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Monitoring of Chinese Citrus fly, *Bactrocera minax* (Enderlein) Pupae in Sweet Orange (*Citrus sinensis* (L.) Obseck) Orchards as a Pest Management Decision Tool in Nepal

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Abstract

Sweet orange is considered a high value crop in the mid-hill of Nepal. Insect pests and diseases are the major yield limiting factors in sweet orange orchard, Chinese citrus fly (CCF) being the most considerable pest in eastern to western hills of Nepal. This study aimed to examine the efficiency of the Area Wide Control Program (AWCP) by monitoring the pupal severity in the sweet orange orchards in Sindhuli district in 2021. Pupal density in different level of CCF infested sweet orange orchards (0-20%, 21-40%, 41-60%, 61-80% and 81-100%) were counted at 3 different depths of soil (0.0-5.0 cm, 5.1-10.0 cm and 10.1-15.0 cm). Five sweet orange orchards were selected in each infestation level and pupae were counted per square meter in 5 different sweet orange orchards. Maximum numbers of pupae (19) were recorded in 81-100% infested orchard followed by 61-80% infested orchard (17). Similarly, highest number of pupa (17) was counted at 5.1-10.0 cm depth around the tree canopy. Lowest numbers of pupae (1) were counted in 21-40% infestation level at 10.0-15.0 cm depth of soil ($P < 0.001$). Orchard with proper sanitation had 29.62% less pupa population compared to non-cleaned orchard with same level infestation. AWCP adopted orchard by spraying Great Fruit fly Bait (25% protein hydrolysate and 0.1% abamectin) had four times lesser pupal population compared to non-AWCP adopted orchard. The study shows that adoption of AWCP management strategy with proper sanitation in the orchard is effective to reduce population of Chinese citrus fly.

Keywords: AWCP, CCF, Management, Pupa, Sweet orange

Introduction

Citrus, among fruits, covers 23.6% area of the total fruit cultivated area of Nepal with productivity of 10.03 MT/ha (MoALD, 2020). Citrus occupies the third position in total fruit production and has 3.0% share out of total fruit export by volume from Nepal (Dahal, Shrestha, Bista, & Bhandari, 2020). Citrus cultivation is highly profitable and advantageous in comparison to other crops in hilly regions of Nepal (MoAC, 2011) and contributes in the profitability and income to the farmers of hilly regions. Sweet orange (*Citrus sinensis* (L.) Osbeck (pro. sp.) also known as Junar belonging to the family Rutaceae, is one of the major citrus fruit grown in the mid-hills of Nepal (Adhikari, Thapa, Joshi, Du, & Acharya, 2020; Gautam et al., 2020). It is commonly cultivated in 54 districts of Nepal and the leading districts for sweet orange production are Ramechhap (14.58 MT/ha) and Sindhuli (13.49 MT/ha) (MoALD, 2020).

Citrus crop is affected by several insects causing in the considerable loss in the production and yield. Fruit fly is the major destructive pest in the citrus orchard and farmers are facing huge loss due to infestation of fruit fly (Rai, Sah, Adhikari & Shrestha, 2022). Chinese citrus fly (CCF), *Bactrocera minax* (Enderlin) (Tephritidae: Diptera) is the major pest of Citrus in some of the countries of asia and Sweet orange is the most preferred host crop (Xia, Ma, Hou, & Ouyang, 2018). *B. minax* is an oligophagous and univoltine pest which can cause up to 97% fruit loss in severe infestations causing rotting of the fruits (Sharma, Adhikari, & Tiwari, 2015). The pupa is tolerant to low temperature and can remain in diapause condition for several months untill the onset of favourable condition (Rai et al., 2022). And after the onset of favourable condition, pupa develops into adult fly. The infestation was seen more frequently in the orchards found above 1,100 masl and maximum damage was observed in the Ramechhap and Sindhuli districts of Nepal and these losses have been increased in the past few years (Chauhan, Dhakal, Panthi & Adhikari, 2020) and currently the infestation is seen in the western part of Nepal moving from Eastern part of

Nepal. It reduces the yield and quality of fruits by laying eggs by inserting an ovipositor inside the fruits and later developing maggots which cause rotting of fruit (Adhikari et al., 2020). Due to severe damage by *B. minax*, farmers of eastern hills are compelled to replace sweet orange with mandarin orange on the same piece of land (Acharya & Adhikari, 2019).

