is one of the most exciting advances made in cotton pest management in recent times. The cotton bollworm, *Helicoverpa armigera* (Hubner) is one of the main target pests of *Bt*-cotton technology. In India, it had developed resistance to almost all groups of chemical insecticides because of their intensive use (Kranthi *et al*., 2001; Ramasubramanyam, 2004). The failure of insecticides to control *H. armigera* has been a strong incentive for the development and adoption of transgenic cotton (*Bt*-cotton) expressing a *B. thuringiensis* insecticidal protein. Development of resistance to Cry toxin in bollworm is considered to be an inevitable evolutionary eventuality, considering the intense selection pressure that *Bt*-cotton is likely to impose on the insects due to constitutive expression of toxins throughout the plant for the entire growth season. A progressive increase in the concentration of resistance conferring alleles in pest populations due to sustained selection pressure, results in a concomitant decrease in the pest control efficacy of the transgenic crop. Ultimately a complete control failure is expected when the frequency of resistance alleles in the pest population would reach to 0.5 (Kranthi and Kranthi, 2004). The development of resistance to Bt-toxin by *H. armigera* is now considered as a major threat to the long-term effectiveness of environmentally benign Bt-cotton eventually compromising the benefit of transgenic cotton. Effective resistance management strategies are needed to preserve the long-term utility of *Bt*-cotton. Constitutive expression of genes from *B. thuringiensis* in crop plants can cause continuous production of high doses of toxins, contrast to sprayed insecticides that generally degrade rapidly. Such high doses can make pest resistance functionally recessive (i.e. heterozygotes are killed with high doses but survive with low doses), which is one of the conditions for durable resistance management with a refuge/high dose strategy (Jouanin *et al*., 1998; Gould, 1994; Tabashnik *et al*., 2000). To get prolong benefit from this technology, refuge/high dose strategy is recommended by many scientists. Results from models also indicate delaying the evolution of resistance when resistance alleles are rare and there is

extensive mating between resistant and susceptible adults (Tabashnik, 1994; Caprio, 1998; Gould, 1998). Fitness costs can be another key factor influencing the success of refuges in delaying the evolution of resistance (Lenormand and Raymond, 1998; Carrière and Tabashnik, 2001). Fitness costs occur when the fitness of individuals bearing resistant alleles (RR) is less than that of homozygous susceptible (SS) individuals in the absence of toxin. Because resistance alleles are rare in populations not previously exposed to insecticides, fitness of heterozygous individuals (RS) impacts strongly on the early dynamics of resistance evolution. If fitness costs are not recessive, RS would be less fit than the SS individuals in refuges. With recessive resistance, RS and SS individuals are equally fit in transgenic fields. This suggests that the spread of a recessive RR allele with non-recessive fitness costs could be prevented with an appropriate refuge / high dose strategy (Carrière and Tabashnik, 2001; Andow *et al*., 2000; Bentur *et al*., 2000). Thus, a better understanding of traits influenced by fitness costs and the degree of dominance of such costs could be valuable for devising resistance management strategies. In a previous investigation, we found that resistance to Cry1Ac toxin was inferred to be polygenic, autosomal and inherited as a recessive trait (Kaur and Dilawari, 2011). In this paper we compared resistant and susceptible *H. armigera* strains to identify other traits affected by resistance. We also measured fitness of $F_1$ hybrid progeny between resistant and susceptible strains.

## MATERIALS AND METHODS

### Insect rearing

The larvae of *H. armigera* were collected from different locations in Punjab. Larvae were reared at 27±2° C and 75±5% relative humidity on semi-synthetic diet composed of wheatgerm (165 g), methyl 4-hydroxy benzoate (2.48 g), sorbic acid (1.28 g), cystine (0.15 g), ascorbic acid (4.125 g), streptomycin (0.37 g), agar agar (12.75 g), dried active yeast (39.75 g), vitamin mixture (0.1 g), linseed oil (5.25 ml) and vitamin E (200µl). The ingredients were added to 800 ml of boiled water and homogenized in a blender. The field-collected larvae were allowed to develop into mature adults. Pupae were sexed and kept singly in polycarbonate vials (Polylab, India) containing sterilized moist sand. The sexing was done by viewing abdomen ventrally using 10 x magnification. The pupae were distinguished on the basis of reproductive slit, which is present near the abdominal segment line in females and in the middle of abdominal segment in males. Adult food (5g sugar in 90 ml sterile water with 0.2 g each of ascorbic acid and methyl 4-hydroxy benzoate) was provided in cotton swabs and suspended in the center of muslin.

### *Helicoverpa armigera* strains

We used two strains: BM-R (resistant strain) and HP-S (susceptible strain). HP-S strain had been kept in the laboratory without exposure to Cry1Ac toxins. BM-R was derived by selection with Cry1Ac toxin in diet at concentration of 1 ìg Cry1Ac per ml of diet. The frequency of resistance allele conferring resistance to Bt-cotton was 0.009 in BM-R at that time. Reciprocal cross was made by using female from resistant strain and male from susceptible strain and *vice versa*. The fitness cost characteristics of *H. armigera* were tested at 14$^{th}$ generations of Cry1Ac toxin selection.

### Toxin used for studies

For selection and bioassays we used MVP-II (19.7 per cent Cry1Ac) (Dow AgroSciences (NZ) Ltd, New Plymouth, New Zealand), a liquid formulation containing a hybrid protoxin similar to CryAc that is expressed in Bt-cotton and encapsulated by *Pseudomonas florescens*. Concentrations of Cry1Ac were calculated based on the amount of protoxin per milliliter of liquid formulation.

### Bioassay and fitness cost study

Bioassay was conducted for susceptible, resistant and reciprocal cross progenies to estimate the variability in toxicity of Cry1Ac to different genotypes. The variable developmental parameters were also observed in order to study the fitness cost associated with the toxin. The 14$^{th}$ laboratory generation ($F_{14}$) of BM-R was used in the bioassay and the progeny obtained from this generation was used for the reciprocal crosses. The fitness cost of Cry1Ac on *H. armigera* was analyzed by exposing 8-day-old larvae of different populations to serial concentrations of Cry1Ac toxin and their effect on various development parameters of all the four genotypes – resistant (RR-BM-R), susceptible (SS-HP-S), RS (BM-R male x HP-S female) and SR (HP-S male x BM-R female). The range of serial concentrations was chosen in view of relative susceptibilities of respective genotypes; i.e., for susceptible baseline HP-S strain (0.025 – 2.0 µg/ml), resistant BM-R strain at 14$^{th}$ generation (0.50 – 15 µg/ml), and the two heterozygotes (0.125 – 6 µg/ml). The $LC_{50}$ values of two types of heterozygotes (RS and SR) were statistically at par indicating neither paternal nor maternal influence associated with Cry1Ac resistance in *H. armigera*. Therefore, only one type of heterozygote (RS) was taken into account for observing effect of toxin on different developmental parameters (larval weight, pupal weight, time taken to pupate, pupal duration, adult weight, adult

life span, wing expanse, and sex ratio). For this, 15 individuals from each concentration under respective bioassay were selected and their weight was recorded at seventh day and then at alternate day interval till pupation.

The mortality data were recorded after 7 days and dose-mortality regression was calculated using Probit analysis (Finney, 1971). The data was inferred by computing arithmetic mean and standard deviation of total development parameters, and larval, pupal and adult weight.

## RESULTS AND DISCUSSION

### $LC_{50}$ of susceptible, resistant and heterozygote population

The $LC_{50}$ of baseline susceptible strain (HP-S) at 14th was 0.106 µg Cry1Ac/ml diet. The $LC_{50}$ of the population after continuously rearing for 14 generations with 1 µg Cry1Ac/ml diet was 4.28 µg/ml of diet. The $LC_{50}$ values of hybrid progeny from either cross [(susceptible male x resistant female) SR–0.232 µg/ml (fiducial range – 0.138 to 0.388) or (resistant male x susceptible female) RS–0.228 µg/ml (fiducial range – 0.143 to 0.346)] were statistically at par. These observations suggest that the gene(s) for resistance were autosomal and not sex-linked. Therefore, only one type of heterozygote (RS) was taken into account for observing effect of toxin on different developmental parameters (Table 1).

### Fitness cost in susceptible, resistant and heterozygote strain

Detailed observations on developmental parameters of different genotypes (from larva to adult) in relation to respective concentration of Cry1Ac for the susceptible, resistant and reciprocal cross progeny (14th generation) are given in Tables 2-5. The development of susceptible genotype (HP-S) was arrested by toxin concentration exceeding 0.20 µg/ml of diet, though reciprocal progenies could tolerate 2.5-fold of this concentration. However, as compared to above, complete development of resistant genotype (BM-R) could proceed even at 25-fold concentration compared to one that allowed development of susceptible genotype. The HP-S strain did not survive at 0.40 µg/ml of diet and at this concentration mean larval weight was 74.33 mg whereas at control weight was 419.98 µg. However, mean larval weight of resistant (BM-R) and heterozygote (RS) strain was 316.74 mg and 207.04 mg at 0.50 µg/ml diet, respectively. The growth of heterozygote strain was inhibited by toxin concentration exceeding 0.50 ug/ml of diet. The BM-R strain survived to larval stage at 8.0 ug/ml of diet but the development was arrested by toxin concentration exceeding 5.0 µg/ml of diet. The total developmental period was 59.51 days at 0.40 µg/ml diet and 52.62 days at 0.50 ug/ml diet in HP-S and RS strain, respectively. However, BM-R strain completed total developmental period in 53.78 days at 5.0 µg/ml diet. This indicates that all strains irrespective of their resistance status responded similarly with variation to different levels of toxins. It is clear from data that irrespective of genotype, the larvae – or at least a significant fraction of population could survive on a much higher level of toxin compared to levels that allowed complete development. The data also showed that presence of toxin in semi-synthetic diet had an inhibitory effect on all the developmental parameters and this level of inhibition related directly to the concentration of toxin.

### Quantified estimation on inhibition of developmental parameters

In order to have a quantified estimate on the level of inhibition of different parameters, per cent change in all the developmental parameters (relative to respective controls), in the presence of respective maximum concentrations of Cry1Ac (that could support complete development) for all the three genotypes was derived and is summarized in Table 5.

A critical analysis of the mean values on the per cent change in different developmental parameters (keeping aside the mortality cause and the genotype) shows that the presence of toxin exerted a similar but, inhibitory influence on all the developmental stages, though to different levels in the different populations. This effect was visualized as an enormous decrease in larval weight (62.5%), pupal weight (56.6%), adult weight (57.1%),

**Table 1. Comparative $LC_{50}$ of susceptible (HP-S), resistant (BM-R) and heterozygote (SR and RS) strain of *Helicoverpa armigera* at 14th generation**

| $LC_{50}$ (µg/ml diet) | | | |
|---|---|---|---|
| 0.106 | 4.280 | 0.232 | 0.228 |
| (0 .015 – 0.185) | (3.431 – 5.146) | (0.138 – 0.388) | (0.143 – 0.346) |

Figures in parentheses are respective fiducial limits

**Table 2. Developmental parameters of HP-S strain (susceptible) of *Helicoverpa armigera* from larvae to pupae bioassayed on different concentrations of Cry1Ac incorporated semi-synthetic diet**

| Concentration of Cry1Ac (µg/ml diet) | Mean larval weight after 17±1.68 days* (mg) | Pupal weight (mg) | Time taken to pupate (days) | Pupal duration (days) | Adult weight (mg) | Wing expanse (cm) | Adult life span (days) | Sex-ratio (male: female) | Total developmental period (days) |
|---|---|---|---|---|---|---|---|---|---|
| 0.025 | 262.83 | 225.25 | 25.00 | 9.67 | 125.55 | 3.00 | 7.61 | 2.04 | 42.28 |
| 0.050 | 245.32 | 221.47 | 26.13 | 10.44 | 117.35 | 3.01 | 7.35 | 1.85 | 43.92 |
| 0.100 | 194.85 | 175.45 | 33.43 | 14.15 | 87.40 | 2.85 | 6.50 | 1.59 | 54.08 |
| 0.200 | 131.64 | 102.75 | 36.01 | 17.66 | 83.75 | 2.80 | 5.85 | 1.57 | 59.51 |
| 0.400 | 74.33 | – | – | – | – | – | – | – | – |
| 0.800 | 13.10 | – | – | – | – | – | – | – | – |
| 1.000 | 5.46 | – | – | – | – | – | – | – | – |
| 2.000 | 4.94 | – | – | – | – | – | – | – | – |
| Control | 419.98 | 302.75 | 19.13 | 9.46 | 181.40 | 3.20 | 8.75 | 1.98 | 37.34 |
| CD ($P = 0.05$) | 37.82 | 46.98 | 4.14 | 2.12 | 8.91 | NS | 1.03 | NS | 4.25 |

**Table 3. Developmental parameters of BM-R strain (resistant) of *Helicoverpa armigera* from larvae to pupae bioassayed on different concentrations of Cry1Ac incorporated semi-synthetic diet**

| Concentration of Cry1Ac (µg/ml diet) | Mean larval weight after 17±1.68 days* (mg) | Pupal weight (mg) | Time taken to pupate (days) | Pupal duration (days) | Adult weight (mg) | Wing expanse (cm) | Adult life span (days) | Sex-ratio (male: female) | Total developmental period (days) |
|---|---|---|---|---|---|---|---|---|---|
| 0.50 | 316.74 | 256.43 | 26.50 | 9.45 | 154.97 | 2.97 | 7.17 | 1.82 | 43.12 |
| 1.00 | 224.86 | 203.12 | 27.33 | 11.83 | 134.79 | 2.87 | 6.67 | 1.89 | 45.83 |
| 2.00 | 202.62 | 186.37 | 27.83 | 15.80 | 119.16 | 2.80 | 5.75 | 1.84 | 49.38 |
| 5.00 | 119.52 | 105.98 | 32.60 | 16.67 | 57.01 | 2.67 | 4.50 | 1.83 | 53.78 |
| 8.00 | 9.10 | – | – | – | – | – | – | – | – |
| 10.00 | 7.41 | – | – | – | – | – | – | – | – |
| 15.00 | 7.54 | – | – | – | – | – | – | – | – |
| Control | 391.98 | 296.33 | 18.80 | 8.22 | 179.04 | 3.20 | 8.55 | 2.16 | 35.57 |
| CD ($P = 0.05$) | 48.57 | 32.22 | 3.62 | 1.51 | 15.87 | 0.26 | 1.48 | NS | 3.97 |

**Table 4. Developmental parameters of heterozygote strain (RS) of *Helicoverpa armigera* from larvae to pupae bioassayed on different concentrations of Cry1Ac incorporated semi-synthetic diet**

| Concentration of Cry1Ac (µg/ml diet) | Mean larval weight after 17±1.68 days* (mg) | Pupal weight (mg) | Time taken to pupate (days) | Pupal duration (days) | Adult weight (mg) | Wing expanse (cm) | Adult life span (days) | Sex-ratio (male: female) | Total developmental period (days) |
|---|---|---|---|---|---|---|---|---|---|
| 0.125 | 294.37 | 243.11 | 24.36 | 10.14 | 94.67 | 3.10 | 7.57 | 1.73 | 42.07 |
| 0.250 | 246.57 | 224.10 | 26.12 | 12.00 | 86.31 | 3.01 | 7.00 | 1.97 | 45.11 |
| 0.365 | 226.15 | 198.10 | 30.29 | 12.50 | 82.41 | 3.04 | 6.83 | 1.78 | 49.62 |
| 0.500 | 207.04 | 181.01 | 33.90 | 13.12 | 71.82 | 2.85 | 6.60 | 1.75 | 53.62 |
| 0.750 | 127.21 | 101.14 | 37.78 | – | – | – | – | – | – |
| 1.000 | 12.81 | – | – | – | – | – | – | – | – |
| 1.500 | 10.86 | – | – | – | – | – | – | – | – |
| 2.000 | 4.72 | – | – | – | – | – | – | – | – |
| 3.000 | 4.33 | – | – | – | – | – | – | – | – |
| 4.000 | 3.79 | – | – | – | – | – | – | – | – |
| 4.500 | 3.73 | – | – | – | – | – | – | – | – |
| 5.000 | 3.78 | – | – | – | – | – | – | – | – |
| 6.000 | 3.95 | – | – | – | – | – | – | – | – |
| Control | 407.41 | 299.65 | 19.86 | 8.50 | 176.20 | 3.18 | 9.15 | 2.17 | 37.52 |
| CD ($P = 0.05$) | 40.68 | 14.85 | 3.58 | 2.79 | 12.65 | NS | 0.98 | NS | 3.37 |

wing expanse (12.5%), adult life span (36.3%) and sex-ratio (17.2%), coupled with increase in time taken to pupate (77.4%), pupal duration (81.4%) and total developmental period (51.2%) (Table 5) The variation was observed in pupal and adult size with respect to different concentrations. The size of pupae and adult reared at 5 µg/ml diet was smaller as compared to that at 1 and 2 µg/ml of diet, and the control. Majority of pupae and adults possessed different types of abnormalities (larval-pupal intermediate; pupal-adult intermediate; no adult emergence; long abdomen in adults; wingless or deformed wings). Thus, Cry1Ac toxin exerted a high cost of fitness on *H. armigera* and in this context susceptible and heterozygous genotypes were most affected, which highlight the potential importance of refuge crops in delaying resistance development in the field.

Our results show that selection with Cry1Ac in *H. armigera* under laboratory conditions was associated with several undefined metabolic and morphological abnormalities and the individuals of different genetic makeup responded differently to the Cry toxin.

The present studies also inferred that toxin in semi-synthetic diet had inhibitory effect on all the developmental parameters and this level of inhibition related directly to the concentration of toxin. Selection with Cry1Ac toxin results in several undefined metabolic and developmental abnormalities in *H. armigera* which interferred with its normal development and growth. Similarly, Konasale and Moar (2007) suggested high level of fitness cost associated with Cry1Ac resistance in *Helicovepa zea* Boddie. The percentage of mating success in AR (laboratory-selected Bt-resistant strain) significantly reduced in treated population as compared with untreated parental strain. Results also revealed that the reciprocal cross progeny of resistant and susceptible strain had fitness costs in terms of significantly longer larval and pupal periods with deformed adult production as compared to individuals on normal diet. Carrière *et al*. (2001) supported the results of high fitness cost in pink bollworm associated with Cry1Ac toxin and observed reduced survival on non Bt-cotton (51.5%) in resistant individuals relative to susceptible ones. They recorded weight and survival of larvae from RS x SS and SS x RS, which were 16.74 and 12.53 mg and 49 and 60 per cent, respectively. Earlier reports showed poor hatching (less than 1%) of eggs after the eight generations of selection and reported that $IC_{50}$ values (concentration producing 50% inhibition of larval development to 3rd instar) ranged between 0.020 and 0.105 µg/ml, 0.016 and 0.099 µg/ml, and 0.016 and 0.080 µg/ml in 1998, 1999 and 2000, respectively (Moar, 2005; Wu *et al*., 2002). Fitness cost associated with Cry1Ac toxin was observed in the form of reduced fecundity, egg hatchability and adult viability in resistant Hawaiian strains of diamondback moth (DBM), *Plutella xyllostella* to Bt. A Japanese strain of this pest also showed lower egg hatchability, longer larval and pupal durations, lower larval, pupal, and adult survivorship and lower fecundity (Groeters, *et al*., 1994; Shirai *et al*., 1998). Variable fitness has been implicated as a factor that has contributed to delayed resistance in insect species, despite the continued widespread use of transgenic cotton (Wu *et al*., 2002; Burd *et al*., 2003). The continuous selection with Cry1Ac improved the adaptability of *H. armigera* with decline in mortality and other deformities with the progression of each generation.

**Table 5. Change in different developmental parameters of susceptible (SS), resistant (RR) and heterozygote (RS) strain of *Helicoverpa armigera* on a concentration of toxin permitting full development into adults**

| Genotype | Cry1Ac (µg/ml diet)* | Per cent change over control | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LW | PW | TTP | PD | AW | WE | ALS | SR | TDP |
| Susceptible | 0.20 | –68.7 | –66.1 | 88.5 | 86.3 | –43.8 | –12.5 | –33.0 | –20.0 | 59.5 |
| Reciprocal | 0.50 | –49.2 | –39.6 | 70.4 | 54.1 | –59.3 | –9.4 | –28.3 | –14.3 | 42.9 |
| Resistant | 5.0 | –69.5 | –64.2 | 73.4 | 103.7 | –68.2 | –15.6 | –47.7 | –18.2 | 51.1 |
| Mean ** Change (%) | | –62.5 | –56.6 | 77.4 | 81.4 | –57.1 | –12.5 | –36.3 | –17.2 | 51.2 |

LW – larval weight (mg); PW – pupal weight (mg); TTP – time taken to pupate (days); PD – pupal duration (days); AW – adult weight (mg); WE – wing expanse (cm); ALS – adult life span (days); SR – sex-ratio (female: male); TDP – total development period (days)

* Maximum concentration of toxin permitting full development into adults

** Values are relative to respective control populations

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**Research Article**

# First report of *Callodicopus* Ogloblin (Mymaridae) from India and new records of some Chalcidoidea (Hymenoptera) from Andaman and Nicobar Islands

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**ABSTRACT**: The mymarid genus *Callodicopus* Ogloblin and the species *Mymar pulchellum* Curtis are reported for the first time from India. New distribution records of Encyrtidae (20 species in 15 genera), Mymaridae (22 species in 13 genera), and Aphelinidae (six species in four genera) and Mymarommatidae (one species belonging to the genus *Mymaromma* Girault) are reported from Port Blair and Diglipore districts of Andaman and Nicobar Islands.

**KEY WORDS**: Hymenoptera, Chalcidoidea, Encyrtidae, Mymaridae, Aphelinidae, Mymarommatidae, *Callodicopus*, *Mymar pulchellum*, Andaman & Nicobar Islands

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## INTRODUCTION

Hayat (1998) reported seven species of aphelinid parasitoids under five genera from Andaman Islands. Similarly, in 2006, he reported 27 species representing 20 genera of encyrtid parasitoids from the same region while compiling the Indian Encyrtidae. However, no literature is available on mymarids and mymarommatids from this region. This paper deals with records of parasitoids representing Encyrtidae, Mymaridae and Aphelinidae of Chalcidoidea and Mymarommatoidea from Andaman and Nicobar Islands.

## MATERIALS AND METHODS

Extensive survey was conducted in two districts, *viz.,* Port Blair (Jawaharlal Nehru Rajkeeya Mahavidyalaya (JNRM) college premises and Bathu Basthi) and Diglipore (Kalighat and Ramnagar) of Andaman Islands for chalcid parasitoids using Malaise traps, yellow pan traps and net sweeps during May, 2012. Collections made from Institute of Wood Science and Technology (IWST) campus, Bengaluru, Karnataka, during March, 2012 were also studied and included. The recovered parasitoids were processed using Hexamethyldisilazane (Brown, 1993) and then card / slide mounted for identification. The identified parasitoids were deposited in EDAU (Entomology Department, Annamalai University), Parasitoid Taxonomy and Biocontrol Laboratory, Faculty of Agriculture, Chidambaram, Tamil Nadu, India.

## RESULTS AND DISCUSSION

In the surveys, the superfamilies Mymarommatoidea (one family) and Chalcidoidea (12 families) were documented. Parasitoid species composition and new distributional records of Encyrtidae, Mymaridae and Aphelinidae of Chalcidoidea and Mymarommatoidea are detailed in this paper. The mymarid genus, *Callodicopus* Ogloblin and the species, *Mymar pulchellum* Curtis are first reported from India. Further, the survey resulted in the following additional distributional record of 20 species under 15 genera of encyrtids, 22 species under 13 genera of mymarids, six species under four genera of aphelinids and the genus *Mymaromma* Girault of Mymarommatidae.

### Superfamily Chalcidoidea

### ENCYRTIDAE

***1. Adelencyrtus coxalis* Hayat, Alam & Agarwal**

**Specimen examined:** INDIA, Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, net sweep, weedy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**2.** ***Adelencyrtus orissanus*** **Hayat**

**Specimen examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 1 female, Malaise trap, forest land, 27.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Orissa (Hayat, 2006), Andaman & Nicobar Islands (new record).

**3.** ***Anagyrus*** **nr.** ***almoriensis*** **Shafee, Alam & Agarwal**

**Specimens examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 5 females, yellow pan trap, weedy field, 28.v.2012; Port Blair, Bathu Basthi, 2 females, yellow pan trap and net sweep, weedy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Uttarakhand (Hayat, 2006), Andaman & Nicobar Islands (new record).

**4.** ***Anagyrus kamali*** **Moursi**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 3 females, yellow pan trap and net sweep, weedy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Delhi, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**5.** ***Anagyrus shahidi*** **Hayat**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, net sweep, grassy field, 31.v.2012; Diglipore, Kalighat, Ramnagar, 2 females, Malaise trap, forest land, 27.v.2012; Port Blair, JNRM College premises, 2 females, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Karnataka, Tamil Nadu, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**6.** ***Anagyrus thailandicus*** **(Myartseva)**

**Specimens examined:** India: Andaman & Nicobar Islands Port Blair, Bathu Basthi, 1 female, net sweep, weedy field, 31.v.2012; Port Blair, JNRM College premises, 1 female, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Assam, Kerala, Tamil Nadu (Hayat, 2006), Andaman & Nicobar Islands (new record).

**7.** ***Anomalicornia tenuicornis*** **Mercet**

**Specimens examined:** India: Andaman & Nicobar Islands Port Blair, Bathu Basthi, 1 female, net sweep, grassy field, 30.v.2012; Port Blair, JNRM College premises, 1 female, yellow pan trap, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Delhi, Karnataka, Kerala, Rajasthan, Tamil Nadu (Hayat, 2006), Andaman & Nicobar Islands (new record).

**8.** ***Aphycus sapporoensis*** **(Compere & Annecke)**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, JNRM College premises, 1 female, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**9.** ***Blepyrus insularis*** **(Cameron)**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, net sweep, grassy field, 31.v.2012; Diglipore, Kalighat, Ramnagar, 3 females, yellow pan trap, weedy field, 27.v.2012; Port Blair, JNRM College premises, 1 female, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution :** India: Delhi, Goa, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Uttarakhand, Uttar Pradesh, West Bengal (Hayat, 2006), Andaman & Nicobar Islands (new record).

**10.** ***Callipteroma sexguttata*** **Motschulsky**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 5 females, net sweep and yellow pan trap, grassy field, 31.v.2012; Diglipore, Kalighat, Ramnagar, 3 females, yellow pan trap, weedy field, 27.v.2012; Port Blair, JNRM College premises, 4 females, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Delhi, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, Tamil Nadu, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**11.** ***Coccidencyrtus shafeei*** **(Hayat, Alam & Agarwal)**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, Malaise trap, grassy field, 31.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Kerala, Rajasthan, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**12. *Mahencyrtus ranchiensis* (Fatima & Shafee)**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, yellow pan trap, weedy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Goa, Jharkhand, Kerala, Maharashtra, Tamil Nadu, Uttarakhand, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**13. *Metaphycus* sp.**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, Malaise trap, grassy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution :** India: Andhra Pradesh, Assam, Bihar, Himachal Pradesh, Karnataka, Kerala, Madya Pradesh, Maharashtra, Punjab, Rajasthan, Tamil Nadu, Uttarakhand, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**14. *Neastymachus* sp.**

**Specimen examined:** India: Andaman & Nicobar Islands Port Blair, Bathu Basthi, 1 female, yellow pan trap, grassy field, 31.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Assam, Bihar, Delhi, Karnataka, Maharashtra, Manipur, Tamil Nadu, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**15. *Ooencyrtus papilionis* Ashmead**

**Specimen examined:** India: Andaman & Nicobar Islands, JNRM College premises, 1 female, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Karnataka, Orissa (Hayat, 2006), Andaman & Nicobar Islands (new record).

**16. *Ooencyrtus utetheisae* (Risbec)**

**Specimen examined:** India: Andaman & Nicobar Islands, JNRM College premises, 1 female, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar. **Distribution:** India: Assam, Karnataka, Kerala, Meghalaya, Tamil Nadu, Uttarakhand, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**17. *Paraclausenia herbicola* Hayat**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, Malaise trap, grassy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Assam, Himachal Pradesh, Jharkhand, Kerala, Maharashtra, Rajasthan, Tamil Nadu (Hayat, 2006), Andaman & Nicobar Islands (new record).

**18. *Protyndarichoides indicus* Singh & Agarwal**

**Specimens examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 3 females, yellow pan trap, weedy field, 28.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Arunachal Pradesh, Assam, Uttar Pradesh (Hayat, 2006), Andaman & Nicobar Islands (new record).

**19. *Rhopus* sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, net sweep and yellow pan trap, grassy field, 31.v.2012; Diglipore, Kalighat, Ramnagar, 6 females, yellow pan trap and Malaise trap, weedy field and forest land, 28 and 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andaman & Nicobar Islands (new record).

**20. *Rhytidothorax* sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 1 female, Malaise trap, forest land, 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Assam, Uttarakhand (Hayat, 2006), Tamil Nadu (Manickavasagam and Rameshkumar, 2010), Andaman & Nicobar Islands (new record).

**MYMARIDAE**

**1. *Callodicopus* sp. (Fig. 1)**

***Diagnosis:*** Mandible bidentate; female funicle 7-segmented, F2 either ring like or not; Fore wing with posterior margin straight, the wing widening gradually towards apex; Proximal and distal macrochaeta present and moderately long; Procoxae widely separated by the broad anterior apex of prosternum; Tarsi 5-segmented; Mesophragma extending into gaster; Propodeal foramen broad, almost touching metacoxal foramen; Petiole apparently absent.

**Fig. 1:** ***Callodicopus*** **Ogloblin**

**Specimen examined:** India: Karnataka, Bengaluru, IWST campus, 1 female, yellow pan trap, forest land, 15.iii.2012, coll. S. Manickavasagam & A. Rameshkumar.

**Distribution:** Brazil (Ogloblin, 1955), Argentina (Yoshimoto, 1990), USA (Florida), Central and South America and Southern Africa (Huber and Lin, 1999), India (new record).

**Host:** unknown (Huber and Lin, 1999).

**Comments:** This genus was erected by Ogloblin in 1955 with *crassula* as type species along with three more species *viz., cursor* Ogloblin, *longicornis* Ogloblin and *silvestriana* Ogloblin. Annecke (1961) erected the genus *Decamymar* with *mangiclavae* as the type species which was later synonymized with *Callodicopus* by Huber and Lin (1999). The collected specimen does not match any of the five described species and efforts are on to recover more specimens to describe this.

**2.** ***Anagrus (Anagrus)*** **sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 4 females, 2 males, yellow pan trap and Malaise trap, grassy field, 30 and 31.v.2012; Diglipore, Kalighat, Ramnagar, 3 females, Malaise trap, weedy field and forest land, 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Himachal Pradesh, Delhi, Orissa (Subba Rao and Hayat, 1983), Kerala (Rameshkumar *et al*., 2011a), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**3.** ***Anagrus (Paranagrus)*** **sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 4 females, yellow pan trap and Malaise trap, grassy field, 31.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Himachal Pradesh, Delhi, Orissa (Subba Rao and Hayat, 1983), Kerala (Rameshkumar *et al*., 2011a), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

***4.*** ***Alaptus*** **sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 3 females, 2 males, yellow pan trap and Malaise trap, grassy field, 31.v.2012; Diglipore, Kalighat, Ramnagar, 5 females, Malaise trap, forest land, 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Delhi, West Bengal, Tamil Nadu (Subba Rao and Hayat, 1983), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**5.** ***Anaphes*** **sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 2 females, Malaise trap, forest land, 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Uttar Pradesh, Himachal Pradesh (Subba Rao and Hayat, 1983), Kerala (Rameshkumar *et al*., 2011a), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

***6.*** ***Arescon*** **sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, Malaise trap, grassy field, 30.v.2012; Diglipore, Kalighat, Ramnagar, 1 female, Malaise trap, forest land, 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: New Delhi, Bihar, Gujarat, Himachal Pradesh, Karnataka, Maharashtra (Subba Rao and Hayat, 1983), Kerala (Rameshkumar *et al*., 2011a), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**7. *Camptoptera* sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 13 females, Malaise trap and yellow pan trap, grassy and weedy field, 30.v.2012; Diglipore, Kalighat, Ramnagar, 7 females, yellow pan trap and Malaise trap, weedy field and forest land, 28 and 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Uttar Pradesh, Himachal Pradesh (Subba Rao and Hayat, 1983), Kerala (Rameshkumar *et al*., 2011a), Andaman & Nicobar Islands (new record).

***8. Dicopomorpha* sp.**

**Specimens examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 2 females, Malaise trap, forest land, 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Tamil Nadu (Manickavasagam and Rameshkumar, 2011), Andaman & Nicobar Islands (new record).

