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Management of Litchi Fruit and Shoot Borer (*Conomorpha sinensis*) in Three Litchi Varieties in Samtenling, Bhutan

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ABSTRACT

In Bhutan, litchi production is constrained by insect pest damage. Among insect pests, the litchi fruit and shoot borer (Conopomorpha sinensis Bradley) is an emerging pest of economic importance in the subtropical regions of Bhutan. Up until now, there is documented research on its biology and management in Bhutan. Therefore, this study was undertaken to study the efficacy of two different management methods against litchi fruit borer infestation in three litchi varieties (Bhur litchi 1, Bhur litchi 2 & Hong houy). The management methods consisted of three treatments; T1-Azadirachtin followed by Imidacloprid, Azadirachtin and Cypermethrin; T2-Azadirachtin followed by Imidacloprid, Azadirachtin and Azadirachtin; T3-Untreated control. The result showed that the fruit infestation at harvest was significantly lower in management method T1 (34.74%) as compared to T2 (78.07%) and untreated control (89.98%). In terms of the variety, the lowest fruit infestation at harvest was observed for Hong houy (56.02%) as compared to Bhur Litchi 1 (73.28%) and Bhur Litchi 2 (73.45%). *There were no significant differences in fruit drop percent and percent infestation of dropped fruits between the treatments and varieties tested. Heavy fruit drop was observed in the third week, due to unknown reasons, which then decreased over the fruit development stages. Fruit borer infestation seems to increase rapidly after the fifth and eighth weeks indicating that most of the fruit borer damage occurs at the fruit maturity stage after fruit colour break. Therefore, the last application of cypermethrin 10% EC at the fruit colour break stage was found necessary to reduce fruit infestation during this vulnerable pest infestation period besides scheduled application of Azadirachtin and Imidacloprid at the early fruit development period.*

Keywords: *Litchi fruit and shoot borer; Emerging pest; Pest management*

1. Introduction

Litchi (*Litchi chinensis Sonn*.) is an important subtropical evergreen fruit crop belonging to the family Sapindaceae. Litchi fruits are prized on the world market for their flavour, semi-translucent white aril, and attractive red skin (Yang, Wang, Prasad, Pan et al., 2011). About 95% of litchi production is in Southeast Asia, with China, Vietnam, Thailand, India, Bangladesh, and Nepal being the most important producers in the world (Menzel & Waite,

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2005). It contributes significantly to the livelihood of several million people throughout Southeast Asia (Menzel & Waite, 2005).

In Bhutan, litchi is grown in fifteen districts with a total production of 384.69 metric tons (RSD, 2020). Sarpang, Samtse, Samdrup Jongkhar, Dagana and Chhukha are the top five producers of litchi, contributing about 95% of the total production. Currently, there are two released varieties (Bhur Litchi 1 and Bhur Litchi 2) and 3 notified varieties (Bhur Selection 1, Shahi and Early Bedena) in Bhutan (DoA, 2021). Although litchi production is gaining momentum in the subtropics of Bhutan (BBSC, 2012; Rai, 2017), the incidence of insect pests and diseases have become a major constrain while growing this crop.

Litchi fruit and shoot borer (*Conopomorpha sinensis* as described by Bradley (1986) is becoming one of the major pests of economic importance in Bhutan (ARDC, 2018). In India, litchi fruit borer damage is estimated between 48% to 74% in West Bengal (Chakraborti & Samanta, 2005), 13.6% to 64.9% in Himachal Pradesh and 24% to 32% in Bihar (Alam, Patra, & Samanta, 2019). The insect lays eggs on the calyx end of the fruit and the developing larva bores into the fruit and feeds on it. The larvae also damage newly emerged shoots from September to October resulting in the failure of the shoots to bloom. Further, it punctures the fruits from April to May resulting in severe losses through fruit drop and deteriorates the fruit quality due to the presence of larva feeding in fruit and frass in funicle while eating (Kumar, Kumar, & Nath, 2011; Srivastava, Patel, Kumar, Pandey et al., 2017).

