Journal of Biological Control, 31(1): 5-9, 2017, DOI: 10.18311/jbc/2017/15594

Research Article

Toxicity of insecticides to *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat), a solitary endoparasitoid of *Phenacoccus solenopsis* Tinsley on Bt cotton under semi-field conditions

PRASUN KARMAKAR and P. S. SHERA*
Department of Entomology, Punjab Agricultural University, Ludhiana – 141004, Punjab, India
**Corresponding author E-mail:* psshera@pau.edu

ABSTRACT: The toxicity of different insecticides was assessed against *Aenasius arizonensis* (Girault) (=*Aenasius bambawalei* Hayat) (Hymenoptera: Encyrtidae), a key endoparasitoid of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on Bt cotton under semi-field conditions at the Entomological Research Farm, Punjab Agricultural University, Ludhiana during *kharif*, 2015. Eight insecticides, viz. profenophos 50 EC, thiodicarb 75 WP, buprofezin 25 EC, imidacloprid 17.8 SL, flonicamid 50 WG, spiromesifen 240 SC, diafenthiuron 50 WP and sulfoxaflor 24 SC were sprayed at recommended field doses on potted cotton plants having parasitized mealybugs (mummies). Based on the per cent reduction in adult emergence over control, spiromesifen (11.11 %), buprofezin (11.11 %) and flonicamid (12.11 %) were categorized as harmless; imidacloprid (28.28 %) as slightly harmful; diafenthiuron (55.56 %) and thiodicarb (70.70 %) as moderately harmful, whereas, profenophos (100.0 %) and sulfoxaflor (100.0 %) were highly toxic to the parasitoid. Insecticides like spiromesifen, buprofezin and flonicamid can be viable option for use in integrated pest management strategies against sucking insect pests in cotton ecosystem.

KEY WORDS: *Aenasius arizonensis*, adult emergence, insecticides, mummies, *Phenacoccus solenopsis,* toxicity

(Article chronicle: Received: 14.02.2017; Revised: 22.03.2017; Accepted: 28.03.2017)

INTRODUCTION

Solenopsis mealybug, *Phenacoccus solenopsis* Tinsley has emerged as a serious threat to cotton cultivation in India over the past decade. It was first documented as a pest of cotton in Texas, United States (Fuchs *et al*., 1991) and now has been widely distributed in various ecological zones of the globe (Ben-Dov and Miller, 2016). *Phenacoccus solenopsis*, which was hitherto not reported to occur in India, is an exotic species and now widespread on cotton in almost all the cotton growing states of the country (Nagrare *et al*., 2009). In Punjab, it appeared in a few pockets of Bathinda, Ferozepur and Muktsar districts during 2006, but in 2007, it also spread to other cotton growing areas of Punjab and emerged in a serious proportion causing 30 to 40 per cent losses in the yield of cotton (Dhawan *et al*., 2007). Due to waxy material covering the mealybug body and its high rate of reproduction, it becomes difficult to manage this pest with insecticides. As an alternative, biological control of mealybug has been found to be one of the most effective means of its management (Ram and Saini, 2010).

Encrytids are the most successful natural enemies used in the biological control programs against mealybugs (Noyes and Hayat, 1994). Among these, an endoparasitoid *Aenasius bambawalei* is the most prominent and key mortality factor of *P. solenopsis* under field conditions across India (Dhawan *et al*., 2011; Kumar *et al*., 2009; Ram and Saini, 2010). Field parasitization to the extent of 90 per cent has been documented in *P. solenopsis* by this parasitoid (Ram *et al*., 2009). It was first time reported by Mahmood (2008) in Pakistan and later, was described and named as *A. bambawalei* by Hayat (2009). However, Fallahzadeh *et al*. (2014) from Iran reported that this species falls closer to *A. arizonensis* (Girault) and reported *A. bambawalei* to be junior synonym of *A. arizonensis*.

Adoption of Bt cotton has led to significant decrease in insecticide usage in cotton ecosystem, but it is still receiving insecticidal sprays against sucking insect pests including mealybugs. Insecticides belonging to three major groups, viz. organophosphates (profenophos, acephate, chlorpyriphos and quinalphos), carbamates (thiodicarb and carbaryl) and insect growth regulator (buprofezin) have been recommended the management of cotton mealybug in Punjab (Anonymous, 2015). Apart from these, insecticides from neonicotinoids, pyridine carboxamid, spirocyclic tetronic acids and thiourea derivative groups are also being used in the cotton ecosystem against other sucking insect pests like whitefly and leafhopper. However, the use of these insecticides may have disruptive influence upon the parasitoid. Enhancing the role of biological control within insecticide dominated management systems requires insecticides that have little and/or no impact on natural enemies. Hence, present study was conducted under semi-field conditions to identify safer insecticides against *A. arizonensis* which may ideally fit in integrated pest management program against sucking pests in the cotton ecosystem.