**9. *Gonatocerus bouceki* Zeya**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, yellow pan trap, grassy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Bihar, Uttar Pradesh (Zeya and Hayat, 1995), Kerala (Rameshkumar *et al*., 2011a), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**10. *Gonatocerus narayani* (Subba Rao & Kaur)**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, yellow pan trap, grassy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Bihar, Himachal Pradesh, Kerala, Tamil Nadu, Uttar Pradesh (Zeya and Hayat, 1995), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**11. *Gonatocerus pahalgamensis* (Narayanan)**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 4 females, net sweep and yellow pan trap, grassy field, 30 and 31.v.2012; Diglipore, Kalighat, Ramnagar, 3 females, yellow pan trap, weedy field, 28.v.2012; Port Blair, JNRM College premises, 2 females, yellow pan trap, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Andhra Pradesh, Assam, Bihar, Jammu & Kashmir, Kerala, Maharashtra, Orissa, Tamil Nadu (Zeya and Hayat, 1995), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**12. *Gonatocerus tamilanus* Mani & Saraswat**

**Specimen examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 1 female, yellow pan trap, weedy field, 29.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Kerala, Tamil Nadu, Uttar Pradesh (Zeya and Hayat, 1995), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

***13. Gonatocerus tarae* (Narayanan & Subba Rao)**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 6 females, net sweep and yellow pan trap, grassy field, 30 and 31.v.2012; Diglipore, Kalighat, Ramnagar, 5 females, yellow pan trap, weedy field, 28.v.2012; Port Blair, JNRM College premises, 1 female, net sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Assam, Andhra Pradesh, Bihar, Jammu & Kashmir, Kerala, Karnataka, Maharashtra, Meghalaya, Tamil Nadu, Uttar Pradesh (Zeya and Hayat, 1995), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**14. *Himopolynema indicum* Hayat & Basha**

**Specimens examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 1 female, yellow pan trap, weedy field, 28.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Assam (Hayat *et al*., 2003), Andaman & Nicobar Islands (new record).

**15. *Mymar pulchellum* Curtis**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 7 females, yellow pan trap, grassy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** Russia, Japan, Belgium, Denmark, France, England, Canada, USA, Europe (Triapitsyn and Berezovskiy, 2001), India (new record, Andaman & Nicobar Islands).

**16. *Mymar schwanni* Girault**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, Malaise trap,

weedy field, 31.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Uttar Pradesh (Subba Rao and Hayat, 1983), Tamil Nadu (Manickavasagam and Rameshkumar, 2011), Kerala (Rameshkumar *et al*., 2011a), Andaman & Nicobar Islands (new record).

**17.** ***Mymar taprobanicum*** **Ward**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, 4 males, yellow pan trap, grassy field, 31.v.2012; Diglipore, Kalighat, Ramnagar, 2 males, yellow pan trap, weedy field, 28.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Delhi, Tamil Nadu, Rajasthan (Hayat, 1977), Kerala (Rameshkumar *et al*., 2011a), Pudhucherry (Rameshkumar *et al*., 2011b), Andaman & Nicobar Islands (new record).

**18.** ***Narayanella thornypoda*** **(Narayanan & Subba Rao)**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, Malaise trap, grassy field, 30.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Tamil Nadu, Andhra Pradesh, Kerala (Rameshkumar *et al*., 2011a, b & c), Andaman & Nicobar Islands (new record).

**19.** ***Palaeoneura bagicha*** **(Narayanan, Subba Rao and Kaur)**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 1 female, Malaise trap, weedy field, 31.v.2012; Port Blair, JNRM College premises, 1 female, yellow pan trap, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Delhi (Huber, 2003), Kerala (Rameshkumar *et al*., 2011a), Andaman & Nicobar Islands (new record).

**20.** ***Palaeoneura indopeninsularis*** **(Mani and Saraswat)**

**Specimen examined:** India: Andaman & Nicobar Islands, Port Blair, JNRM College premises, 1 female, yellow pan trap, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Uttar Pradesh, Tamil Nadu (Huber, 2003), Kerala (Rameshkumar *et al*., 2011a), Andaman & Nicobar Islands (new record).

**21.** ***Polynema brevicarinae*** **Annecke & Doutt**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, JNRM College premises, 1 female, 1 male, sweep, weedy field, 25.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Bihar, Delhi, Karnataka, Kerala, Maharashtra, Orissa, Pudhucherry, Tamil Nadu, Uttar Pradesh (Anis and Hayat, 1999), Andaman & Nicobar Islands (new record).

**22.** ***Polynema mendeli*** **Girault**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 11 females, 2 males, yellow pan trap, net sweep and Malaise trap, weedy and grassy field, 30 and 31.v.2012; Diglipore, Kalighat, Ramnagar, 1 female, yellow pan trap, weedy field, 28.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Assam, Bihar, Kerala, Pudhucherry, West Bengal (Anis and Hayat, 1999), Tamil Nadu (Manickavasagam and Rameshkumar, 2011), Andaman & Nicobar Islands (new record).

**23.** ***Stethynium empoascae*** **Subba Rao**

**Specimen examined:** India: Andaman & Nicobar Islands, Diglipore, Kalighat, Ramnagar, 1 female, yellow pan trap, weedy field, 28.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Delhi (Subba Rao and Hayat, 1983), Andaman & Nicobar Islands (new record).

**APHELINIDAE**

**1.** ***Ablerus williamsi*** **(Annecke & Insley)**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, Malaise trap, weedy field, 30.v.2012; coll. S. Manickavasagam and A. Rameshkumar.

**Distribution**: India: Assam, Orissa (Hayat and Khan, 2010), Andaman & Nicobar Islands (new record).

**2.** ***Coccobius mirus*** **Hayat**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, yellow pan trap, weedy field, 30.v.2012; Diglipore, Kalighat, Ramnagar, 4 females, Malaise trap and yellow pan trap, forest land, 27.v.2012; coll. S. Manickavasagam and A. Rameshkumar.

**Distribution**: India: Andhra Pradesh, Assam, Kerala, Orissa, West Bengal (Hayat & Khan, 2010), Andaman & Nicobar Islands (new record).

**3. *Coccophagus cowperi* Girault**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, yellow pan trap, weedy field, 30.v.2012; Diglipore, Kalighat, Ramnagar, 2 females, Malaise trap and yellow pan trap, forest land, 27.v.2012; coll. S. Manickavasagam and A. Rameshkumar.

**Distribution**: India: Andhra Pradesh, Goa, Kerala, Karnataka, Maharashtra, Pudhucherry, Tamil Nadu (Hayat, 1998), Andaman & Nicobar Islands (new record).

**4. *Encarsia azimi* Hayat**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 3 females, yellow pan trap and malaise trap, weedy field, 30.v.2012; Diglipore, Kalighat, Ramnagar, 2 females, Malaise trap, forest land, 27.v.2012; coll. S. Manickavasagam and A. Rameshkumar.

**Distribution**: India: Karnataka, Tamil Nadu, Uttar Pradesh (Hayat, 1998), Andaman & Nicobar Islands (new record).

**5. *Encarsia hitam* Hayat**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, yellow pan trap, weedy field, 30.v.2012; Diglipore, Kalighat, Ramnagar, 1females, Malaise trap and yellow pan trap, forest land, 27.v.2012; coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Assam, Meghalaya, Uttar Pradesh, Uttarakhand (Hayat, 2011), Andaman & Nicobar Islands (new record).

**6. *Encarsia tinctoriae* Krishnan & David**

**Specimens examined:** India: Andaman & Nicobar Islands, Port Blair, Bathu Basthi, 2 females, Malaise trap, weedy field, 30.v.2012; coll. S. Manickavasagam and A. Rameshkumar.

**Distribution**: India: Tamil Nadu (Hayat, 1998), Andaman & Nicobar Islands (new record).

**Superfamily Mymarommatoidea**

**1. *Mymaromma* sp.**

**Specimen examined:** India: Andaman & Nicobar Islands Port Blair, Bathu Basthi, 1 female, Malaise trap, weedy field, 31.v.2012, coll. S. Manickavasagam and A. Rameshkumar.

**Distribution:** India: Tamil Nadu (Manickavasagam, 2011), Andaman & Nicobar Islands (new record).

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**Research Article**

# Influence of rice cultivars on the parasitization efficiency of *Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead

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**ABSTRACT**: Trichogrammatids are efficient egg parasitoids in rice agroecosystem and two species of *Trichogramma* viz., *Trichogramma chilonis* Ishii and *T. japonicum* Ashmead were reported from several species of rice stem borers and other lepiodpterous pests. Physicochemical variations between the cultivars of crops often interfere with the efficiency of the *Trichogramma* spp. The response of *T. chilonis* and *T. japonicum* to the variations in the volatile profile of rice cultivars was investigated. The results indicated that the parasitization efficiency of both *T. chilonis* and *T. japonicum* was influenced by the volatiles of rice cultivars. While in some cultivars, such as Kadamba, MTU-1010, KMT 148, KCP-1, the response of *T. chilonis* was very high, in some of the cultivars like CTH-1, MTU 1010, VTT-5204, the response by *T. japonicum* was high. The highest overall response of 83.89 % was recorded in the variety Kadamba by *T. chilonis*. Volatile profile of the cultivars indicated the presence of 9,12,15 octadecatrienoic acid and 9-octadecenal might have played positive role in the attraction of *T. chilonis* to specific cultivars while hexadecane, heptadecane, petadecane and hexadeconic acid might be responsible for the attraction of *T. japonicum*

**KEY WORDS**: *Trichogramma chilonis, Trichogramma japonicum, rice cultivars, volatile*

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## INTRODUCTION

The *Trichogramma* of egg parasitoids play a significant role in the management of lepidopteran pests. *Trichogramma chilonis* Ishii and *Trichogramma japonicum* Ashmead are effective egg parasitoids used in the management of *Chilo* spp. and *Scirpophaga incertulas* Walker, respectively, on sugarcane and rice (Jalali *et al*., 2003).

Physicochemical characters of plants such as trichomes and leaf volatiles often influence the efficiency of the parasitism by trichogrammatids (Tandon, 2001). Inter species and intraspecies variation often play an important role in the trichogrammatid searching behaviour on plants (Khan and Tiwari, 2001; Shankarganesh and Khan, 2006a; 2006b; Balakrishnan *et al.,* 2006; Singh *et al.,* 2001; Virk *et al.,* 2004; Dandale *et al.,* 2002; Hakeem *et al*., 2006b; Basit *et al.,* 2001; Tandon and Bakthavatsalam, 2001; 2002; 2004; 2005). While the trichomes and their exudates negatively influenced the searching behaviour of *T. chilonis* on the chickpea (Romeis *et al.,* 1999), chemicals such as heneicosane in the vegetative phase and heneicosane and tricosane in the flowering phase in chickpea attracted trichogrammatids (Srivastava *et al.,* 2004).

In our studies, the parasitization response of *T. chilonis* and *T. japonicum* to the variations in the volatile profile of rice cultivars was investigated.

## MATERIALS AND METHODS

The experiments were conducted under laboratory conditions of 27±2°C and RH 70±10%, during 2008-2010.

### Plant extracts

Leaf samples of cultivars of rice in the vegetative phase (Table 1) were collected 30 days after they were transplanted from the VC farm, Mandya, (Karnataka) and the samples were brought in sealed polythene containers to the laboratory. For each determination, 5 grams of leaf material was kept in a conical flask, 50 ml of methanol was added, and mixed in a rotary shaker at 80–90 rpm, at ambient temperature for 24 hours. The extract was filtered through Whatman filter paper and additional

**Table 1**: **Percentage parasitization of *Trichogramma chilonis* and *Trichogramma japonicum* as influenced by the methanol extract of rice**

| Rice variety | Percent parasitization | |
|---|---|---|
| | *T. chilonis* | *T. japonicum* |
| BASMATI 370 | 50.14 (44.97) | 26.33 (30.82) |
| CTH-3 | 33.23 (35.04) | 47.62 (43.62) |
| CTH-1 | 34.70 (35.80) | 55.69 (48.51) |
| KMT-105 | 28.12 (31.79) | 31.72 (33.99) |
| KMT-148 | 56.00 (48.53) | 37.59 (37.64) |
| KCP-1 | 52.99 (46.71) | 38.13 (38.03) |
| MANGLA | 42.94 (40.49) | 29.24 (32.58) |
| RASI | 33.26 (35.30) | 21.87 (27.69) |
| MTU-1010 | 58.27 (49.80) | 55.55 (48.24) |
| JYOTI | 36.13 (36.66) | 22.58 (27.93) |
| KMP-149 | 38.05 (37.96) | 18.24 (24.99) |
| JR-20 | 18.23 (23.29) | 23.63 (28.96) |
| IR-64 | 44.56 (41.69) | 30.25 (33.23) |
| IR-30864 | 32.75 (34.68) | 42.32 (40.56) |
| KRH-2 | 36.02 (36.39) | 41.41 (40.05) |
| KRH-3 | 36.29 (36.88) | 17.46 (24.41) |
| TANU | 0.64 (3.63) | 32.59 (34.75) |
| MTU-1001 | 27.55 (31.52) | 30.85 (33.56) |
| IET-8116 | 30.1 (33.11) | 17.45 (24.37) |
| VILIRAJAMUNDI | 34.45 (38.96) | 47.43 (43.50) |
| MANDYA VIJAYA | 39.91 (39.16) | 28.88 (32.46) |
| RP-BIO-326 | 32.87 (34.90) | 32.32 (34.53) |
| VTT-5204 | 36.19 (36.84) | 50.36 (45.18) |
| KADAMBA | 83.89 (66.57) | 32.59 (34.72) |
| JAYA | 26.37 (54.40) | 34.59 (35.98) |
| BR2-655 | 43.27 (33.66) | 29.79 (33.01) |
| TN1 | 41.675 (40.16) | 38.62 (37.16) |
| PTB-33 | 41.98 (40.27) | 25.39 (30.14) |
| TRIGUNA | 31.23 (33.92) | 29.45 (32.76) |
| CONTROL | 24.14 (29.42) | 24.15 (29.42) |
| CD at 5% | 8.96 | 6.45 |
| CV % | 20.78 | 16.1 |

methanol was added to bring the volume to 100 ml and the extract was stored in a refrigerator (5°C).

**Insect cultures**

The cultures of the egg parasitoids, *T. chilonis* and *T. japonicum* were maintained on the eggs of *Corcyra cephalonica* (Stainton), at the Entomophagous Insect Behaviour Laboratory. Two-day old adult females were used in all the experiments. Fresh eggs of *C. cephalonica* used in the experiments were obtained from the Mass Production Laboratory of this Institute.

**Wind tunnel bioassay**

The bioassays were conducted in a plastic wind tunnel made of transparent, non-adsorbent, non-odorant acrylic sheet (4mm thick), with a trap chamber (25 cm dia.) and a test chamber (25 cm dia.) connected through a tunnel (15 cm dia.). The length of the wind tunnel was 100 cm. A rubber septa impregnated with 0.2 ml samples of the methanol extracts of the respective rice varieties was positioned at a distance of 50 cm from the test chamber, along with a small piece of card board containing 50 UV radiated fresh eggs of *C. cephalonica.*

One hundred adults of *T. chilonis* or *T. japonicum* were released into the test chamber and a flow of filtered (passed through the activated charcoal) air at 25 cm per second was maintained from the trap chamber to the test chamber. After 60 minutes, the egg cards were collected and kept in a small vial for observation. The parasitization, which is directly related to the number of adults visiting the egg cards, was counted once the eggs turned black after 5 days of experiment. Six replications were maintained for each treatment. The per cent of eggs parasitized was computed from these observations. The percentage values were transformed into arcsine values and then the data were subjected to analysis of variance (ANOVA).

**Volatile analysis**

Volatile analysis of the rice samples was conducted in an Agilent GC-MS. The inlet temperature was kept at 250°C, with a constant flow of helium of 99.999% purity @ 1ml/min. A phenyl siloxane column (30 m x 0.25 um) (HPMS–5 Column) was used as the stationary phase. The oven program was set at an initial temperature of 40°C $min^{-1}$ with a 2 minute hold and a ramp of 6°C $min^{-1}$ until 180°C, held for 2 min, with a column flow of 1 ml/min, and the mass spectral detector was maintained at a temperature of 280°C. The mass spectra created using the MS was compared with those in the Wiley Mass Spectral Library.

**RESULTS AND DISCUSSION**

Both *T. chilonis* and *T. japonicum* showed parasitization response to cultivars of rice. However, there were variations between the cultivars. While, in some cultivars like Kadamba, MTU-1010, KMT 148, KCP-1, the response by *T. chilonis* was very high, in cultivars like CTH-1, MTU 1010, VTT-5204 the response by

*T. japonicum* was high. Strikingly, the highest response of 83.89% was recorded in the variety Kadamba by *T. chilonis*. This result revealed that *T. chilonis* can also be effectively used in the selected cultivars of rice (Table 2), which confirms earlier studies (Wakil, 2011; Chakraborty, 2011).

The volatile profile for the different varieties of rice cultivars indicated that KMT-148, MTU-1010 and KCP-1 showed the presence of 9,12,15 octadecatrienoic acid and KCP-1 showed the presence of 9-octadecenal, which should have acted as attractants for *T. chilonis*. For *T. japonicum*, the commonality of chemicals in the preferred cultivars, like CTH-1, MTU-1010 and VIT 5204, include hexadecanes heptadecane, petandeance and hexadeconic acid. The presence of pentadecane, ocatdecane and nonadecane was noticed in cultivar 5204. Additionally, 9,12,15 octadecatrienoic acid was present in MTU-1010.

**Table 2. Volatile profile of rice cultivars using GCMS**

| Rice variety | Volatiles identified |
|---|---|
| BASMATI 370 | Heptadecane, 13-tetradecanal, 2,4–dioctylphenol, hexadecanoic acid, 9,12,15-octadecatrienoic acid |
| CTH-3 | Heptadecane, 13-tetradecanal |
| CTH-1 | Hexadecane, eicosane, heptadecane, 2 propanone, hexadecanoic acid |
| KMT-105 | 2-Propanone, hexadecanoic acid |
| KMT-148 | 2-Propanone, hexadecanoic acid, 9,12, 15-octadecatrienoic acid, ethyl linoleolate |
| KCP-1 | Pentadecane, octadecane, 9-octadecenal, 13-tetradecenal, tetradecanoic |
| MANGLA | 1-Octene, heptadecane, 13-tetradecenal, 14-methyl-8-hexadecyn-1-ol, hexadecanoic acid, 9,12, 15-octadecatrienoic acid |
| RASI | Heptadecane, 9-octadecenal, 13-tetradecenal, tetradecanoic, hexadecanoic acid, 9,12, 15-octadecatrienoic acid |
| MTU-1010 | 1-Hexadecanol, hexadecane, heptadecane, pentadecane, 13-tetradecanal, nonadecane, hexadecanoic acid, 9,12,15-octadecatrienoic acid. |
| JYOTI | 13-Tetradecenal, 1, 12-tridecadiene, 9-hexadecenoic acid, 9,12,15-octadecatrienoic acid. |
| KMP-149 | 2-Propanone, hexadecanoic acid, 9,12, 15-octadecatrienoic acid, ethyl linoleolate |
| JR-20 | Heptadecane, octadecane, 13-tetradecanal ,hexadecanoic acid, 9,12,15-octadecatrienoic acid |
| IR-64 | Pentacosane, octadecane, 9-octadecenal, 13-tetradecenal, tetradecanoic |
| IR-30864 | 2-Propanone, heptadecane, 13-tetradecenal, hexadecanoate, hexadecanoic acid, 9,12, 15-octadecatrienoic acid. |
| KRH-2 | Hexadecane, octadecane, Geranyl linalool isomer, Styrene, 5-Hydroxymethylfurfural, 2, 6-dimethoxyphenol |
| KRH-3 | Heneicosane, octacosane, pentacosane, heptadecane, hexadecanoic acid |
| TANU | Hexadecane, eicosane, heptadecane, pentadecane, octadecane, nonadecane |
| MTU-1001 | Heptadecane, Phytol, methyl linoleate, Neophytadiene, Methyl octadecanoate |
| IET-8116 | Hexacosane, Tricosane, Farnesol, Isocaryophyllene, (E,E)-Farnesyl acetone, Triacontane, Pentacosane, Nonacosane, Octacosane |
| VILIRAJAMUNDI | Hexadecanoic acid, 9.12,15- octadecatrienoic acid |
| MANDYA VIJAYA | Hexadecanoic acid, 9,12,15-octadecatrienoic acid |
| RP-BIO-326 | Nonadecane, hexadecanoic acid, 9.12,15- octadecatrienoic acid |
| VTT-5204 | Pentadecane, octadecane, nonadecane |
| KADAMBA | Hexadecane, eicosane, heptadecane, 9,12, 15-octadecatrienoic acid. |
| JAYA | Thunbergol, Neophytadiene, Octacosane, Geranyl linalool isomer, Cyclohexane, Styrene, 5-Hydroxymethylfurfural, 2, 6-dimethoxyphenol, Heptadecane, Octadecane, Eicosane, trans-Phytol |
| BR2-655 | Hexadecane, heptadecane, nonadecane, Pentacosane, Octacosane |
| TN1 | 5-(Hydroxymethyl)furfural, 4-vinyl-2-methoxy-phenol, 1,4-dihydro-1-naphthalenone, (E)-isoeugenol, isopropylpseudocumene, cyclohexane, thunbergol, farnesyl acetate, heptacosane, eicosane, hexacosane, tricosane, farnesol, methyl palmitate, isocaryophyllene, (E,E)-farnesyl acetone, triacontane, pentacosane, nonacosane, octacosane |
| PTB-33 | Heptacosane, eicosane, hexacosane, tricosane, hexadecanoic acid |
| Triguna | Phytol, methyl linoleate, neophytadiene, nethyl palmitate, hexadecanoic acid, methyl octadecanoate |

Madhu *et al.* (2000) indicated that nonadecane in sorghum elicited higher parasitisation by *T. japonicum*. Rani *et al.* (2007) inferred that pentadecane, hexadecane and nonadecane deterred oviposition. Pentadecane, octocosane and heptadecane were considered to be favourable hydrocarbons for *T. exiguum* and not for *T. brasiliensis* (Paul *et al.*, 2002). Generally, trichogrammatid preferred compounds having higher number of carbon atoms. However, to establish the preference of cultivars by different species, individual compounds and combinations of compounds need to be tested.

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**Research Article**

# Intraguild predation and biosafety of entomopathogenic nematode, *Heterorhabditis bacteriophora* Poinar *et al.,* and its bacterial symbiont, *Photorhabdus luminescens,* to parasitoid, *Trichogramma chilonis* Ishii and predator *Chrysoperla zastrowi sillemi* (Esben, Petersen)

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**ABSTRACT**: Intraguild predation (IGP) appears to be pervasive among communities of biocontrol agents associated with nematode sharing the host with trophic interaction. Entomopathogenic nematode (*Heterorhabditis bacteriophora)* and its associated bacterium (*Photorhabdus luminescens*); an egg parasitiod, *Trichogramma chilonis* and a predator *Chrysoperla zastrowi sillemi* were selected for present study. There was no adverse effect of *H. bacteriophora* and *P. luminescens* observed on adult emergence of *T. chilonis.* Microscopic examination of eggs, larvae and adults of *T. chilonis* and *C. z. sillemi* treated with *H. bacteriophora, P. luminescens* and cell-free culture filtrates of *P. luminescens,* exhibited no deformity, discoloration or infection of organisms. Similarly, *H. bacteriophora, P. luminescens* or the cell-free culture filtrates exhibited no adverse activity on egg hatching or larvae of *C. z. sillemi* indicating that there was no intraguild competition under artificial epiphytotic conditions between the organisms under report, viz., *H. bacteriophora,* its associated bacterium (*P. luminescens*), *T. chilonis* and *C. z. sillemi*.

**KEY WORDS**: Intraguild predation, entomopathogenic nematode, *Heterorhabditis bacteriophora*, *Photorhabdus luminescens*, *Trichogramma chilonis*, *Chrysoperla zastrowi sillemi*

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## INTRODUCTION

Intraguild predation (IGP) occurs when two species that share a host or also engage in a trophic interaction with each other (parasitism or predation), different entomopathogenic nematode-bacterium complexes, biology, life cycle and vertebrate safety and related legislative issues, exchange of germplasm, commercial aspects, post application persistence, transgenic and defined the boundaries with pathogenic bacteria of medical, veterinary or agronomic importance and sustainability of wild and transgenic entomopathogenic nematode-bacterium complexes in the field were well studied (Jansson, 1993; Rosenheim *et al*., 1995; Richardson, 1996; Rizvi *et al*., 1996; Boemare *et al*., 1996, Ehlers, 1996; Smits, 1996; Gaugler *et al*. 1997). Kaya (1978) reported susceptibility of adults *Apanteles militaris* (Hymenoptera: Braconidae), parasitoid of the armyworm, *Pseudaletia unipuncta* and its larvae. Similar results were recorded during his study with the tachinid parasite *Compsilura concinnatau* (Diptera: Tachinidae) to *Neoaplectana carpocapsaeu* (Nematoda: Steinernematidae) and its associated bacterium, *Xenorhabdus nematphilus* in 1984. Haag & Boucias (1991) reported in their study to test the infectivity of the insect pathogens to weed control agent *Neochetina eichhorniea* that 2 strains of *Steinernema carpocapsae* resulted in 60–70% adult mortality.

Among several parasitoids and predators recorded as natural enemies of several insect pests, *Trichogramma* and *Chrysoperla* have the distinction of reaching commercial use against several lepidopteran pests. These parasitoids are most widely used for biological control in more than 30 countries, with use in recent years covering a total area of 32 million ha of agricultural and forestry land (Li, 1994). The chrysopid larvae are predaceous, feeding on the eggs and neonate larvae of lepidopterans, nymphs and adults of whiteflies, aphids and other homopterans. Among 69 species of chrysopids recorded in India, *Chrysoperla zastrowi sillemi* is the most common species (Jalali *et al*., 2003).

Beneficial organisms including entomopathogenic nematodes, their associated bacteria, chrysopid predator, trichogrammatid parasitoid share common insect species as hosts, although vary in infectivity/predation/parasitism to life-cycle stages of insect hosts. Hence, their field

success as biological control agents either individually or in combinations, depends also on their cross-infectivity and suppressivity. In other words, the biological control agents preferably must be non-inhibitory and minimal in IGP.

The field evaluations proved the insecticidal virulence of the *Photorhabdus luminescens* bacterium against the cabbage white butterfly, *Pieris brassicae* (Linnaeus) (Mohan *et al*., 2003), mango mealybug, *Drosicha mangiferae* (Green) (Mohan *et al*., 2004) and the pupae of the diamond-back moth, *Plutella xylostella* (Linnaeus) (Razek-Abdel, 2003). The bacterium is reported to be non-toxic to humans and mammals and differs genetically from the human clinical isolate *P. asymbiotica* (Fischer-Le Saux *et al*., 1999). Subsequently, Mohan and Sabir (2005) reported that *P. luminescens* from *H. bacteriophora* adversely affected trichogrammatids. The results suggest conflicting report of its safety. Therefore, in the present study laboratory screening of *P. luminescens* against *T. chilonis* and *C. z. sillemi* to examine the toxicity and biosafety in pure culture, its culture filtrate and in natural association with its nematode host, using two protocols for comparison – Standard IOBC protocol and in comparison method adopted by Mohan and Sabir (2005) in order to avoid experimental differences.

## MATERIALS AND METHODS

### Nematode

Monoxenic infective juveniles (IJs) of *Heterorhabditis bacteriophora* (strain PDBC Hbb1) were established by collecting freshly emerged infective juveniles from *Galleria mellonella* cadavers and washing them 5 times in sterile $dH_2O$, followed by surface sterilizing with 0.1% Hymine (methyl benzothionium chloride) solution and several rinses with sterile distilled water.

### Bacterial cultures and insect infection

### Isolation of symbiotic bacterium, *P. luminescens*

Pure culture of *P. luminescens* was isolated from haemolymph of *G. mellonella* cadavers infected with *H. bacteriophora* on Mac Conkey medium as per Akhurst (1980). Five healthy and robust 5$^{th}$ instar larvae of *G. mellonella* were inoculated with 100 monoxenic infective juveniles (IJs) of *H. bacteriophora* by moist filter paper method in sterile Petri plates and incubated at 28°C. After 72h of inoculation, the cadavers of *G. mellonella* were surface-sterilized with 70% ethanol for 1 min., ignited and plunged in sterile $dH_2O$. The surface-sterilized cadavers of *G. mellonella* were punctured with a sterile needle and the haemolymph was streaked out onto NBTA medium (Akhurst, 1980). Colonies of *P. luminescens* were identified by their cell and colony morphology and matched with primary phase characteristics as described by Akhurst (1980). Single cell colonies of the bacterium were then transferred to autoclaved 2% proteose peptone medium ($PP_3$) and incubated for 48 hours at 28°C on a rotary shaker (Sciegenics Make) at 90 rpm in dark. Bacterial cells of *P. luminescens* from 48 hours-old proteose peptone medium ($PP_3$) were obtained separately by spinning at 4,000 x g for 5mins. The bacterial cells were re-suspended in phosphate buffered saline (PBS), washed thrice before finally making a stock of bacterial suspension in phosphate buffered saline (PBS) and used for further experimental treatments. In another set, cell free culture filtrates were obtained by ultra filtration using 0.23µm filter paper and then using the culture filtrate for treatments.

### Natural enemy selection and maintenance

Two freshly collected and identified species of natural enemies *T. chilonis* and *C. z. sillemi* used for testing against bacterium were reared on *Corcyra cephalonica* Stainton eggs in the laboratory for the past 20 years and were designated as susceptible. Both species were maintained at 26±1ºC and 65±5% relative humidity.

### Bacterium and its preparation

### Testing protocol

Six different treatments were screened against *T. chilonis* and *C. z. sillemi* in the present study. Two methods were employed to test the effect on adult emergence, adult mortality and parasitism by female of *T. chilonis*; egg hatching, larval and adult survivability of *C. z. sellimi*. In the first method IOBC protocol was followed as suggested by Hassan *et al.* (1985). The treatments imposed were:

$T_1$ No treatment

$T_2$ Dry filter paper

$T_3$ Freshly emerged *H. bacteriophora* NBAIIHbb1 infective juveniles (5000 IJs)

$T_4$ Nutrient broth

$T_5$ *P. luminescens* cell suspension

$T_6$ Cell-free cuture filtrate of *P. luminescens*

$T_7$ Sterile distilled water (SDW)

To test the effect of treatments on immature stages (pupal stage of *T. chilonis* and egg and larval stages of *Chrysoperla*) and adults, a clear plastic container (6 x 6 x 2cm$^3$) was modified into a testing unit. One window on four sides was cut, and fine brass wire-mesh (80 mesh size) was heat-sealed across them to provide aeration. A layer of foam was fixed on all sides of the lid to make the testing unit insect escape-proof. The area of the testing unit was calculated 72cm$^2$ and the prepared solution (0.05 ml) was sprayed with an atomizer over *C. cephalonica* eggs parasitized by *T. chilonis* 1, 2, 3, 4, 5, 6 and 7 days after parasitisation. Sample card containing 100 parasitized eggs of each day was considered per replication. The egg cards were kept in the testing units sprayed with various treatments. The testing units containing sprayed egg cards were sealed tightly and kept in incubator maintained at 28°C.

For immature stages of *C. z. sillemi*, 1, 2 and 3 days old eggs and 1$^{st}$, 2$^{nd}$ and 3$^{rd}$ instar stage larvae were tested in a similar manner as described for *T. chilonis* except for each stage 10 eggs or 10 larvae were used per replication. Toxicity to adults of *T. chilonis* and *C. z. sillemi* was tested as suggested in IOBC protocol (Hassan, 1980, 1985; Elzen, 1998). A Borosil glass tube opened both sides was used a the testing unit and was sprayed with the solutions and allowed to shade dry. One end of the dried tube was closed tightly with double layered black cloth and adults were allowed to move inside the tube from the other end. Movement of adequate number of adults was followed by closing of the end by double layered black cloth to permit the test organism in continuous surface contact with the treated surface and to avoid death of the adult due to suffocation. Fine streak of 50% diluted honey was provided. Hundred adults of *T. chilonis* and 10 adults of *C. z. sillemi* were introduced in each unit. Adult mortality was recorded after 24 h of constant exposure. Subsequently, observations on percentage parasitism, emergence and mortality of natural enemies on various treatments were recorded. Each treatment was replicated ten times. The evaluation categories for testing the effect of bio-pesticide were based on IOBC protocol as suggested by Hassan (1985).

In the second method, protocol as suggested by Mohan and Sabir (2005) was followed. The observations were similar to IOBC protocol. In the laboratory screening test, scores were assigned based on per cent mortality of *T. chilonis* and *C. z. sillemi,* after 24h of constant exposure (Table 1).