According to Meng, Hu, Li, Dai et al. (2018), *C. sinensis* is difficult to manage with chemical control due to its cryptic feeding behaviour and overlapping generations. The vulnerable period between egg hatching and fruit penetration, where the larvae are exposed to chemical treatments outside the fruits, is very short to attain the required control (Schulte, Martin, & Sauerborn, 2007). However, Upadhyay, Aryal, Bhusal, and Chaudhary (2020) tested five insecticides applied at ten-day interval against *C. sinensis* and found that all the insecticides were effective as compared to the control. Schulte et al. (2007) suggested weekly applications of insecticides such as permethrin for effective control of fruit borer. However, heavy reliance on insecticides can lead to several adverse effects such as development of insecticide resistance, outbreak of secondary pests, harmful effects on beneficial organisms and problems of pesticide residue. Kumar et al. (2011) recommend integrated pest management methods such as the use of pheromone trap, biocontrol agents, removal, and destruction of dropped fruits, prophylactic spray of neem-based insecticides and need-based application of chemical insecticides for controlling litchi fruit and shoot borer infestation.

With limited knowledge about this pest and its management in Bhutan, this study was conducted to evaluate the efficacy of two spray regimes by combining different insecticides in three litchi varieties to effectively manage this pest in litchi crop.

2. Materials and Method

2.1 Experimental site

This experiment was conducted in the litchi germplasm block of the Agriculture Research and Development Centre (ARDC), Samtenling (26°54’14” N, 90°26’20” E), Sarpang during the litchi fruiting season (March to May) of 2021. The site is located at an altitude of 375 meters above sea level (masl) which falls under the “wet sub-tropical” agro-ecological zone. The experiment was laid out in a split-plot design. The main plot consisted of three litchi varieties (Bhur Litchi 1, Bhur Litchi 2 and Hong houy) and each variety had three trees per replication. The subplot consisted of three management methods including untreated control (see Section 2.3 below) which were replicated three times per variety making a total of nine trees per replication. The age of litchi plants used in this study was 15 years with full fruit-bearing potential.

2.2 Treatments

The management methods were: (T1) One foliar application of Azadirachtin 300 ppm (@ 6 mL/L of water) during the time of panicle emergence before flowering (33 days before flowering) followed by second spray with Imidacloprid 20% SL (@1 mL/L of water) at clove sized fruit stage (14 days after flowering (DAF)), third spray of Azadirachtin 300 ppm (@ 6 mL/L of water) when the fruits attained about 50% of the final size (45 DAF) and fourth spray of Cypermethrin 10% EC (@1 mL/L) at fruit colour break stage (61 DAF) as described by Wei et al. (2013); (T2) with a foliar spray of Azadirachtin 300 ppm (@ 6 mL/L of water) during the time of panicle emergence before flowering (33 days before flowering) followed by second spray of Imidacloprid 20% SL (@1 mL/L of water) at clove sized fruit growth stage (14 DAF) followed by third and fourth spray of Azadirachtin 300 ppm (@ 6 mL/L of water) when the fruits attained about 50% of the final size (31 DAF) and at fruit colour break stage (61 DAF); (T3) Untreated control. Each treatment was applied using a separate battery-operated knapsack sprayer (Neptune, NF-708).

Table 2. Description of different insecticides used in the experiment; Source: (IRAC, 2021)

S.N.	Common name	Trade name	Mode of action	Formulation	Dose
1.	Azadirachtin	Neem Baan	UN*	300 ppm	6 mL/L
2.	Imidacloprid	Termitox	Nicotinic Acetylcholine Receptor (NACHR) competitive modulators	20% SL	1 mL/L
3.	Cypermethrin	Cypermethrin	Sodium channel modulator	10% EC	1 mL/L
4.	Wetting agent	Anuvit	-	-	2 mL/L

