Impact of Pesticides on Mealybug Parasitoid *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae)

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Abstract: *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae) is solitary endoparasitoid associated with mealybugs. Parasitoid having very good biocontrol potential against *Phenacoccus solenopsis* Tinsley and *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae). The parasitoid affected by several pesticides sprayed on vineyards for the control of pest species. Bioassay of nine different pesticides through dry film method was conducted in the laboratory by using adults and pupae of parasitoid. Among tested pesticides Spirotetramate and Horti-Impact caused high mortality of adult parasitoid and similarly these pesticides also found toxic towards adult emergence of parasitoid. Whereas, drenching of Imidacloprid and Clothianidin cause moderate mortality of parasitoids emerged from cocoons. The emerged parasitoids from treated cocoons were tested for their longevity. Even 24 hours after treatment (HAT) Agroclean (10.00%), Azadirachtin 5% (16.66%), Spida (20.00%), Brigade (30.00%) and Azadirachtin 1% (40.00%) did not cause adverse effect at the recommended dose for adult mortality and likewise both Agroclean and Spida showed 80.00% emergence of parasitoid from treated cocoons. The objective of the present work is to analyse the pesticides which are compatible to parasitoids which initiate the biocontrol programme. This will help to develop baseline data to evaluate efficacy of pesticides on biocontrol agents.

Key Words: Coccidoxenoides perminutus, Mealybugs, Bioassay, Biocontrol, Phenacoccus solenopsis, Maconellicoccus hirsutus.

Introduction:

Use of broad spectrum pesticides for control of pests is the choice of farmers, which affect the population of biocontrol agents and also pollinating insects. The need of modern agriculture is to develop awareness among farmers towards eco-friendly management of pest species and diminish the threat to biocontrol agents. Impact of pesticides on vineyards is initiated with mortality of pests and continues to the residue problem on vine, which finally shows high response to biocontrol agents which lower down longevity, fecundity and also ability to find hosts [1]. The searching and selection of hosts by parasitoid counts the success of biocontrol programme of any pest species [2].

Coccidoxenoides perminutus (Timberlake) (Hymenoptera: Encyrtidae) is small solitary parasitoid of *Phenacoccus solenopsis* Tinsley and *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae). The parasitoid showed potent role as biocontrol agent for suppression of grape mealybug [3]. Efficient use of *C. perminutus* in vineyard ultimately minimizes the disruptive use of pesticides. Similarly, heavy dose of pesticides reduces the population of parasitoids in the field and causes interruption in natural control of pests [3].

Several pesticides were tested for effect on biocontrol agents of apple as well as citrus pests and found compatible pesticides with natural enemies [4]. Likewise, impact of pesticide residue on a parasitoid *Aphelinus mali* Haldeman of woolly aphid, *Eriosoma lanigerum* (Hausmann) from apple orchards and analyse the safer pesticides for biocontrol agents [5]. Similar attempts have been made to evaluate the eco-friendly pesticides.

Substantial dose of pesticides on vineyard for controlling mealybugs and other pests may lead to severe problem like residue due to which the table grapes and wine are rejected from international market. This is only because there is growing concern on environment and health. Hence, there is need to develop the strategies that minimizes their disruptive effects of pesticides on vineyard and surpasses the threats by keeping the pest population under check and biocontrol plays a vital role. In table 1, the characteristics of pesticides used to study their effect on biocontrol agent are tabulated.

Pesticides are applied based on Economic Threshold Level (ETL) of pest species but lack of concern towards population of natural enemies while application of pesticides. Thus, found disruption in natural establishment of parasitoid (fig. I). Therefore, there is need of modern agriculture to pay attention towards level of natural enemies and spraying should be done accordingly. The attempts has been made to evaluate the pesticidal effect on adult longevity of parasitoid when sprayed on crops and also analyse the adult emergence from treated cocoons that help to develop compatible pesticides for biocontrol programme [6], [7]. After application of pesticides, mummies of mealybugs containing pupae of parasitoid observing for the impact of ingestion of pesticide through adult exit [8].

The objective of the study is to analyse the impact of pesticide on longevity and emergence of *C. perminutus*. This also leads to found the compatible pesticides for parasitoids and also timing of parasitoid release in biocontrol programme to minimize pesticidal mortality of parasitoids in vineyards.