**Table 1: Scoring chart for screening the bio-safety of insecticidal pathogens**

| Mortality of test organism recorded (%) | Category | Score |
|---|---|---|
| <50 | Harmless | 1 |
| 50-79 | Slightly harmful | 2 |
| 80-99 | Moderately harmful | 3 |
| >99 | Harmful | 4 |

Data was transformed by arcsine transformation; subjected to ANOVA and drawn conclusions following the Scoring chart.

## RESULTS AND DISCUSSION

Systematic studies on the biosafety of *P. luminescens* (symbiotic bacterium associated with *H. bacteriophora* HIP) to the common beneficial insects which are commercialized, viz. *T. chilonis* and *C. z. sillemi* were carried out and the results are presented under different aspects.

### Emergence pattern of *T. chilonis* from parasitized eggs of *C. cephalonica* that received the treatments

The emergence pattern of *T. chilonis* adults from the parasitized eggs of *C. cephalonica* was recorded at 24 hours interval for 7 days in treated conditions. The percentage emergence of *T. chilonis* adults on first day ranged between 90.3 and 97.3 in different treatments as recorded by IOBC protocol, while the emergence ranged between 88.8 and 95.8 by the second protocol, with no significant differences among treatments (Table 4). The adult emergence pattern recorded on 2$^{nd}$, 3$^{rd}$, 4$^{th}$, 5$^{th}$, 6$^{th}$ and 7$^{th}$ days by both the protocols was more or less similar and statistically non-significant, clearly indicating that the treatments, including cell-free culture filtrates, *P. luminescens* cells and *H. bacteriophora,* had no discernable adverse effect on emergence of *T. chilonis* adults. However, Mohan and Sabir (2005) recorded significant reduction in the per cent adult emergence upto 84%.

### Adult mortality of *T. chilonis* and its parasitism as influenced by the treaments

A maximum adult mortality of (20.4%) in *T. chilonis* was recorded in nutrient broth (check), followed by

13.2% in *H. bacteriophora*, 9.4% in cell-free culture filtrate of *P. luminescens* treated condition and 6.7% in *P. luminescens* cells alone, while there was no mortality of adults recorded in untreated and sterile water tested conditions. Although adult mortality in *T. chilonis* was observed in 4 treated conditions, the respective values correspond to the score of 1 (i.e., <50% morality of the test organism) as per the mortality scoring chart of test organism, which accordingly come under the category of 'harmless'. Parasitism by *T. chilonis* ranged between 94.3 and 97.5% in treated conditions which was statistically on par with the untreated check (97.5%). Treatment with nutrient broth and cell-free culture filtrate of *P. luminescens* marginally reduced the parasitism of *T. chilonis* on the eggs of laboratory host, *C. cephalonica,* which were statistically not significant in comparison to the parasitism by *T. chilonis* in untreated and water treated control. These observations clearly indicated that there was no treatment effect on parasitism by *T. chilonis* or on adult mortality, thus showing that the *P. luminescens* associated with *H. bacteriophora* NBAII Hbb1 and its culture filtrate was biologically safe to *T. chilonis* (Table 2).

**Egg hatching, larval mortality and survival of adults of *C. z. sillemi* under treated conditions**

Biosafety of cells, cell-free culture filtrates of *P. luminescens* and the nematode, *H. bacteriophora,* to the beneficial insect, *C. z. sillemi*, was evaluated in term of their effect on egg hatching, larval mortality and survival of *C. z. sillemi at* 24 hours interval for 3days.

Percentage of hatching (95.2 – 100%) and survivability (80 – 100%) of 4 days old larvae of *C. z. sellimi* recorded after 1-3 days of spraying in different treatments were more or less similar between the two protocols (Table 5). Survivability of adults of *C. z. sellimi* ranged from 96 to

**Table 2: Effect of various treatments on adults of *Trichogramma chilonis* and its parasitizing ability**

| Treatments | Adult mortality (%) | Category | Parasitism (%) |
|---|---|---|---|
| No treatment | 0.0 (1.3)[b] | Harmless | 97.5 (81.7) |
| Filter paper with 100µl SDW | 0.0 (1.3)[b] | Harmless | 97.2 (81.0) |
| *Heterorhabditis bacteriophora* 5000 IJs (50µl) | 13.2 (18.5)[a] | Harmless | 95.7 (78.4) |
| Nutrient broth (50µl) | 20.4 (25.5)[a] | Harmless | 94.3 (76.6) |
| *Photorhabditis luminescens* cells alone (50 µl) | 6.7 (14.7)[a] | Harmless | 97.5 (81.3) |
| Cell- free culture filtrate of *P. luminescens* | 9.4 (16.3)[a] | Harmless | 96.5 (79.5) |
| Sterile distilled water | 0.0 (1.3)[b] | Harmless | 97.1 (81.0) |
| SEM± | 3.48 | – | 1.54 |
| CD at 5% | 10.2 | – | NS |
| **CD at 1%** | **13.8** | – | NS |

**Table 3: Effect of various treatments on survival of adults of *Chrysoperla zastrowi sillemi***

| Treatments | Adult survival (%) | Category |
|---|---|---|
| No treatment | 100.0 (90.0) | Harmless |
| Filter paper with 100µl sterile water | 100.0 (90.0) | Harmless |
| Freshly emerged *Heterorhabditis bacteriophora* 5000 IJs (50µl) | 96.0 (84.7) | Harmless |
| Nutrient broth (50µl) | 100.0 (90.0) | Harmless |
| *Photorhabditis luminescens* cells alone (50 µl) | 100.0 (90.0) | Harmless |
| *P. luminescens* supernatant cell-free culture filtrate of *P. luminescens* | 96.0 (84.7) | Harmless |
| Sterile distilled water | 100.0 (90.0) | Harmless |
| SEM± | 2.58 | – |
| CD at 5% | NS | – |
| CD at 1% | NS | – |

**Table 4: Emergence pattern of *Trichogramma chilonis* from treated parasitized eggs of *Corcyra cephalonica* in various treatments**

| Treatments | Emergence (%) from treated parasitized eggs after days– IOBC Protocol | | | | | | | Emergence (%) from treated parasitized eggsdays – Mohan and Sabir, (2005) Protocol | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| No treatment | 92.3 (75.6) | 91.9 (74.0) | 83.6 (66.7) | 87.4 (70.0) | 92.1 (74.9)[a] | 84.9 (68.9) | 75.3 (59.9)[a] | 92.3 (76.3) | 92.3 (74.0) | 83.6 (66.7) | 87.4 (70.0) | 92.1 (74.9)[a] | 84.9 (68.8) | 74.6 (59.9) |
| Filter paper with 100ml sterile water | 93.3 (76.9) | 97.0 (83.8) | 88.3 (72.8) | 82.1 (65.2) | 78.8 (63.5)[b] | 81.3 (64.5) | 74.3 (59.4)[a] | 88.8 (77.3) | 90.3 (76.4) | 91.3 (73.8) | 87.0 (69.2) | 89.8 (72.2)[a] | 75.0 (60.2) | 74.4 (60.1) |
| Freshly emerged *H. bacteriophora* 5000 infective juveniles (50µl) | 90.3 (72.3) | 92.7 (76.1) | 83.7 (66.4) | 81.1 (65.1) | 74.4 (60.0)[b] | 78.1 (64.9) | 67.3 (54.9)[a] | 89.9 (76.3) | 93.3 (80.5) | 92.4 (77.8) | 81.8 (65.1) | 80.6 (64.4)[b] | 72.5 (58.6) | 73.2 (58.9) |
| Nutrient broth (50µl) | 90.3 (74.2) | 91.9 (75.6) | 93.2 (76.6) | 81.2 (64.7) | 84.4 (67.4)[a] | 73.2 (59.1) | 61.3 (51.6)[b] | 92.1 (80.3) | 95.3 (82.0) | 90.2 (74.0) | 75.8 (60.7) | 84.0 (67.1)[a] | 75.2 (60.4) | 73.6 (62.1) |
| *P. luminescens* cells alone (50 µl) | 94.3 (76.7) | 94.9 (81.8) | 87.8 (69.9) | 82.7 (68.2) | 75.8 (61.0)[b] | 81.6 (67.6) | 67.3 (54.8)[a] | 94.4 (78.3) | 96.3 (82.8) | 91.6 (73.4) | 87.4 (72.3) | 81.0 (64.5)[b] | 78.3 (63.1) | 85.1 (68.4) |
| *P. luminescens* supernatant cell free culture filtrate of *P. luminescens* | 90.3 (73.0) | 85.7 (68.5) | 91.2 (74.6) | 88.3 (72.8) | 75.9 (61.0)[b] | 76.5 (61.1) | 71.3 (57.5)[a] | 95.8 (81.3) | 87.3 (70.5) | 91.5 (75.1) | 84.4 (67.3) | 76.4 (61.1)[b] | 70.7 (57.7) | 78.2 (62.4) |
| Sterile distilled water | 97.3 (80.9) | 86.9 (71.5) | 86.1 (70.7) | 85.8 (68.3) | 75.7 (61.0)[b] | 74.9 (60.1) | 73.3 (58.6)[a] | 93.6 (76.3) | 87.3 (69.5) | 84.9 (67.5) | 80.8 (64.9) | 77.8 (62.5)[b] | 75.3 (60.5) | 74.8 (60.0) |
| SEM± | 3.29 | 4.28 | 3.90 | 4.04 | 3.05 | 4.09 | 1.81 | 5.09 | 4.45 | 3.57 | 3.34 | 2.89 | 3.16 | 3.83 |
| CD ($p = 0.05$) | NS | NS | NS | NS | 8.9 | NS | 5.3 | NS | NS | NS | NS | 8.4 | NS | NS |
| Category | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less | Harm-less |

Figures in parentheses are arcsine transformed values, In the columns letter followed by different letter is significantly different at $p = 0.05$, NS = Non-significant.

100% at different treatments which was statistically not significant (Table 3). Microscopic examination of the treated eggs, adults and larvae exhibited no morphological or physical changes.

The effect of entomophilic nematodes on the natural enemies of some parasitoids and predators of insect pests was investigated in the laboratory and field in Poland (Jaworska *et al*., 1995). No effects of *Heterorhabditis bacteriophora* or *Steinernema carpocapsae* on Ichneumonidae or predatory Carabidae were recorded. In another study the effect of entomopathogenic nematodes on non-target arthropods in the laboratory, field soils, and a stream were assessed (Georgis *et al*., 1991). In the laboratory, adult predators were less susceptible to the nematodes, *S. carpocapsae* and *H. bacteriophora* than the immature stages. In field tests, entomopathogenic nematodes that had significantly suppressed pest populations (Japanese beetle) *Popillia japonica* Newman, *Scapteriscus vicinus* Scudder, tawny mole cricket, (black vine weevil) *Otiorhynchus sulcatus* (F.), (cabbage maggot), *Delia radicum* (L.) and (western corn rootworm) *Diabrotica virgifera* LeConte did not adversely affect the numbers of non-target soil arthropods in comparison with the untreated control.

Experiments of Mrácekand and Spitzer (1983) revealed that *S. kraussei* was not a normal parasite of the predators (*Thereva* spp., *Rhagio* spp.) and parasitoids (Tachinidae: Ichneumonidae) of sawfly *Cephalcia abietis*; no reduction in the impact of the predators and parasitoids on *C. abietis* populations. Even though they recorded some invasion by *S. kraussei* in *Thereva handlirschi* and *Rhagio* spp., none occurred in the parasitoids. It was stated that

**Table 5. Effect of various treatments on eggs and larvae of *Chrysoperla zastrowi sillemi***

| Treatments | Egg hatching (%) after treatment after days – IOBC Protocol | | | Larval mortality (%) after treatment after instars – IOBC Protocol | | | Egg hatching (%) after treatment after days – Mohan and Sabir, (2005) Protocol | | | Larval mortality (%) after treatment after instars – Mohan and Sabir, (2005) Protocol | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1st | 2nd | 3rd | 1 | 2 | 3 | 1st | 2nd | 3rd |
| No treatment | 98.0 (86.3) | 98.0 (86.3) | 100.0 (90.0)[a] | 92.0 (79.4) | 96.0 (84.7)[a] | 98.0 (68.9) | 98.0 (86.3) | 98.0 (86.3) | 100.0 (90.0) | 92.0 (79.4)[b] | 96.0 (84.7) | 98.0 (86.3) |
| Filter paper with 100µl sterile water | 96.0 (84.7) | 98.0 (86.3) | 100.0 (90.0)[a] | 100.0 (90.0) | 96.0 (84.7)[a] | 100.0 (64.5) | 96.0 (82.6) | 96.3 (83.0) | 100.0 (90.0) | 96.0 (84.7)[b] | 100.0 (90.0) | 100.0 (90.0) |
| Freshly emerged *H. bacteriophora* 5000 infective juveniles (50µl) Nutrient broth (50µl) | 96.6 (83.3) | 96.8 (83.4) | 95.2 (80.3)[b] | 80.0 (66.2) | 96.0 (84.7)[a] | 88.0 (64.9) | 96.5 (83.2) | 96.5 (83.1) | 95.1 (81.9) | 84.0 (68.8)[b] | 92.7 (79.9) | 92.0 (79.4) |
| *P. luminescens* cells alone (50 µl) | 96.2 (82.8) | 98.0 (86.3) | 94.7 (81.5)[b] | 83.0 (70.9) | 96.0 (84.7)[a] | 100.0 (67.6) | 98.0 (86.3) | 97.8 (86.1) | 98.0 (86.3) | 74.0 (59.6)[b] | 92.0 (79.4) | 92.0 (79.4) |
| *P. luminescens* supernatant cell free culture filtrate of *P. luminescens* | 98.0 (86.3) | 98.0 (86.3) | 100.0 (90.0)[a] | 89.0 (72.8) | 100.0 (90.0)[a] | 98.0 (61.1) | 96.0 (84.7) | 98.0 (86.3) | 98.0 (86.3) | 90.0 (75.7)[b] | 94.0 (81.0) | 94.0 (81.0) |
| Sterile distilled water | 96.0 (84.7) | 96.5 (83.2) | 100.0 (90.0)[a] | 92.0 (79.4) | 96.0 (84.7)[a] | 100.0 (60.1) | 98.0 (86.3) | 96.0 (82.6) | 98.0 (86.3) | 92.0 (79.4)[b] | 95.0 (84.0) | 100.0 (90.0) |
| SEM± | 4.57 | 3.23 | 2.57 | 5.28 | 4.77 | 4.09 | 3.97 | 3.96 | 3.58 | 5.16 | 5.60 | 5.23 |
| CD ($p = 0.05$) | NS | NS | 7.5 | NS | 8.9 | NS | NS | NS | NS | 15.1 | NS | NS |

Figures in brackets are arcsine transformed values, In the columns letter followed by different letter is significantly different at $p$ = 0.05%, NS = Non-significant.

neither the predators nor the parasitoids of *C. abietis* serve as supporting hosts for the development of *S. kraussei* in focuses of *C. abietis.*

Results of the present studies also revealed similar trend and are in concurrence with the reports of the above researchers.

## CONCLUSION

Results indicated that *P. luminescens*, its culture filtrate and *H. bacteriophora* NBAII Hbb1 did not cause any physical changes in eggs, larvae and adults of *T. chilonis* and *C. z. sellimi* and did not exhibit any significant reduction in egg hatching, emergence and parasitism by these natural enemies and was found to be safe without any intraguild effects.

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**Research Article**

# Control of black rot disease of tea, *Camellia sinensis* (L.) O. Kuntze with mycoflora isolated from tea environment and phyllosphere

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**ABSTRACT**: The potential of some aeromycoflora and the tea phyllosphere micro organisms to control black rot disease of tea (causal organism – *Corticium theae* Bernard) was evaluated. Fungal microorganisms isolated from the tea plantation environment and phyllosphere of 11 clones of tea were evaluated. The fungal genera most frequently trapped from the environment of tea plantation were *Aspergillus flavus, Aspergillus niger, Curvularia* sp., *Penicillium* sp. and *Trichoderma atroviride*. The most frequently recovered mycoflora from the tea phyllosphere are *Aspergillus flavus, Aspergillus niger, Penicillium* sp., *Trichoderma atroviride* and *Trichoderma citrinoviride*. Experiment was carried out to assess the possible use of these micro organisms as biocontrol agents against the black rot disease of tea causing organism i.e. *Corticium theae* under *in vitro* and field conditions. The aqueous solution of the antagonists which showed maximum inhibition of the pathogen *in vitro* was applied under field conditions as foliar spray. The percentage symptom and senility index was found to be lowest in the plots sprayed with *A. niger* followed by *T. atroviride* and *T. citrinoviride*, respectively.

**KEY WORDS**: Aeromycoflora, antagonists, *Corticium theae,* phyllosphere

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## INTRODUCTION

The microbial diversity of phyllosphere communities is influenced by plant age, species, micro- and macro-habitat, changes to environmental regimes and position of leaf on the plant (Kinkel 1997; Talley *et al*., 2002; Behrendt *et al*., 2004). Plant genera growing in close proximity have their own characteristic mycota (Kinkel, 1997) which is conditioned by the nature of the plant exudates, microclimate and by other members of the mycota (Goodman *et al*., 1986; Lucas and Knights, 1987; Osono and Mori, 2004).

Population of saprophytic micro-organisms in soil, leaf surface and air-borne propagules has been studied by different workers (Last, 1955; Ruinen, 1961; Dixit and Gupta, 1980; Satpute *et al.,* 1987). The report of the intensive investigations on leaf surface mycoflora has been reported by Last and Deighton (1965). The importance of studies on air spora over crop field to understand the dissemination and spread of microbes, especially the pathogen in the atmosphere have been emphasized by many workers (Pady *et al.,* 1965; Kaiser and Lukezie, 1966; Schnek, 1968; Tilak and Babu, 1981). Bordoloi and Baruah (1967) studied and reported the distribution of mycoflora in tea plantation, soil and air.

A number of studies have identified the ecological relationships between microbes and host plants (Baker and Cook, 1974). Knowledge of the occurrence of air-borne pathogens is helpful in controlling the disease. Some aerobiological studies conducted in India during early decades revealed the qualitative and quantitative features of air flora in different parts of the country (Rajan *et al.,* 1952; Lakhan pal and Nair, 1958; Shivpuri *et al.,* 1960; Bhati and Gaur, 1979; Satpute *et al.,* 1987). The possibility that tea may serve as vehicle for pathogen has been reported earlier (Ekanayaka *et al.,* 1987).

The mycoflora present in the air, phyllosphere and soil of the tea plantations may be interlinked and they may play important positive and or negative role in relation to disease development/control. Toxin producing organisms, if any, can be regarded under the negative role; on the other hand biological control measures of some specific tea diseases may be possible by using some of

the mycoflora trapped from the atmosphere of tea plantations (i.e. from the air and phyllosphere). No systematic study has been made on this aspect till date, especially under the agro-climatic conditions of Cachar district, Assam. Therefore, in the present work an attempt has been made to investigate the same.

## MATERIALS AND METHODS

### Study Area

Rosekandy Tea Estate is situated in the Barak valley which is surrounded by N. C. hills and Jaintia hills in the North, in the east by the state of Manipur, in the south by Mizoram and in the west by the state of Tripura and Sylhet district of Bangladesh (altitude of 26–30 m above main sea level and 24°8'N latitude and 29°15' E longitude.

### Media for isolation of culturable fungi from phyllosphere and air

Rose Bengal agar media for the isolation of aeromicrobes and for the isolation of microbes from the phyllosphere, Czapek Dox Agar media (Tsao, 1964) were used**.**

### Isolation of culturable fungi from tea phyllospheres

Eleven numbers of Tocklai (Tocklai Experimental Station, Jorhat, Assam) released tea varieties were selected for the experiment. For the isolation of leaf surface mycoflora the modified leaf washing technique of Dickinson (1971) was adopted for phyllosphere study. The tea varieties selected were TV-1, TV-9, TV-20, TV-23, TV-26, TV-27, TV-29, TV-30, S-3 A-3, Heelika and Paanitola.

The leaves collected for the isolation of phyllospheric micro organisms were of the same age/ flush. Discs of 4 mm diameter were cut randomly from five leaves of the same variety with a sterile cork borer. Fifty discs were placed in 250 ml conical flask containing 100 ml sterile distilled water and shaken for 20 minutes to get a homogenous suspension of the fungal propagules. From this, 1 ml suspension per plate (9 cm diameter) was poured in Petri plates. Czapek's Dox agar medium was poured into them and mixed thoroughly. Total mycobial population per square cm of leaf surface for each variety of tea was calculated separately using the following formula, after seven days of incubation. The experiment was repeated thrice.

$$\text{Total no. of fungi} = \frac{\text{Total no. of fungi in 1 ml x 100}}{\text{Total area of 50 discs x 2}}$$

### Isolation of culturable fungi from air

Two methods were adopted for the isolation of aeromycoflora in the tea environment. Gravity Petri plate exposure method was followed by simply exposing the Petri plates containing media at 165 to 180 cm height in the tea field. Another way of trapping the air microbes was by using the two stage Andersen sampler. The two stage Andersen air sampler is a form of cascade impactor in which a two stage model provides a cut-off between respirable and non-respirable particles. The plates have progressively smaller holes from the upper most plate. Air was drawn through the sampler at 28.3 litres / min and air-borne bio – particles were deposited on the plates containing Rose Bengal Agar Medium, according to their aero – dynamic size. During the process, spores got impacted into sterile medium, which were kept for incubation at a temperature of 25°C ± 2°C (for 5–7 days). The sampler is run by AC current.

The total number of colonies isolated was correlated to the nearest count with the help of the correction factor table given by Andersen (1958) and the count was expressed as colony forming units per cubic meter of air ($CFU/m^3$). The correction factor was calculated as per the formula given below:

$$\text{Correction factor } (CFU/m^3) = \frac{(x + y) \times 1000}{28.5 \times 10}$$

Where x = total number of colonies in the top.

y = total number of colonies in the bottom.

### Determination of fungal population

Populations of fungal microbes were determined by counting the number of colonies which appeared on the plates during incubation.

### Identification of the isolated microorganism

After the isolation, the fungi were subcultured on potato dextrose agar (PDA) slants and identified consulting the literature (Raper, K. B. and Fennel, 1973; Gilman, 1956; Barnett and Hunter, 1972; Nagamani *et al*., 2002).

### Antagonism studies

To ascertain whether antagonism existed between the test fungi and the pathogens, dual culture method (Wood, 1951) was employed. A 4 mm disc of the antagonistic fungi from 7 days old culture plate was placed in the petridishes containing sterile PDA medium at 2 cm apart from the pathogen. Three replicates were

prepared for each fungus. Respective controls were also made without the test fungi. All the plates were separately incubated at 25 ± 1°C for 7 days and the antagonistic colony interaction were examined thereafter. The kind and degree of antagonism was determined according to the classification of Skidmore and Dickinson (1976).

The colony growing on both sides i.e. towards and opposing each other from loci was measured. The parameters used for the assessment of colony interaction were degree of inhibition or intermingled zone between both the colonies. The inhibition of radial growth was calculated by using the formula of Fokkema (1973):

$$\text{Inhibition } \% = \frac{100 \times r_1 - r_2}{r_1}$$

$r_1$= radial growth of the pathogen in control

$r_2$= radial growth of *Corticium theae* in dual inoculation.

**Field experiment**

A field experiment was conducted to assess the efficacy of antagonistic microorganisms against black rot disease of tea in a randomized block design with six treatments and three replications. Each replicate consists of five tea bushes each; in one treatment fifteen bushes were taken under observation for each treatment. The treatment consisted of five microorganisms and an unsprayed control. The micro-organisms were sprayed on the heavily disease infested plots. The spray was repeated for three times at two weeks interval, while the control was sprayed only with water.

**Field disease assessment**

The experimental plants were examined for disease symptom and senility index. The tea bush plucking table was divided into four equal parts and values were assigned to each, proceeding from the infected part of the plucking table. Symptom expression in one-fourth of the plucking table was given the value 1; if half of the table was affected then the value 2 was given; if three quarter of the plucking table of the bush was affected value 3 was given, and if the symptoms were found throughout the plucking table or the plants showing symptoms of total defoliation/ death due to black rot disease the value 4 was given. A modified symptom and senility index described earlier by Dutta, (1981) was used for calculating for each group of plants in a single treatment as a percentage figure.

$$\text{Symptom \& Senility index} = \frac{\text{Sum of the individual rating value} \times 100}{4 \times \text{no of plants assessed}}$$

**RESULTS AND DISCUSSION**

The results of phyllosphere and aeromycoflora survey showed that the tea garden atmosphere was always with abundant with fungal spores. A total of 8 exposures were carried out by using Andersen air sampler**.** The total number of trapped micro-organisms ranged from 88.33 to 413.42 (Table 1). *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Penicillium* sp. and *Trichoderma atroviride* were found to be dominant in the atmosphere of tea garden. Moderate population was shown by *Alternaria humicola*, *Fusarium* sp., *Penicillium rubrum* and least population of *Aspergillus aureus*, *Helminthosporium* sp., *Penicillium* sp. and *Aspergillus* sp. were recorded (Table 2). The total no of microbes ranges from 33.09 to 1259.8 in the phyllosphere. Maximum microbes were recovered from TV 27 clone, while the minimum was recovered from S 3 A 3 (Table 3). *A. niger* was found to be dominant in all the clones of tea followed by *A. flavus* while the least dominance was exhibited by *A. nidulans*, *Cladosporium* sp and *Trichoderma citrinoviride*. The antagonistic fungus grew over the colony of *C. theae* and completely inhibited its growth. The interaction was rated as Bii. *A. niger and T. atroviride* inhibited the growth of *C. theae* by 74.26% and 72.05% respectively (Table 5). The mycelial growth measurement of *C. theae* and the nine antagonists against each other on PDA on the seventh day after inoculation and percent inhibition of *C. theae* are summarized in Table 6. It can be seen that percentage symptom and senility index were found to be minimum in the black rot infested plots sprayed with aqueous extract of *A. niger* followed by *T. atroviride* and *T. citrinoviride* and maximum symptom and senility percentage was exhibited by the plots sprayed with *A. flavus* as compared to control.

Satyanarayana (1968) reported the presence of spores of *Corticium* and *Cephaleuros* in tea aerosphere. Maximum number of red rust spores was encountered during April and May and it was observed to reach its peak in the month of May. The population of leaf surface propagules has also drawn considerable attention. It is also known that these organisms play significant

**Table 1: Number of microorganisms trapped in aerobiological survey done with 2- stage Anderssen sampler**

| Observation (no of Petri plates) | No of colonies | | CFU / $m^3$ |
|---|---|---|---|
| | Top | Bottom | |
| 1 | 17 | 8 | 88.33 |
| 2 | 24 | 21 | 159.01 |
| 3 | 89 | 28 | 413.42 |
| 4 | 21 | 19 | 141.34 |
| 5 | 14 | 5 | 67.13 |
| 6 | 9 | 5 | 49.46 |
| 7 | 24 | 22 | 162.54 |
| 8 | 21 | 28 | 173.14 |

**Table 2: List of the organisms trapped from the aerobiological survey**

| Name of the trapped organisms | Relative abundance | Diversity index |
|---|---|---|
| *Aspergillus flavus* | +++ | |
| *Aspergillus niger* | +++ | |
| *Aspergillus fumigatus* | ++ | |
| *Aspergillus* sp. | + | |
| *Curvularia lunata* | +++ | |
| *Alternaria humicola* | ++ | 2.42549 |
| *Fusarium* sp. | ++ | |
| *Penicillium rubrum* | ++ | |
| *Penicillium* sp. (green) | +++ | |
| *Penicillium* sp. (yellow) | + | |
| *Trichoderma atroviride* | +++ | |
| *Trichoderma citrinoviride* | ++ | |
| *Helminthosporium* sp. | + | |

+ – small population, ++ – moderate population, +++ – large population

**Table 3: Phyllosphere mycoflora (per $cm^2$ of leaf) in different varieties of tea**

| Variety* | **Phyllosphere mycoflora (per cm) |
|---|---|
| TV 1 | 79.54 |
| TV 9 | 61.07 |
| TV20 | 75.28 |
| TV 23 | 696.02 |
| TV 26 | 567.77 |
| TV 27 | 1259.8 |
| TV 29 | 113.63 |
| TV 30 | 113.63 |
| S 3 A 3 | 33.09 |
| Heelika | 828.26 |
| Paanitola | 994.31 |

* TRA released clones
** Mean of five replicates

**Table 4**: ***In vitro*** **colony interaction of the antagonists with the test fungus (*Corticium theae*)**

| Name of the antagonist | Type of colony interaction |
|---|---|
| *Trichoderma atroviride* | Bi |
| *Trichoderma citrinoviride* | Bi |
| *Penicillium* sp (greyish green colony) | A |
| *Penicillium* sp (fluorescent green) | D |
| *Aspergillus niger* | C |
| *Aspergillus flavus* | Bii |
| *Aspergillus fumigatus* | Bii |
| *Curvularia* sp. | A |
| *Fusarium* sp. | D |

**A**: Mutual intermingling growth, **Bi**: Overgrowth by antagonism, **Bii**: Intermingling growth in which the test fungus under observation has ceased growth and is overgrown by another colony, **C**: Light inhibition, **D**: Not detected
*Type of colony interaction as per Skidmore and Dickinson, 1976

role in the resistant mechanism of plants from air borne plant pathogens. The reports of the intensive investigations on the leaf surface mycoflora are given by Last and Deighton (1965). A significant inhibitory activity was observed for *A. niger* and *T. viride* isolated from the phylloplane of rubber plant against *Corynespora cassiicola,* causal organism of *Corynespora* leaf fall disease of rubber (Evueh *et al*., 2011). Interestingly, the important issue that must be noticed in the present work is the effectiveness of *A. niger*, which appears to be the most effective antagonist in reducing the black rot disease in tea under *in vitro* and field conditions in barak valley, South Assam.

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**Table 5: *In vitro* antagonism of fungal spp. against *Corticium theae***

| Sl. No | Test mycoflora | Control (mm) | Interaction (mm) | % growth inhibition of *Corticium theae* |
|---|---|---|---|---|
| 1. | *Trichoderma atroviride Karsten* | 81.6 (±0.49) | 22.8 (±0.18) | 72.05 |
| 2. | *Trichoderma citrinoviride* | 81.6 (±0.49) | 23.5 (±0.91) | 71.20 |
| 3. | *Penicillium* sp (greyish green colony) | 81.6 (±0.49) | 25.3 (±1.20) | 68.99 |
| 4. | *Penicillium* sp (fluorescent green) | 81.6 (±0.49) | 60.3 (±2.10) | 26.47 |
| 5. | *Aspergillus niger* | 81.6 (±0.49) | 21.00 (±0.57) | 74.26 |
| 6. | *Aspergillus flavus* | 81.6 (±0.49) | 28.5 (±0.75) | 65.07 |
| 7. | *Aspergillus fumigatus* | 81.6 (±0.49) | 24.00 (±1.65) | 60.58 |
| 8. | *Curvularia* sp | 81.6 (±0.49) | 30.00 (±0.04) | 63.23 |
| 9. | *Fusarium* sp | 81.6 (±0.49) | 28.8 (±0.88) | 64.70 |
| | CD at 1% | | | 14.47 |
| | CD ($p = 0.05$) | | | 10.43 |

*Mean of three replications, the experiment is significant at 5 % level of significance
Calculation done as per Fokkema (1973)

**Table 6**: **Effect of foliar spray on the symptom and senility index of black rot disease of tea caused by *Corticium theae***

| Treatments | Pre treatment | Percent symptoms during treatment | | | Post treatment |
|---|---|---|---|---|---|
| | | 1st | 2nd | 3rd | |
| *Aspergillus niger* | 51.66 | 35 | 16.67 | 11.66 | 8.33 |
| *Aspergillus flavus* | 53.33 | 53.33 | 40 | 28.33 | 25 |
| *Penicillium* sp. (greyish green colony) | 56.66 | 45 | 43.33 | 31.66 | 20 |
| *Trichoderma atroviride* Karsten | 56.66 | 53.33 | 33.33 | 25 | 10 |
| *Trichoderma citrinoviride* | 55 | 53.33 | 45 | 35 | 10 |
| Control | 68.33 | 71.66 | 75 | 85 | 90 |
| F – test | 78.35 | 45.12 | 35.32 | 30.45 | 20.78 |

*mean of 15 plants. The experiment is significant at 5% level.

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**Research Article**

# Mass production of *Beauveria bassiana* (Bals.)Vuill. for the management of rice hispa, *Dicladispa armigera* (Olivier)

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**ABSTRACT**: Potato broth, rice gruel, coconut water, rice husk, sawdust and rice bran were evaluated for mass culturing of *Beauveria bassiana* (Bals.) Vuill (Strain AAU-09). Amongst the solid media rice husk in addition of 2% dextrose was superior to others in terms of spore production ($6.25 \times 10^7$ conidia/ml) and pathogenicity (86.67%) to *Dicladispa armigera* (Olivier) adults. Likewise, amongst the liquid media potato broth supplemented with synthetic chitin (2%), dextrose (2%) and peptone (2%) supported maximum spore production.