*Compounds of unknown or uncertain mode of action

2.3 Data collection

Data were collected from all the nine trees selected for each of the treatments making a total of 27 trees. From each tree, 120 fruits were randomly harvested during the fruit maturity stage (27th May) and examined for fruit borer infestation damage. To determine the fruit drop rate and borer infestation over the growing period, all the dropped fruits per tree were collected weekly over the fruit development stages (7 weeks) and dissected with the help of a sharp knife. The fruits were assigned as infested if larvae were present inside the fruit, or if there were the presence of entry and exit holes or insect excreta in the fruits. Percent fruit infestation at harvest, percent fruit drop and percent infestation of dropped fruit were calculated as;

$$\text{Percent fruit infestation at harvest (\%)} = \frac{\text{Number of infested fruit per tree}}{\text{Number of fruits examined per tree (120)}} * 100 \quad \ldots\ldots\ldots (1)$$

$$\text{Percent fruit drop (\%)} = \frac{\text{Number of dropped fruit per tree per week}}{\text{Total number of fruits per tree}} * 100 \quad \ldots\ldots\ldots (2)$$

Where; Total number of fruits per tree was calculated as sum of total fruit bearing at harvest and total number of fruits dropped

$$\text{Dropped fruit infestation percent (\%)} = \frac{\text{Number of infested dropped fruits per week}}{\text{Total number of dropped fruit per week}} * 100 \quad \ldots\ldots\ldots (3)$$

In addition, the length and width of the fruits were measured at each sampling point, using a standard vernier calliper to correlate fruit infestation rate with fruit size over the fruiting season.

2.4 Statistical analysis

The distribution of the variables was determined using the Shapiro-Wilk test and the data on percent fruit drop, percent infestation of dropped fruit and percent fruit infestation at harvest were largely normal and variance were homogenous. The data were subjected to a two-way

ANOVA using the Statistical Tool for Agriculture Research (STAR) (Version: 2.0.1) software package. The relationship between percent fruit drop, dropped fruit infestation percent and fruit size amongst the varieties was tested using Pearson correlation.

3. Results and Discussion

3.1 Fruit drop percent

The result showed no significant difference in percent fruit drop between the three management methods tested on three varieties during the entire fruit development period (Table 2). It was observed that 70% to 78% of the fruits per tree dropped during the entire fruiting season and only 21% to 30% of the fruits were retained on the tree at harvest. Among the three varieties, the lowest percent fruit drop was recorded in Hong houy variety (71.35%) with the lowest fruit drop observed for management method T1 (70.35%). The growth of litchi fruit (length and width) followed a sigmoid curve. A weekly observation showed the highest fruit drop percent (56.8%) at the third week after the fruit set when the fruit attained a size of 1.15 cm in length and 0.61 cm in width (Figure 1). Fruit drop percent decreased to 2.7% by the fourth and fifth week and increased gradually to 7.4% in the sixth week and subsequently decreased towards the last two weeks when the fruits t matured.

Table 3. Fruit drop percent in three varieties of litchi with three treatments during fruiting period in 2021

Factors	Percent fruit drop (%)							Total fruit drop (%)	Fruits retained on tree (%)	Total No. of fruits/ tree
Main Plot (Variety)	Weeks after fruit set (WAFS)									
	3	4	5	6	7	8	9			
Bhur Litchi 1	64.22	5.88	12.35	29.65	4.47	2.26	10.08	78.28	21.72	3500
Bhur Litchi 2	55.32	17.01	14.26	32.57	6.77	2.27	8.24	74.80	25.20	3233
Hong houy	50.82	11.24	19.31	26.75	7.65	2.08	8.20	71.35	28.65	3161
Sub plot (Treatments)										
T1	52.97	2.02	3.47	7.97	1.79	0.46	1.67	70.35	29.65	3011
T2	56.93	2.93	4.36	7.01	1.49	0.49	2.04	75.26	24.74	3570
T3	60.46	3.15	3.44	7.22	1.48	0.59	2.47	78.82	21.18	3314
P>F Variety	0.21	0.12	0.35	0.59	0.14	0.93	0.98	0.13	0.13	0.73
Treatments	0.42	0.56	0.74	0.86	0.85	0.75	0.11	0.06	0.06	0.74
Variety*Treatment	0.17	0.12	0.32	0.21	0.37	0.35	0.25	0.43	0.43	0.15
Std. Error Mean	2.57	0.49	0.59	0.73	0.23	0.07	0.18	1.42	84.62	279.05

