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## Evaluation of Ecofriendly Control Methods for Management of Mealybug, *Phenacoccus solenopsis* Tinsley in Cotton

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### ABSTRACT

Eco-friendly management of mealybug, *Phenacoccus solenopsis* Tinsley, was important to reduce reliance on insecticides and conservation of natural enemies. The study was conducted to evaluate a set of eco-friendly biopesticides, biorationals and insecticides against mealybug, *Phenacoccus solenopsis* Tinsley. The treatments were applied thrice in mealybug infested field at 15 days interval and the mortality data both for mealybug and generalist predators were recorded. Similarly the mortality data on 5 day old mealybug nymphs was also obtained under laboratory condition. The insecticides, acephate and chlorpyrifos proved effective in reducing the population of mealybug by 72.34 and 68.60%, respectively after 3 spray applications. The insecticides were found statistically superior over biopesticides and biorationals in reducing the mealybug population. The biopesticides and biorationals insecticides reduced 18.52 to 41.42% mealybug population after three spray applications under field condition. The biopesticides and biorationals are least toxic to generalist predators and can be integrated with insecticides for sustainable management of the mealybug.

**Key words:** Bacterial symbiont of nematode, biorational, entomopathogens, mealy quit, predator

### INTRODUCTION

Mealybugs are sap-sucking insects and some species cause severe economic damage to a wide range of vegetable, horticultural and field crops. In cotton, mealybugs were never considered as pest of economic importance (APCoAB, 2006). The infestation of the mealybug on cotton in North India has been reported (Monga *et al.*, 2009a) during, 2006. Widespread infestation of the species *Phenacoccus solenopsis* (Tinsley), originally described from the US (Tinsley, 1898), causes a great concern in India (Nagrare *et al.*, 2009). This pest species, considered as exotic (Hayat, 2009) have enough potential to create havoc, not only on cotton, but on other crops as well. The potential loss recorded due to infestation of mealybug in cotton was 15.60% at 1st grade severity, 31.78% at 2nd grade severity, 36.18% at 3rd grade severity and 52.55% at 4th grade severity index (Anonymous, 2009). The incidence of mealybug reported in North cotton growing zone of India was 38.58% during 2008 and 15.90% during 2009 with severity index of 1.54 and 1.32, respectively, during 2008 and 2009 (Kumar *et al.*, 2011). The wide host range and absence of any naturally occurring predators, parasitoid or pathogen helped this pest to establish easily on cotton in India. A large number of hosts including overwintering and source for off season survival of mealybug *P. solenopsis* has been reported from South East Asian cotton growing countries (Abbas *et al.*, 2010;

Rishi *et al.*, 2009). Having a skewed and clumped distribution mealybugs are easily managed by predators and parasitoid (Afifi *et al.*, 2010). As Bt cotton is attacked by sucking pests (Abro *et al.*, 2004), presence and abundance of large number of predators associated with sucking pests have been reported in cotton.

The management interventions with insecticides disturb the naturally occurring control of mealybugs through predators and especially the *Aenasius bambawalei* a potential natural parasitoid. The biorationals/biopesticides were earlier reported as effective against insect pests, safe to natural enemies and to environment (Shonouda *et al.*, 2008; Irshad, 1999; Duraimurugan and Regupathy, 2005) but could not find any place in recommendations for mealybug management. So, the study was carried out to evaluate a set of eco-friendly strategies including biopesticides, biorational and insecticides for sustainable management of mealybug.

## MATERIALS AND METHODS

The experiments were carried out at experimental farm of Central Institute for Cotton Research (CICR), Regional Station, Sirsa (Longitude 75° 02' 11.05" E and Latitude 29° 32' 44.3" N). The cotton crop was sown during the second week of May during the year 2008 and 2009. The experiment was conducted in randomized block design with a plot size of 5.4×4 m and three replications. Three spray applications were given during the peak infestation of mealybug at 15 days interval with the help of knapsack sprayer. The first spray application was done on 20th and 30th August during 2008 and 2009, respectively followed by two more sprays at 15 days interval. The total numbers of treatments applied are listed in Table 1.