Area Wide Control Program (AWCP) is an integrated and sustainable approach for the management of CCF over a large area. Spraying with protein bait, use of trap, sanitation of the orchard by removal of rotten fallen fruits with proper disposal, removal of pupa from the soil by soil ploughing etc. are commonly adopted in area-wide control program (Adhikari et al., 2020). This study helps to identify the pest population in different depths of the soil in the orchard and compares the pupal density in the orchard with and without AWCP. The study also examines the effectiveness of sanitation in the orchard for the management of the pest.

Materials and Methodology

Study site

The experiment was carried out in the Sindhuli district in 2021. The study locations were Gulanjor Rural Municipality ward no. 5 and Sunkoshi rural municipality ward no. 2 of Sindhuli district. The sites were chosen purposively to compare the orchard with and without AWCP.

Experiment details and Data record

Citrus orchards of the Sindhuli district were grouped into different categories based on the percentage of maggot infestation in the fruit in the orchard. The infestation level in the orchard is 0-20%, 21-40%, 41-60%, 61-80% and 81-100%. Five orchards from each infestation group were selected and 5 sampling spots around the tree canopy from each orchard were demarcated. The pupal number was counted from each sampling spot. The pupal count was carried out by using quadrant by making a quadrant of 1 square meter around the tree canopy. In each quadrant, pupal count was done from three depths (0.0-5.0 cm, 5.1-10.0 cm and 10.1-15.0 cm) assuming a similar percentage of moisture in all the depths of soil. Data of the count was recorded separately to determine the depth of maximum pupal count. For comparing the number of pupae in the field with and without AWCP adoption, pupa was counted from AWCP implemented location (Gulanjor Rural Municipality ward no. 5) and from the location where AWCP is not implemented (Sunkoshi rural municipality ward no.2) in Sindhuli district. The data collected was analyzed using R-studio. The comparison data were analyzed using an independent t-test and two-factor ANOVA by comparing means.

Results and Discussion

Pupa count based on depth and infestation level in the orchard

The number of pupae in the soil was significantly affected by the depth of the soil and the level of infestation in the orchard. The maximum number of CCF pupae per meter square (18.83) were recorded in the sweet orange orchard infested in the range of 81-100% followed by 61-80% level of infestation. The pupal population at 81-100% level of infestation was significantly at par with the density of pupa at 61-80% level of infestation in the orchard. The least population of pupa was found at 21-40% infestation. The population of pupa with 21-40% infestation was slightly lower than that of 0-20% infestation level. This might be due to the good tillage practice in the field. In addition to field sanitation, the farmers perform tillage and expose the pupa to direct sunlight.

The highest number of pupae (16.64) were found at depth of 5.1-10.0 cm followed by 0.0-5.0 cm (11.90) depth of soil. With the increase in the level of infestation in an orchard, the pupal count was recorded in the increasing trend. In three different depths of the soil, the number of pupae was different with the least value of (3.84) at 10.1-15.1 cm depth (Table 1). The highest number of pupae was found at the depth of 5-10 cm and pupa were found up to 15 cm. Thapaliya, Adhikari and Bhattarai (2020) reported that there was a direct relation between the infestation level in the orchard and the number of pupae found per square meter.

Table 1. Pupal count in different soil depths of orchards having different infestation levels in sweet orange orchards in Sindhuli District

Treatments	Pupa count
Depth	
0.0-5.0cm	11.90 (3.45) [b]
5.1-10.0cm	16.64 (4.08) [a]
10.1-15.0cm	3.84 (1.96) [c]
LSD (0.05)	0.293
SEM ±	0.002
F value	110.79***
CV%	16.35
Infestation level	
0-20%	5.19 (2.28) [c]
21-40%	4.41 (2.10) [c]
41-60%	9.12 (3.02) [b]
61-80%	16.56 (4.07) [a]
81-100%	18.83 (4.34) [a]
LSD (0.05)	0.378
SEM ±	0.001
F value	57.99***
CV%	16.35
Grand mean	3.168 (10.03)

Note: *** significance at 0.1% level, ** significance at 1% level, * significance at 5% level, ns= non-significance, LSD= Least Significance Difference, CV= Coefficient of variation, SEM= Standard Error of Mean, number in parenthesis shows square root transformed value

Table 2 shows that the highest number of pupae was from 81-100% infested orchard at 5-10 cm depth. The least infestation was recorded from 21-40% infested orchard at 10-15cm depth. The number of pupae at 41-60% infestation at a depth 0-5 cm was similar to the depth of 5-10 cm. The study result is similar with Xia et al., (2018) reported that the majority of pupa population was found at the depth of 4-6 cm.