**KEY WORDS**: Entomopathogenic fungi, *Beauveria bassiana*, mass production, pathogenicity, spore production

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## INTRODUCTION

In a biocontrol programme production of good quality inoculum of any microorganism is prerequisite for receiving excellent result. The production of entomopathogenic fungi had been successfully accomplished in many semisynthetic and synthetic media (Campbell *et.al.,* 1978, Smith and Grula, 1981). While, for harvesting of dried blastospores of the fungus, Ferron (1978) and Alvas and Periera (1989) utilized liquid media such as rice guel, coconut water and potato broth, supplemented with different carbon and nitrogen sources. Aquino *et al.* (1977), Filho *et al*. (1988*)* and Pandit and Som (1988) utilized rice grains and soyaben chunk for mass culturing of *Beauveria bassiana* (Bals.) Vuill. Rice husk, rice barn, and sawdust are agricultural and industrial wastes which cause serious problems in their disposal resulting in environmental pollution as such if these wastes can be managed by utilizing them as media for mass production of entomopathogenic fungi, we may contribute toward economic waste management. Therefore, the present study was undertaken to evaluate these wastes as economically viable substrates for mass production of *B. bassiana* and to evaluate their pathogenicity against a serious major pest of rice, the rice hispa. *Dicladispa armigera* (Oliver).

Similalry, attempt for utilizing liquid media such as potato broth, rice gruel and coconut water has also been made in this study.

## MATERIALS AND METHODS

### Solid media

Rice husk, rice bran, saw dust were collected from the local mills. One hundred gm of each of the solid media soaked in 25 ml distilled water were taken in an autoclaveable polypropylene bag (24 cm x 14 cm) sterilized by autoclaving at 121°C and 15 lb for 20 minutes, and the process was repeated for two consecutive days and $p^H$ was adjusted to 7 (Mazumder *et al.,* 1994). Each bag was condsidered as a replicate and 3 bags were used for each treatment.

### Liquid media

One hundred ml each of rice gruel, coconut water and potato broth were poured in 250 ml conical flask. To each of the flask streptomycin sulphate @ 0.5gm/l was added and all the flasks were autoclaved at 121°C under 15 lb pressure for 20 min. Each flask of containing a medium was inoculated with 1 ml of spore suspension

and incubated at 25±1°C for 15 days and was replicated thrice.

**Inoculation of media**

Conical flasks containing medium and polypropylene bags as described were inoculated under laminar air flow chamber with 1 ml of pure culture (14-day-old) of conidial suspension of *B. bassiana* as per the method described Puzari and Hazarika (1994). These bags were then incubated at 25°C ± 1°C for 15 days. To avoid clumping after 7 days of inoculation, the bags/flasks were shaken to separate the substrate and to break the mycelial mat.

**Conidial Density**

After 15 days of incubation, 10 gm homogenous solid sample was drawn from each replicate which were transferred to 100 ml sterilized distilled water containing Tween 80 (0.023%) solution in 250 ml conical flask. The suspension was filtered through double layered muslin cloth and then through filter paper (Whatman No. 1). Spore count was made after the serial dilution of the suspension using double ruled Neubauer haemocytometer for determining the number of conidia per gm or ml of medium

**Supplementation of carbon, nitrogen and minerals**

Starch, casein, synthetic chitin, yeast extract, dextrose, peptones $MgSO_4$, $CaC0_3$ and manitol were added individually to 250 ml flask containing 100 ml of either potato broth or rice gruel or coconut water at a concentration of 2%. Each treatment was replicated five times. All the supplement were obtained from the Hi Media Laboratories Private Limited.

The liquid media such as potato broth, rice gruel and coconut water were supplemented with sucrose, manitol, starch, dextrose, casein, peptone, chitin, yeast extract, $MgSO_4$, $CaCO_3$ and were added to find out their effect on conidial density and pathogenecity.

**Bioassay of *B. bassiana* strains against *D. armigera* adults**

Adults of *D. armigera* were bioassayed at room temperature (30±1°C, RH 80-85%) as per the method of Puzari *et al*. (1994). Pathogenecity of the strain, AAU-09 was assayed in the laboratory at a concentration of 1 x $10^7$ conidia/ml (Puzari and Hazarika 1991, 1992; Hazarika and Puzari, (1995). Four seedlings of twenty-day-old (*var*. Ranjit) were grown in plastic pots (510 ml capacity) containing soil mixed with manure and fertilizers. Ten laboratory reared pre-starved adults (2-day-old) were released into each pot and caged in paired lantern chimneys one kept atop another. The open end of the top chimney was fitted with a muslin cloth. One day after the release of the test insects, *B. bassiana* at a concentration of $10^7$spores /ml in water mixed with Tween 80 @ 0.023% was sprayed @ 20 ml/pot with a glass atomizer. Each treatment was replicated thrice in CRD. Control pots were treated with water mixed with tween 80. Mortality of adults due to infection was recorded after 7 and 10 days of inoculation and data were subjected to ANOVA.

**RESULTS AND DISCUSSION**

Table 1 shows the mean spore production of *B. bassiana* as grown in three solid media and per cent pathogenicity of these spores against *D. armigera* adults. Though, three substrates favoured the conidial production and infectivity of the fungus, rice husk alone and in combination with 2% dextrose showed highest inoculum density (6.25 x $10^7$conidia/ml) and pathogenicity (86.67%) than rice bran and saw dust alone or in combination with 2% dextrose. Nutrient composition of a medium selected for mass production is of key importance for the growth, sporulation and infectivity of entomo-pathogenic fungi (Ferron, 1981). Rice husk contains lignin (20-42%) and cellulose (30-45%), which supported a proliferated growth of this fungus (Mazumder *et al*., 1995, Puzari *et al.* 1997; Sharma *et al*., 2002). Many attempts to utilize rice bran and husk as sole nutrient source had produced satisfactory results in terms of production of good quality inoculum of *B. bassiana* (Sahayaraj *et al.*, 2008; Pham *et al*., 2009). Poor growth of the fungus in saw dust and rice bran individually may be due to absence of sufficient cabrohydrate and protein in order to support sporogenesis. Sawdust contains phenolic compounds which may act as growth inhibitors (Mazumder *et al*., 1995). However, as a result of addition of dextrose and peptone to saw dust and rice bran, productivity of the media improve significantly with concomitant increase in potentiality and ignificant growth. In terms of potentiality, sawdust and rice brane alone were the poorest media, spores harvested out of which caused only 12.00% and 50.00% mortality, respectively.

Coconut water was significantly superior to potato broth and rice gruel in terms of spore counts and pathogenecity. However, when media were supplemented with carbon and nitrogen compounds, peptone in potato broth produced significantly higher number of propagules (5.16 x $10^7$ conidia/ml) followed by dextrose (4.32 x $10^7$ conidia/ml) and synthetic chitin (4.24 x $10^7$ conidia/ml). Similarly, in case of coconut water and rice gruel, there was no significant effect of supplemented nutrients.

**Table 1: Mean spore production (x$10^7$) and pathogenicity (%) of *B. bassiana***

| Treatments | Spore Production x $10^7$ | Mortality (%) |
|---|---|---|
| Rice husk | 5.25 | 50.00 |
| Saw dust | 0.19 | 12.00 |
| Rice bran | 5.03 | 50.00 |
| Rice husk + dextrose | 6.25 | 86.67 |
| Saw dust + dextrose | 4.31 | 32.33 |
| Rice bran + dextrose | 6.33 | 83.51 |
| CD ($p = 0.05$) | 0.46 | 2.67 |

**Table 2: Effect of medium on mean spore production of *B. bassiana* (AAU-09) and mean pathogenicity (%)**

| Treatments | Spore Production x $10^7$ | Mortality (%) |
|---|---|---|
| Coconut water | 3.80 | 53.33 |
| Potato Broth | 3.52 | 40.00 |
| Rice gruel (Rice cooked water) | 3.27 | 36.67 |
| CD ($p = 0.05$) | 0.20 | 1.05 |

Table 2 shows the mean spore prduction of *B. bassiana* in liquid media and mean percent virulence of the spores' poduced in those media. Out of the three media tested, coconut water served as the best medium in terms of spore production and pathogenicity. Abundance of glucose and mineral present in coconut water may enhance the growth and spore production of *B. bassiana* (Dangar *et al.,* 1991; Sahayaraj *et al.*, 2008). Though, coconut water is proved to be a rich medium for mass production of *B. bassiana* fulfilling all the characteristics necessary for this purpose, availability and cost may prevent it to become commercially viable medium for any biocontrol agent in raw form. It is known that some liquid media, such as rice wash (Sahayaraj *et al.*, 2008), rice gruel (Sudharma and Peethambaran, 2000) produce mycotoxin and quality spores having greater viability and virulence (Akbar *et al*., 2005).

Nutrient supplementation is also an important area of entomopathogenic fungal nutrition; as such experiments conducted in this respect were presented in Table 3. Potato broth supplemented with peptone, a nitrogen source supported maximum sporulation (5.16 x $10^7$ conidia/ml) having virulence (66.67%). Addition of peptone in all the liquid media brought about similar changes as observed in this study. Pham *et al*. (2009) observed maximum spore yield (16.5 x $10^7$ spores/ml) in corn meal by adding 2% peptone as nitrogen source. Likewise, dextrose, yeast extract and synthetic chitin

**Table 3: Effect of supplements on spore production and pathogenecity (%)**

| Treatments | Spore Production x $10^7$ | Mortality (%) |
|---|---|---|
| Potato broth + 2% casein | 3.76 | 53.33 |
| Potato broth + 2% synthetic chitin | 4.24 | 56.67 |
| Potato broth + 2% yeast extract | 4.14 | 56.67 |
| Potato broth + 2% dextrose | 4.32 | 66.67 |
| Potato broth + 2% peptone | 5.16 | 66.67 |
| Potato broth + 2% starch | 4.00 | 46.33 |
| Potato broth + 2% $MgSO_4$ | 3.76 | 43.33 |
| Potato broth + 2% $CaCO_3$ | 3.81 | 43.33 |
| Potato broth + 2% manitol | 3.84 | 42.00 |
| Rice gruel + 2% casein | 3.52 | 40.00 |
| Rice gruel + 2% synthetic chitin | 3.68 | 53.33 |
| Rice gruel + 2% yeast extract | 3.60 | 56.67 |
| Rice gruel + 2% dextrose | 4.20 | 53.33 |
| Rice gruel + 2% peptone | 4.26 | 50.60 |
| Rice gruel + 2% starch | 3.52 | 39.90 |
| Rice gruel + 2% $MgSO_4$ | 3.60 | 46.67 |
| Rice gruel + 2% $CaCO_3$ | 3.52 | 40.00 |
| Rice gruel + 2% manitol | 3.60 | 43.33 |
| Coconut water + 2% casein | 3.44 | 43.33 |
| Coconut water+ 2% synthetic chitin | 3.84 | 50.00 |
| Coconut water + 2% yeast extract | 3.52 | 43.33 |
| Coconut water+ 2% dextrose | 4.16 | 56.67 |
| Coconut water+ 2% peptone | 4.20 | 56.67 |
| Coconut water + 2% starch | 3.44 | 40.00 |
| Coconut water + 2% $MgSO_4$ | 3.52 | 46.67 |
| Coconut water + 2% $CaCO_3$ | 3.68 | 40.00 |
| Coconut water + 2% manitol | 3.68 | 43.33 |
| CD ($p = 0.05$) | 0.26 | 1.10 |

also enhanced sporulation. Mazumder *et al.* (1995) reported potato broth supplemented with 2% dextrose showed maximum spore production of *B. bassiana* (4.3 x $10^7$ conidia/ml) causing 76.30% mortality to the adults of *D. armigera.* Potato dextrose liquid broth medium and Richard's medium were best media for mycelia growth (Manisegarane and Letchoumanane, 1996). Similarly, bean broth, rice broth and potato broth were shown as good liquid media for *B. bassiana* spore production, and it was interesting to note that 96% of these spores germinated (Batista-Filho *et al*., 1985). Thus, it is clear that liquid media need supplementation with carbon and nitrogen sources to enhance growth and potentiality of *B. bassiana*. From this study it is clear that though *B. bassiana* can grow in a wide variety of agricultural products of both solid as well as liquid state, quantity and quality of nutrients favour diferentially on sporogenesis and mycotoxin production (Latge and Sanglier, 1985), sometimes these being highly specific to carbohydrates

(Campbell *et al*., 1983), amino acids (Campbell *et al*., 1978) and peptones (Barnes *et al.,* 1975). This is an area which requires further studies. Potentiality of conidia grown in different media may vary from one medium to another due to the variability in production of viable conidia and biochemical constituents of conidia. The variation in virulence of pathogen and susceptibility of the host are dependent upon several intrinsic factors in the host pathogen interaction including capacity of the pathogen to produce lethal dose of toxins responsible for causing pathogenesis in the host and also capacity of the host to counteract the same, however, optimization of culture medium through manipulation of nutrients and physical environment can enhance virulence of *B. bassiana* to a great extent (Samsinakova *et al*., 1981).

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**Research Article**

# Evaluation of epiphytic microflora as antagonists of red rot pathogen, *Colletotrichum falcatum* in sugarcane under subtropical conditions

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**ABSTRACT**: Epiphytic microflora isolated from different plant parts of healthy sugarcane plants at different growth stages were assessed for their ability to control fungal red rot pathogen of sugarcane both *in vitro* and *in vivo*. Microbial population was higher in the month of August with highest on cane stalk followed by buds and dry leaves. A total of 112 bacterial isolates representing 16 morphotypes, 63 fungal and 35 actinomycete isolates representing 7 morphotypes each and 21 mould isolates of 3 morphotypes were purified from the collection of isolates obtained from different plant parts at different periods of plant growth stage and environment condition. These 231 microbial isolates were screened *in vitro* for their antifungal activity against *Colletotrichum falcatum* using dual culture technique. None of the mould isolates could inhibit *C. falcatum* growth, while 12 bacterial isolates, 4 fungal isolates and 5 actinomycete isolates were found to be antagonistic to *C. falcatum*. Biochemical and 16SrRNA partial sequence characterization of 12 bacterial isolates led to identification of *Pseudomonas*, *Serratia, Bacillus*, *Paenibacillus*, *Gluconacetobacter*, *Serratia marscens* (DQ144501) and *Paenibacillus macerans* (DQ144502). Based on colony morphology, spore arrangement and structure characteristics the fungal and actinomycete isolates were identified to of *Aspergillus*, *Trichoderma* and *Penicillium* and of *Streptomyces* and *Saccharopolyspora* sp., respectively. The strongest inhibition of *C. falcatum* was obtained with actinomycete isolates and *Serratia marscens* both under *in vitro* and *in vivo* conditions, that can serve to be part of integrated disease management (IDM) of red rot in sugarcane in subtropics.

**KEY WORDS**: Epiphytes, Biocontrol, *Colletotrichum falcatum, Serratia marscens*, *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp., *Streptomyces* sp., S*accharopolyspora* sp., sugarcane

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## INTRODUCTION

Sugarcane (*Saccharum* spp.), is cultivated in an area of around 4.0 million ha with average productivity of 60 t $ha^{-1}$ and provides major economic security to the farmer. Red-rot caused by the fungus *Colletotrichum falcatum* is the prime disease of sugarcane in tropical belts and is the major production constraint for sugarcane production in the country (Alexander and Viswanathan, 1996). The changing races of fungus makes the commercial sugarcane varieties susceptible to red rot. In India, due to red rot outbreaks, many high sugar commercial varieties of sugarcane (Co312, Co997, Co1148, CoJ64, CoC671 etc.) have been eliminated. The sugar industry in India suffers losses of more than US$ 500 million every year due to red rot disease (Padmanaban *et al*., 1996; Viswanathan *et al.,* 1997). Disease is mainly sett-borne with secondary infections through leaf lamina and buds. Moist hot air therapy (MHAT), dilute acid treatments have been tried, but with little success as the presence of tough external rind makes the cane unamenable for chemicals to enter and control the pathogen.

Resistance to red-rot is monitored by inoculating *C. falcatum* spores in the growing canes at 6-month-old stage by plug-method (Singh and Singh, 1989) and spread of disease is monitored after 6 weeks for assigning disease related character. The susceptible varieties are not recommended for cultivation, however, there are certain susceptible varieties like CoJ 64 and CoS 767 which are cultivated by farmers due to their high sugar (18% brix) and early maturing (10 month) characteristics. Such varieties have been showing some kind of unexplained field resistance to secondary stage infection of *C. falcatum*. Since, secondary stage infections are mainly through leaf-lamina, buds and root-primordia on the nodal region, we at Indian Institute of Sugarcane Research, Lucknow started working on a hypothesis that probably epiphytic micro-

flora of such varieties may have some role in checking secondary infection of *C. falcatum*. Since sugarcane plant houses a plethora of micro-organisms in its rhizosphere and as endophyte and epiphytes, the present work aims to (i) isolate the predominant naturally occurring epiphytic microbial isolates that can check the proliferation of *C. falcatum*, (ii) identify and utilize them for managing the red rot disease.

## MATERIALS AND METHODS

### Epiphytic micro-flora of sugarcane

Sugarcane variety CoJ 64 growing at farm of Indian Institute of Sugarcane Research, Lucknow (located at 2605'N, 8006'E, 111m above sea level) was selected for sampling different plant parts (Sehgal *et al.,* 1990). Sugarcane leaf is attached to stalk through leaf sheath with a transverse mark at the joint of leaf lamina and leaf sheath. The first leaf in which this transverse mark becomes visible is called LTM (Lateral Transverse Mark) leaf and is considered as an indicator leaf for physiological/biochemical studies. Other leaves on sugarcane stalk are numbered relative to LTM leaf. The planting of the crop was done in February 2005 and the plant samples were drawn at 3 stages of crop growth representing different seasonal temperatures of 40–45°C (May; 3 month crop), 30–35°C (August; 6 month crop) and 4–7°C (December 15, 10 month crop). Ten intact plants were uprooted and brought to laboratory. After giving a mild washing with sterilized water for removing dust, different leaves (–2, LTM, +2, +4, last green leaf and dried leaf), buds, stem and root pieces were cut using a sterilized knife or a pair of scissors and were carefully transferred to aseptic sealable plastic bags. The experiment was started by composting the samples and three replicates were taken with 5 leaves of each kind, 20 buds and 5 canes for each replicate.

### Processing of leaf samples

Five leaf pieces measuring 3 $cm^2$ were cut from each leaf randomly covering up to the tip and in total 25 such pieces from 5 leaves were transferred to a sterilized 500 ml Erlenmeyer flask containing 100 ml of saline–$PO_4$ buffer. Cane pieces measuring 5 cm were cut with sterilized sharp knife and the ends were sealed with paraffin film. Five such cane pieces were transferred to 50 ml of saline–$PO_4$ buffer. Similarly, 20 buds and five grams of roots were transferred to 50 ml and 100 ml of saline-$PO_4$ buffer, respectively. All the flasks were shaken for 1hr at 100 rpm using an incubator shaker for preparing suspension of epiphytic micro-flora.

### Isolation of epiphytic micro-flora associated with cane parts

Serial dilutions of suspension containing epiphytic micro-flora of leaf, bud, stem and roots were plated on Nutrient Agar (NA), Trypton Yeast-Extract Mannitol (TYM), King's B (KB), Tryptic Soy Agar (TSA) growth media for bacterial enumeration, Czapkdox Agar (CA) and Potato Dextrose Agar (PDA) for fungal and Actinomycete Agar for actinomycete counts. Three dilutions for each sample in duplicate were plated and the plates were incubated at 30°C for 3–7 days. Observations were recorded for total microbial counts and the fraction of most predominant ones. Colonies of bacteria, fungi and actinomycete appearing different on the basis of color, size and colony margin pattern were purified by streaking on the respective media and maintained on same culture plates as well as at –80°C with 40% glycerol. For *in vitro* assays the suspensions of different microbial isolates containing $10^7$–$10^8$ cells 10 ($L^{-1}$) were prepared by suspending isolates in saline–$PO_4$ buffer.

### Culturing of *Colletotrichum falcatum*

A virulent isolate of *Colletotrichum falcatum* (Cf 09), from IISR, Lucknow was used for this studies. The culture was grown on oat meal agar media plates, containing oatmeal 30 g and agar-agar 16g $L^{-1}$, at 28°C for 15 days for complete sporulation. Spore suspension was prepared by adding 5 ml of saline–$PO_4$ buffer to the Petri plate, shaking and collecting it in a sterilized vial. The spore counts in the suspension were done by haemocytometer and by dilution plating on oat-meal agar. Depending upon counts, the spore suspension was diluted to maintain approximately $10^7$ spores per $10^{-1}$ and of suspension.

### *In vitro* screening of microbial isolates for antifungal activity

Dual inoculation method was followed for initial screening. The common medium on which both *C. falcatum* and different microbial bioagents could grow efficiently was found to be TYM and King's B agar. Therefore, these two growth media have been used for all *in vitro* assays. Fungal spores absorbed on disc of sterilized Whatman filter paper (No. 10) was kept in the center of the medium plate and after incubating for 24hr at 30°C, the bacterial suspension discs were placed on either side of the fungal disc. The plates were incubated at 30°C for 7–10 days and the inhibition of growing fungal culture was taken as positive antifungal activity of different bacterial, fungal and actinomycete isolates.

The extent of antifungal activity of bioagents was estimated by plate and broth culture inhibition assays. For plate inhibition assay 0.1 ml of *C. falcatum* spore suspension was plated on King's B agar plate and after it dried, the microbial disc was placed in the center. Fungal inhibition zone around the bacterial disc was measured after incubating at 30°C for 7 days. For broth culture inhibition assay 20ml of King's B broth was inoculated with different bioagents. After incubation of 10 days at 30°C the cultures were centrifuged at 6000-g for 10 min and passed through a 0.2m filter. The culture filtrate was inoculated by 0.1ml *C. falcatum* spore suspension and the flasks were incubated for 10 days at 30°C. The developed fungal mycelium of *C. falcatum* was separated by filtering it through pre-weighed Whatmann (no. 1) filter paper and thus the weight of the developed mycelia was recorded. All the assays were performed three times in duplicate. Direct microscopic observation of the plates or temporary slides of the *C. falcatum* mycelia was performed using Leica phase contrast microscope.

**Enzyme assays in culture filtrate**

Culture supernatant was assayed for carboxymethyl cellulase (CMCase), chitinase, and protease activities. For CMCase activity, 50mM 2-(Nmorpholino) ethanesulfonic acid (MES) buffer, pH 5.0 was mixed with 0.2% CMC and incubated at 37°C. Reducing sugars liberated due to CMCase activities were determined by the method of Nelson (1944) with a glucose standard. One unit of CMCase activity was defined as the amount of enzyme that released 1mM glucose reducing equivalent $min^{-1}$ $ml^{-1}$ culture. Chitinase activity was determined by incubation of culture supernatant with 50mM citrate buffer, pH 5.0, and 0.2% chitin at 37°C. Liberated N-acetylglucosamine equivalents were determined by the method of Reissing *et al.* (1955). One unit of chitinase activity was the amount of enzyme that released 1mM N-acetylglucosamine equivalent $min^{-1}$ $ml^{-1}$ culture. Protease activity was determined by incubating culture filtrate in 100mM potassium phosphate buffer, pH 7.0, plus 0.4% azocoll at 37°C (Chavira *et al.,* 1984). One unit of protease activity was the amount of enzyme that increased absorbance at 520nm 1 unit $hr^{-1}$ $ml^{-1}$ culture filtrate. Lower limits of detection were 2.3U for CMCase and 0.3U for chitinase activity, and 0.001U for protease activity.

**Characterization of potent biocontrol agents and developing antibiotic resistant variant of bio-agents**

Potential bacterial biocontrol agents were characterized using morpho-physical and biochemical techniques as per Bergey's Manual of Determinative Bacteriology (1984). Further, by using MicroSeqTm500-16SrDNA bacterial sequencing kit, 16SrDNA amplification and sequencing was done using Eppendorf thermal cycler and capillary based ABI Prism sequencer and its data software, respectively. Upon blasting the DNA sequences in existing database and based on the sequence matching, the bacterial cultures were identified. Colony, mycelial, sporangium and biochemical characteristics were used for fungal (Pitt and Cocking, 1986) and actinomycete identification (Bergey's Manual of Determinative Bacteriology, 1984). Selection from naturally occurring variants of a culture was used to develop the antibiotic resistant marker for the survival studies. It was achieved by plating bacterial isolates on King's B agar containing kanamycin (100g $ml^{-1}$) as most of the isolates were sensitive to 50g $ml^{-1}$ concentration. For fungal and actinomycete, sodium azide (200g $ml^{-1}$) was used as selection pressure in their respective growth media. All the antibiotic resistant marked bioagents were tested again for their antifungal activity under *in vitro* condition.

***In vivo* testing of biocontrol agents for suppressing red rot disease**

**Crop culture and treatments**

Two set of experiments were performed for studying the effect of bio-agents on red-rot disease development under pot culture. Soil for filling earthen pots was collected from 0 to 15 cm layer of five different fields at IISR farm. Soil of the IISR farm belong to fine loamy, non-calcareous, mixed hypothermic udic ustochrepts. Before filling the pots, considering the standard recommendation of fertilizer application for sugarcane (per hectare N:P:K:150:60:60) 0.15 g nitrogen $kg^{-1}$ soil in the form of urea, 0.3 g phosphorous $kg^{-1}$ soil through single super phosphate and 1.6 g potassium $kg^{-1}$ soil through murate of potash was mixed in the soil. Each pot was filled with 8.5 kg of soil and approximately 60% available soil moisture was maintained through out the growth cycle by watering pots as and when required. Pots were placed completely randomized in a net-house under natural light from February to September,. The mean ambient temperature during this period was 24.5°C with a mean minimum of 15.9°C and mean maximum of 33.1°C. The mean period of bright sunshine was 7.5 h $day^{-1}$ with solar radiations of approximately 19.5 MJ $m^{-2}$ $day^{-1}$.

**Experiment I**

Healthy canes were given moist hot air treatment (MHAT) at 54°C for 2 hrs. As a common practice for

disease induction, single bud sets were treated with *C. falcatum* spore suspension ($10^8$ spores $ml^{-1}$) by mixing the setts with fungal inoculum in sterilized poly-bags. After 48 hr of incubation at 30°C, the *C. falcatum* inoculated setts were treated with bioagents by overnight dipping the setts in culture suspension ($10^8$ cells $ml^{-1}$) of different bio-agents. At planting, 5 ml of culture suspension was also added in the adjacent soil of the setts. Autoclaved fungal suspension and subsequently water was used for control treatment.

**Experiment II**

Red rot infected canes of sugarcane variety CoJ 64 having fungal infection in its buds were taken from the IISR field trial. Infected single bud setts were treated with bioagents by overnight dipping the setts in culture suspension ($10^8$ cells $ml^{-1}$) of different bio-agents. At planting, 5 ml of culture suspension was also added in the adjacent soil of the setts. Water was used for control treatment.

Eight single bud cane setts were planted horizontally in each pot. In all, there were 39 pots with 13 treatments (12 bioagents + 1 control) in 3 replications. Different treatments were- T1: *Serratia marcens*; T2: *Pseudomonas fluorescens*; T3: *Paenibacillus macerans*; T4: *Bacillus subtilis*; T5: *Lactobacillus* spp.; T6: *Gluconacetobacter diazotrophicus*; T7: *Aspergillus terreus*; T8: *Trichoderma reesi*; T9: *Trichoderma harzianum;* T10: *Penicillium* spp. T11: *Streptomyces* spp; T12: *Saccharopolyspora* spp. and T13: Control. The growing plants in pots were sprayed with the same bioagent suspension after 3 months of planting using mist sprayer and 25 ml culture broth mixed with 50 ml of sterilized water was used for spraying each pot.

**Red rot disease development and its severity index**

Canes growing in pots were harvested by cutting from the base and were split open to note the disease development. The severity of the disease was ranked using severity index that depends on parameters such as condition of green top, transgression of disease through internodes, development of white patches and nodal necrosis. It is ranked on a 0-9 scale where 0-2 is for resistant phenotype (R), 2-4 for moderate resistant (MR) 4-6 for moderate susceptible (MS), 6-8 for susceptible (S) and >8 is highly sensitive (HS) phenotypes.

**Population of biocontrol agents in soil, on setts and plant surface**

Before planting two setts from each bioagent treatment were shaken with 50 ml of saline-$PO^4$ buffer and similarly at harvest different plant parts (leaf, stalk and bud) and soil attached to roots (5g each) were also suspended in 50 ml saline $PO^4$ buffer. All the suspensions were diluted and plated on respective culture growth medium with kanamycin/sodium azide. Microbial counts were made after incubating the plates at 30°C for 5-7 days. Re-isolated biocontrol agents were tested for their *in vitro* antifungal property and other biochemical/ genetic characteristics.

**Statistical analyses**

Data are expressed on an oven-dry soil or plant weight basis. One way analysis of variance with Duncan Multiple Range Test (DMRT) as post hoc analysis was used to compare the means (Snedecar & Cochran, 1967). Microbial count data were log transformed before analysis of variance.

## RESULTS AND DISCUSSION

**Selection of biocontrol agents suppressing *Colletotrichum falcatum***

Variable number and type of microbial population was observed at different plant parts and growth stages (Table 1). Invariably, the microbial population was more in the month of August with highest on cane stalk followed by buds and dry leaves. A total of 112 bacterial isolates representing 16 morphotypes, 63 fungal and 35 actinomycete isolates representing 7 morphotypes each and 21 mould isolates of 3 morphotypes were purified from the collection of isolates obtained from different plant parts at different periods of plant growth stage and environment condition (Table 1). These 231 microbial isolates were screened in vitro for their antifungal activity against *Colletotrichum falcatum* using dual culture technique. None of the mould isolates could inhibit *C. falcatum* growth, while 12 bacterial isolates, 4 fungal isolates and 5 actinomycete isolates were found to be antagonistic to *C. falcatum*. Upon biochemical and 16SrRNA partial sequence characterization 12 bacterial isolates were identified to 6 genera namely *Pseudomonas, Serratia, Bacillus, Paenibacillus* and *Gluconacetobacter* and the partial sequence of 2 genera *Serratia marscens* (DQ144501) and *Paenibacillus macerans* (DQ144502) have been submitted to NCBI Genbank (Suman *et al.,* 2005a and b). Based on colony morphology, spore arrangement and structure characteristics the fungal and actinomycete isolates were identified to 3 (*Aspergillus, Trichoderma* and *Penicillium*) and 2 (*Streptomyces* and *Saccharopolyspora*) genera, respectively. The strongest inhibition of *C. falcatum* was observed by actinomycete isolates and *Serratia marscens*.

**Table 1: Sugarcane associated microbial population as influenced by plant parts and seasonal temperature**

| Plant parts | Bacteria | | | Fungi | | | Actinomycete | | | Mould | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3MAP (43°C) | 6MAP (30°C) | 10MAP (5°C) | 3MAP (43°C) | 6MAP (30°C) | 10MAP (5°C) | 3MAP (43°C) | 6MAP (30°C) | 10MAP (5°C) | 3MAP (43°C) | 6MAP (30°C) | 10MAP (5°C) |
| Leaf [1] | | | | | | | | | | | | |
| –2 | 28 | 32 | 4 | 2 | 2 | 5 | 3 | 2 | 2 | 3 | 4 | ND |
| LTM | 30 | 43 | 6 | 3 | 3 | 6 | 3 | 5 | 2 | 37 | 39 | 2 |
| +2 | 97 | 134 | 28 | 5 | 8 | 6 | 6 | 5 | 5 | 51 | 58 | 5 |
| +4 | 98 | 129 | 32 | 3 | 6 | 6 | 8 | 10 | 7 | 59 | 57 | 10 |
| Last green | 66 | 93 | 12 | 4 | 7 | 7 | 14 | 12 | 8 | 42 | 37 | 17 |
| Dry-trash | 740 | $1.02x10^3$ | 725 | 40 | 52 | 44 | 32 | 38 | 22 | 72 | 58 | 51 |
| Stalk[2] | $1.12x10^3$ | $1.43x10^3$ | $8.46x10^3$ | $1.58x10^3$ | $5.41x10^3$ | $3.98x10^3$ | 12 | 14 | 10 | 40 | 37 | 35 |
| Bud[3] | $1.17x10^3$ | $1.23x10^3$ | $7.50x10^3$ | 645 | $1.04x10^3$ | 906 | 10 | 12 | 7 | ND | ND | ND |
| Root[4] | $3.67x10^6$ | $6.89x10^7$ | $1.92x10^7$ | $3.62x10^3$ | $4.65x10^3$ | $4.25x10^3$ | 635 | 689 | 609 | $1.9x10^3$ | $6.4x10^3$ | $3.6x10^3$ |

* Mean of 3 experiments; MAP: months after planting of cane setts; 1 and 2: counts $cm^{-2}$; 3: counts $bud^{-1}$; 4: counts $g^{-1}$ root

**Antifungal potential of biocontrol agents**

*C. falcatum* inhibition zone formed by different microbial isolates ranged from 10 to 32.7 mm (Table 2). The maximum zone of inhibition was shown by actinomycete isolates and *Serratia marscens* (30-32.7mm) and minimum by *Gluconacetobacter diazotrophicus* (10mm). *C. falcatum* inhibition in broth culture was assessed by measuring its mycelia weight in the culture filtrates of biocontrol agents (Table 2). Mycelia weight was lowest with actinomycete isolates (1.2–1.3 mg) followed by *Serratia marscens* and *Pseudomonas fluorescens* isolate (2.7–3.13 mg) compared to 35.6mg in the control where no biocontrol agent was cultured. The mycelia weight varied from 8.4–10.5 mg in the treatments where fungal bioagents were used.