**Preparation for spray solution of insecticides:** Two insecticides from organophosphate group acephate and chlorpyrifos were applied at 4 g L<sup>-1</sup> and 6.0 ml L<sup>-1</sup> volume of water, respectively, purchased from Excel crop science. Triton-X-100 sticker was also added at the rate 1 ml L<sup>-1</sup> of spray solutions for prolonged action for all the treatments excluding entomopathogens. Total volume of water required for three plots was calibrated.

**Preparation of spray formulation of entomopathogen (Biopesticides):** Entomopathogen fungi were obtained from National Centre for Integrated Pest Management (NCIPM), New Delhi, India. Talc based formulation of *Verticillium lecanii*, *Beauveria bassiana* and *Metarrhizium*

Table 1: Details of treatments applied

Name of treatment*	Dose/liter	Mode of action
Acephate 75% SP**	5 mL	Systemic organophosphate
Chlorpyrifos 20% EC***	8 mL	Contact organophosphate
5% NSKE	50 g seed kernel	Ovipositional deterrent, Repellent
Neem oil (2.5 L ha <sup>-1</sup> ) + Nirma powder (0.1%)	6.25 ml L <sup>-1</sup> + 1 g L <sup>-1</sup>	Ovipositional deterrent, Repellent
<i>Verticillium lecanii</i>	5 g	-
<i>Beauveria bassiana</i>	5 g	-
<i>Metarrhizium anisopliae</i>	5 g	-
Bacterial symbiont of nematode	1: 4 (Water)	-
Fish Oil rosin Soap (FORS)	20 g	Stomach
New botanical (Mealy Quit)	10 g	Contact and stomach
Control water spray	---	---

\*Both for laboratory and field evaluation, \*\* WHO Class-III Slightly hazardous, \*\*\*WHO Class-II Moderately hazardous

*anisoplae* containing  $10^8$  conidia  $g^{-1}$  of formulation were added to water in the spray tank at the dose of  $5 g L^{-1}$  and the solution was stirred mechanically. For each species the slurry at  $g L^{-1}$  of water was added to spray tank during application of entomopathogenic fungi to facilitate the germination of spores on plants after spraying. The conidia count  $mL^{-1}$  was confirmed using a haemocytometer. Plots receiving treatments of the fungus were also pre-sprayed with water at one liter water per plot to create higher level of humidity, to facilitate the spore germination. The entomopathogen once in contact with host start germinating in the body of host and eventually the infected host dies.

Bacterial symbiont of nematode, an entomopathogen was developed and formulated at Crop Protection Division, Central Institute for Cotton Research (CICR), Nagpur and obtained for testing under Technology Mini Mission on Cotton (TMC-MM-1-3.1) project. One milliliter of bacterial symbiont of nematode was diluted with 1:4 ratio of water as advised by the concerned nematologist.

**Preparation of Neem Seeds Kernel Extract (NSKE), neem oil, mealy quit and Fish Oil Resin Soap (FORS):** The neem seeds for preparation of NSKE were obtained from neem trees available at Experimental Farm of CICR, Regional Station, Sirsa. 500 g dried and powdered neem seeds were soaked in 10 L of water overnight for preparation of 5% NSKE. The suspension was filtered through muslin cloth and applied with the help of knapsack sprayer. The readymade neem oil was obtained from local market and was applied at the rate of  $6.25 ml L^{-1}$  of water; the liquid soap at a dose of  $1 g L^{-1}$  was added to the spray tank as a wetting agent with neem oil.

The Fish Oil Resin Soap (FORS) procured from Crop Protection Division, CICR Nagpur was used at the rate of  $20 g L^{-1}$  of water. The fish oil resin soap has earlier been tested and found effective against other species of mealybug. Mealy Quit is herbal insecticide formulated by Crop Protection Division, Central Institute for Cotton Research especially for mealybug and is in process of registration with Central Insecticide Board (India) and was applied at the rate of  $10 g L^{-1}$  of water.