Table 2. Pupal count at different infestations in different depths of soil in the orchard

Infestation Depth	0-20% Infestation	21-40% infestation	41-60% Infestation	61-80% infestation	81-100% infestation
0.0-5.0cm	(5.24) 2.29^{fgh}	(6.20) 2.49^{efg}	(13.17) 3.63^{cd}	(14.79) 3.84^{c}	(24.9) 4.99^{b}
5.1-10.0cm	(7.39) 2.72^{ef}	(7.56) 2.75^{ef}	(13.98) 3.74^{cd}	(28.3) 5.32^{ab}	(34.81) 5.9^{a}
10.1-15.0cm	(3.42) 1.85^{gh}	(1.14) 1.07^{i}	(2.78) 1.67^{hi}	(9.36) 3.06^{de}	(4.62) 2.15^{fgh}
LSD (0.05)	0.65				
F-value	7.25*** (P < 0.001)				
CV%	16.35				
SEM ±	0.005				
Grand Mean	3.168 (10.03)				

*Note: *** significance at 0.1% level, ** significance at 1% level, * significance at 5% level, ns non-significance, LSD = Least Significance Difference, CV = Coefficient of variation, SEM = Standard Error of Mean, number in parenthesis shows square root transformed value*

Pupa population based on the sanitation in the orchard

Table 3 shows the number of pupae in different levels of infestation in the orchard has a significant influence on the extent of sanitation. The number of pupae was 29.62 % lower in the sanitized field in comparison to the field without sanitation having a similar infestation level (81-100%). This indicates that field sanitation directly influenced the number of pest present in the orchard. Sanitation includes removal and management of fallen rotten

fruits from the orchard. Gautam et al. (2020) also revealed that one of the causes of loss in the orchard was poor sanitation. This result supports Hasyim, Muryati and De Kogel, (2008) which revealed that the fruit fly population was directional proportional to the number of damaged fruits left in the orchard and there was 20% decrease in the population of insects with field sanitation in comparison to the field without sanitation. The study result supports Huan et al. (2019) which suggests sanitation in the orchard for maintaining the *Bactrocera spp.* Population below the economic injury level.

Table 3. Number of pupae in the orchard with and without sanitation at same infestation level

Sanitation in orchard	Mean number of pupae ± SE
Orchard with sanitation	21.40 ± 0.18
Orchard without sanitation	27.74 ± 0.29

Pupa of CCF in AWCP implemented and non-AWCP orchard

An independent t-test was performed to know the differences between the pupa number in two different kinds of orchards i.e., AWCP adopted and non-AWCP adopted orchards. There was a significant difference in the number of pupae between the AWCP adopted and non-adopted field (t=3.37, p=0.007) (Table 4). Since the area wide management approaches (use of protein bait, field sanitation) were introduced in the field, the number of pupae was 4 times higher in the area where area wide control program was not applied. The use of AWCP along with sanitation decreased the pupal dynamics in the soil which supports the result of Adhikari et al. (2020) where the percentage of infestation was lower after the application of AWCP with proper sanitation.

Table 4. Number of pupal counts under AWCP and non AWCP conditions in Sindhuli district

Management practices	Mean (No of pupa)	P-value	Std
AWCP	6.57	0.007	4.42
Non AWCP	24.15		14.98

P = Significance value

Conclusion

Chinese citrus fly infestation is the major problem faced by the farmers in Sweet orange cultivation causing great loss in production. The number of pupae in the field is directly related to the loss in the yield so monitoring of pupa in the field is very essential to estimate the population of the pest. The pupa population was highest near the soil surface around the tree canopy and the number of pupae decreased at a deeper depth of soil. The pupa population was significantly different in the orchard with and without AWCP implementation. Field sanitation including the collection of fallen rotten fruits and safe disposal also plays role in reducing the pupa population in the orchard which eventually decreases the number of adult CCF infestations in sweet orange. Sustainable management of CCF is very essential for the production of sweet orange in the orchard as it is the major yield loss factor for the farmers. Area-wide control program along with sanitation of the field can be an effective integrated approach to helping in the management of the CCF in the field.

Author's Contribution

S. Sharma is involved in conducting the study, analyzing the result and preparing the manuscript. S. Tiwari, D. Adhikari and B. Gautam are involved in monitoring and supervising the work, sharing guidelines for results and manuscript.

Declaration of Conflict of Interest and Ethical Approval

The authors have gone through the manuscript before submission and declare that there is no any type of competing interest regarding the current manuscript. The article does not include any human participants or animals by authors and has taken prior approval if applicable.

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