3.2 Percent infestation in dropped fruits

There was no significant difference in percent infestation of dropped fruits among the management methods as well as the tested varieties throughout the fruiting season except at the third week after fruit set. In the third week, a significant difference (P = 0.03, F = 5.00, df = 2) was observed in percent infestation of dropped fruit between the two management methods and the control. There was zero percent infestation of dropped fruit in both the treatments T1 (00.00 %) and T2 (0.00%), but significantly higher percent infestation of dropped fruits in the untreated control T3 (0.56 %) (Table 3). Dropped fruit infestation was recorded as early as the third week after fruit set (12th April) with an infestation rate of 0.19%. The trend increased gradually following sigmoid curve with the highest infestation rate at fruit maturity (69.09 %) (Figure 1 & 3).

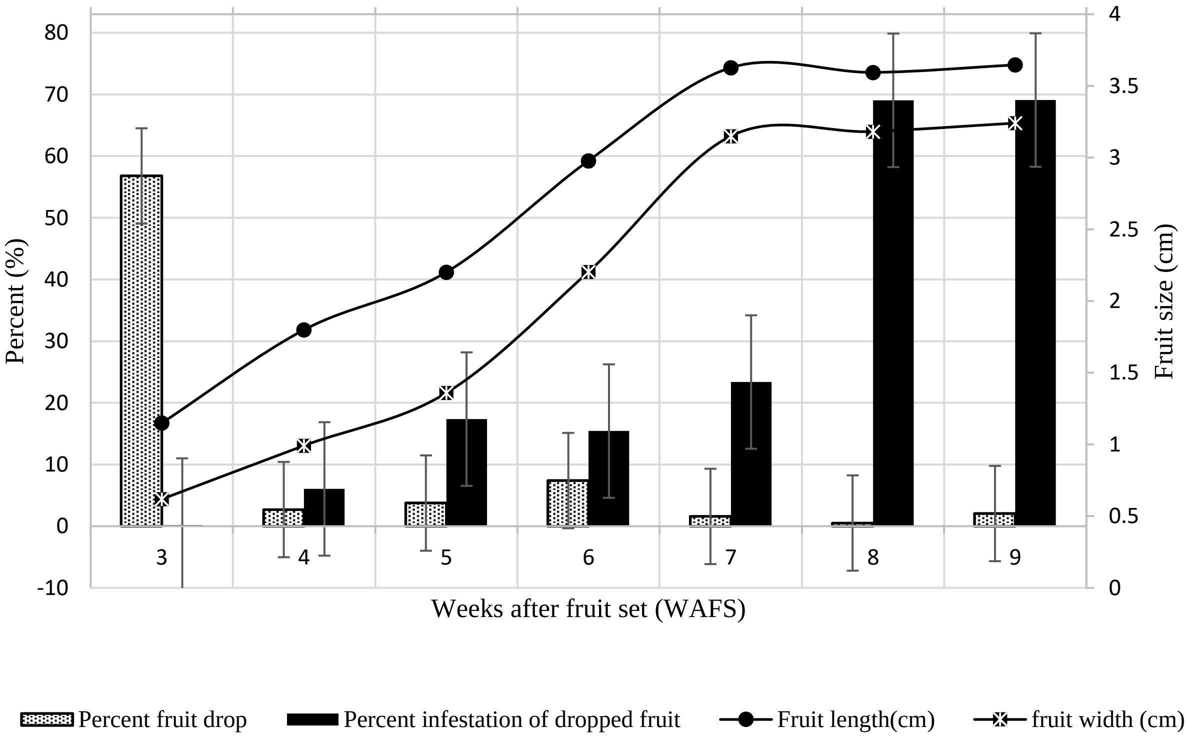

Figure 3. Percent fruit drop (%) and percent infestation of dropped fruits by *C. sinensis* during the fruit development period from 12th April to 24th May 2021

Table 4. Percent infestation of dropped fruit in three varieties of litchi with three treatments during fruiting period of 2021