**Measurements of mealy bug population for observations under field experiments:** As the experiment was conducted on the plants having mealybug severity grade II or more, the population of mealy bug was counted from the five centimeter portion of infested shoot/branch of cotton plant. The grading system was followed as given by Monga *et al.* (2009a) (0-No Mealybug; I-About 1-10 Mealybug scattered over the plant; II-One branch infested heavily with mealybug; III-Two or more branches infested with mealybug, up to 50% plant affected and IV-Complete plant affected).

The mealy bug population was counted both pre and after application of different treatments. The populations of generalist predators like spiders, green lace wing and lady bird beetle per plant were also recorded to study the harmful effect of the treatments applied. These predators are associated with all sucking pests including mealybug. The mummified mealybug bodies due to *A. bambawalei* were also recorded as this parasitoid was specific to mealybug, *P. solenopsis* Tinsley.

**Laboratory bioassay:** The laboratory bioassay was carried out against 5 day old nymphs of mealybug using the spray solutions prepared for field conditions by leaf dipping method. Cotton leaves (RCH-134) were collected from plants and surface sterilized with alcohol before treatment. Treated leaves were kept on sterile moist filter paper in petriplates and the stalk end of the leaf was put in eppendorf tube containing sucrose solution to maintain its turgidity. The eppendorf tube was plugged with cotton and cello tape. Five day old 120 nymphs were released on each treated leaf in

a petriplate. To avoid any error in counting due to escape or other settling problem, the nymphs were first allowed to settle on treated leaf for 12 h, the leaf was then taken out of the petriplate and transferred to another fresh and sterilized petriplate and the settled nymphs of mealybug were counted carefully. The petriplate containing treated leaf with settled mealybug nymphs was sealed with paraffin film to further reduce the chances of escape. Petriplates were stored at  $25\pm 2^{\circ}\text{C}$  in BOD incubator. The data on nymphal mortality was recorded at 48 h after inoculation. To verify mortality or survival a fine hair brush was used to touch the body of insect to induce or detect movement. Individual were considered dead based on the absence of leg movement after stimulus or colour change. In case of biopesticides (entomopathogens) treated insects cadavers were surface sterilized in absolute alcohol and placed on SDYA (Sabouroud Dextrose Yeast Agar) medium for growth of fungi. But for nematodes the conventional method of touch and observe was used.

**Statistical analysis:** The % reduction in mealy bug population was calculated and the pre and post treatment population of mealybug was subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT) using SAS (Statistical Analysis System).

## RESULTS

Three sprays of all the insecticides, biopesticides (entomopathogens) and biorational were applied at 15 days interval. Insecticides acephate (Class III-slightly hazardous declared by World Health Organization) proved effective in reducing the population of mealybug by 72.34% after 3 spray applications. The pre treatment population of mealybug recorded in acephate treated plot (pooled population of 2008 and 2009) was 29.25 mealybugs/plant. The population recorded after 1st spray was 12.25 mealybugs/plant, after 2nd spray it was 10.06 mealybugs/plant and after 3rd spray it was 8.09 mealybugs/plant. The chloropyriphos reduced the population of mealybug by 68.60% after three spray applications. The pre-treatment population of mealybug recorded was 39.05 mealybugs/plant, after 1st spray 19.33 mealybugs/plant, after 2nd spray 16.42 mealybugs/plant and 12.26 mealybugs/plant after 3rd spray application, respectively (Table 2).