A time course investigation of the interaction between biocontrol agents and *C. falcatum* by light microscopy of the culture plates showed the formation of the abnormal forms of mycelia such as swelling, curling, multiple branching, degraded protoplasm and cell wall. These ultimately led to the hyphal death as observed by the clear zone formed between biocontrol agent and the fungus.

**Biocontrol agents mediated suppression of sugarcane red rot**

There was only 12% germination in *C. falcatum* inoculated setts (Cf–Control) compared to 100% in healthy cane sett treatment (blank) (Table 3). Treatment with biocontrol agents improved the germination of infected setts by 67 to 96%. The germination vigor, which takes care of germination% and plant height, was high and varied from 1704 to 3648 units in biocontrol agents' treatments compared to 216 in Cf-Control and 3500 in blank treatment. All the germinated setts of Cf-Control treatment developed red rot disease and the disease severity index was ranked as highly sensitive (Table 3), whereas disease induction was 14 to 65% in different biocontrol agents treatment. The disease was also less severe as disease severity index varied from sensitive in *Lactobacillus* spp. treatment to resistant in the case of *S. marscens, P. fluorescens, G. diazotrophicus* and *T. harzianum* treatments.

In the other experiment where infected cane setts were used, the germination was only 10% in Cf-Control treatment, whereas the treatment of infected cane setts by different biocontrol agents improved the germination up to 32% (Table 4). The *Lactobacillus* and actinomycete isolates treatments could not improve the germination. The germination vigor varied from 170 to 864 units in different bioagent treatments compared to Cf-Control treatment, where the germinated plants developed red rot disease and succumbed immediately. Induction of red rot disease varied from 12 to 100% in different biocontrol agents' treatments. Disease severity index was highly sensitive (HS) in *Lactobacillus* and sensitive (S) to moderated resistant (MR) in different bioagent treatments (Table 3). Overall among the different biocontrol agents *S. marscens, P. fluorescens, G. diazotrophicus, T. reesi* and *Penicillium* treatments were found to be the best

**Table 2: Antifungal activity of different biocontrol agents against *Colletotrichum falcatum***

| Sl. No. | Biocontrol agents | *Colletotrichum falcatum* | |
|---|---|---|---|
| | | Inhibition zone (mm) | Mycelial weight (mg) |
| 1 | *Serratia marscens* | 30.0$^{ab}$ ± 0.58 | 2.70 ± 0.10 (92%) |
| 2 | *Pseudomonas fluorescens* | 27.7$^{b}$ ± 0.33 | 3.13 ± 0.15 (91%) |
| 3 | *Paenibacillus macerans* | 20.7$^{c}$ ± 0.33 | 5.90 ± 0.26 (83%) |
| 4 | *Bacillus* spp. | 19.0$^{c}$ ± 0.58 | 6.67 ± 0.35 (81%) |
| 5 | *Lactobacillus* spp. | 13.7$^{d}$ ± 0.88 | 7.80 ± 0.30 (78%) |
| 6 | *Gluconacetobacter diazotrophicus* | 10.0$^{e}$ ± 0.58 | 10.8 ± 0.26 (70%) |
| 7 | *Aspergillus terreus* | 20.3$^{c}$ ± 0.88 | 9.63 ± 0.40 (73%) |
| 8 | *Trichoderma reesi* | 19.0$^{c}$ ± 1.15 | 10.4 ± 0.40 (72%) |
| 9 | *Trichoderma harzianum* | 20.0$^{c}$ ± 1.15 | 10.5 ± 0.56 (70%) |
| 10 | *Penicillium* spp. | 20.3$^{c}$ ± 1.33 | 8.40 ± 0.40 (76%) |
| 11 | *Streptomyces* spp. | 30.3$^{ab}$ ± 1.33 | 1.20 ± 0.20 (97%) |
| 12 | *Saccharopolyspora* spp. | 32.7$^{a}$ ± 1.43 | 1.30 ± 0.20 (96%) |
| 13 | Control | 0.00 | 35.6 ± 0.72 |

**Table 3: Effect of biocontrol agents on *C. falcatum* infected healthy sugarcane setts**

| Sl. No. | Biocontrol agents | Cf-infected healthy setts | | Red Rot Disease | |
|---|---|---|---|---|---|
| | | Germination (%) | Germination vigor | Induction (%) | Reaction |
| 1 | *Serratia marscens* | 92$^{a}$ | 3588$^{a}$ | 23$^{ab}$ | R |
| 2 | *Pseudomonas fluorescens* | 96$^{a}$ | 3648$^{a}$ | 17$^{a}$ | R |
| 3 | *Paenibacillus macerans* | 75$^{c}$ | 2400$^{c}$ | 39$^{c}$ | MS |
| 4 | *Bacillus* spp. | 79$^{bc}$ | 2765$^{b}$ | 47$^{cd}$ | MS |
| 5 | *Lactobacillus* spp. | 71$^{c}$ | 1704$^{e}$ | 65$^{d}$ | S |
| 6 | *Gluconacetobacter diazotrophicus* | 92$^{a}$ | 3588$^{a}$ | 14$^{a}$ | R |
| 7 | *Aspergillus terreus* | 71$^{c}$ | 1917$^{d}$ | 29b | MR |
| 8 | *Trichoderma reesi* | 79$^{bc}$ | 2765$^{b}$ | 26$^{b}$ | MR |
| 9 | *Trichoderma harzianum* | 67$^{c}$ | 2490$^{c}$ | 20$^{a}$ | MR |
| 10 | *Penicillium* spp. | 83$^{b}$ | 2490$^{c}$ | 20$^{a}$ | MR |
| 11 | *Streptomyces* spp. | 87$^{b}$ | 2088$^{d}$ | 48$^{cd}$ | MS |
| 12 | *Saccharopolyspora* spp. | 83$^{b}$ | 2241$^{cd}$ | 45$^{cd}$ | MS |
| 13 | Control–*Cf* | 12$^{d}$ | 216$^{f}$ | 100$^{e}$ | HS |
| 14 | Control-blank | 100$^{a}$ | 3500$^{a}$ | – | – |

S: Sensitive; MS: Moderate Sensitive; HS: Highly Sensitive; R: Resistant; MR: Moderate Resistant
Control-*Cf*: Healthy sugarcane setts infected by *Cf* but not treated by any biocontrol agent
Control-blank: Healthy setts not treated by Cf and/or any biocontrol agent
Values in each column with same letter do not differ significantly at $p \leq 0.05$ by Duncan's Multiple Range Test.

**Table 4: Effect of biocontrol agents on *Colletotrichum falcatum* diseased sugarcane setts**

| Sl. No. | Biocontrol agents | Cf-infected healthy setts | | Red Rot Disease | |
|---|---|---|---|---|---|
| | | Germination (%) | Germination vigor | Induction (%) | Reaction |
| 1 | *Serratia marscens* | 32[b] | 864[b] | 12[a] | MR |
| 2 | *Pseudomonas fluorescens* | 30[b] | 840[b] | 14[a] | MR |
| 3 | *Paenibacillus macerans* | 20[c] | 380[e] | 60[c] | S |
| 4 | *Bacillus* spp. | 24[c] | 504[d] | 50[c] | S |
| 5 | *Lactobacillus* spp. | 10[d] | 170[f] | 100[d] | HS |
| 6 | *Gluconacetobacter diazotrophicus* | 22[c] | 660[c] | 20[a] | MR |
| 7 | *Aspergillus terreus* | 18[cd] | 378[e] | 25[b] | MR |
| 8 | *Trichoderma reesi* | 20[c] | 500[d] | 20[a] | MR |
| 9 | *Trichoderma harzianum* | 24[c] | 576[d] | 17[a] | MR |
| 10 | *Penicillium* spp. | 25[c] | 700[c] | 14[a] | MR |
| 11 | *Streptomyces* spp. | 10[d] | 140[f] | 50[c] | MS |
| 12 | *Saccharopolyspora* spp. | 10[d] | 170[f] | 33[b] | MS |
| 13 | Control - *Cf* | 0 | – | – | HS |
| 14 | Control - blank | 97[a] | 3492[a] | – | – |

*Cf: Colletotrichum falcatum*; S: Sensitive; MS: Moderate Sensitive; HS: Highly Sensitive; R: Resistant; MR: Moderate Resistant
Control-*Cf*: Healthy sugarcane setts infected by *Cf* but not treated by any biocontrol agent
Control-blank: Healthy setts not treated by Cf and/or any biocontrol agent
Values in each column with same letter do not differ significantly at $p \leq 0.05$ by Duncan's Multiple Range Test.

in checking red rot disease and improving germination vigor and plant growth.

**Survival of biocontrol agents**

Survival of potent 5 microbial biocontrol agents (*S. marscens, P. fluorescens, G. diazotrophicus, T. reesi* and *Penicillium*) on different plant parts and soil was studied at the harvest stage in December. Sufficient number of microbial population was present on all the plant surfaces and even in the adjoining rhizospheric soil around the setts (Table 4). The re-isolated biocontrol agents showed positive *in vitro* antifungal property and were similar to the original isolates based on biochemical/ genetic characteristics.

In the recent years many reports concerning epiphytes with an adverse effect on plant pathogens have been published (Campant *et al.,* 2005; Janisiewicz *et al.,* 1992; Sholberg *et al.,* 1995; Sinigaglia *et al.,* 1998). The antagonistic effect of the microflora is explained not only by their production of antibiotic substances/ inhibitory allelochemicals, but also by competition for ecological niche, nutrients and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens and/or abiotic stresses (Chalutz *et al.,* 1988; Droby *et al.,* 1989; Janisiewicz 1987; Chand-Goyal and Spotts 1996, 1997). Interactions among epiphytes can play an important role in a plant's defense responses. The nature of the interactions depends, among other things on the qualitative-quantitative composition of epiphytic communities. In an attempt to identify potential biocontrol agent for controlling red rot disease in sugarcane our results demonstrate that a variety of bacterial, fungal and actinomycete isolates colonize different parts of the sugarcane plant. A total of 231 different microbial isolates representing 33 morphotypes were purified from different plant parts of sugarcane. A notable feature of epiphytic microbial population is their variation in size or type even on different leaves of same age and having identical appearance of the same plant (Kinkel, 1997). Availability of nutrients, sugars and selected metabolites on the plant parts govern the variable distribution of the epiphytic microbial population (Mercier and Lindow, 2000). Therefore, the microbial population of the plants could be modified by changing the nutrient status of the plant surface and this has implication for the biological control of plant

pathogens. In this study, approximately 64% of the isolates purified as epiphytic microflora strongly inhibited *C. falcatum* under *in vitro* condition. Six identified bacterial isolates belonged to *Pseudomonas, Serratia, Bacillus, Paenibacillus* and *Gluconacetobacter* sp. some of which have been used as biocontrol agents in other crops also. Four fungal agents were identified to *Aspergillus, Trichoderma* and *Penicillium* group and two actinomycete isolates were placed in *Streptomyces* and *Saccharopolyspora* group. These microbial isolates are known to produce a variety of bioactive compounds which makes a firm basis for their antagonistic principle against pathogens. Co-culturing of *C. falcatum* and biocontrol agents was avoided as in case of fungal and actinomycete biocontrol agents it is difficult to segregate the contribution of each in the total mycelial weight observed. Rather culturing of *C. falcatum* in the culture filtrate of different biocontrol agents observed a reduction of 70 to 97% in the mycelial growth, indicating that production of bioactive compounds was mainly responsible for inhibiting *C. falcatum* growth and not the direct contact as evidenced by the effect of culture filtrates on *C. falcatum* growth. Nautiyal *et al.* (2006) have shown that co-culturing of *Bacillus* individually and a microbial consortium of 3 isolates with *Fusarium moniliformis* and *C. falcatum* inhibited fungal growth ranging from 47 to 70%.

Light microscopy observations of the clearing zone formed between biocontrol agents and *C. falcatum* indicated the development of abnormal forms in the *C. falcatum* mycelium. There were alteration and distortion of the hyphal cell wall resulting in the formation of swelling, curling, branching and ultimately lysed or empty tube like structure. Someya and Kataoka (1999) have shown the development of such abnormal forms in Cyclamen pathogen *Fusarium oxysporum* and *Rhizoctonia solani* by an isolate of *Serratia marscens*. Ultimately loss of cytoplasmic content and cell lysis was responsible for its death. In a prolonged interaction of pathogen and biocontrol agent, the cells of *Serratia marscens* were seen in the empty tube like mycelium of *C. falcatum*. Nautiyal *et al.* (2006) have demonstrated a similar mechanism in the lysis of *Fusarium moniliformis* causing sugarcane wilt by the *Bacillus* isolates from cow milk. The production of extracellular enzymes and bioactive compounds are mainly involved in the lysis of cell wall of phytopathogenic fungi (Glick and Bashan, 1997; Raajmakers *et al.,* 2002). Using light microscopy Huang and Chan (2008) have shown that the chitinase acitivity of *Bacillus elliptica* alone and in synergism with fungicides could inhibit conidial germination of *A. brassicicola, A. longipes* and *C. gloeoporioides*. Viswanathan and Samiyappan (1999 a,b) have also demonstrated the early and increased expression of peroxidase and chitinase enzymes by fluorescent pseudomonas probably mediated induced systemic resistance in sugarcane and resulted in significant disease suppression

Biopriming plants with plant growth promoting microorganisms (PGPM) can also provide systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal, bacterial, and viral origin and in some instances even damage caused by insects and nematodes can be reduced after application of PGPM. Manifestation of ISR is dependent on the combination of host plant and bacterial strain. PGPM-elicited ISR was first observed on carnation (*Dianthus caryophillus*) with reduced susceptibility to wilt caused by *Fusarium* sp. and on cucumber (*Cucumis sativus*) with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare*. Some other similar examples where ISR was triggered by biological agents are: *P. fluorescens* EP1 against red rot caused by *Colletotrichum falcatum* on sugarcane, *Burkholderia phytofirmans* PsJN against *Botrytis cinerea* on grapevine and *Verticllium dahliae* on tomato, *P. denitrificans* 1-15 and *P. putida* 5-48 against *Ceratocystis fagacearum* on oak, *P. fluorescens* 63-28 against *F. oxysporum* f. sp. *radicis-lycopersici* on tomato and *Pythium ultimum* and *F. oxysporum* f. sp. *pisi* on pea roots and *Bacilluspumilus* SE34 against *F. oxysporum* f. sp. *pisi* on pea roots and *F. oxysporum* f. sp. *vasinfectum* on cotton roots. *Trichoderma viride* isolated from cowpea phylloplane hyperasitised the mycelium of *Colletotrichum truncatum*, causal agent of brown blotch disease of cowpea *in vitro*. *T. viride* treatment in the form of a seed dip in a spore suspension and soil drenching with a spore suspension were very effective in reducing infection from brown blotch infected seeds.

Presence of high population of these biocontrol agents as estimated, using intrinsic antibiotic/drug resistant marker, on different plant parts at harvest stage (approximately after 8 months) clearly indicate that these microbial forms have well colonized the sugarcane epiphytic plane and are habituated of the sugarcane ecosystem. Mercier and Lindow (2000) have shown the role and specificity of leaf sugars in colonization of plants by bacterial epiphytes. Plants with variable sugar exudation pattern vary in their epiphytic microbial population carrying capacity as pea and corn which had the lowest amount of leaf surface sugars had the lowest counts of epiphytic microflora compared to beans and tomato, which harbored high amounts of sugars. The proportion of antagonistic microflora in whole epiphytic

communities was higher in the resistant clones and the hybrid than in the susceptible clones, with the microflora having a more restrictive effect on the development of the pathogen.

To sum up, irrespective of the mechanisms underlying interactions among the plant, saprophytic epiphytes, and the pathogen, several epiphytic bacterial and fungal isolates of sugarcane were able to check the red rot pathogen, *C. falcatum*, both under *in vitro* and in vivo condition. Further, detailed study on their inoculation effects in field trials both on disease management and in turn on plant growth and productivity shall make these bioagents be part of integrated disease management (IDM) of red rot in sugarcane, which is the prime concern for sugarcane growing farmers in subtropics.

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**Research Article**

# Management of pigeonpea wilt caused by *Fusarium udum* Butler through integrated approaches

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**ABSTRACT**: Integrated Disease Management (IDM) approach was carried out to combat pigeonpea wilt with a combination of fungicides, bio agents, organic amendments and different cropping systems in *kharif* seasons of four years *viz*., 2006, 2007, 2008 and 2009. Based on the performance, four treatments were identified as best practices for the management of pigeonpea wilt *viz*., carbendazim seed treatment @ 2 g/kg of seeds + *Trichoderma viride* @ 2.5 kg/ha in FYM @ 50 kg / ha recorded lowest mean wilt incidence of 11.38 per cent with highest mean yield of 969.18 kg/ha, followed by carbendazim seed treatment @ 2g/Kg of seeds + $ZnSo_4$ @ 25 kg/ha which recorded mean wilt incidence of 11.75 per cent with mean yield 951.25 kg/ha. However, *Trichoderma viride* seed treatment @ 5g/kg of seeds + *Trichoderma viride* @ 2.5 kg/ha in FYM @ 50 kg/ha which recorded mean wilt incidence of 11.97 per cent and yield of 929.63 kg/ha and pigeonpea intercrop with sorghum @ 1:1 recorded mean wilt incidence of 17.62 per cent and yield of 632.18 kg/ha were also found effective in controlling wilt incidence and increasing yield compared to untreated control which recorded highest wilt incidence of 53.04 per cent and lowest yield (314.95 kg/ha).

**KEY WORDS**: Pigeonpea, wilt, *Fusarium udum,* fungicide, bio-agent

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## INTRODUCTION

Pigeonpea *(Cajanus cajan* (L. Mill) is one of the most important pulse crops in the semi-arid tropics. The largest producer of pigeonpea in the world is India, where it is widely cultivated with minima1 input of nutrients and pest management measures. The main constraints in boosting the yield of the crop, are susceptibility to diseases and insects. Pigeonpea is known to be affected by more than hundred pathogens (Nene *et al*., 1989a). *Fusarium* wilt (*Fusarium udum* Butler) is an important soil borne disease, which causes significant yield losses in susceptible cultivars throughout the pigeonpea growing areas. (Reddy *et al.,* 1990). The pathogen is primarily a soil inhabitant; hence controlling the disease is very difficult. Application of carbendazim has been successful in controlling the disease, but to a limited extent and also it is not economical. Bio-control approaches have been initiated by using antagonistic microorganisms to combat the wilt disease in pigeonpea. Many control measures have been suggested (Sharma and Nene, 1990; Mishra, 1992; Whitehead, 1998; Chaudhary and Kumar, 1999), but, cost-effective options for the management of these pests, especially under rain-fed conditions, have not been developed. Therefore, an attempt was made to provide inexpensive and effective control measures. Studies were conducted to evaluate bio-pesticides, bio-agents, resistant varieties and chemicals as seed treatments. Keeping this in view, present investigations were envisaged with the development of integrated management approaches for pigeonpea wilt disease.

## MATERIAL AND METHODS

### Field experimental details

Field experiment was conducted at AICRP on pigeonpea, ZARS, GKVK, Bangalore under *Fusarium* wilt sick plot, during *Kharif* 2006, 2007, 2008 and 2009 with a combination of fungicides, bio agents, organic amendments and different cropping systems (Table 1, 2, 3 and 4).

**Experimental details**

| | | |
|---|---|---|
| Location | : | AICRP on pigeonpea, ZARS,GKVK, Bangalore |
| Season | : | *Kharif,* 2006, 2007, 2008 and 2009 |
| Variety | : | TTB-7 |
| Plot size | : | 3.5 x 3 sq.m |
| Treatments | : | 15 |
| Replications | : | 3 |

Periodically observations were recorded and mean wilt incidence was calculated as year wise treatments.

$$\text{Wilt incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants examined}} \times 100$$

**Mass multiplication of *Trichoderma viride***

Present investigation was undertaken for mass multiplication of *T. viride*. Giant culture of antagonist was prepared in the proportion of 95:5 w/w sand, maize meal mixture and moistened with sterile water to 20 per cent of volume in order to get maximum inoculants. The mixture was filled to autoclavable poly propylene bags and the bags were sealed by using rubber band and autoclaved at 15 lbs pressure at 121.6°C for 20 minutes for two consecutive days. After autoclaving, 8–10 mycelial discs of 5 mm were cut from the margin of actively growing fungal antagonist and transferred aseptically to the polypropylene bags and were incubated at 28 ± 1°C for 15 days. The bags were carefully shaken periodically in order to obtain uniform growth mycelia.

**Mass multiplication of bacterial antagonists**

Bacterial antagonists such as *Bacillus subtilis* and *Pseudomonas fluorescens* were multiplied on nutrient broth as per the procedure given by Sivamani and Gnanamanickam, (1988).

**RESULTS AND DISCUSSION**

**Integrated approaches for wilt management during *Kharif* 2006**

Results of integrated disease management of pigeonpea wilt were recorded during *Kharif* season, 2006 by employing twenty treatments ( Table 1). Among the treatments employed, combination of seed treatment of carbendazim @ 2 g/kg of seeds + soil application of *T. viride, B. subtilis* + *P. fluorescens* each @ 2.5 kg/ha in FYM @ 50 kg/ha recorded significantly lowest wilt incidence of 5.32 per cent with highest yield of 1473 kg/ha, followed by combination of carbendazim seed treatment @ 2 g/kg of seeds + soil application of *T. viride* @ 2.5 kg/ha in FYM @ 50 kg/ha recorded wilt incidence of 9.30 per cent and yield of 1398 kg/ha, seed treatment of carbendazim @ 2 g/kg of seeds + soil application of $ZnSO_4$ @ 25 kg/ha., recorded wilt incidence of 9.37 per cent and yield of 1376 kg/ha. Among, the intercrops, pigeonpea intercropped with sorghum @ 1:1 recorded significantly less wilt incidence of 9.44 per cent and yield of 1213 kg/ha; followed by pigeonpea intercropped with marigold @ 1:1, which recorded wilt incidence of 9.55 per cent with yield of 1173 kg/ha; while the untreated control showed highest wilt incidence of 62.20 per cent with lowest yield of 554 kg/ha.

**Integrated approaches for wilt management during *Kharif,* 2007**

During *Kharif* season 2007, 15 treatments were imposed along with the effective treatments of the previous year (Kharif, 2006) (Table 2). Among the 15 treatments employed for the disease management, combination carbendazim seed treatment @ 2 g/kg of seeds + soil application of *P. fluorescens* (2.5 kg/ha) + *T. viride* @ 2.5 kg/ha in FYM @ 50 kg/ha., recorded lowest wilt incidence of 9.18 per cent with highest yield of 933.33 kg/ha, followed by carbendazim seed treatment @ 2 g/kg of seeds + *T. viride* soil application @ 2.5 kg/ha in FYM @ 50 kg/ha., recorded wilt incidence of 10.27 per cent with yield of 910.80 kg/ha, seed treatment of Carbendazim 2 g/kg of seeds + $ZnSO_4$ @ 25 kg/ha soil application recorded wilt incidence of 10.45 per cent with a seed yield of 796.19 kg/ha and seed treatment of *T. viride* @ 5 g/kg of seeds + *T. viride* @ 2.5 kg/ha in FYM @ 50 kg/ha soil application recorded wilt incidence of 10.84 per cent with yield of 928.89 kg/ha. Among, the intercrops, pigeonpea mixed crop with sorghum @ 3:1 recorded significantly less wilt incidence of 10.66 per cent and yield of 360.64 kg/ha, whereas untreated control showed wilt incidence of 64.86 per cent with lowest yield of 171.43 kg/ha.

**Integrated approaches for wilt management during *Kharif,* 2008**

Results of integrated pigeonpea wilt disease management conducted during *kharif* season 2009 by imposing 15 treatments are presented in the Table 3. Seed treatment of *T. viride* @ 5 g/kg seeds + *T. viride* @ 2.5 kg/ha in 50 kg FYM / ha recorded lowest wilt incidence of 10.13 per cent and highest yield of 978 kg/ha, followed by *T. viride* seed treatment 5 g/kg of seeds, pigeonpea intercrop with maize @ 1:1 recorded wilt incidence of

**Table 1: Treatments impact under integrated approaches for wilt management during *Kharif* 2006**

| Sl. No. | Treatments | Wilt Incidence (%) | Yield (kg/ha) |
|---|---|---|---|
| 1 | T-1: Carbendazim seed treatment @ 2 g/kg of seeds + carbendazim soil application @ 1g/liter 60 days after sowing | 12.08* | 1086 |
| 2 | T-2: Carbendazim seed treatment @ 2 g/kg of seeds + hexaconazole soil application @ 2ml/liter 60 days after sowing | 22.80 | 860 |
| 3 | T-3: Carbendazim seed treatment @ 2 g/kg of seeds + *T. viride* soil application @ 2.5 kg/ha in FYM @ 50 kg/ha | 9.30 * | 1398** |
| 4 | T-4: Carbendazim seed treatment @ 2 g/kg of seeds + *T. viride* soil application @ 2.5 kg/ha in sheep drops @ 50 kg/ha | 11.51* | 1120 |
| 5 | T-5: Carbendazim seed treatment @ 2 g/kg of seeds + *T. viride* soil application @ 2.5 kg/ha in poultry manure @ 50 kg/ha | 21.97 | 868 |
| 6 | T-6: Carbendazim seed treatment @ 2 g/kg of seeds + *P. fluorescens* soil application | 19.09 | 937 |
| 7 | T-7: Carbendazim seed treatment @ 2 g /kg of seeds + soil application of *T. viride, Bacillus subtilis* + *Pseudomonas fluorescens* each @ 2.5 kg/ha in FYM @ 50 kg/ha | 5.32 * | 1473** |
| 8 | T-8: Carbendazim seed treatment @ 2 g/kg of seeds + soil application of *Rhizobium* @ 5 kg/ha + *B. subtilis, P. fluorescens* each @ 2.5 kg/ha in FYM @ 50 kg/ha | 14.24 | 1060 |
| 9 | T-9: Carbendazim seed treatment @ 2 g/Kg of seeds + *Mycorhiza* soil application @ 5 kg/ha | 27.20 | 846 |
| 10 | T-10: Carbendazim seed treatment @ 2 g/kg of seeds + soil application of Zn $SO_4$ @ 25 kg/ha | 9.37 * | 1376** |
| 11 | T-11: Carbendazim seed treatment @ 2 g/kg of seeds + thiophanate methyl soil application @ 1g/liter 60 days after sowing | 30.41 | 710 |
| 12 | T-12: *T. viride* seed treatment @ 5 g/kg of seeds + *T. viride* soil application @ 2.5 kg/ha in FYM @ 50 kg/ha | 11.77* | 1088 |
| 13 | T-13: *T. viride* seed treatment @ 5 g/kg of seeds +*B. subtilis* soil application @ 2.5 kg/ha in FYM @ 50 kg/ha | 19.35 | 910 |
| 14 | T-14: *B. subtilis* seed treatment @ 5 g/kg of seeds + *B. subtilis* soil application @ 2.5 kg/ha in FYM @ 50 kg/ha | 21.24 | 929 |
| 15 | T-15: Zn $SO_4$ soil application @ 25 kg/ha | 20.28 | 982 |
| 16 | T-16: Pigeonpea inter crop with sorghum @ 1:1 | 9.44 * | 1213** |
| 17 | T-17: Pigeonpea inter crop with marigold @ 1:1 | 9.55 * | 1173 |
| 18 | T-18: Pigeonpea intercrop with *Citronella* grass @ 1:1 | 18.10 | 977 |
| 19 | T-19: Vitavax seed treatment @ 2 g/kg of seeds + vitavax soil application @1g/liter 60 days after sowing | 15.16 | 1003 |
| 20 | T-20: Control | 62.20 | 554 |
| | S.Em $\pm$ | 2.99 | 94.62 |
| | CD ($p = 0.05$) | 8.56 | 270.84 |

**Note: *** T2, T3, T6, T7, T10, and T14 are on par for wilt.

** T1, T2, T3, T4, T5, T6, T7, T13 and T14 are on par for yield.

**Table 2: Treatments impact under integrated approaches for wilt management during *Kharif* 2007**

| Sl. No. | Treatments | Wilt Incidence (%) | Yield (kg/ha) |
|---|---|---|---|
| 1 | T1: *Trichoderma viride* seed treatment @ 5g/kg of seeds | 16.88 | 715.55** |
| 2 | T2: Carbendazim seed treatment @ 2 g/kg of seeds + *T. viride* soil application @ 2.5 kg/ha in FYM @ 50 kg/ha | 10.27* | 910.80** |
| 3 | T3: Carbendazim seed treatment 2 g/kg of seeds+ soil application of *Pseudomonas fluorescens* + *T. viride* @ 2.5 kg/ha in FYM @ 50 kg/ha. | 9.18* | 933.33** |
| 4 | T4: Carbendazim seed treatment 2 g/kg of seeds+$ZnSO_4$ @ 15 kg/ha soil application. | 14.80 | 705.08** |
| 5 | T5: Carbendazim seed treatment 2 g / kg of seeds+$ZnSO_4$ @ 20 kg/ha soil application. | 13.25 | 765.08** |
| 6 | T6: Carbendazim seed treatment 2 g / kg of seeds+$ZnSO_4$ @ 25 kg/ha soil application. | 10.45* | 796.19** |
| 7 | T7: *T. viride* seed treatment @ 5 g/kg of seeds + *T. viride* @ 2.5 kg/ha in FYM @ 50 kg/ha soil application. | 10.84* | 928.89** |
| 8 | T8: Pigeonpea intercrop with sorghum @ 1:1. | 18.58 | 435.24 |
| 9 | T9: Pigeonpea intercrop with sorghum @ 1:2. | 14.37 | 212.07 |
| 10 | T10: Pigeonpea mixed crop with sorghum @ 3:1. | 10.66* | 360.64 |
| 11 | T11: Pigeonpea intercrop with ragi @ 1:1. | 14.63 | 549.52 |
| 12 | T12: Pigeonpea intercrop with maize @ 1:1 | 12.81 | 342.86 |
| 13 | T13: Soil application of neem cake @ 20 kg /3 guntas (667 kg/ha). | 28.39 | 706.67** |
| 14 | T14: *T. viride* soil application @ 2.5 kg/ha in FYM 50 kg /ha. | 11.39* | 752.38** |
| 15 | T15: Control. | 64.86 | 171.43 |
| | S.Em ± | 1.18 | 91.03 |

**Note: *** T2, T3, T6, T7, T10, and T14 are on par for wilt.

** T1, T2, T3, T4, T5, T6, T7, T13 and T14 are on par for yield.

10.47 per cent with 813, 462 kg/ha, respectively. Carbendazim seed treatment 2g/Kg of seeds + *T. viride* @ 2.5 kg /ha in 50 kg FYM / ha recorded wilt incidence of 12.73 per cent with yield of 818 kg/ha, whereas control treatment showed wilt incidence of 32.44 per cent with lowest yield of 356 kg/ha.

**Integrated approaches for wilt management during *Kharif,* 2009**

Results of integrated pigeonpea wilt disease management conducted during *kharif* 2009 by imposing 15 treatments, along with the effective treatments during previous year revealed that (Table 4), seed treatment of carbendazim @ 2g/Kg of seed + soil application of *T. viride* @ 2.5 kg /ha in FYM @ 50 kg / ha recorded significantly lowest wilt incidence of 13.20 per cent and highest yield of 748.70 kg/ha, followed by seed treatment of *T. viride* @ 5g/kg of seed + soil application of *T. viride* @ 2.5 kg /ha in FYM @ 50 kg / ha as recorded wilt incidence of 15.17 per cent with yield of 722.50 kg/ha. Among the intercrops tested, pigeonpea intercroped with sorghum @1:2 recorded significantly lesser wilt incidence of 15.77 per cent and yield of 228.60 kg/ha; followed by pigeonpea mixed crop with sorghum @ 3:1 which recorded wilt incidence of 18.61 per cent with yield of 362.60 kg/ha. While, untreated control showed highest wilt incidence of 52.66 per cent with lowest yield of 178.80 kg/ha.