Factors		Percent infestation of dropped fruit (%)						
		Weeks after fruit set (WAFS)						
Main Plot (Variety)		3	4	5	6	7	8	9
	Bhur Litchi 1	0.11	7.55	14.18	18.17	24.72	62.04	63.62
	Bhur Litchi 2	0.00	4.55	24.69	15.49	30.68	84.56	73.23
	Hong houy	0.44	6.04	13.23	12.63	14.73	60.52	70.42
Sub Plot (Treatments)								
	T1	0.00 b	5.59	14.70	14.44	21.63	65.18	72.25
	T2	0.00 b	5.02	15.35	17.01	19.99	64.87	66.78
	T3	0.56 a	7.53	22.05	14.84	28.51	77.08	68.24
P>F								
Variety		0.145	0.570	0.296	0.504	0.199	0.291	0.757
Treatments		0.026	0.476	0.056	0.841	0.062	0.610	0.864
Variety*Treatments		0.090	0.701	0.023	0.944	0.613	0.530	0.073
Std. Error Mean (±)		0.11	0.83	2.19	1.65	2.22	5.61	4.68

Means with the same letter are not significantly different at $P \leq 0.05$

3.3 Correlation analysis

There was no positive correlation observed between percent fruit drop and fruit size (r = -0.71 & r = -0.62, $P \leq 0.05$). Similarly, no positive correlation was observed between percent fruit drop and percent dropped fruit infestation level (r = -0.49, $P \leq 0.26$). However, there was a strong positive correlation (r = 0.79, $P \leq 0.03$ & r= 0.82, $P \leq 0.02$) between percent dropped fruit infestation and fruit size (length, width) (Table 4).

Table 5. Pearson correlation analysis between fruit drop rate, percent infestation of dropped fruit and fruit size

	Percent fruit drop (PFD)	Dropped fruit infestation percent (DFIP)	Fruit length (FL)	fruit width (FW)
(PFD)	1	-0.49	-0.71	-0.62
(DFIP)	-0.49	1	0.79*	0.82*
(FL)	-0.71	0.79	1	0.99
(FW)	-0.62	0.82	0.99	1

*****Correlation is significant at the 0.05 level (2- tailed)

3.4 Fruit infestation at harvest

Percent fruit infestation at harvest was significantly lower ($P \leq 0.001$, F = 34.06, df = 2) in management method T1 (34.74%) as compared to T2 (78.07%) and untreated control (89.98%). Although no significant difference was observed among the varieties, the lowest fruit infestation percent at harvest was found in variety Hong houy (56.02%) as compared to Bhur Litchi 1 (73.28%) and Bhur Litchi 2 (73.45%) (Figure 2).