Among botanical/biorational pesticides 5% NSKE, Neem oil ( $2.5 \text{ L ha}^{-1}$ )+Nirma powder (0.1%), FORS and Mealy quit were applied. In case of Mealy quit, the population of mealybug reduced from 41.43 mealybugs/plant (pre-treatment) to 27.74 mealybugs/plant after 3rd spray, accounting for 33.04% reduction in the population. The population of mealybug in FORS treated plots was 44.15 mealybugs/plant before spray application but reduced to 31.8 mealybugs/plant after the 3rd spray (27.97% reduction). The population reduction recorded in 5% NSKE and Neem oil+Nirma powder treated plots were 21.77% and 18.52%, respectively (Table 2). The population of mealybug/plant after three spray applications was significantly less in insecticidal treatments than the biorationals. The botanicals and biorationals do not proved much effective in reducing the mealy bug population as compare to the chemical insecticides (Table 2). But the biorationals (mealyquit or FORS) can be applied at early stage of crop growth as observed comparatively effective against mealybug and safer to natural enemies.

In addition to chemical insecticides and botanical, four entomopathogen i.e., *V. lecanii*, *B. bassiana*, *M. anisoplae* and Bacterial symbiont of nematode were also applied. Among the entomopathogens *M. anisoplae* resulted into maximum reduction (41.42%) of mealy bug population where the pretreatment population recorded was 33.56 followed by 19.66 mealybugs/plant after

Table 2: Effect of insecticides, biopesticides and biorational on mealybug population under laboratory and field conditions\*\*\*

Particulars	Pre-treatment population/plant*	Population after spray			Population reduction after 3rd spray (%)	Mortality under laboratory condition (%) **
		1st	2nd	3rd		
Acephate	29.25	12.25 <sup>a</sup>	10.16 <sup>a</sup>	8.09 <sup>a</sup>	72.34 <sup>a</sup>	58.66 <sup>a</sup>
Chlorpyrifos	39.05	19.33 <sup>bc</sup>	16.42 <sup>ab</sup>	12.26 <sup>ab</sup>	68.60 <sup>a</sup>	61.33 <sup>a</sup>
5% NSKE	46.21	40.34 <sup>d</sup>	39.21 <sup>ef</sup>	36.15 <sup>fg</sup>	21.77 <sup>de</sup>	26.33 <sup>d</sup>
Neem oil (2.5 L ha <sup>-1</sup> ) + Nirma powder (0.1%)	51.61	49.31 <sup>f</sup>	46.37 <sup>g</sup>	42.05 <sup>g</sup>	18.52 <sup>de</sup>	29.66 <sup>cd</sup>
<i>Verticillium lecanii</i>	42.34	36.19 <sup>d</sup>	29.67 <sup>cd</sup>	25.47 <sup>de</sup>	39.84 <sup>b</sup>	45.66 <sup>b</sup>
<i>Beauveria bassiana</i>	29.78	23.08 <sup>bc</sup>	20.55 <sup>b</sup>	18.56 <sup>bc</sup>	37.68 <sup>b</sup>	44.33 <sup>b</sup>
<i>Metarhizium anisopliae</i>	33.56	27.10 <sup>bc</sup>	22.46 <sup>bc</sup>	19.66 <sup>cd</sup>	41.42 <sup>b</sup>	44.66 <sup>b</sup>
Bacterial symbiont of nematode	53.19	47.80 <sup>ef</sup>	43.18 <sup>fg</sup>	41.34 <sup>g</sup>	22.28 <sup>de</sup>	25.66 <sup>d</sup>
Fish Oil Rosin Soap (FORS)	44.15	41.05 <sup>de</sup>	38.26 <sup>c</sup>	31.8 <sup>ef</sup>	27.97 <sup>bcde</sup>	34.33 <sup>c</sup>
New botanical (Mealy Quit)	41.43	37.10 <sup>d</sup>	31.09 <sup>d</sup>	27.74 <sup>e</sup>	33.04 <sup>bc</sup>	29.33 <sup>cd</sup>
Control water spray	37.02	39.17 <sup>d</sup>	34.39 <sup>de</sup>	32.06 <sup>ef</sup>	13.40 <sup>e</sup>	4.00 <sup>e</sup>
CD (p = 0.05)	NS	5.05	6.97	6.68	12.08	5.33
SEm	-	2.42	2.43	2.61	4.11	1.74