**Pooled data of effective treatments for management of pigeonpea wilt**

Four effective treatments along with untreated control from four seasons of integrated disease management were analyzed statistically in order to identify best treatments

**Table 3: Treatments impact under integrated approaches for wilt management during *Kharif* 2008**

| Sl. No. | Treatments | Wilt Incidence (%) | Yield (kg/ha) |
|---|---|---|---|
| 1 | T-1: *Trichoderma viride* seed treatment 5 g/kg of seeds | 10.47 | 813 |
| 2 | T-2: Carbendazim seed treatment 2 g/kg of seeds + *T. viride* @ 2.5 kg/ha in 50 kg FYM / ha soil application | 12.73 | 818 |
| 3 | T-3: Carbendazim seed treatment 2 g/kg of seeds + *Pseudomonas fluroscens* + *T. viride* @ 2.5 kg/ha in 50 kg FYM / ha soil application | 18.09 | 516 |
| 4 | T-4: Carbendazim seed treatment 2 g/kg of seeds + $ZnSO_4$ @ 15 kg/ha soil application | 19.99 | 540 |
| 5 | T-5: Carbendazim seed treatment 2 g/kg of seeds + $ZnSO_4$ @ 20 kg/ha soil application | 16.52 | 869 |
| 6 | T-6: Carbendazim seed treatment 2 g/kg of seeds + $ZnSO_4$ @ 25 kg/ha soil application | 13.15 | 882 |
| 7 | T-7: *T. viride* seed treatment @5 g/kg seeds + *T. viride* @ 2.5 kg/ha in 50 kg FYM / ha soil application | 10.13 | 978 |
| 8 | T-8: Pigeonpea intercrop with sorghum @ 1:1 | 20.30 | 405 |
| 9 | T-9: Pigeonpea intercrop with sorghum @ 1:2 | 19.62 | 450 |
| 10 | T-10: Pigeonpea mixed crop with sorghum @ 3:1 | 14.40 | 180 |
| 11 | T-11: Pigeonpea intercrop with ragi @ 2:1. | 15.66 | 570 |
| 12 | T-12: Pigeonpea intercrop with maize @ 1:1 | 10.74 | 462 |
| 13 | T-13: *P. f.* seed treatment @ 5 g/kg seed+ *P.f* @2.5 kg in 50 kg FYM / ha soil application | 22.71 | 640 |
| 14 | T-14: *T. viride* soil application 2.5 kg/ha in FYM 50 kg/ha. | 18.20 | 716 |
| 15 | T-15: Control | 32.44 | 356 |
| | S.Em ± | 3.17 | 41 |
| | CD ($p = 0.05$) | 5.41 | 116 |

for the management of pigeonpea wilt and their yield performance (Table 5). Among the four treatments, Carbendazim seed treatment @ 2 g/kg of seeds + *T. viride* @ 2.5 kg/ha in FYM @ 50 kg/ha recorded lowest mean wilt incidence of 11.38 per cent with highest mean yield of 969.18 kg/ha. Followed by carbendazim seed treatment @ 2 g/kg of seeds + $ZnSO_4$ @ 25 kg/ha which recorded mean wilt incidence of 11.75 per cent with mean yield of 951.25 kg/ha. However, seed treatment of *T. viride* @ 5 g/kg of seeds + *T. viride* @ 2.5 kg/ha in FYM @ 50 kg / ha with mean wilt incidence of 11.97 per cent and yield 929.63 kg/ha and pigeonpea intercrop with sorghum @ 1:1 with mean wilt incidence 17.62 per cent and yield of 632.18 kg/ha were also effective in controlling wilt incidence and increasing yield compared to untreated control which recorded highest mean wilt incidence of 53.04 per cent and lowest yield 314.95 kg/ha. In the present study, systemic fungicide; bio-control agent and FYM application was found the most effective treatment which may be recommended on large scale management of pigeonpea wilt disease.

The results of the present study are in agreement with Ingole *et al.* (2005) who observed a combination of carbendazim + thiophanate (0.15 + 0.10%) was found effective in reducing the *Fusarium* wilt disease. The results of present study received support from Somashekhara *et. al.* (2000), who recorded reduced pathogen population and with 13.3% wilt incidence in *T. viride* amended soil. Similarly, Naik *et al.* (1997) observed a significant reduction in wilt incidence at ICRISAT when sorghum (cv. CSH 9) was intercropped with pigeonpea compared with sole pigeonpea. The reduced wilt incidence in sorghum intercropped with pigeonpea was attributed to fungi-toxic exudates secreted by sorghum roots. Rangaswami and Balasubramanian (1963) observed secretion of hydrocyanic acid by sorghum roots, when spores of *Fusarium*

**Table 4: Treatments impact under integrated approaches for wilt management during *Kharif* 2009**

| Sl. No. | Treatments | Wilt Incidence (%) | Yield (kg/ha) |
|---|---|---|---|
| 1 | T-1: *Trichoderma viride* seed treatment 5 g/kg of seeds. | 20.16 | 602.30 |
| 2 | T-2: Carbendazim seed treatment 2 g/kg of seeds + *T. viride* soil application 2.5 kg/ha in FYM 50 kg/ha | 13.20 | 748.70 |
| 3 | T-3: *T. viride* seed treatment 5 g/kg of seed + *T. viride* 2.5 kg /ha in FYM 50 kg/ha soil application. | 15.17 | 722.50 |
| 4 | T-4: Carbendazim seed treatment 2 g/kg of seeds + $ZnSO_4$ 15 kg/ha soil application. | 18.60 | 654.20 |
| 5 | T-5: Carbendazim seed treatment 2 g/kg of seeds + $ZnSO_4$ 20 kg/ha soil application. | 18.31 | 685.80 |
| 6 | T-6: Carbendazim seed treatment 2 g/kg of seeds + $ZnSO_4$ 25 kg/ha soil application. | 13.88 | 704.00 |
| 7 | T-7: Pigeonpea intercrop with groundnut @ 1:1 | 26.20 | 538.60 |
| 8 | T-8: Pigeonpea intercrop with sorghum @ 1:1 | 22.19 | 474.70 |
| 9 | T-9: Pigeonpea intercrop with sorghum @ 1:2 | 15.77 | 228.60 |
| 10 | T-10: Pigeonpea mixed crop with sorghum @ 3:1 | 18.61 | 362.60 |
| 11 | T-11: Pigeonpea intercrop with castor @ 1:1. | 20.86 | 286.90 |
| 12 | T-12: Pigeonpea intercrop with maize @ 1:1 | 25.60 | 263.60 |
| 13 | T-13: Pigeonpea intercrop with avare @ 1:1 | 31.78 | 308.40 |
| 14 | T-14: *T. viride* soil application 2.5 kg/ha in FYM 50 kg/ha. | 18.19 | 639.60 |
| 15 | T-15: Control | 52.66 | 178.80 |
| | S. Em ± | 3.25 | 44.06 |
| | CD ($p = 0.05$) | 9.47 | 127.30 |

**Table 5: Pooled data of wilt incidence and yield in the best treatments in integrated pigeonpea wilt disease management**

| Treatment | % wilt incidence | | | | | Yield (kg/ ha) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2006 | 2007 | 2008 | 2009 | Mean | 2006 | 2007 | 2008 | 2009 | Mean |
| T1 – Carbendazim ST @ 2 g/kg of seeds + *T. v* @ 2.5 kg/ha in FYM @ 50 kg/ha SA | 9.3 | 10.3 | 12.7 | 13.20 | 11.38 | 1399 | 911 | 818 | 748.70 | 969.18 |
| T2 – Carbendazim ST @ 2 g/kg of seeds + $ZnSO_4$ @ 25 kg/ha SA | 9.4 | 10.5 | 13.2 | 13.88 | 11.75 | 1423 | 796 | 882 | 704.00 | 951.25 |
| T3 – Pigeonpea intercorp with sorghum @ 1:1 | 9.4 | 18.6 | 20.3 | 22.19 | 17.62 | 1214 | 435 | 405 | 474.70 | 632.18 |
| T4 – *T. v* ST @ 5 g/kg of seeds +*T. v* @ 2.5 kg/ha in FYM @ 50 kg/ha SA | 11.8 | 10.8 | 10.1 | 15.17 | 11.97 | 1089 | 929 | 978 | 722.50 | 929.63 |
| T5 – Control | 62.2 | 64.9 | 32.4 | 52.66 | 53.04 | 554 | 171 | 356 | 178.80 | 314.95 |

*moniliforme* treated with sorghum root exudates showed delayed germination. Similar results were obtained by Goudar and Srikant Kulkarni (1998) the observed that seed pelleting with *T. viride* either alone or in combination with carbendazim reduced the plant infection besides enhancing the growth. Effective disease management control by integrated method could be due to the synergistic effect of the fungicide and biocontrol agents on the pathogens (Haider *et al*., 1979). Mahesh *et al*. (2010) showed that based on two years performance of different treatments, four effective treatments were identified as pooled analysis for the management of pigeonpea wilt.

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**Research Article**

# Bio-management of Fusarium wilt disease complex with *Pseudomonas fluorescens* and *Aspergillus niger*

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**ABSTRACT**: Flue Cured Virginia (FCV) tobacco is a major rain-fed crop grown in light soil regions of Karnataka. Fusarium wilt disease complex caused by *Fusarium oxysporum* f. sp. *nicotianae* in association with Root-knot nematodes, *Meloidogyne incognita* is a major threat to the successful production and also for its sustainability in the region. The tobacco crop affected with above malady exhibit stunted growth, severe wilt symptoms, drying of leaves on one side of the plant and with conspicuous galls on the infected roots resulted in heavy yield and quality loss. Fungicides and nematicides are being used against this wilt disease complex with limited efficacy. Extensive use of pesticides of chemical origin especially in higher doses for disaese control has to be avoided due to higher costs and associated hazards to the environment. Replicated trials were conducted with antagonistic bacterium, *Pseudomonas fluorescens* and antagonistic fungi, *Aspergillus niger* singly and in combinations against fusarium wilt disease complex in FCV tobacco under sick field conditions. Results revealed that application of *P. fluorescens* @ 1g/plant in combination with *A. niger* enriched with FYM @ 100 g /plant at the time of planting resulted in 61.0% reduction in fusarium wilt disease incidence at 70 DAT compared to untreated check. There was significant reduction in root knot nematode incidence in terms of RKI (Root-Knot Index) to 1.93 and final soil nematode population to 72.5 as compared to RKI of 3.71 and final soil nematode population of 140 in untreated check. Subsequent increase in total cured leaf and bright grade yield was 1311 kg/ha and 926 kg/ha respectively as compared to 1042 kg/ha and 615 kg/ha respectively in untreated check.

**KEY WORDS**: *Pseudomonas fluorescens*, *Aspergillus niger*, Fusarium wilt complex, *Meloidogyne incognita*, FCV tobacco

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## INTRODUCTION

Flue-Cured Virginia (FCV) tobacco is an important rainfed commercial crop grown in Karnataka light soils with lot of export potentiality. The produce is preferred internationally due to its ideal chemistry with below detectable levels of TSNA (Tobacco specific nitrosamines) compounds. Wilt disease caused by *Fusarium oxysporum* f. sp. *nicotianae* is a major threat to FCV tobacco production under field conditions (Shenoi *et al*., 2004). Moreover, it is widely reported that fusarium wilt disease occurs in association with root-knot nematodes causing wilt disease complex. Plant parasitic nematodes often play a major role in disease interactions. Infection by one pathogen usually alters host response to subsequent infection by another pathogen. Mostly, root decay and necrosis of root-knot nematode infected plants are due to the association of nematodes with numerous pathogenic fungi and bacteria (Powel, 1971). Ramakrishnan *et al.* (2008) reported that root knot nematode, *Meloidogyne incognita* predisposes FCV tobacco crop to wilt disease caused by *F. oxysporum* f. sp. *nicotianae* contributing to significantly reduced yields of FCV tobacco in Karnataka. Similar to wilt disease, root knot nematode, *M. incognita* is also a major limiting factor for the successful production of tobacco, both in nursery and main field (Hussaini, 1983; Ramakrishnan *et al.,* 2001). The conspicuous symptoms of the wilt disease complex of FCV tobacco under field conditions are gradual yellowing, wilting and drying of leaves on one side of the plant. Underground symptoms exhibit infected roots turning black and in many cases with conspicuous galls caused by root knot nematodes. For the management of wilt disease complex in FCV tobacco, application of a fungicide or a nematicide alone will be effective only against the target organisms. Moreover, chemical pesticides proved to be not cost effective and many effective chemicals were withdrawn from the market due to their ill-effects and hazards they pose to environment. Hence, bio-management of wilt disease complex with antagonists is an alternative, cost effective and eco-friendly approach. Keeping in view of

export demand of the tobacco crop, efforts were made to evolve safe and eco-friendly management strategies against wilt disease complex in field crop through the use of farm yard manure enriched bio-agents, *Pseudomonas fluorescens* and *Aspergillus niger* in rational combinations.

## MATERIALS AND METHODS

The field experiments were conducted for two seasons during 2008-09 in kharif at CTRI Research Station, Hunsur in a field sick with the pathogens, *F. oxysporum* and *M. incognita.* The soil type in experimental site is red sandy loam and the mean initial population of infective juveniles of *M. incognita* was 150 second staged infective juveniles/ 100g soil. The *P. fluorescens* was locally isolated, multiplied by using King's B medium and brought into talc formulation with load of 2.5 x $10^8$ cfu $g^{-1}$ and the other fungal antagonist, Kalisena, the commercial formulation of *A. niger* (strain AN 27) obtained from IARI were evaluated either singly and in combinations against *F. oxysporum* complex in FCV tobacco under field conditions. The slurry form of the bio-agent (Kalisena SL) was applied through enriched FYM. The enrichment of FYM with Kalisena SL $20g^{-1}$ was done by heaping the FYM with optimum soil moisture. The heap was turned at regular intervals and allowed for 10 days incubation before application in the field.

The treatment details are as follows, T1-*Pseudomonas fluorescens* (talc formulation) @ 1g/plant at planting, T2 – *Aspergillus niger* enriched FYM @ 100g/plant at planting, T3 – *P. fluorescens* (talc formulation) @ 1g/ plant at planting + *A. niger* enriched FYM @ 100g/plant at planting, T4 – *P. fluorescens* (talc formulation) @ 1g/ plant at planting + *A. niger* enriched FYM @ 100g/plant at 30 DAT, T5 – *P. fluorescens* root dip (5% solution) for six hours before planting, T6 – *P. fluorescens* root dip (5% solution) for six hours before planting + *A. niger* enriched FYM @ 100g/plant at planting and T7 = Untreated check. All the seven treatments were replicated four times in a randomised block design.

*P. fluorescens* in talc formulation and *A. niger* enriched FYM were applied at the time of planting to each plant hole, as root dip and at 30 DAT as per treatment schedule. All other management and cultural practices were followed as recommended (Shenoi, 1998). Data on FCV tobacco yield parameters (green leaf yield, bright grade yield, medium grade yield, low grade yield and total cured leaf yield), Root Knot Index (RKI) at 0-5 scale at the time of final harvest, initial and final soil nematode population per 100g soil and fusarium wilt disease incidence at 60 and 70 DAT were recorded. Pooled data of the two season trials were statistically analysed and critical differences determined (Gomez and Gomez, 1984).

## RESULTS AND DISCUSSION

Pooled results of two seasons data on evaluation of *P. fluorescens* and Kalisena enriched FYM singly and in combinations against fusarium wilt and root-knot nematode disease complex revealed that application of *P. fluorescens* @ 1g/plant in combination with *A. niger* enriched FYM @ 100 g /plant at the time of planting resulted in 71.7 and 61.4 percent decrease in wilt disease incidence at 60 & 70 DAT respectively, compared to untreated check (Table 1). At 70 DAT, wilt disease incidence in untreated plots was 51.5%, whereas in bio-agents treated plots, disease incidence ranged from 19.9 to 39 per cent. Applications of *P. fluorescens* as seedling root dip (5% solution) for six hours before planting reduced the wilt disease incidence to the extent of 24.3 per cent as compared to untreated check. It is clear from the present investigations, soil applications of bio-agents gave significantly better disease control as compared to seedling root-dip applications. Earlier, Shenoi and Sreenivas (2007) indicated that Kalisena SL was effective against soil borne fungal diseases such as damping-off, blight and black shank also significantly increased the healthy transplants count in FCV tobacco nurseries. Barua and Bora (2008) studied the efficacy of *T. harzianum* and *P. fluorescens* against *M. incognita* and *R. solanacearum* complex in brinjal. Brinjal plants when treated with *T. harzianum* and *P. fluorescens* significantly reduced wilt disease incidence and RKI in brinjal. In case of bacterial population in soil the highest reduction was observed in the treatment with *T. harzianum* and *P. fluorescens*.

In the case of root knot nematode incidence, combined application of *P. fluorescens* @ 1g/plant with *A. niger* enriched FYM @ 100 g /plant at the time of planting recorded significantly reduced RKI to 1.93 and final soil nematode population was 72.5 infective juveniles / 100g soil as compared to RKI of 3.71 and final soil nematode population of 140 infective juveniles/100 g soil respectively, in untreated check. But, application of *P. fluorescens* (talc formulation) alone @ 1g/plant at planting and *A. niger* enriched FYM alone @ 100g/plant at planting reduced the RKI and final soil nematode population to the tune of 36.4% and 40.76 and 46.0 per cent respectively, compared to untreated check. Reduction in root knot nematode incidence in terms of RKI and final soil nematode population in bio-agents treated plots ranged from 23.2 to 48.04 per cent and 15.7 to 48.2 per cent respectively, compared to untreated check. Sobita Devi

**Table 1: Effects of bioagents on Fusarium wilt disease incidence and root knot nematode population in FCV tobacco field crop**

| Sl. No. | Treatments | Wilt at 60 DAT | | Wilt at 70DAT | | Root-knot Index (0-5 Scale) at 90 DAT100 | | Nematode population / g. soil | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | B | A | B | A | B | A | B |
| 1 | *Pseudomonas fluorescens* @1g/plant at planting | 19.4 (11.04) | 47.7 | 29.3 (24.0) | 43.1 43.1 | 2.36 2.36 | 36.4 36.4 | 83.0 83.0 | 40.7 40.7 |
| 2 | *Aspergillus niger* enriched FYM@ 100g/ plant at planting | 16.9 (8.43) | 54.4 | (34.35) (34.35) | 30.3 30.3 | 2.36 2.36 | 36.4 36.4 | 75.5 75.5 | 46.0 46.0 |
| 3 | *P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@ 100g/plant at planting | 10.5 (3.32) | 71.7 71.7 | 19.9 (11.55) | 61.4 61.4 | 1.93 1.93 | 48.0 48.0 | 72.5 72.5 | 48.2 48.2 |
| 4 | *P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@100g/plant at 30DAT | 17.5 (9.01) | 52.8 52.8 | 35.9 (34.45) | 30.3 30.3 | 2.14 2.14 | 42.0 42.0 | 90.5 90.5 | 35.3 35.3 |
| 5 | *P. fluorescens* root dip(@ 5% solution) for 6hrs. before planting | 18.8 (10.34) | 49.3 49.3 | 39.0 (39.55) | 4.3 24.3 | 2.85 2.85 | 23.2 23.2 | 118.0 118.0 | 15.7 15.7 |
| 6 | *P. fluorescens* root dip (@ 5% solution) for 6hrs. before planting + *A. niger* enriched FYM@ 100g/plant at planting | 15.9 (7.55) | 57.1 57.1 | 32.1 (28.27) | 37.7 37.7 | 2.55 2.55 | 31.3 31.3 | 120.0 120.0 | 14.2 14.2 |
| 7 | Untreated check | 37.1 | – | 51.5 | – | 3.71 | – | 140.0 | – |
| | | (36.47) | – | (61.28) | – | 3.71 | – | 140.0 | – |
| | S.Em | 1.33 | | 1.72 | | 0.08 | | 0.72 | |
| | CD at ($p = 0.05$) | 3.70 | | 4.78 | | 0.13 | | 2.31 | |
| | CV% | 37.67 | | 27.43 | | 7.98 | | 9.20 | |
| | Seasons mean 2005-06 | 10.99 | | 31.55 | | 2.48 | | 102.3 | |
| | 2006-07 | 27.88 | | 38.0 | | 2.63 | | 97.5 | |
| | S.Em | 1.38 | | 1.80 | | 0.04 | | 1.91 | |
| | CD at ($p = 0.05$) | 4.79 | | 6.24 | | 0.13 | | 6.30 | |
| | CV% | 19.41 | | 14.01 | | 8.64 | | 10.50 | |
| | S x T interaction S.Em | 1.89 | | 2.44 | | 0.11 | | 2.46 | |
| | CD at ($p = 0.05$) | 5.23 | | NS | | NS | | NS | |

A = Incidence (%); B – Percent Incidence over untreated check Figures in parenthesis are arc sine transformed values

and Pandey (2001) also had studied the field application of *P. fluorescens* in chick pea crop against *M. incognita* and *Fusarium oxysporum* f.sp. *ciceri* on chickpea and observed significant reduction in root knot disease incidence in field in terms of reduced gall formation and soil nematode population.

Beside reduction in Fusarium wilt disease and root knot nematode incidence, application of *P. fluorescens* @ 1g/plant in combination with *A. niger* enriched FYM @ 100 g/plant at the time of planting significantly improved FCV tobacco yield parameters under field conditions (Table 2). There was 20.5% increase in total cured leaf yield and 33.6% increase in bright grade out turn compared to untreated check. Application of *P. fluorescens* alone and *A. niger* enriched FYM alone recorded the cured leaf yield of 1215 and 1213 kg/ha respectively. Whereas, combined application of *P. fluorescens* and *A. niger* enriched FYM recorded significantly improved yield of 1311 kg/ha as compared to 1042 kg/ha in untreated check plots. Improvement in total cured leaf yields in plots treated with bio-agents ranged from 13.2 to 20.5% compared to untreated check. Similar increase in plant growth and yield parameters due to application of *P. fluorescens* @ 2.5 kg/ha against root knot nematode in various crop plants were reported

**Table 2: Effects of bioagents on yield parameters of FCV tobacco (Kg ha$^{-1}$)**

| Sl. No. | Treatments | | Yield Parameters (kg/ha) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Green leaf | Low grade | Medium grade | Bright grade | % increase over check | Total cured leaf | % increase over check |
| 1 | *Pseudomonas fluorescens* @1g/plant at planting | | 13235 | 130 | 295 | 791 | 22.3 | 1215 | 14.2 |
| 2 | *Aspergillus niger* enriched FYM @100g/plant at planting | | 13587 | 156 | 276 | 801 | 23.2 | 1213 | 14.1 |
| 3 | *P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@100g/plant at planting | | 13299 | 142 | 244 | 926 | 33.6 | 1311 | 20.5 |
| 4 | *P. fluorescens* @1g/plant at planting + *A. niger* enriched FYM@100g/plant at 30DAT | | 13681 | 138 | 281 | 782 | 21.4 | 1200 | 13.2 |
| 5 | *P. fluorescens* root dip @5% solution for 6hrs. before planting | | 12728 | 144 | 186 | 698 | 11.9 | 1028 | – |
| 6 | *P. fluorescens* rot dip (@ 5% solution for 6hrs. before planting + *A. niger* enriched FYM@ 100g/plant at planting | | 12712 | 186 | 239 | 782 | 21.4 | 1207 | 13.7 |
| 7 | Untreated check | | 12436 | 229 | 202 | 615 | – | 1042 | – |
| | | S.Em | 330 | 11.47 | 11.82 | 14.0 | – | 18.28 | – |
| | | CD ($p = 0.05$) | 1040 | 31.80 | 32.75 | 38.81 | – | 63.26 | – |
| | | CV% | 12 | 21.3 | 15.72 | 5.43 | – | 8.22 | – |
| | Seasons mean | 2005-2006 | 13789 | 164 | 246 | 828 | – | 1238 | – |
| | | 2006-2007 | 14404 | 157 | 246 | 713 | – | 1115 | – |
| | | S.Em | 300 | 6.46 | 7.30 | 7.91 | – | 18.28 | – |
| | | CD ($p = 0.05$) | 1040 | NS | NS | 27.36 | – | 63.26 | – |
| | | CV% | 7 | 20.22 | 13.58 | 5.14 | – | 4.76 | – |
| | S x T interaction | S.Em | 466 | 16.23 | 16.71 | 19.80 | – | 28.03 | – |
| | | CD ($p = 0.05$) | NS | NS | NS | NS | – | NS | – |

**Table 3: Cost economics of Bio management of Fusarium wilt disease complex per ha**

| Particulars | Bio management by *Pseudomonas fluorescens* @1g/plant at planting + *Aspergillus niger* enriched FYM@100g / plant at planting | Check (Un treated) |
|---|---|---|
| Cost of Cultivation (Rs. /-) | 75000 | 75000 |
| Crop protection measure for wilt disease complex with application cost | 6500 | – |
| Yield (kg/ha) | 1311 | 1042 |
| Bright Grade (kg/ha) | 926 (106628.9)* | 615 (70817.25)* |
| Medium Grade (kg/ha) | 244 (19654.2) | 202 (16271.1) |
| Low Grade (kg/ha) | 142 (7206.5) | 229 (11621.75) |
| Gross returns (Rs. /-) | 133489.6 | 98710.1 |
| Net Profit (Rs. /-) | 51989.6 | 23710.1 |
| Additional income over check | 28279.5 | – |
| ICBR | 1:4.4 | – |

Cost of FYM = Rs. 800 / Tone
Cost of Bio agents = Rs. 5200/-
Cost of Bio agent application = Rs. 500/-
Market price of tobacco on average (Rs./-) (Bright = 115.15, Medium 80.55 and Low = 50.75)
* Figures in parenthesis are amount realised at the market

earlier (Santhi and Sivakumar, 1995; Kavitha *et al*., 2007). Mani *et al*. (1998) also reported significant inhibition of potato cyst nematode in potato crop in Nilgiris with *P. fluorescens* application under field conditions. Ramakrishnan *et al*. (2009) had evaluated *P. fluorescens* under FCV tobacco nursery conditions against root knot nematodes and obtained reduced incidence of root knot index and final soil nematode population and also subsequent increase in root knot free and healthy transplants count of FCV tobacco seedlings. It is concluded from the study that the schedule involving *P. fluorescens* @1g/plant + *A. niger* enriched FYM@100g/plant at planting resulted in cost effective management of Fusarium wilt – root-knot disease complex with a better ICBR of 1:4.4 (Table 3).

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**Research Note**

# Mud wasp, *Sceliphron madraspatanum* (Fabricius) (Hymenoptera: Sphecidae): A threat or nature's regulation of spider fauna in the vegetable agroecosystem?

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**ABSTRACT**: The biology and behaviour of mud wasp, *Sceliphron madraspatanum* (Fabricius) and its possible role in insect pest management were studied under vegetable agroecosystem. The gravid female lays a single egg (2.75 ± 0.33 mm in length) in a mud chamber that is provisioned almost exclusively with orb-weaver spiders, wolf spiders and jumping spiders available in the vegetable agroecosystem. Total numbers of spiders provisioned in each cell was negatively correlated with their mean body weight. However, the wasps avoided provisioning of lynx spider, the most predominant spider in this ecosystem, due to its short and reduced abdomen and also the presence of large, strong and erect setae over its body. In the agroecosystem, mud wasp was found to constitute the third trophic level in the food chain comprising of the vegetable crops – insect pests – spiders – *S. madraspatanum* and thereby their role could be detrimental for the pest management.

**KEY WORDS**: *Sceliphron madraspatanum,* biology, prey spiders, tritrophic interactions

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The genus *Sceliphron* is a cosmopolitan wasp comprising of more than thirty species. They are commonly referred to as mud wasp or mud daubers. *Sceliphron madraspatanum* (Fabricius) has been reported widely from the Indian sub-continent. Billberg (1820) first reported this species as *Pelopaeus madraspatanus* from southern Malabar, India. Later, this species was also recorded from West Bengal and Nepal by Paiva (1907). Nurse (1914) documented it from Assam, India. More recently, the medicinal and aesthetic values of this species was reported from Panch Pargana area of Jharkhand, India (Kumari and Kumar, 2009). This wasp is locally called as Kunkal or Kumhar poka and its mud nest is used for folk medicine. The paste of the mud nest is applied on fore-head as a cure against migraine while for dyspepsia and frequent thirst, the paste is applied on the navel. These solitary wasps are strong fliers, generally not aggressive and do not sting unless mishandled or disturbed. However, there has been no information about its role in agriculture. Therefore, an effort was made to study the role of this mud wasp in agriculture more specifically in pest management, if any.

**Occurrence and feeding behaviour of the mud wasps**

During June to September, 2010 many such mud nests of *S. madraspatanum* were observed in and around the farm of Indian Institute of Vegetable Research, Varanasi, Uttar Pradesh, India. An attempt was made to study their biology and behaviour and their role as predators of spiders in the vegetable ecosystem. Numerous nests of these wasps were observed, mainly in shaded places of the farm. Interestingly, very few mud nests were recorded from the border trees. The adult female was observed to make around 10–13 round trips to construct each cell (2.38±0.19 cm x 0.975±0.083 cm) carrying soft mud collected mainly from frequently irrigated fields or nearby irrigation channels with their mandibles and fore legs. Initially, a single cell was constructed to which cells were added one by one on each side. Commonly each nest consists of 8-13 such cells. The wasp usually took about one to two days to construct a single cell and incompleted cells were closed temporarily before sunset and opened the very next day. Freeman and Johnston (1978) also observed that incompletely stored cells of *S. assimile* were closed with an externally concave

lamella of mud at the onset of rain or at 16.00 – 17.00 hours E.S.T. and never reopened the same day.

The identity of mud wasp encountered in the present study was confirmed as *S. madraspatanum* from the taxonomists of Division of Entomology, Indian Agricultural Research Institute, New Delhi and diagnosed by the following taxonomic characters, *viz*., body length 17 ± 3.5 mm; body black with yellow pattern on thorax; narrow slender waist yellow in colour; scape below except base, top of collar, tegulae, part of post scutellum, petiole, nearly apical half of fore and mid femora and basal half of hind femora, whole of fore and mid tibiae and basal two thirds of hind tibiae, first tarsomere of hind legs except base and apex above, and trochanters yellow; wings hyaline with a brownish tint (Fig. 1).

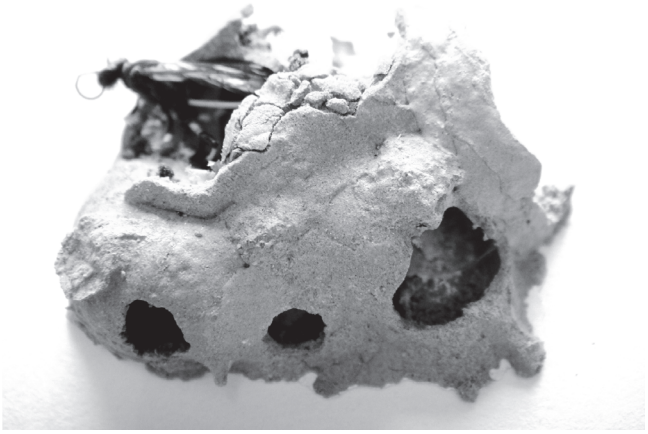
Critical observations revealed that female wasps practised mass provisioning the food substrate for their developing young ones. Each cell was provisioned with 9-13 dead or moribund immobile spiders gathered from the nearby vegetable fields. However, it was also observed that total numbers of spiders provisioned in each cell was negatively correlated with their mean body weight. Thereafter, the gravid female laid whitish grey eggs singly in each cell. The eggs measured 2.75 ± 0.33 mm in length and glued to the abdominal portion of one large spider and the cell was sealed with mud. The full grown wasp larva was about 8.5 ± 0.62 mm long, creamy white in colour with a peculiar hammer shaped head. The larva fixed its head to the abdomen of the spider's body and started feeding. Later, on the other body parts *viz*., legs, thorax etc., were devoured.