Materials and Method:

1. Pesticides spray for *P. solenopsis*

Experiment was carried out on Hibiscus plants (*Hibiscus rosasinensis* L.) for control of *P. solenopsis*. The heavily infested plants along with mummies of mealybugs (fig. I) were treated with different pesticides (Table 1). The treatments were repeated at one week interval and replicated three times for confirming the result. Application of nine different pesticides which were used to control mealybugs with different mode of action and their recommended doses were sprayed on plants.

2. Collection and rearing of *C. perminutus*

The cocoons of parasitoids were collected from the untreated field and they kept in test tube for adult emergence. The emerged adults were used for mortality experiment. The adult parasitoids supplied with 50% honey as food [9]. Simultaneously in vitro mass rearing of parasitoids was carried out by exposing the mealybugs towards the females of *C. perminutus* and culture was maintained in the laboratory to fulfil the requirement of parasitoids.

3. Adults of *C. perminutus* exposed to pesticides

Adults of *C. perminutus* were exposed directly to pesticides on treated test tubes for 24 hrs and replicated three times. For each treatment test tube was treated with recommended pesticidal does sprayed with the help of atomizer and cotton wool soaked with 50% honey water solution was provided to the parasitoids as a food source [10], [11]. Treatments consisted of 9 pesticides and one with water sprayed as control.

The clean test tubes were treated with pesticides dried at room temperature to evaporate the water and releasing adults of *C. perminutus*. The mortality of parasitoid was checked under the laboratory conditions 25±2^{O}C, 70±5% RH and 12:12 (L:D) photoperiod.

Observations were taken after 6, 12, 18 and 24 hours of parasitoid introduction. They were supposed to be dead when their movement was not observed even after touch with needle. A trinocular stereozoom microscope Leica M-165-C (Leica Microsystem Ltd., Germany) with 1.0x lens was used to examine parasitoid for the experiment. Mortality of parasitoid was calculated by using Abbott's formula [12].

4. Emergence of adults from treated cocoons

The cocoons of parasitoid were collected after 24 hrs of spraying and kept in test tubes for observing the adult emergence. Effect of pesticides on the adult emergence was calculated by decrease in numbers of adult emerged from the treated cocoons. The emerged adults of *C. perminutus* were fed with 50% honey [9].

Statistical analysis was made by using ANOVA, Tukey's HSD test in the computer programme SAS version 9.3.

Results:

1. Pesticides spray for *P. solenopsis*

Population of *P. solenopsis* from the field found to be minimized with treatment of pesticides (Table 1). Mealybugs along with cocoons were densely sprayed with pesticides. Three applications of pesticide were required to check the mealybug population under control whereas; some treatments like Horti-impact, Spirotetramate, Imidacloprid control the pest population after single application. Clothianidin, Spida, Brigade, Agroclean required two applications but in general after third application the pest population could controlled effectively.

2. Collection and rearing of *C. perminutus* Emergence of adults from treated cocoons

The number of adult emerged from the treated cocoons were dependent on toxicity of chemical, as toxicity increases the number of adult emergence decreases and vice versa. The results showed in table 2 and fig. II indicated that, Agroclean and Spida showed best effect with 80% of adult emergence from each treatment whereas; Horti-impact and Spirotetramate, found hazardous towards biocontrol agents with 4, 10, 10, 16.66 and 22 % of adult emergence respectively. From the results obtained we concluded that the protective covering of pupae cannot cause any interference with mortality of parasitoids. Even if the parasitoid emerged, it may die earlier as compared to control that may because of adult at the time of breaking of cocoon to exposed out, the pesticide taken place which cause mortality.

3. Adults of *C. perminutus* exposed to pesticides

Adult of *C. perminutus* were exposed directly towards the treated test tubes and found that the mortality of parasitoid may dependent on toxicity of pesticides. The results are tabulated in table 3 showed that the adults of *C. perminutus* having maximum longevity towards biopesticides as compared to synthetic pesticides. From the results it concluded that Agroclean, Spida and Brigade found to be potent for spraying while for drenching Neemazal 5% showed minimum adult mortality so these were best suited in biocontrol programme.

Discussion:

Adult emergence and mortality has to be lowered as compared to control which is probably because of impact of pesticidal residue on developmental pattern of parasitoid. However, in field condition the pesticide residue on vineyard may be lower. Similar findings were observed and calculated toxicity of pesticide residues on glass plates and also on field, and found toxicity of pesticides on glass plates are higher as compared to field and finally concluded that it may because, insects in the field search their shelter where the pesticides may not reach [6]. Results may be also varied because of large area and migrating nature of insects.