\*Population recorded from 5 cm infested shoot of the plant and population count does not include the crawlers' population. \*\*Experiment conducted with only five day old nymphs. \*\*\*Pooled data of 2008 and 2009. Mean with same superscript alphabets are not significantly different from each other according to Duncan's Multiple Range test at p = 0.05

Table 3: Percent reduction in predator's population by various biopesticides and insecticides after application of three sprays\*

Treatments	Spider	<i>Chrysoperla</i>	Coccinellid
Acephate	16.67	42.86	48.66
Chlorpyrifos	33.33	52.73	50.00
5% NSKE	0.00	26.32	-23.08
Neem oil (2.5 L ha <sup>-1</sup> ) + Nirma powder (0.1%)	-45.45**	8.70	-7.69
Nirma powder 0.1%	-83.33	-11.76	-27.78
<i>Verticillium lecanii</i>	0.00	-9.09	-66.67
<i>Beauveria bassiana</i>	0.00	-13.04	-50.00
<i>Metarhizium anisopliae</i>	-17.65	-35.29	-50.00
Bacterial symbiont of nematode	-12.50	9.09	0.00
Fish Oil Resin Soap (FORS)	-20.00	-40.00	0.00
New botanical (Mealy Quit)	-29.41	-50.00	-50.00
Control water spray	-35.29	-30.00	25.00

\*Pooled data of 2008 and 2009; \*\*Negative sign (-) means no reduction in population

the 3 sprays. In case of *B. bassiana* the pretreatment population recorded was 29.78 mealybugs/plant and 18.56 mealybugs/plant after the 3rd spray application (37.68% reduction in population). In *V. lecanii*, pre treatment population observed was 42.34 mealybugs/plant and reduced to 25.47 mealybugs/plant after 3rd spray applications (39.84% reduction in population). Bacterial symbiont of nematode reduced 22.28% population of mealybug after 3 sprays applied at an interval of 15 days (Table 2). All the treatments were statistically superior over control but the mealybug population in Bacterial symbiont of Nematode treated plot was statistically at par with control after 3 spray application.

The data collected after three applications of various treatments on predators revealed that both chlorpyrifos and acephate has significant adverse impact on the population of predators as compare to biorationals and biopesticides (recorded with almost negligible mortality to predators) (Table 3). Acephate recorded 16.67, 42.86 and 48.66% reduction in population of spider, lacewing

(*Chrysoperla*) and coccinellid beetle which was found comparatively safer to chlorpyrifos (recorded 33.33, 52.33 and 50.00% reduction of above mentioned predators, respectively after the 3rd spray application) (Table 2). The data on *A. bambawalei* a specific parasitoid could not be recorded because of its unavailability in the mealybug colonies.

**Laboratory evaluations:** The population of nymphs in the bioassay conducted under laboratory condition was recorded after 48 h where insecticides acephate with 58.66% and chlorpyrifos with 61.33% mortality were the best treatment (Table 2). Among the entomopathogens (% reduction in population after 48 h) like *V. lecanii* (45.66%), *B. bassiana* (44.33%) and *M. anisopliae* (44.66%). Bacterial symbiont of nematode (25.66%) were found reasonably well in management of mealybug. In biorationals 5% NSKE (26.33%), Neem oil (2.5 L ha<sup>-1</sup>) + Nirma powder (0.1%) (29.66%), FORS (34.33%) and Mealy Quit (29.33%) were also effective in reduction of mealybug population (Table 2).