**Composition of food substrate in mud wasp nests**

The spiders collected from the mud wasp nests could be identified as *Neoscona odites* (Simon) (Araneidae) commonly known as orb-weaver spiders; *Lycosa* spp. (Lycosidae) or wolf spiders; and *Marpissa* spp. (Salticidae) or jumping spiders all of which were found abundantly in the vegetable ecosystem. Landes *et al*. (1987) from Missouri, USA reported that Araneidae was the most numerous spider family and exhibited a pattern of seasonal increase in abundance as prey of mud-dauber, *Chalybion californicum*. Interestingly, another predominant spider, *Oxyopes lineatipes* (Lynx spider) abundant in the okra and brinjal fields was not observed in the nest of the mud wasp. The probable reason for its absence can be linked to its morphology. It was observed that the abdominal portion of these spiders were proportionately smaller than the thorax which is the largest part of its body. Besides, the setae present on the body of this lynx spider were large, strong and erect which might keep its predators at bay. Since, it was observed that the *Sceliphron* grub preferred the swollen abdomen of its host, lynx spiders with its small sized abdomen and strong and erect setae over its body could be the probable reason for its non-preference as a suitable host by the mud wasp. Similar observation was also confirmed by Elgar and Jebb, (1999) that mud-dauber wasp, *S. laetum* generally avoided the provisioning of *Gasteracantha* spiders due to its hard integument that restricted the penetration by its larvae.

In the vegetable agroecosystem, *S. madraspatanum* was found to constitute the third trophic level in the food chain comprising of the vegetable crops (*i.e.,* okra / brinjal) – insect pests (jassids / whitefly / neonate larvae

**A**



**B**

*Fig. 1: A. Adult mud wasp (Sceliphron madraspatanum F.) and B. Nest of mud wasp with emerging holes.*

of fruit borer) spiders – *S. madraspatanum*. In natural biological control, spiders play an important role in controlling many insect pests. In a parallel study it was estimated that in unsprayed brinjal and okra plots the spider populations were 2.90 and 2.54 per plant, respectively. Further, a single spider can prey about 53 to 86 jassids per day under confined condition. Our present observation also is in conformity with the observation that a single wolf spider can eat up to 20 brown plant hoppers [*Nilaparvata lugens* (Stål)] adults a day in the rice ecosystem (Rajan and Shukla, 1996). Sahu *et al*. (1996) studied the feeding potential of wolf spider (*Lycosa pseudoannulata*) in rice ecosystem and confirmed that they preyed more on green leaf hopper (43.3%) followed by rice hispa (6.67%), stem borers (3.3%) and rice leaf folder (3.3%). In the cotton ecosystem, a spider consumed cotton aphid, *Aphis gossypii* Glover, cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) through out its growing period (Nyffeler *et al.,* 1989) and consumed 5 or 6 worms per day, halting infestation early. Another American study revealed that wolf spiders, jumping spiders, and crab spiders, have lessened pest damage up to 70% over vegetable fields (Rajan and Shukla, 1996). There is growing awareness about conservation of these tiny creatures. China had already adopted extensive use of spiders to control pests in cotton and rice (Sunderland, 1999).

Literature indicated that there are very few insects which devour spiders for their survival. So far, preying mantis, flies (Acroceridae), daddy long-leg (*Pholcus phalangioides*) in rice ecosystem (Jackson, and Brssington 1987) and spider wasp (Pompilidae) have been reported to feed on spiders. But, the present studies concluded that the mud wasp, *S. madraspatanum* is a predator of spiders.

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**Research Note**

# Biology of *Cardiochiles nigricollis* Cameron, a larval endo-parasitoid of *Cnaphalocrocis medinalis* (Guen.) and *Marasmia exigua* Butler

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**ABSTRACT**: The parasitoid *Cardiochiles nigricollis* Cameron usually parasitize the fourth instar larvae of *Cnaphalocrocis medinalis* and *Marasmia exigua*. Four instars were identified during the development of the parasitoid. The first instar larva is un-segmented and slightly curved. In the second instar segmentation appeared and tracheal system could be observed faintly. In the third instar head was well demarcated and the larva further grew in size. Six to eight days after egg laying the full grown larva came out of the host. The average pupal period lasted for 5.3 days in females and 6.7 days in males in the laboratory. Average incubation period, larval duration and pupal duration were 1.2 days, 10 days and 6.7 days respectively. *C. nigricollis* entered diapause at pupal stage and it started from the end of August and increased steadily till the first week of December when the entire population entered diapause. This phenomenon was observed only during wet season. On an average male emerged after 213 days and females after 224 days from the diapausing pupae. The gradual increase in the number of *C. nigricollis* entering diapause in the field population could possibly be due to occurrence of a heterogenous population of *C. nigricollis* in the field which responded gradually to fall in atmospheric temperature. *Brachymeria* sp., *Orgilus* sp., *Trichomalopsis* (*Eupteromalus*) *parnarae* Gahan and *Elasmus* sp. were reared as hyper-parasitoids on pupae of *C. nigricollis*.

**KEY WORDS**: *Cardiochiles nigrocollis, Cnaphlocrocis medinalis, Marasmia exigua, biology, rice*

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*Cardiochiles* sp. was recorded on the rice leaf folder, *Cnaphalocrocis medinalis* (Guen.) as a larval parasitoid at Siripur, Orissa (Rao *et al.*, 1970) and China (Hu and Wu, 1987). Ayyar (1927) recorded *Cardiochiles* sp. on the same host in India. *C. philippinensis* Ashm. was recorded on *C. medinalis* in the Philippines (Barrion *et al.,* 1979). The genus *Cardiochiles* has been reported on *Hymenia recurvalis* F. (Singh and Prasad, 1970), *Heliothis virescens* (Fabricius) (Martin *et al.,* 1982), *Neomarasmia suspicalis* Wlk. (Banarjee and David, 1982). *Cardiochiles* sp. (near C. *philippinensis*) has been reported as a common parasitoid of *Cnaphalocrocis medinalis,* (Gurr *et al.*, 2012). *C. philippinensis* Ashm. was reported to cause 3.4% larval parasitism at Aruppukottai, Tamil Nadu, India (Baby Rani *et al*., 2007). Life history of *C. philippinensis* was studied by Yu-jie *et al*. (1991) in the Philippines. *C. philippinensis* completed its life cycle in 22.7 days. Total number of larvae parasitized by *C. philippinensis* was 16.7±0.2. Information on the biology and activity of *C. nigricollis* on *C. medinalis* is not available. Hence, an attempt was made to study the reproductive biology and bionomics of *C. nigricollis* Cameron on *C. medinalis*, the dominant leaffolder species on rice.

*C. nigricollis* was reared from field collected *C. medinalis* larvae maintained on cut rice leaves in glass vials in the laboratory. Adult parasitoids from the parasitized larvae and field collected adults were used in the study. Larvae of *C. medinalis, M. exigua*, and pupae of *C. nigricollis* were collected from the field and reared in the laboratory to study the extent of parasitism by *C. nigricollis* in the field. Besides, a pair of freshly emerged (1 male + 1 female) *C. nigricollis* adults were released on potted rice plants (variety TN 1) with ten third instar *C. medinalis* larvae and covered with mylar cages to study its biology in the greenhouse. The potted plants with ten leaffolder larvae were replaced everyday till the female parasitoid was dead. Ten such pots were used in a batch in completely randomized design. Parasitized larvae were dissected periodically and examined under stereoscopic binocular microscope to study the development of the parasitoid inside the body of the host larva.

Two days old males and females mated in the laboratory. Mating lasted for 2-3 minutes. Egg laying was observed on the second day after mating. Field observations indicated that the female hovered above the infested rice plant and located the folded leaf. Later, it perched directly on the leaf fold and probed the roll with its ovipositor. While this process continued, the larva also moved inside the fold and at times fell into the water below. After locating the larva inside the roll, the female inserted its ovipositor through the leaf and pricked the host larva.

One to three parasitoid larvae per host larva were observed developing in the body cavity corresponding to the last four segments of the host larva. However, only one fully developed parasitoid larva emerged from one host larva indicating cannibalism or competition at early stages of development. Tillman and Mullinix (2003) studied the host searching and ovipositional behaviour of *C. nigriceps* Viereck in *Heliothis virescens* and reported that only one egg was deposited in the host. Usually, third and fourth instar larva of *C. medinalis* and *M. exigua* were parasitized by *C. nigricollis*. Four instars were identified during the development of the parasitoid (Table 1). The first instar larva is un-segmented and slightly curved. In the second instar segmentation appeared and tracheal system could be observed faintly. In the third instar head was well demarcated and the larva further grew in size. Six to eight days after egg laying the full-grown parasitoid larva emerged out of the host. The host larva continued to look normal and fed regularly until the full-grown *C. nigricollis* larva came out. The full-grown larva looked pale yellow with prominent fat granules and spiracles. Soon after emergence, the larva moved about on the leaf and spun a translucent, off-white oval cocoon on leaf surface or on the walls of the rearing tube in the laboratory. Examination of field samples revealed that majority of the cocoons of *C. nigricollis* (88.2%) was formed bottom of the rice plant.

**Table 1: Life history of *Cardiochiles nigricollis* Cameron on *Cnaphalocrocis medinalis***

| Attribute | Range | Average | SD |
|---|---|---|---|
| Mating (minute) | 2-3 | 2.5 | 0.4 |
| Age at mating (days) | | | |
| Male | 2-3 | 2.5 | 0.4 |
| Female | 2-3 | 2.8 | 0.4 |
| Oviposition by mated female (No. of eggs/female) | | | |
| Pre-oviposition period (hrs.) | 12-18 | 14.5 | 1.5 |
| Oviposition period (days) | 3-5 | 3.7 | 0.9 |
| Eggs/Host larva | 1-4 | 2.6 | 1.1 |
| No. host larva parasitized | 5-12 | 6.5 | 2.7 |
| Incubation period (days) | 1-1.5 | 1.2 | 0.2 |
| Larval duration (days) | | | |
| 1st instar | 1.5-2.5 | 2.0 | 0.5 |
| 2nd instar | 2.0-3.5 | 2.5 | 0.7 |
| 3rd instar | 2.5-3.5 | 3.0 | 0.5 |
| 4th instar | 2.0-3.0 | 2.5 | 0.5 |
| Pupal duration (days) Male | 4-7 | 5.3 | 1.2 |
| Female | 6-8 | 6.7 | 0.9 |
| Diapause duration (days) | | | |
| Male | 191-223 | 213.0 | 13.2 |
| Female | 211-265 | 222.4 | 23.8 |
| Longevity (days) | | | |
| Male | 2-4 | 3.7 | 0.8 |
| Female | 2-7 | 4.5 | 2.0 |
| Total life cycle (days) | | | |
| Male | 13-21 | 15.3 | 3.34 |
| Female | 15-22 | 16.5 | 3.08 |

The average pupal period lasted for 5.3 days in females and 6.7 days in males in the laboratory. In a few cases, the pupal period was extended and adult parasitoids emerged after 191 to 265 days of cocoon formation indicating occurrence of diapause. Males emerged after an average of 213 days and females after 224.4 days of formation of pupa of *C. nigricollis*. *C. nigricollis* adults emerging from such cocoons behaved like normal parasitoids in the laboratory. Such behaviour of the parasitoid was observed both in the field-collected samples and in the laboratory culture. Population of *C. nigricollis* enterered diapause from the end of August and continued till the first week of December when the entire population entered diapause (Table 2). This phenomenon was observed only during wet season (kharif).

**Table 2: Progress of diapause in *Cardiochiles nigricollis* in the field during kharif**

| Month | % of larvae entering diapause |
|---|---|
| August | 5.55 |
| September | 16.66 |
| October | 25.00 |
| November | 94.11 |
| December | 100.00 |

When fed on 10% aqueous honey solution, longevity of unmated male and female *C. nigricollis* was 3.50 and 4.38 day respectively. The sex ratio was even during warmer months but, turned out in favour of females, 1:1.8 during September and October. *C. nigricollis* parasitized larvae of *C. medinalis* and *M. exigua* both during kharif and rabi but more in the former season. Its peak activity (35.0 to 48.7% parasitism) was observed during July and August in kharif season. During rabi season maximum parasitism due to *C. nigricollis* (8.0 to 18.0%) was observed during March and April. Among the three species of rice leaf folders prevalent in Odisha, *C. nigricollis* attacked only *C. medinalis* and *M. exigua* but, not *Brachmia arotraea* Meyer.

During the course of study, *Brachymeria* sp., *Orgilus* sp., *Trichomalopsis* (*Eupteromalus*) *parnarae* Gahan and *Elasmus* sp. were reared as pupal hyper-parasitoids of *C. nigricollis*. The cumulative hyper-parasitism ranged from 0.8 to 2.5%.

Singh and Prasad (1970) observed that four eggs were laid by *Cardiochiles hymeniae* in its host *Hymenia recurvalis,* but only one parasitoid developed. In the present study also up to three developing larvae of *C. nigricollis* were observed in the body cavity of the host larva but, only one parasitoid developed ultimately. They also observed that field population of *C. hyminae* disappeared after first week of September, despite the presence of its host population and concluded that *H. recurvalis* was an alternative host for the parasitoid. However, in the present study field population of *C. nigricollis* disappeared by December as the entire parasitoid population entered diapause. Such over wintering was observed by Lopez (1982) in *C. nigriceps* parasitizing *Heliothis armigera* (Hubner) on *Cajanus cajan* in Texas. He observed over-wintering in the pest as well as the parasitoid. Butler *et al.* (1983) observed that at temperatures below 22.5°C, the insects remained in diapause. In the present study over wintering was noticed in the parasitoid only. The gradual increase in the number of *C. nigricollis* entering diapause in the field population appears to be due to occurrence of a heterogenous population of *C. nigricollis* in the field which responded gradually to fall in atmospheric temperature. Runjie *et al*. (1996) studied the relationship between temperature and functional response in *C. philippinensis* and concluded that the highest attack rate was at 28°C but, was followed by a decrease with increasing temperature. Yu-jie *et al.* (1991) recorded 22.7 days as the duration for completion of life cycle, whereas in the present investigation it was observed to be 16.5 days and 15.3 days for females and males, respectively.

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**Research Note**

# Effect of selected pesticides on the growth parameters of *Metarhizium anisopliae* (Metch.) Sorokin

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**ABSTRACT**: *In vitro* compatibility studies were conducted with the green muscardine fungus, *Metarhizium anisopliae* (Metch.) Sorokin and commonly used pesticides. The test insecticides *viz.,* spinosad, indoxacarb, novaluron and cartap hydrochloride after 21 days recorded 6.42, 5.86, 5.74 and 5.30 cm radial growth of *M. anisopliae*, respectively and all these treatments were on par with each other and with that of control. The conidial concentration per cm of *M. anisopliae* in spinosad amended media was highest and lowest in cartap hydrochloride. The conidial viability of *M. anisopliae* in spinosad, indoxacarb, novaluron and cartap hydrochloride treated media was 89.2, 84.6, 80.4 and 77.4 per cent, respectively. No significant difference in radial growth of *M. anisopliae* was found with tebuconazole, azoxystrobin and chlorothalonil, whereas total inhibition of radial growth was observed in propiconazole treated medium. Significant reduction of *M. anisopliae* conidial concentration per cm was recorded in tebuconazole and azoxystrobin treated media.

**KEY WORDS**: *Metarhizium anisopliae,* spinosad, indoxacarb, novaluron, cartap hydrochloride, tebuconazole, azoxystrobin, chlorothalonil, propiconazole

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It is reported that 700 (Charnley, 1989) to 750 species (Carruthers and Soper, 1987) of fungi are pathogenic to insects, but only about a dozen species have been exploited so far for insect control. They can perpetuate in target insect (Ferron, 1978) while their safety to non target organisms, ease and economical feasibility for *in vitro* mass culture makes them one of the preferred options among microbials. Under Indian conditions four entomopathogenic fungi were reported promising *viz., Beauveria bassiana* (Bals.) Vuillemin, *Metarhizium anisopliae* (Metch.) Sorokin, *Nomuraea rileyi* F Samson and *Verticillium lecanii* Viegas.

The *M. anisopliae* is widely distributed fungus with a broad host range. Over 100 species of insects belonging to different orders are known to be infected by this fungus. Chemical pesticides being synergistic/ antagonistic among themselves, may have antagonistic or synergistic effects on the potentiality of *M. anisopliae* and may influence natural epizootics. Such situation warrants only the compatible insecticides and/or fungicides to be used in combination with these microbial agents to derive the fullest potential of the organism with least environmental pollution along with cost effectiveness. Therefore, for successful establishment of entomopathogenic fungi in IPM programmes, its compatibility with insecticides and fungicides is very important to manage the insect pests.

The strains of *M. anisopliae* were obtained from National Bureau of Agriculturally Important Insects (NBAII), Bangalore and single spore isolation procedure was followed till pure cultures were established on the Sabouraud's Dextrose Agar medium with yeast extract (SDAY). The pure cultures thus obtained were further cultured and preserved on SDAY slants as well as in Paraffin oil for further experimental purpose (Rombach *et al.,* 1986).

### Inoculation of the medium with mycelial mat

Circular discs of 10 mm diameter were cut from vigorously grown culture of *M. anisopliae* using a sterile cork borer and such discs were placed in the middle of each Petri plate on the medium amended with insecticide at recommended concentration. Medium inoculated with

the fungus without insecticide served as control. All these steps were carried out under aseptic conditions inside an inoculation chamber. These plates were incubated at 25 ± 1°C for 21 days. The *M. anisopliae* cultured on SDAY medium was studied for various biological properties such as radial growth, conidia per unit area, viability of conidia and time taken for germination of 50 per cent spores.

**Radial growth**

The radial growth of *M. anisopliae* was measured using a measuring scale at an interval of 7, 14 and 21 days and compared with control.

The radial growth, number of conidia per unit area and viability of conidia were also recorded following the procedures and compared with control using the formula.

$$R = \frac{C - T}{C} \times 100$$

Where,

R = Per cent reduction of radial growth / conidia per unit area / conidial viability.

C = Radial growth / conidia per unit area / conidial viability of fungi grown on control or untreated medium.

T = Radial growth / conidia per unit area / conidial viability of fungi grown on insecticide treated medium.

**Conidia per unit area**

The circular disc of 10 mm diameter was cut randomly from the ten days old uniformly grown culture plates. Each disc was placed in a test tube containing 10 ml of distilled water. The spores present in the disc were allowed to disperse uniformly in the water by rotating the test tube on a vortex for one minute. Proper care was taken to avoid spillage of the suspension while rotation. The suspension was serially diluted and the spores were counted with the help of an improved Neubaur Haemocytometer under a compound microscope at 40 x magnification and number of spores present per ml was calculated using the formula.

No. of spores / ml = Total no. of spores in 5 randomly selected big squares of haemocytometer x 5 x $10^4$ (Aneja, 1996).

The readings thus obtained were computed to 10 ml to determine the number of conidia per unit area of 10 mm diameter disc.

**Conidial viability and time taken for 50 per cent germination of spores**

The conidia were harvested from the uniformly grown culture plates with the help of a fine camel hair brush into sterile distilled water and filtered through double layered muslin cloth.

A 500 µl of the spore suspension was placed in the cavities of a cavity slide containing 1000 µl of SDAY medium. The slides were placed in a Petri plate containing a moistened filter paper at its bottom and were incubated at a temperature of 25 ± 1°C and RH of 95 per cent. The slides were observed after every 2 interval under microscope till 50 per cent of the conidia visible in any of the focused region germinated. This was recorded as $TG_{50}$, the time taken for germination of 50 per cent of spores. The germination of conidia was recorded after 24 hrs of incubation and the per cent spore germination was calculated using the formula

$$G = \frac{N}{T} \times 100$$

Where,

G = Per cent spore germination.

N = Number of spores germinated.

T = Total number of spores observed.

**Effect of selected insecticides on growth parameters of *M. anisopliae***

**Radial growth**

The radial growth of *M. anisopliae* recorded at 7 Days after inoculation (DAI) on media amended with spinosad, indoxacarb and novaluron were 4.62, 4.34 and 4.16 cm, respectively, and they were on par with each other, while the cartap hydrochloride amended medium recorded lowest radial growth of 3.58 cm which was significantly different from other treatments including control (4.80 cm) and was on par with novaluron (Table 1).

After 14 DAI, *M. anisopliae* recorded a radial growth ranging from 5.28 to 7.00 cm. The highest radial growth among treatments was in control (7.00 cm) which significantly differed from other insecticides *viz.,* spinosad (6.26 cm) indoxacarb (5.52 cm), novaluron (5.50 cm)

**Table 1: Effect of selected insecticides on the growth of *Metarhizium anisopliae***

| Insecticides | Field recommended concentration *(%)* | Radial growth (cm) | | | | Conidial concentration / cm x $10^7$ | Conidial viability (%) | $TG_{50}$ (hrs) |
|---|---|---|---|---|---|---|---|---|
| | | 7 days | 14 days | 21 days | Mean | | | |
| Indoxacarb | 0.015 | 4.34 (12.01)[a] | 5.52 (13.58)[c] | 7.72 (16.12)[b] | 5.86 (13.91)[a] | 3.02 (10.00)[d] | 84.6 (66.96)[c] | 11.0 |
| Spinosad | 0.0135 | 4.62 (12.39)[a] | 6.26 (14.48)[b] | 8.40 (16.84)[a] | 6.42 (14.58)[a] | 3.78 (11.21)[b] | 89.2 (70.93)[bc] | 9.5 |
| Novaluron | 0.0100 | 4.16 (11.75)[ab] | 5.50 (13.55)[c] | 7.56 (15.95)[b] | 5.74 (13.76)[a] | 3.65 (11.01)[bc] | 80.4 (63.97)[cd] | 11.0 |
| Cartap hydrochloride | 0.0500 | 3.58 (10.87)[b] | 5.28 (13.27)[c] | 7.06 (15.40)[c] | 5.30 (13.19)[a] | 3.57 (10.89)[c] | 77.4 (61.71)[d] | 13.2 |
| Control | | 4.80 (12.64)[a] | 7.00 (15.34)[a] | 8.66 (17.11)[a] | 6.82 (15.03)[a] | 4.79 (12.64)[a] | 94.8 (77.49)[a] | 7.6 |
| SEm ± | | 0.30 | 0.21 | 0.16 | 1.25 | 6.80 | 1.69 | |
| C.D. *P* = 0.05 | | 0.90 | 0.62 | 0.48 | 3.96 | 0.20 | 4.92 | |

Figures in parentheses are angular transformed values
Figures indicated by same alphabets are not significantly different from each other as per DMRT
SEm± : Standard Error of Mean

and cartap hydrochloride (5.28 cm). The radial growth of *M. anisopliae* on media treated with spinosad differed significantly from indoxacarb, novaluron and cartap hydrochloride that are on par with each other.

Spinosad with 8.40 cm radial growth was found to be significantly superior over other insecticidal treatments at 21 DAI. That was on par with control (8.66 cm), whereas indoxacarb, novaluron and cartap hydrochloride treated media recorded a radial growth of 7.72, 7.56 and 7.06 cm respectively. Indoxacarb and novaluron treatments were on par with each other, wherea cartap hydrochloride was significantly lower when compared to other insecticidal treatments.

The overall radial growth of *M. anisopliae* on spinosad, indoxacarb, novaluron and cartap hydrochloride treated media recorded 6.42, 5.86, 5.74 and 5.30 cm respectively and all these treatments were on par with each other and also with that of control which recorded 6.82 cm radial growth.

The overall per cent inhibition of radial growth over control was lowest on spinosad (5.77%) and highest in cartap hydrochloride (22.82%). All the insecticidal treatments were on par with each other and also to that of control which indicates that all the insecticides were exhibiting similar effect on per cent inhibition of radial growth of *M. anisopliae.*

**Conidial concentration**

*M. anisopliae* on spinosad and novaluron contaminated media recorded a conidial concentration of 3.78 and 3.65 x $10^7$ /cm respectively and were on par with each other, whereas cartap hydrohchloride and indoxacarb recorded conidial concentration of 3.57 and 3.02 x $10^7$ /cm, respectively as against 4.79 x $10^7$ /cm in control (Table 1). The conidial concentration on cartap hydrochloride treated medium with *M. anisopliae* was on par with novaluron treatment, whereas, indoxacarb treated media was significantly lower when compared with other treatments. The lowest per cent reduction of conidial concentration over control was recorded in spinosad (21.08%), whereas highest was in indoxacarb (36.95%).

**Conidial viability**

The per cent conidial viability obtained in spinosad and indoxacarb treated media was 89.2 and 84.6 per cent respectively, which were on par with each other and differed significantly from control (94.8%). The conidial viability of 80.4 and 77.4 per cent respectively was recorded in novaluron and cartap hydrochloride treated

media which were on par with each other and significantly differed with other treatments.

**Time taken for 50 per cent spore germination**

Among the media amended with insecticide, *M. anisopliae* recorded a lowest duration of 9.50 hrs in spinosad when compared with other insecticidal amendments ndicating the rapid germination of conidia (Table 1). The indoxacarb + *M. anisopliae and* novaluron + *M. anisopliae* treatment recorded 11.0 hrs for their 50 per cent spore germination. The longest duration of 13.2 hrs ($TG_{50}$) was recorded in cartap hydrochloride treatment when combined with *M. anisopliae* as against the least duration of 7.6 hrs in control.

These results are in concurrence with the findings of Mietkiewski and Gorski (1995), who reported that dimethoate and pirimicarb inhibited the growth of *M. anisopliae* at ten times the recommended doses, while the fungal colonies obtained were 85 per cent of the size of control even at the highest concentration of alpha – cypermethrin indicating lesser inhibitory action.

Gupta *et al.* (2002) also found *M. anisopliae* to be tolerant to azadirachtin at all the concentrations (10, 1000 and 2000 ppm), while the fungus was found to be sensitive to insecticides *viz.,* chlorpyriphos, endosulfan, monocrotophos and quinalphos, wherein fungal inhibition occurred with the increasing concentrations of insecticides. Neves *et al*. (2001) reported that the neonicotinoid insecticides (acetamiprid, imidacloprid and thiamethoxam) did not affect the conidial germination, conidial production and vegetative growth of *M. anisopliae*.

**Effect of selected fungicides on growth parameters of *M. anisopliae***

**Radial growth**

The radial growth of *M. anisopliae* recorded at 7 DAI showed no significant difference in tebuconazole (1.82 cm) and azoxystrobin (1.62 cm) contamination, while, chlorothalonil amended medium recorded 1.04 cm radial growth which was significantly lower than the above treatments (Table 2). The *M. anisopliae* radial growth was totally inhibited in propiconazole amended medium. However, all the fungicidal amendments differed significantly with control (2.42 cm).

At 14 days after the fungicidal contamination the radial growth of *M. anisopliae* was 2.94 and 2.80 cm in tebuconazole and azoxystrobin treated media, respectively and were on par, while, chlorothalonil contamination recorded 1.96 cm radial growth which was significantly different with the above treatments. The radial growth was totally inhibited in case of propiconazole contaminated medium as against 4.16 cm in control.

The tebuconazole and azoxystrobin recorded 3.80 and 3.64 cm radial growth of *M. anisopliae* respectively which were on par with each other and differed significantly with control (7.18 cm) and chlorothalonil (3.06 cm). The total inhibition of *M. anisopliae* radial growth with propiconazole fungicide treated media continued even after 21 DAT which is significantly lower when compared to above treatments.

The overall radial growth of *M. anisopliae* recorded was 2.85, 2.68 and 2.02 cm in tebuconazole, azoxystrobin and chlorothalonil treatments respectively and there was no significant difference among them and also with that of control. The total inhibition of *M. anisopliae* radial growth was recorded in propiconazole treated medium only, whereas control recorded 4.58 cm overall radial growth which significantly differed with the above treatments.

**Conidia per unit area**

Tebuconazole and azoxystrobin treatments recorded 2.39 and $2.19 \times 10^7$ / cm respectively of conidial concentration, while chlorothalonil recorded $1.97 \times 10^7$ / cm conidial concentration as compared to control ($4.21 \times 10^7$ /cm). In propiconazole treated medium 100 per cent inhibition of conidial germination was recorded. There was significant difference among all the treatments tested against *M. anisopliae* (Table 2).

The cent per cent reduction of conidial concentration over control was recorded in propiconazole, whereas lowest reduction of 43.23 per cent was recorded in tebuconazole treated medium.

**Conidial viability**

The per cent conidial viability obtained in tebuconazole (73.8%), azoxystrobin (64.8%) and chlorothalonil (60%) amended media differed significantly among them and was significantly lower than the control (85.8%). The propiconazole recorded 100 per cent loss of conidial viability (Table 2).

**Time taken for 50 per cent spore germination**

The $TG_{50}$ for conidia of *M. anisopliae* in media amended with fungicides was found to be lowest in chlorothalonil (12.8 hrs) followed by tebuconazole (13.2 hrs) and azoxystrobin (13.8 hrs). No spore germination was recorded for *M. anisopliae* in propiconazole amendment (Table 2).

**Table 2: Effect of selected fungicides on the growth of *Metarhizium anisopliae***

| Insecticides | Field recommended concentration *(%)* | Radial growth (cm) | | | | Conidial concentration / cm x $10^7$ | Conidial viability (%) | $TG_{50}$ (hrs) |
|---|---|---|---|---|---|---|---|---|
| | | 7 days | 14 days | 21 days | Mean | | | |
| Propiconazole | 0.1 | 0.00 (0.00)[d] | 0.00 (0.00)[d] | 0.00 (0.00)[d] | 0.00 (7.99)[a] | 0.00 (0.00)[e] | 0.00 (0.00)[e] | – |
| Chlorothalonil | 0.2 | 1.04 (5.51)[c] | 1.96 (7.94)[c] | 3.06 (10.07)[c] | 2.02 (7.99)[a] | 1.97 (8.06)[d] | 60.0 (50.80)[d] | 12.8 |
| Azoxystrobin | 0.1 | 1.62 (7.27)[b] | 2.80 (9.62)[b] | 3.64 (10.99)[b] | 2.68 (9.31)[a] | 2.19 (8.50)[c] | 64.80 (53.62)[c] | 13.8 |
| Tebuconazole | 0.1 | 1.82 (7.72)[ab] | 2.94 (9.86)[b] | 3.80 (11.24)[b] | 2.85 (9.62)[a] | 2.39 (8.89)[b] | 73.80 (59.25)[b] | 13.2 |
| Control | | 2.42 (8.94)[a] | 4.16 (11.7)[a] | 7.18 (15.53)[a] | 4.58 (12.08)[a] | 4.21 (11.853)[a] | 85.80 (67.94)[a] | 10.7 |
| SEm ± | | 0.49 | 0.31 | 0.11 | 1.21 | 7.38 | 1.00 | |
| C.D. ($P = 0.05\%$) | | 1.46 | 0.93 | 0.33 | 3.81 | 0.21 | 2.96 | |

Figures in parentheses are angular transformed values
Figures indicated by same alphabets are not significantly different from each other as per DMRT
C.D. (0.05%): critical difference at 5 per cent level
SEm± : Standard Error of Mean

The results are concurrent with the findings of Gardner and Kinard (1998), James and Elzen (2001), Xu *et al*. (2002) and Bhattacharya *et al*., (2004) wherein they observed no detrimental effects of imidacloprid on *B. bassiana*. The expression of low inhibition in the biological properties of *B. bassiana* strains may be due to the presence of emulsifiers and other additives in the formulated products of insecticides. Generally, wettable powders and flowable formulations cause no inhibition and often increase colony counts whereas, emulsifiable concentrate formulations frequently inhibit *B. bassiana* germination (Anderson *et al*., 1989). Adjuvants in wettable powders and flowable formulations may act as mild abrasives and break up agglomerations of conidia, which would improve the field performance of *B. bassiana*.

The results of the present study suggest that the insecticides spinosad and indoxacarb can be used with *M. anisopliae* in pest management. This combination would give an added advantage where the insecticide pathogen mixtures introduce multiple mortality factors against the target pest with insecticide making the insect physiology weak to a desired degree which makes it much more susceptible to the attack of the entomopathogens (Fedorinehik, 1974) and also delay the chances of expression of resistance to new insecticides (Georghiou, 1983). This approach in pest management was explored by Steinkraus (1996) and Brown *et al*. (1997) who found that the combination of imidacloprid and *B. bassiana* yielded greater control of adult tarnished plant bugs in cotton over the use of either of them alone. Ali *et al.* (2007) reported that imidacloprid was highly compatible with *B. bassiana* (isolate DEB1008) and flufenoxuran is not compatible with *B. bassiana.*

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**Research Note**

# Classical biocontrol of papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink in Kerala using the parasitoid, *Acerophagus papayae* Noyes and Schauff (Hymenoptera: Encyrtidae)

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**ABSTRACT**: Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willlink (Hemiptera: Pseudococcidae) was first observed in Kerala during 2009. It was found infesting on papaya, mulberry, brinjal, tomato, cowpea, jack, plumeria, hibiscus, ocimum, raulfia, teak and rubber. *Acerophagus papayae* Noyes and Schauff (Hymenoptera: Encyrtidae) was mass reared and released @ 25-100 nos. / plant in Thrissur and Ernakulam districts of Kerala. Before the release of the parasitoid the incidence was above 60 per cent and intensity was medium to very high. A rapid survey carried out in the released areas showed that the percentage of infestation came down to below five and intensity of infestation was medium to very low. In other districts the incidence was low with the activity of *A. papayae*

**KEY WORDS**: Papaya mealybug, *Paracoccus marginatus*, *Acerophagus papayae*, Kerala

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Papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) was observed in Kerala during 2009 and was first reported on papaya during 2010 (Lyla and Philip, 2010). Severe incidence of the pest was observed on mulberry plants (Krishnakumar and Rajan, 2009). It was found infesting on brinjal, tomato, cowpea, jack, *Plumeria*, hibiscus, ocimum, raulfia, teak, rubber and parthenium (Chellappan, 2010).