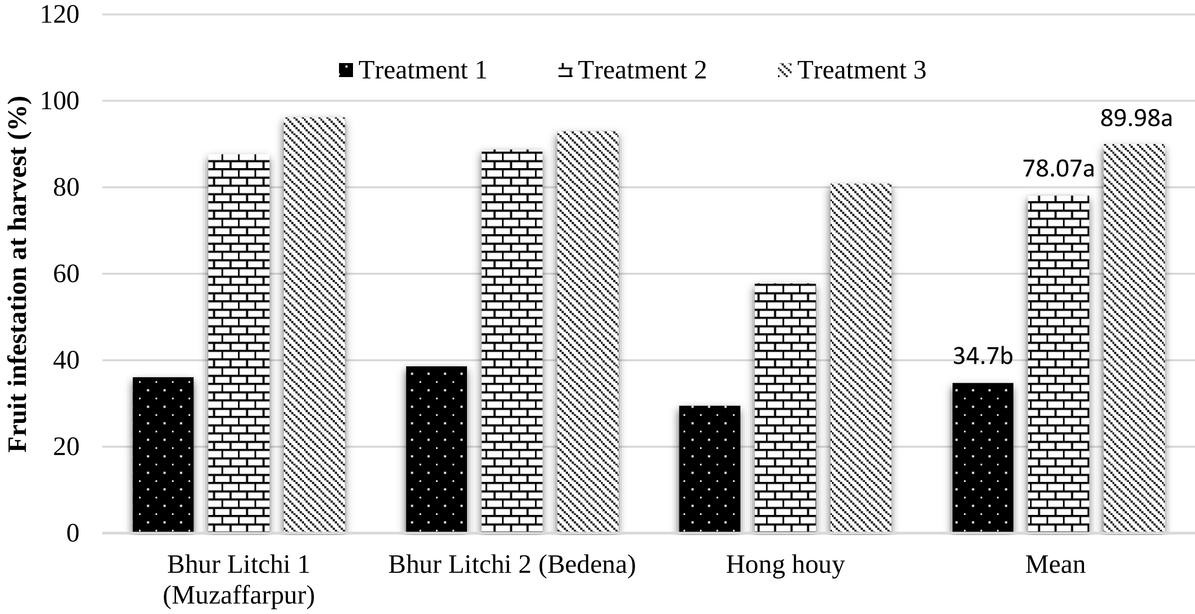

Figure 2. Fruit infestation percent at harvest by *Conopomorpha sinensis* in three varieties of litchi treated with three management methods (Treatments) during the fruiting period in 2021 at ARDC- Samtenling

Table 6. Analysis of variance of the fruit infestation percent at harvest between three varieties tested with three management methods (Treatments)

Source of variation		*DF*	*SS*	*MS*	*F*	*p*
Main plot	Rep	2	570.56	285.28	0.87	0.485
	Variety	2	1805.20	902.60	2.76	0.177
	Main plot Error	4	1309.95	327.49		
Sub Plot						
	Treatment	2	15232.83	7616.41	34.06	<0.0001
	Variety*Treatment	4	574.76	143.69	0.64	0.643
	Sub plot Error	12	2683.79	223.65		
	Total	26	22177.09			

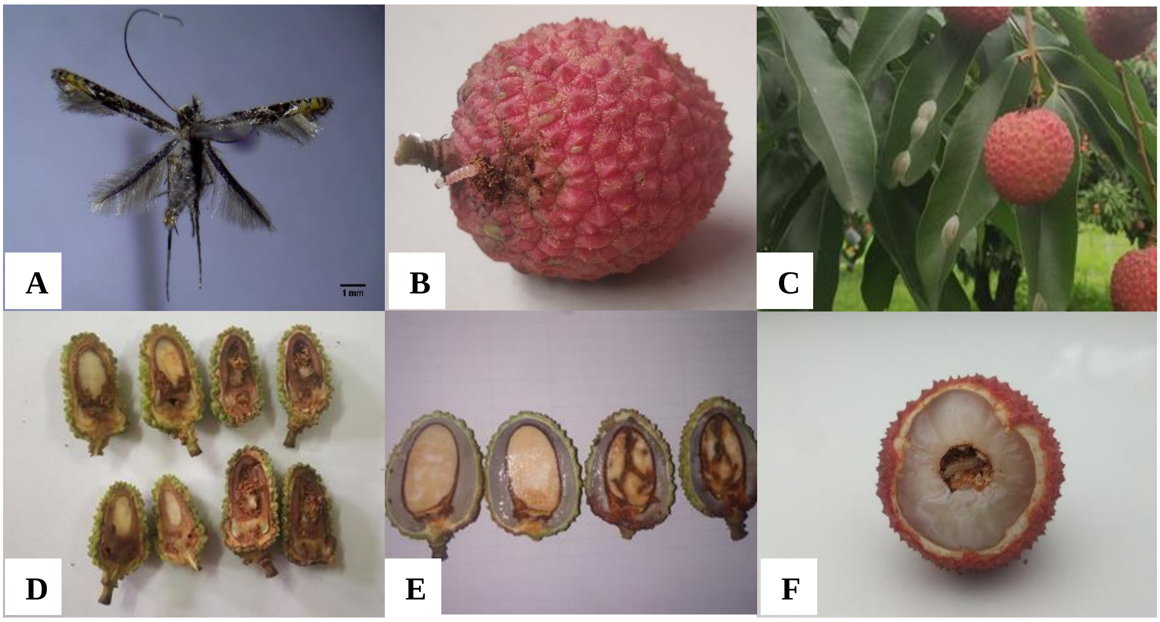

Figure 3. (A) Adult moth of Litchi Fruit borer, (B) Larva exiting from exit hole at the base of fruit, (C) Pupation on leaves after exiting from the fruit, (D) Fruit infestation at early fruit development stage, (E) Fruit infestation and damage at fruit maturity, (F) Poor fruit quality due to insect feeding frass at the base of fruit