Several workers were contributed to analyze the role of pesticides on biocontrol agents. Stenersen screened toxicological study of chemical pesticides with their mode of action on arthropods [13]. Izawa *et al* conducted experiment on inhibition of chitin biosynthesis by buprofezin analogs with their relation for controlling *Nilapavata lugens* Stal [14]. However, effect of buprofezin also studied on survival of *Harmonia axyridis*, *Stethorus punctum picipes, Orius tristicolor* and *Geocoris* sp and found that buprofezin is compatible with IPM programs using parasitic wasps and predators [15].

Nalini and Manickavasagam, evaluated safety of insecticides to mealybug parasitoids, *Aenasius bambawalei* Hayat and *Aenasius advena* Compere by using dry film method at 1, 3, 6, 12, 18 and 24hrs after treatment (HAT) [16]. Results showed that endosulfan, monocrotophos, profenofos and dimethoate caused 100% mortality therefore to be termed as hazardous to biocontrol agents. Horti-Impact, Spirotetramate and Imidacloprid showed high toxicity to the parasitoids therefore, may not compatible in the IPM programme conducted for mealybug control in vineyards. Avoid use of these chemicals where there is establishment of parasitoids. Likely, Walton and Pringle, stated hazardous nature of Cypermethrin to *C. perminutus* and they discontinued spraying of Cypermethrin at the time of parasitoid release for establishment [3].

The cocoon case of parasitoid acts as barrier for entry of pesticide to the pupa of parasitoid [8]. But it is observed that, the adults died as they masticate the case by making exit hole with their mandibles. The partial exit of adult parasitoid also found due to high toxicity of pesticides like Horti-Impact and Spirotetramate. In the field situation these pesticides also contaminate the food source as well as shelter places of parasitoids hence, the performance of parasitoids may adversely affected [17]. In present findings, the adult stage of parasitoid was more sensitive to pesticides than immature stage (pupa) developing in mummies, as they directly exposed towards pesticides whereas, pupa must be safer in the case which acts as barrier for pesticides. Several workers were contributed to the effect of pesticides on the lifecycle and behavioural pattern of parasitoids [18], [19], [20].

Low persistence pesticides may be used to attain maximum longevity of biocontrol agents [21]. The place and time of pesticide application plays a crucial role in pest management strategies [22], [23]. Stem application of pesticides in high infested fields minimizes mortality of parasitoids which is due to proper shelter for parasitoids and ultimately achieve maximum parasitism. According to Grafton-Cardwell *et al*, for in vitro study, some insecticides showed little negative impact on parasitoids but, in vineyards they can reduce parasitoid population indirectly by reducing the pest infestation [24]. Similar findings were observed during present study. Both Neemazal 1% and 5% showed delayed adult emergence, this may found to reducing the ability of parasitoid to parasitize the mealybug. Horti-Impact, Spirotetramate and Imidacloprid showed high toxicity to mealybug parasitoid while Agroclean, Spida, Brigade, Neemazal 1% and Neemazal 5% should be safer to parasitoid which did not cause significant mortality at the recommended dose. The present study will be helpful for mass rearing and mass release of parasitoid in biocontrol programme. However, application of compatible pesticides for control of pest population helps to establish biocontrol agents in the field.

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Table 1: Toxicological characteristics of pesticides used for experiment

Treatment	Name of Pesticide		Name of Manufacturing company	Recommended Dose
	Trade Name ®	Active ingredient		
T1	Agroclean™	Botanical Agrobase	Shukla Ashar Impex Private Limited	3.3 mL/lit.
T2	Horti-Impact™	Antibacterial Agrobase	Green Vision Life Sciences Pvt. Ltd	2.0 mL/lit.
T3	Movento™	Spirotetramate 240 SC	Bayer CropScience Limited	0.625 mL/lit.
T4	Admire™	Imidacloprid 70WG	Bayer CropScience Limited	0.6 g/lit.
T5	Dantotsu™	Clothianidin 50WDG	Sumitomo Chemical India Pvt. Ltd.	0.275 g/lit.
T6	Spida™	Botanical extract	Kan Biosys Pvt. Ltd.	1.0 mL/lit.
T7	Brigade™	Plant Probiotic	Kan Biosys Pvt. Ltd.	7.5 mL/lit.
T8	Neemazal 5% W/W™	Azadirachtin 5%	EID Parry India Limited	1.25 mL/lit.
T9	Neemazal 1% EC™	Azadirachtin 1%	EID Parry India Limited	3.0 mL/lit.
T10	Untreated Control	-		-