In Kerala, despite the use of water spray and chemicals the incidence of *P. marginatus* on papaya plants was severe, necessitating the use of biological control agents.

**Survey on papaya mealybug**

Surveys on papaya mealybug and their natural enemies were carried out during October – November months, 2010 from randomly selected villages of different districts of Kerala. Twenty five randomly selected plants from each village were surveyed and the incidence recorded in a scale of 1–5 (1 – very low, 2 – low, 3 – medium, 4 – high and 5 – very high). During the survey it was found that above 60 per cent of the papaya plants were infested with the mealybug and the intensity of damage ranged from medium to very high (grade 3 to 5). The mealybug infested garden plants like hibiscus and *Plumeria*. Mulberry plants in three districts of Kerala – Idukki, Wayanad and Palakkad were also infested with *P. marginatus* .

Three exotic parasitoids, *Acerophagus papayae* Noyes and Schauff, *Anagyrus loecki* Noyes and *Pseudleptomastix mexicana* Noyes and Schauff (Hymenoptera Encyrtidae) were received from National Bureau of Agriculturally Important Insects (NBAII, Bangalore during October, 2010.

Among them, *A. papayae* was multiplied in the laboratory on potato sprouts containing immature stages of *P. marginatus*. Mature potatoes with eyes were collected, washed in water to remove the dirt and disinfected using five per cent sodium hypochlorite solution. A slight incision was made in the eye free area of potato and treated with gibberlic acid 100 ppm solution for half an hour. The treated potatoes were air dried and sown on moist sand in plastic basins and covered with black cloth. The potatoes were infested with *P. marginatus* females with ovisacs. Males can be easily identified from 4$^{th}$ instar onwards by their long and slender nature with well developed wings in adults. Sprouted

potatoes with mealybugs were placed in cages and *A. papayae* were released for egg laying. After 20 days, *A. papayae* adults were collected from cages for field releases. Papaya seedlings were also used for the multiplication of *A. papayae*.

State level release of the parasitoid was made at Krishi Vygyan Kendra, Malappuram on 09-12-2010 and supplied the parasitoid to farmers from different parts of Kerala. The adults of *A. papayae* were released @ 25 to 100 nos/plant depending upon the intensity of mealy bug incidence. During February – March 2012, a random survey was carried out in different districts of Kerala and the results indicated drastic reduction in the incidence of *P. marginatus* with the good activity of *A. papayae* (Table 1). The percentage of infestation came down to below five and intensity of infestation was medium to very low. Similar results were observed in Maharashtra (Nakat *et al.,* 2010), Karnataka (Shylesha *et al.,* 2010) and Tamil Nadu (Kalyanasundaram *et al.,* 2010). During 2009, the incidence was very severe throughout Kerala. The timely release of the parasitoid during 2010 suppressed the population of *P. marginatus* and the parasitoid persisted in the field even eighteen months after release.

The parasitoid emergence from the random samples drawn from different locations of Thrissur and Ernakulam district revealed that the emergence of parasitoids ranged from 18 to 75 depending on the severity of incidence of *P. marginatus* (Table 2).

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**Table 1: Survey for the incidence of *Paracoccus marginatus* and *Acerophagas papayae* in different locations at Kerala**

| No. of villages covered in each district | % infested plants | Infestation grading | Incidence of *A. papayae* |
|---|---|---|---|
| Thrissur – 10 | 2.2 | Low – Medium | Present |
| Ernakulam – 4 | 3.7 | Low – Medium | ” |
| Palakkad – 4 | 1.6 | Very low | ” |
| Malappuram – 6 | 3.1 | Very low | ” |
| Wayanad – 6 | 1.5 | Very low | ” |
| Kozhikkode – 4 | 1.6 | Low | ” |

**Table 2: Incidence of *Paracoccus marginatus* and *Acerophagas papayae* at Thrissur and Ernakularm**

| Location | Mealybug infestation (Grading) | *A. papayae* emerged/ 30 $cm^2$ leaf area |
|---|---|---|
| Thrissur | | |
| 1 | Medium | 55 |
| 2 | Medium | 75 |
| 3 | Low | 18 |
| 4 | Medium | 41 |
| 5 | Medium | 48 |
| Ernakulam | | |
| 1 | Medium | 33 |
| 2 | Low | 18 |
| 3 | Medium | 64 |
| 4 | Medium | 38 |
| 5 | low | 11 |

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**Research Note**

# Natural colonization of Australian ladybird beetle, *Cryptolaemus montrouzieri* Mulsant in papaya plantation infested with *Paracoccus marginatus* Williams and Granara de Willink in Tamil Nadu

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**ABSTRACT**: The role of *Cryptolaemus montrouzieri* as an effective predator of *Paracoccus marginatus* Williams and Granara de Willink was often doubtful. Natural colonisation of *C. montrouzieri* was observed on papaya at Sathyamangalam (Tamil Nadu). The number of larvae were 18 to 30 per leaf. The massive colonisation of *C. montrouzieri*, will help in its effective utilisation against *P. marginatus*.

**KEY WORDS**: *Paracoccus marginatus, Cryptlolaemus montrouzieri*

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The papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), native of Mexico, was reported in Coimbatore (Tamil Nadu) in July 2008. *P. marginatus* sucks the sap from the leaf resulting in leaf distortion. Fruits covered with mealybugs and sooty mould lose market value (Suresh *et al*., 2010; Tanwar *et al.,* 2010). Chemicals were used desperately when there was outbreak of *P. marginatus* which gave short-term control. However, chemical control is difficult and requires repeated application of the insecticides (Ayyasamy and Regupathy, 2010). Alternatively, there was good scope for the biological control of the *P. marginatus* using parasitoids.

A total of 22 natural enemies were known to attack *P. marginatus* in different countries (Mani *et al.,* 2012). During October 2010, the parasitoid *Acerophagus papayae* Noyes and Schauff obtained from Puerto Rico through National Bureau of Agriculturally Important Insects, Bangalore was released in the farmer's field of papaya in Coimbatore district. There was substantial reduction of *P. marginatus* density to a very low level upto three months of its introduction (Kalyanasundaram *et al.,* 2010).

The incidence of papaya mealybug and its biological control programme was monitored by making periodical field visits in Coimbatore district. During the visit in July 2012, large number of *Cryptolaemus montrouzieri* Mulsant were found feeding on *P. marginatus* on papaya plantation located at Sathyamangalam. All stages of *C. montrouzieri* were found amongst the mealybug colonies indicating colonization on papaya mealybug. Number of larvae ranged from 18 to 30 per papaya leaf. Similarly, they were found feeding on the mealybugs on fruits, trunk and flower panicles. The other predators like *Mallada boninensis* (Okamoto) and *Spalgis epeus* Westwood were observed in negligible numbers besides *A. papayae*.

*Cryptolaemus montrouzieri* has been reported as a minor predator of *P. marginatus* in Malaysia (Mastoi *et al.*, 2011), Palau (Muniappan *et al.*, 2008), Hawaii (Ronald *et al*., 2007) and Florida (Walker *et al.*, 2011) besides India (Shylesha *et al.,* 2011).

In the present investigation, massive colonization of *C. montrouzieri* was reported for the first time on *P. marginatus* in India and elsewhere.

The predator readily settles in large mealybug colonies and feeds on the eggs and larger female mealybugs whereas the parasitoid attacks the mealybug nymphs and scattered low populations (Panis, 1979). *C. montrouzieri* can supplement the exotic parasitoid *A. papayae* in

bringing down the mealybug population quickly. Being a polyphagous predator, the added advantage is that it can feed on the other species of mealybugs including *A. papayae* on papaya.

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**Research Note**

# Evaluation of toxicity of agrochemicals on *Trichoderma* isolates *in vitro*

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**ABSTRACT**: Thirty nine agrochemicals comprising eighteen fungicides, eleven insecticides, six fertilizers and four herbicides were evaluated at different concentrations against two isolates of *Trichoderma* spp., and $EC_{50}$ and $EC_{90}$ values were calculated based on inhibition in radial growth. Isolate *T. virens* TV9, obtained from citrus orchard – relatively less exposed to agrochemicals was found more sensitive compared to isolate *T. harzianum* Th4, obtained from cotton ecosystem relatively more exposed to agrochemicals. Among fungicides, benzimidazoles showed higher toxicity followed by chlorothalonil and triazoles when mean $EC_{50}$ values were compared. Wettable sulphur, Bordeaux mixture, azoxystrobin and mancozeb were found to be less toxic to *Trichoderma* spp. Among insecticides, organophosphorous group was found more toxic while carbofuran followed by spinosad were least toxic. Among fertilizers Zinc sulphate and diammonium phosphate were found highly toxic whereas potassium nitrate, muriate of potash and ammonium sulphate showed less toxicity. All the four test herbicides, *i.e*., pendimethalin, alachlor, glyphosate and 2, 4 – D were found to be toxic to *Trichoderma* spp.

**KEY WORDS**: Toxicity, *Trichoderma* spp., fungicides, insecticides, fertilizers, herbicides, $EC_{50}$ and $EC_{90}$

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Soil borne plant pathogenic fungi such as *Fusarium, Phytophthora, Pythium, Rhizoctonia, Sclerotium etc.* are the causal agents for various diseases in most of the economically important crops. Chemical means of managing the diseases caused by these pathogens are not practicable owing to high cost of chemicals and environmental pollution. Biological control offers a novel approach when applied either alone or in combination with other management practices without the demerits of chemical control (Papavizas, 1985 and Mukhopadhyay, 1987). *Trichoderma* is one of the most common soil inhabitants and extensively studied biocontrol agent in the management of soil borne plant pathogens (Elad *et al.,* 1980). Applied in the soil *Trichoderma* proliferates in the soil and protects the crop from pathogens for a longer time.

Species of *Trichoderma* are being used either through seed treatment or soil application to manage several soil borne plant diseases. Once in the soil, the antagonist is continuously exposed to different agrochemicals applied to the field. Agrochemicals are likely to influence the efficacy of native or applied *Trichoderma*. The present investigation is aimed at evaluating the toxicity of agrochemicals at different concentrations on two isolates of *Trichoderma* spp.

Two isolates of *Trichoderma* spp., *viz*., *T. virens* – Tv9 (obtained from citrus orchard where in relatively lower amount of agrochemicals are used) and *T. harzianum* Th4 (obtained from cotton ecosystem where in usage of agrochemicals is more) were used in the present investigation. Eighteen fungicides, *viz.,* copper oxychloride, Bordeaux mixture, wettable sulphur, mancozeb, thiram, captan, carbendazim, benomyl, carboxin, metalaxyl, propiconazole, hexaconazole, tricyclazole, tridemorph, fosetyl-Al, azoxystrobin, chlorothalonil and dinocap; eleven insecticides *viz.,* endosulfan, chlorpyrifos, quinalphos, dimethoate, indoxacarb, carbofuran, imidacloprid, fipronil, thiamethoxam, emamectin benzoate and spinosad; seven inorganic fertilizers *viz.,* urea (46% N), muriate of potash (60% $K_2O$), ammonium sulphate, diammonium phosphate (DAP), potassium nitrate and zinc sulphate; and four herbicides, *viz*., pendimethalin, glyphosate, alachlor and 2,4-D sodium salt were used to assess their toxicity at different concentrations on *Trichoderma* isolates *in vitro* by using the poisoned food technique (Nene and Thapliyal, 1993). Radial growth of the test *Trichoderma* isolates were recorded after 48 h of incubation and per cent inhibition of growth was calculated using the following formula:

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

I – per cent inhibition

C – growth in unamended medium

T – growth in fungicide amended medium

Different concentrations of individual chemicals were assessed in order to arrive at $EC_{50}$ and $EC_{90}$ values calculated using probit analysis programme, *viz*., EPA Probit Analysis Programme in M STAT C.

Both the isolates of *Trichoderma*, *viz*., *T. harzianum* Th4 and *T. virens* Tv9, grew equally well and attained a radial growth of 9.0 cm after 48 h of incubation at 28±1°C on unamended PDA plates. In agrochemicals amended medium when $EC_{50}$ values were calculated based on inhibition per cent, all the agrochemicals showed varied inhibitory effect on radial growth Tables 1 to 4). Variation also existed between the two test *Trichoderma* isolates in their sensitivity. Chemicals with $EC_{50}$ values above the recommended field concentration were considered highly toxic and less than filed concentrations were considered as less toxic.

When mean $EC_{50}$ values of different fungicides were compared, benzimidazoles, *viz*., carbendazim and benomyl were found to be highly toxic ($EC_{50}$ 0.5 and 0.9 ppm and $EC_{90}$ 3.6 and 5.4 ppm respectively) than all the other fungicides (Table 1). Except azoxystrobin (mean $EC_{50}$ 3491.1 ppm), all other systemic fungicides tested such as carboxin, metalaxyl, fosetyl Al, triazoles (hexaconazole, propiconazole and tricyclazole) and tridemorph were found highly toxic when their mean $EC_{50}$ values were compared with respective recommended field concentrations. Among the contact fungicides wettable sulphur (inorganic S with mean $EC_{50}$

**Table 1: Toxicity of fungicides on isolates of *Trichoderma***

| Fungicide | Recommended field concentration (ppm) | Concentration in ppm at 95 FL | | | | | |
|---|---|---|---|---|---|---|---|
| | | $EC_{50}$ | | | $EC_{90}$ | | |
| | | Th 4 | Tv 9 | Mean | Th 4 | Tv 9 | Mean |
| Carbendazim | 1000 | 0.42 | 0.59 | 0.50 | 1.13 | 6.13 | 3.60 |
| Benomyl | 1000 | 0.85 | 0.89 | 0.90 | 2.24 | 8.64 | 5.40 |
| Carboxin | 2000 | 420.00 | 224.50 | 322.30 | 2100.20 | 46831.60 | 24465.90 |
| Metalaxyl | 2000 | 421.70 | 286.60 | 354.20 | 948.80 | 1317.60 | 1133.20 |
| Fosetyl Al | 1500 | 950.50 | 487.80 | 719.20 | 2000.10 | 2878.50 | 2439.30 |
| Hexaconazole | 2000 | 61.20 | 13.60 | 37.40 | 3645.60 | 11254.10 | 7449.90 |
| Propiconazole | 1000 | 1.40 | 6.04 | 3.70 | 20.13 | 4380.30 | 2200.20 |
| Tricyclazole | 600 | 137.00 | 42.35 | 89.70 | 2803.40 | 4634.40 | 3718.90 |
| Tridemorph | 1000 | 3.10 | 5.75 | 4.40 | 124.70 | 148.00 | 136.40 |
| Azoxystrobin | 1000 | 3965.10 | 2873.00 | 3419.10 | 2484611.20 | 85393.30 | 1285002.30 |
| Copper oxychloride | 3000 | 258.00 | 310.30 | 284.20 | 11563.10 | 963.30 | 6263.20 |
| Bordeaux mixture | 10000 | 44692.65 | 28038.20 | 36365.40 | 226620.60 | 167596.20 | 197108.40 |
| Wettable sulphur | 2000 | 91284.10 | 37801.20 | 64542.70 | 731640.70 | 1511692.20 | 1121666.50 |
| Mancozeb | 2500 | 5676.30 | 706.50 | 3191.40 | 253052.20 | 452412.20 | 352732.20 |
| Thiram | 2500 | 614.90 | 312.10 | 483.50 | 1466.30 | 7832.60 | 4649.50 |
| Captan | 2000 | 172.60 | 87.60 | 130.10 | 1673.60 | 2660.40 | 2167.00 |
| Chlorothalonil | 2000 | 2.21 | 0.19 | 1.20 | 1220.40 | 1.90 | 611.20 |
| Dinocap | 1000 | 52.20 | 43.65 | 47.90 | 93224.00 | 18773.00 | 55998.50 |
| | | 8261.90 | 3957.80 | 6109.90 | 212039.90 | 128821.40 | 170430.60 |

64542.7 ppm), Bordeaux mixture ($EC_{50}$ 36365.4 ppm) and mancozeb (organic S with mean $EC_{50}$ 3191.4 ppm) were found less toxic where as copper oxychloride (proprietary copper group), thiram (organic S), captan, chlorothalonil and dinocap were found highly toxic with mean $EC_{50}$ values less than their recommended field concentrations. It may be noted here that all the three most commonly used seed dressing fungicides were found highly toxic to *Trichoderma*, *i.e*., carbendazim, thiram and captan and hence requires cautious approach for their use in integrated disease management as seed dressing chemicals along with *Trichoderma*. When mean $EC_{50}$ values over all the fungicides were compared for individual *Trichoderma* isolate, *T. virens* Tv9 (mean $EC_{50}$ of 3957.8 ppm) was found more sensitive to fungicides than *T. harzianum* Th4 (mean $EC_{50}$ of 8261.9 ppm). Except for propiconazole, tridemorph and copper oxychloride, *T. virens* Tv9 was found sensitive to all other test fungicides compared to *T. harzianum* Th4 which indicated relative tolerance of *T. harzianum* Th4 to fungicides.

Among the eleven insecticides tested and compared for their mean $EC_{50}$ values over both the *Trichoderma* isolates, all the three test organophosphorous compounds such as quinalphos ($EC_{50}$ 34.1 ppm), chlorpyriphos ($EC_{50}$ 185.9 ppm) and dimethoate ($EC_{50}$ 1036.6 ppm), and endosulfan, an organochlorine chemical ($EC_{50}$ 975 ppm) were found highly toxic to *Trichoderma* isolates (Table 2). Carbamate fungicide carbofuran was found least toxic to *Trichoderma* isolates with mean $EC_{50}$ as high as 66529 ppm. Other test insecticides belonging to the new generation chemicals such as fipronil, imidachloprid, thiamethoxam, emamectin benzoate, indoxacarb and spinosad were found less toxic with mean $EC_{50}$ values lower their recommended field concentrations. When comparisons were made between the two test isolates with $EC_{50}$ values over all the insecticides tested, *T. virens* Tv9 was found highly sensitive (mean $EC_{50}$ 13356.3 ppm) compared to *T. harzianum* Th4 (mean $EC_{50}$ 18839.7 ppm). When individual comparisons are made, except for thiamethoxam for all other insecticides *T. virens* Tv9 was more sensitive compared to *T. harzianum* Th4.

As fertilizers are directly applied to the field either through broadcast method or furrow placement there by *Trichoderma* is likely to get direct exposure to these fertilizers, Hence, $EC_{50}$ values were compared with ten times (20000 ppm) the concentration recommended for their foliar spray (2000 ppm). Three of the most commonly used fertilizers, *i.e*., zinc sulphate (mean $EC_{50}$ 1387.3 ppm), diammonium phosphate (7021.1 ppm) and urea (16811.7 ppm) were found highly toxic to

**Table 2: Toxicity of insecticides on isolates of *Trichoderma***

| Fungicide | Recommended field concentration (ppm) | Concentration in ppm at 95 FL | | | | | |
|---|---|---|---|---|---|---|---|
| | | $EC_{50}$ | | | $EC_{90}$ | | |
| | | Th 4 | Tv 9 | Mean | Th 4 | Tv 9 | Mean |
| Endosulfan | 2000 | 1636.2 | 313.8 | 975.0 | 5383.7 | 2694.0 | 4038.9 |
| Chlorpyrphos | 2500 | 181.6 | 190.2 | 185.9 | 1977.8 | 2461.8 | 2219.8 |
| Dimethoate | 2000 | 1521.6 | 551.5 | 1036.6 | 10070.0 | 1584.1 | 5827.1 |
| Quinalphos | 2000 | 48.9 | 19.31 | 34.1 | 898.9 | 173.3 | 536.1 |
| Fipronil | 2000 | 8735.9 | 2330.1 | 5533.0 | 384591.4 | 49434.3 | 217012.9 |
| Imidachloprid | 250 | 8923.9 | 4944.2 | 6934.1 | 21072.6 | 59742.9 | 40407.8 |
| Thiamethoxam | 200 | 9568.8 | 50828.0 | 30198.4 | 38694.6 | 2347723.5 | 1193209.1 |
| Emamectin benzoate | 450 | 6891.9 | 5823.2 | 6357.6 | 686962.6 | 12433.8 | 349698.2 |
| Indoxacarb | 1000 | 13118.7 | 7313.2 | 10215.9 | 192225.5 | 20093.4 | 106159.5 |
| Spinosad | 400 | 85108.2 | 13049.0 | 49078.6 | 617755.5 | 976584.5 | 797170.0 |
| Carbofuran | 2000 | 71501.4 | 61556.6 | 66529.0 | 800705.6 | 8617881.0 | 4709293.3 |
| Mean | | 18839.7 | 13356.3 | 16098.0 | 250939.8 | 1099164.2 | 675052.4 |

*Trichoderma* isolates (Table 3). Remaining three test fertilizers studied, *i.e*., muriate of potash, ammonium sulphate and potassium nitrate were found less toxic. Isolate *T. virens* Tv9 was found more sensitive (mean $EC_{50}$ 26359 ppm) to all the fertilizers tested compared to *T. harzianum* Th4 (31376.7 ppm) when mean $EC_{50}$ values were compared over all the fertilizers and also individually.

Evaluation of $EC_{50}$ values of herbicides over both the *Trichoderma* isolates indicated that alachlor (157.5 ppm), pendemethalin (440.6 ppm) and glyphosate (1782 ppm) were highly toxic where as 2, 4 – D was less toxic (4613.4) when compared to respective recommended field concentrations (Table 4). Of both the isolates tested, *T. virens* Tv9 (mean $EC_{50}$ 1139.5 ppm) was more sensitive than *T. harzianum* Th4 (2357.3 ppm) when comparisons were made over all the herbicides and also individually.

The present study revealed higher sensitivity of *T. virens* Tv9 isolated from citrus orchard where in the isolate was less exposed to several of the xenobiotics available for plant protection but, *T. harzianum* Th4 obtained from cotton ecosystem was more tolerant to agrochemical toxicity owing to its continuous exposure to applied xenobiotics.

Inhibitory effect of different agrochemicals on *Trichoderma* spp. was reported by Akbari and Parakhia (2001), Srinivasulu *et al.* (2002), Reshmy Vijayaraghavan and Koshy Abraham (2004), Upadhyay *et al*. (2004), Tiwari *et al.* (2004), Rai Ajay Kumar *et al.* (2005) and Pandey *et al.* (2006) along with isolate variation. However, a great deal of work done was based on arbitrary concentrations that were less than the recommended field concentrations. In the present study calculation of $EC_{50}$ and $EC_{90}$ values of test agrochemicals against two different *Trichoderma* isolates obtained from two different agroecosystems revealed the range of sensitivity to these test agrochemicals.

**Table 3: Toxicity of fertilizers on isolates of *Trichoderma* spp.**

| Fungicide | Recommended field concentration (ppm) | Concentration in ppm at 95 FL | | | | | |
|---|---|---|---|---|---|---|---|
| | | | $EC_{50}$ | | | $EC_{90}$ | |
| | | Th 4 | Tv 9 | Mean | Th 4 | Tv 9 | Mean |
| Zinc sulphate | 2000 | 1843.6 | 931.0 | 1387.3 | 8247.8 | 2537.9 | 5392.9 |
| Diammonium phosphate | 2000 | 10946.0 | 3096.2 | 7021.1 | 41127.8 | 26924.5 | 34026.2 |
| Urea | 2000 | 17984.4 | 15638.9 | 16811.7 | 40510.7 | 184044.4 | 112277.6 |
| Muriate of potash | 2000 | 48576.7 | 46730.9 | 47653.8 | 101479.1 | 110338.6 | 105908.9 |
| Ammonium sulphate | 2000 | 50780.4 | 42660.2 | 46720.3 | 109040.9 | 243647.6 | 176344.3 |
| Potassium nitrate | 2000 | 58128.8 | 49096.9 | 53612.9 | 157870.3 | 209529.7 | 183700.0 |
| Mean | | 31376.7 | 26359.0 | 28867.9 | 76379.4 | 129503.8 | 102941.7 |

**Table 4: Toxicity of herbicides on isolates of *Trichoderma***

| Fungicide | Recommended field concentration (ppm) | Concentration in ppm at 95 FL | | | | | |
|---|---|---|---|---|---|---|---|
| | | | EC 50 | | | EC 90 | |
| | | TH 4 | TV 9 | Mean | TH 4 | TV 9 | Mean |
| Alachlor | 6000 | 231.3 | 83.64 | 157.5 | 980.9 | 652.7 | 816.8 |
| Pendimethalin | 7000 | 770.4 | 110.8 | 440.6 | 4375.5 | 12591.2 | 8483.4 |
| Glyphosate | 6000 | 2343.7 | 1220.3 | 1782.0 | 23756.3 | 15712.0 | 19734.2 |
| 2,4-D Sodium salt | 4000 | 6083.6 | 3143.1 | 4613.4 | 64730.5 | 120840.0 | 92785.3 |
| Mean | | 2357.3 | 1139.5 | 1748.4 | 23460.8 | 37448.9 | 30454.9 |

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# H. NAGARAJA: The doyen of *Trichogramma* taxonomy passes away

***'Trichogramma* and its numerous species lay hid in night: God said, "Let Nagaraja be!", and all was light.'**

**H. Nagaraja,** who had joined the Commonwealth Institute of Biological Control (CIBC), Bangalore as a Technical Assistant in 1958 and had risen to the next level of Senior Technical Assistant, with the brashness of one with no formal training in either entomology or systematics agreed to take on the task of cracking the '*Trichogramma* riddle' when asked to do so by his mentor and Head V. P. Rao.

Taxonomists had spent decades trying to find traits to distinguish between species of *Trichogramma* and thrown up their hands in despair. To A. B. Gahan, his "memory of *Trichogramma* [was] mostly a memory of frustration". He lamented that he "could never find characters that seemed to be constant" to enable him to distinguish between *Trichogramma* obtained from different sources. Many years after Gahan retired from work, S. E. Flanders, still grappling with the conundrum of distinguishing between species of *Trichogramma* concluded his paper on the status of *Trichogramma* taxonomy thus: "It is apparent that the task ahead for the *Trichogramma* systematist is biologically difficult and agriculturally important" (Flanders and Quednau, 1960).

Working late into the night with a Japanese Everest FX – II microscope – a primitive microscope as compared to those in use today – Nagaraja for the first time discerned minute morphological features that the giants of hymenopteran taxonomy working on *Trichogramma* had failed to see before him. But how was he, a rank novice in the field, to be certain of his momentous findings? A letter together with Nagaraja's elegantly executed figures was dashed off to Charles D. Michener, one of the reigning eminences in the taxonomy of Hymenoptera, for an expert opinion. To Nagaraja's delight, Michener concurred with his findings. And in 1968, in less than a decade after Flanders' pessimistic pronouncement, Nagaraja published his findings jointly with Sudha Nagarkatti in the *Technical Bulletin of the CIBC*. This was followed by papers in *Entomophaga* and *The Bulletin of Entomological Research*, all showing the importance of male genitalic characters in recognising species of *Trichogramma*. This took the world of *Trichogramma* taxonomy by storm. A flurry of activity followed. Many new species were discovered resulting in the description of well over 200 species, of which over 80 per cent were described after 1968.

Hulickal Nagaraja was born on the 12th of August, 1934 in Kamalashile, a tiny mountainous village in Karnataka, India. Having lost his father early, he set off on his own to Mysore to fend for himself and to study. By 1958, he earned his Bachelor's Degree in Biological Sciences from the University of Mysore. The same year he joined the CIBC at Bangalore as a Technical Assistant. For the next 25 years he worked in over a dozen projects on biological control and rose to become the Deputy Chief of the Indian Station of the CIBC. The more notable projects on which he worked were on the natural enemies of a number of harmful insects including *Antonina graminis*, *Triatoma* spp. (vectors of Chagas disease), *Helicoverpa armigera*, *Hypsipyla robusta*, *Phthorimaea operculella* and diaspine scales. It was during his stint at the CIBC that he got his D.Sc. from the University of Mysore on the basis of his work on the 'Biosystematics of *Trichogrammatoidea* species'. He, for the first time synthesised knowledge on *Trichogrammatoidea* by examining species from all over the world, discovering and describing ten new species in the endeavour.

In mid 1983, he left the CIBC, Bangalore to join Cocoa Investors Inc., Hagonoy, Philippines and was involved in formulating a programme for the biocontrol of the cocoa pod borer (CPB). Here, he discovered *Trichogrammatoidea cojuancoi,* an egg parasitoid and initiated a mass breeding and release programme of this parasitoid for the management of the CPB. In the wake of political turmoil in the Philippines resulting in the overthrow of President Marcos, Nagaraja had to return to India and work as a freelance Biocontrol Consultant for two years.

In 1988, he once again left India, this time to work as Biocontrol Consultant for Ramu Sugar Ltd., Gusap, Papua, New Guinea. Here he was involved in the classical biological control of *Sesamia* spp. in sugarcane and the invasive weed *Mimosa diplotricha (M. invisa)*.

On his return to India he set up Biotech International Ltd. at Bangalore, a facility for the production and supply of bioagents for the farming community. Subsequently he took up the assignment of Expert Consultant in the Network Project on Insect Biosystematics (NPIB) at the Project Directorate of Biological Control, Bangalore to resume his work on the taxonomy of his first love *Trichogramma*, to provide a reliable identification service and to train entomologists on *Trichogramma* taxonomy. On completion of his tenure as an Expert Consultant in the NPIB he continued his studies on *Trichogramma* from his residence in Malleswaram. He actively participated in all entomological activities and was always available for consultation.

He passed away suddenly on the night of the 6th of November, 2012 leaving behind his wife Ratnamala, his daughter Rama (an entomologist) and his son Satyadev (an engineer). We share their grief and wish them the strength to bear this irreparable loss.

**Acknowledgement**

We acknowledge our debt to Alexander Pope for his lines meant for Isaac Newton's epitaph that we have taken the liberty to paraphrase as the epigraph.

**Reference**

Flanders S. E. and Quednau W., 1960. Taxonomy of the genus *Trichogramma* (Hymenoptera: Chalcidoidea: Trichogrammatidae)

# GUIDELINES TO AUTHORS

The Journal of Biological Control, published quarterly, accepts papers based on original research in aspects relating to biological suppression of crop pests (insects, mites, nematodes, vertebrates, pathogens and weeds) and vectors of plant and animal diseases.

## GENERAL

Two copies of the manuscript and illustrations should be submitted in the 'proof ready' form to the Chief Editor. The manuscript should not exceed 3500 words or 10 typed pages. The manuscript should preferably pertain to research work carried out during the last five years. Authors must certify that the manuscript has not been submitted elsewhere. Short research notes (not exceeding 1500 words) on a significant finding or a description of new species of natural enemies or a new technique is welcome. All papers will be refereed. The decision of the Editorial Board is final. The authors are requested to furnish names, addresses and email of at least three referees in their respective fields. The editorial committee has full right to alter/modify the content of the manuscript accepted for publication without any intimation to the authors. In view of the shortage of time, the editorial committee will not be able to send the galley proof to the authors before printing.

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Each table should be on a separate sheet. The data should be arranged in such a way so that the table would fit in the normal layout of the page. Tables should carry a short title and standard abbreviations for common headings. The data reported must be subjected to appropriate statistical analysis. Avoid duplication of data in tables and figures.

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Illustrations should be relevant to the article. Line drawings should be in Indian ink or laser print and large enough to permit reduction by 50 per cent. The legends should be typed on a separate sheet. Plates are only accepted where the description can not be illustrated in text

## REFERENCES

References should be cited in the text as Nordlund and Sauls (1981) or (Metcalf *et al*., 1992). References at the end of the text should be given in the following form at:

Nordlund DA, Sauls A. 1981. Aggregation in field parasitoid population: foraging time allocation by a population of *Diadegma* (Hymenoptera: Ichneumonidae). *Eco Entomol.* **8**: 447–453.

Kaya HK. 1993. Entomogenous and entomopathogenic nematodes in biological control, pp. 565–591. In: Evans K, Trudgill DL. and Webster JM. (Eds.). *Plant Parasitic nematodes in Temperate Agriculture*. Commonwealth Agriculture Bureaux International, Cambridge University Press, Cambridge, UK.

Metcalf RL, MetcaIf ER. 1992. *Plant kairomones in insect ecology and control*. Chapman and Hall, New York, USA. 550 pp.

**Research Notes**

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