A study by Schulte et al. (2007) demonstrated that litchi fruit infested by fruit borer at an early fruit growth stage shows higher fruit drop. In this study, although heavy fruit drop was observed in the third week after fruit set, fruit borer infestation level was observed to be very low. Studies have shown physiological fruit drop in litchi during the early fruit growth stage (Wei, Zhang, Li, Xie et al., 2013). Therefore, the early fruit drop, as observed in this study, might primarily be some form of physiological fruit drop than that caused by fruit borer infestation.

Research by Ramakrishnaiah, Damodaram, Rai, Rajendra et al. (2017) showed a positive correlation between the number of dropped fruits and fruits infested with *C. sinensis*, suggesting that borer incidences lead to heavy fruit drop. However, in contrast, there was no strong correlation observed between the percent fruit drop and percent infestation of dropped fruit in this study. This suggests that fruit borer incidence might not be the main factor causing fruit drop. Instead, other factors such as physiological fruit drop and infestation by other pests such as fruit borer (*Deudorix epijarbas*) larva, litchi bugs, birds and other abiotic factors might also have contributed to the observed fruit drop.

Ramakrishnaiah et al. (2017) also found that about 48.4% of the dropped fruit was infested by *C. sinensis* and the infestation decreased as the fruit matured. However, in our study the infestation of dropped fruits increased exponentially up to 69% as the fruit matured. A strong positive correlation between the dropped fruit infestation rate and fruit size was observed which indicates that the percent dropped fruit infestation rate increased with an increase in fruit size. Most of the fruit borer damage occurred at fruit maturity after the fruit colour break stage. Therefore, the results of this study align with the findings of Schulte et al. (2007), where a similar trend between the fruit infestation rate and fruit size was reported.

Schulte et al. (2007) also showed the ineffectiveness of insecticides such as ilorbac, imidacloprid and the three different concentrations of spinosad against *C. sinensis*. This was primarily attributed to the short exposure period between the egg hatching and fruit penetration by the larvae, where the larvae are not fully exposed to the insecticides to attain effective control. Finally, this study found that scheduled and combined application of Azadirachtin, Imidacloprid, Azadirachtin and Cypermethrin were effective in reducing litchi fruit borer infestation.

4. Conclusion

This study reveals the presence of heavy litchi fruit borer infestation regardless of the varieties grown in the sub-tropical region of Sarpang, Bhutan. Based on the result, the management method T1 was effective in managing *C. sinensis* as the lowest fruit infestation at harvest was observed in T1 (34.74%) as compared to T2 (78.07%) and the untreated control (89.98%). In terms of the variety, the lowest fruit infestation at harvest was observed in Hong houy (56.02%) as compared to Bhur Litchi 1 (73.28%) and Bhur Litchi 2 (73.45%).

Although fruit borer infestation in dropped fruit was observed as early as the third week after fruit set, infestation level was found to increase rapidly after the fifth and eighth week indicating that most of the fruit borer damage occurs at the fruit maturity stage after fruit colour break. The initial combined applications of biopesticide (Azadirachtin 300 ppm) and insecticide (Imidacloprid 20% SL) seem to have significantly suppressed the successive generations of litchi fruit borer. Further, the last application of Cypermethrin 10% EC at fruit colour break stage is found necessary to reduce fruit infestation during this vulnerable pest infestation period in addition to the scheduled application of control measures right from the early fruit development period. Further study needs to be carried out on the pest's biology and the effect of these combinations on natural enemies so that such management methods can be effectively used for managing this pest species in litchi.

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