Table 2. Effect of Insecticides over the emergence rate of *C. perminutus*

Treatment No.	Treatments	Dose mL/L or g/L	Percent Emergence of Adult Parasitoids
T1	Agroclean	3.3	80.00±20.00[a]
T2	Horti Impact	3.0	10.00±8.00[b]
T3	Spirotetramate	0.625	16.67±15.25[b]
T4	Imidachloprid	0.6	36.67±20.81[ab]
T5	Clothianidin	0.275	40.00±17.32[ab]
T6	Spida	1.0	80.00±10.00[a]
T7	Brigade	7.5	53.33±5.77[ab]
T8	Neemazal 5%	1.25	73.33±25.17[a]
T9	Neemazal 1%	3.0	60.00±17.32[ab]
T10	Control	-	83.33±28.87[a]
	F value (9,20)	-	6.38 Pr< F (0.003)

Table 3. Effect of Insecticides over the adults of *C. perminutus*

Treatment No.	Treatments	Dose mL/L or g/L	Mean Parasitoid Adult mortality (n=10)
T1	Agroclean	3.3	1.00±0.00[e]
T2	Horti Impact	3.0	7.67±2.31[ab]
T3	Spirotetramate	0.625	8.00±1.73[a]
T4	Imidachloprid	0.6	6.00±1.73[abc]
T5	Clothianidin	0.275	5.00±1.00[abcd]
T6	Spida	1.0	2.00±1.00[de]
T7	Brigade	7.5	3.00±1.00[cde]
T8	Neemazal 5%	1.25	1.67±0.57[de]
T9	Neemazal 1%	3.0	4.00±1.00[bcde]
T10	Control	-	0.33±0.00[e]
	F value (9,20)	-	13.17 P<0.0001

Fig. I: Natural establishment of parasitoid in field

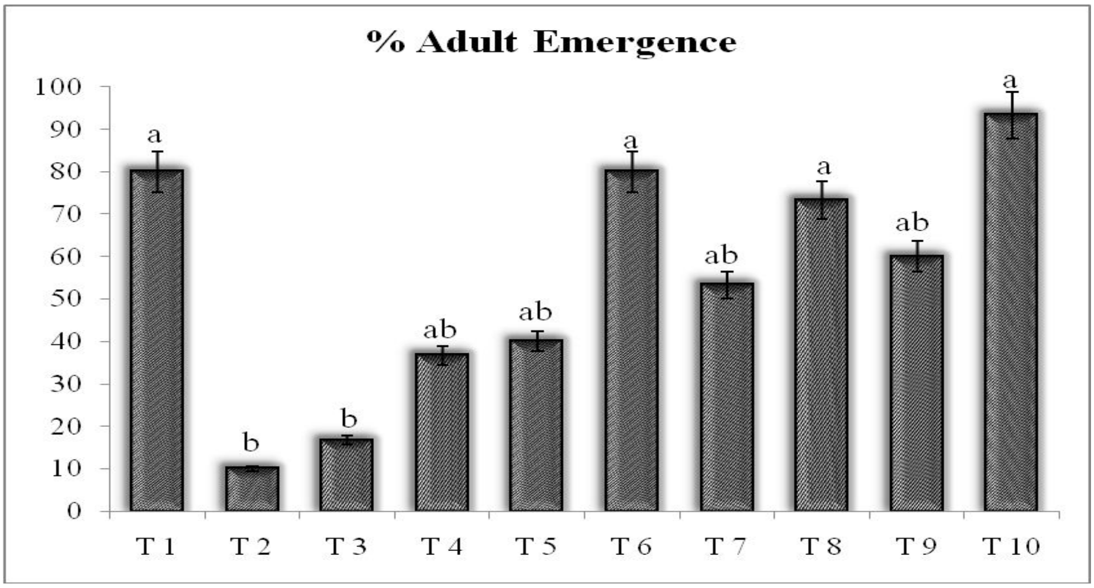

Fig. II: Per cent parasitoid adult emergence after pesticidal treatment