## DISCUSSIONS

Mealybug (Hemiptera: Pseudococcidae) are important pest of cultivated and ornamental crops worldwide. Between insecticides, acephate being safer and systemic in action is effective against the entire sucking pest complex of cotton. Chlorpyrifos after first spray reduced more population of mealybug than acephate but after 3 spray applications acephate proved better but statistically non significant. Among the biorationals, significantly low population was observed in mealyquit and FORS after 3rd spray application as compare to NSKE and Neem oil+Nirma powder. The population in Neem oil+Nirma powder treated plot was at par with control. Contrary to the present finding, two detergents SU 120 and Tecs Fruta were evaluated against long tailed mealybug *Pseudomonas longispinus* under laboratory conditions and found effective against second stage nymphs (Curkovic *et al.*, 2007). The entomopathogen i.e., *V. lecanii*, *B. bassiana* and *M. anisopliae* are good for mealybug management and can be rotated or mixed with insecticides. *V. lecanii* has shown good compatibility with insecticides like acephate and imidacloprid (Monga *et al.*, 2009b). The maximum reduction in population of mealybug, *P. solenopsis* was observed in case of *Metarrhizium anisopliae*. The study lend credence from the findings of Ujjan and Shahzad (2007), who observed up to 100% mortality of different instars of *Maconellicoccus hirsutus*, pink mealybug by various strains of *M. anisopliae*. In line with the present findings, Chavan and Kadam (2009) recorded maximum mortality (82.5%) of mealybug, *M. hirsutus* one day old nymphs in a formulation containing *V. lecanii*+glycerol 8%+Tween 80%. Similarly, among the two naturally occurring fungi, *Beauveria bassiana* was found more effective than *Cladosporium cladosporioides* (Fresenius) de Vries against papaya mealybug, *Paracoccus marginatus* Williams and graver de Willink in cotton when assessed for its pathogenicity (Jeyarani *et al.*, 2011). Entomopathogens are known for their activity against many sucking pests and can be used in integrated pest management also (Abdel-Baky *et al.*, 1998; Abdel-Baky, 2000; Lemawork, 2008). However, there is a need to screen a larger number of isolates to identify potent entomopathogens.

For successful use of pesticides/biopesticides, information is not only needed on the biology and feeding activity of the control agents but also on the most susceptible stage of pest species (Cuthbertson *et al.*, 2003). The laboratory bioassay was carried out against 5 day old nymphs of mealybug using the spray solutions prepared for field conditions by leaf dipping method. In the present study 5 day nymphs were used for bioassay as these were reported susceptible to insecticides and biopesticides by Curkovic *et al.* (2007) and Zettler *et al.* (2002) recorded that the

presence of wax on the cuticle of later nymphal instars and adult might have prevented the entry of insecticide inside the insect body. The findings obtained under field condition in the present study were strengthened through the laboratory evaluations of insecticides, biopesticides and biorationals. Similar to the field condition under laboratory evaluation also insecticides acephate with 58.66% and chlorpyrifos with 61.33% mortality were the best treatment (Table 2). The insecticides were followed by the entomopathogens like *V. lecani*, *B. bassiana* and *M. anisopliae* and Bacterial symbiont of nematode. Among biorationals, FORS (34.33%) and Mealy Quit (29.33%) were effective in reduction of mealybug population (Table 2). The study lend credence from Tadesse (2006) where different insecticidal seed/plant extract treatments were found effective in controlling Enset root mealybugs in the laboratory, in pot experiments and in farmers' fields.

The data collected after three applications of various treatments on generalist predators revealed that both chlorpyrifos and acephate has significant adverse impact on the population of predators as compare to biorationals and biopesticides (recorded with almost negligible mortality to predators). Data on *Aenasius bambawalei* a specific parasitoid could not be recorded because of its unavailability in the mealybug colonies. The data recorded on the biorational insecticides like bio-neem showed very less (10%) mortality against lady bird beetle, *Adonia variegata* extend its support to present finding (Al-Doghairi *et al.*, 2004).

## CONCLUSION

The insecticides are highly effective in controlling the mealybug population but cannot used constantly without any alternate eco-friendly strategies. The mealybug population in field is associated with many species of parasitoids and predators. The eco-friendly biopesticides/biorationals/entomopathogens if rotated with insecticides will give 18-42% reduction in population of mealybug and will also highly helpful in conservations of the natural regulators of mealybug population. Many of the biopesticides/biorationals/entomopathogen were also found compatible with insecticides and can be applied as mixtures as per the reports available.

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