MATERIALS AND METHODS

The experiment to assess the safety of different insecticides to *Aenasius arizonensis* was conducted under semi-field conditions at the Entomological Research Farm, Punjab Agricultural University (PAU), Ludhiana during 2015. Eight insecticides representing different insecticidal groups (Table 1), recommended for the control of various sucking insect pests in Bt cotton were selected for their toxicity against the parasitoid.

Rearing of *Phenacoccus solenopsis*

Phenacoccus solenopsis was reared on sprouted potatoes (*Solanum tuberosum* L.) in an environmental chamber at 27 ± 2^{0}C temperature and 70 ± 5 % relative humidity (Macro Scientific Works Ltd, India). Small to medium sized potato tubers were washed, air dried and held at room temperature in the dark until sprouting. When the potato sprouts were 2.5-5 cm in length, they were placed in glass jars (4-5 tubers/ jar) of size 20 x 15 cm with moist soil at the base to maintain turgidity of the sprouted tubers. The sprouts were inoculated with gravid females with the help of a soft camel hair brush. The jars were then covered with muslin for the establishment of mealybug colonies. The culture of *P. solenopsis* was maintained for multiple generations, which was used for rearing of *A. arizonensis* and for bioassay experiment.

Rearing of *Aenasius arizonensis*

The parasitoid, *A. arizonensis* was reared on the colonies of *P. solenopsis* maintained on sprouted potato tubers in glass jars. Parasitized mealybugs (mummies) were collected from mealybug infested cotton, *Hibiscus* sp. and weed hosts (*Abutilon* sp., *Sida* sp. and *Parthenium* sp.) and brought to the Biocontrol Laboratory. They were kept in glass vials plugged with cotton wool. Naive/virgin parasitoid adults obtained from mummies of mealybug were sexed morphologically on the basis of body size and antennal structure (Hayat, 2009). They were released in glass jars having healthy colonies of mealybug maintained on sprouted potatoes. After parasitization, hard, leathery, brown coloured structures called "mummies" were formed. These mummies were collected and kept in glass vials plugged with cotton wool. These glass vials were provided with honey streak on a paper strip as a source of food for the emerging adult parasitoids. The freshly emerged adults were used for further experimentation.

Bioassay under semi-field conditions

The seeds of Bt cotton hybrid (NCS 855 BG II) were sown in earthen pots and plants were raised as per the practices recommended by PAU, Ludhiana except plant protection measures (Anonymous, 2015). The potted cotton plants were kept in net cages (90 cm x 80 cm x 70 cm) under field conditions. Five plants were kept per treatment in each net cage and single plant was considered as one replication. The potted cotton plants were inoculated with gravid females of *P. solenopsis* with the help of a soft camel hair brush for the establishment of mealybug colonies. Ten pairs of freshly

Table 1. Insecticide treatments used for bioassay on *Aenasius arizonensis* under semi-field conditions

Treatments	Insecticidal Group	Mode of Action (IRAC*)	Dose (g a.i. ha^{-1})
Profenophos 50 EC	Organophosphate	Acetylcholinesterase (AChE) inhibitors	625
Thiodicarb 75 WP	Carbamate	Acetylcholinesterase (AChE) inhibitors	468.75
Buprofezin 25 EC	Insect growth regulator	Inhibitors of chitin biosynthesis	312.50
Imidacloprid 17.8 SL	Neonicotinoid	Nicotinic acetylcholine receptor (nAChR) competitive modulators	17.8
Flonicamid 50 WG	Pyridine carboxamid	Chordotonal organ modulators	100
Spiromesifen 240 SC	Spirocyclic tetronic acid	Inhibitors of acetyl CoA carboxylase	120
Diafenthiuron 50 WP	Thiourea derivative	Inhibitors of mitochondrial ATP synthase	250
Sulfoxaflor 24 SC	Sulfoxamines	Nicotinic acetylcholine receptor (nAChR) competitive modulators	90
Untreated control	-	-	-

* According to IRAC (Insecticide Resistance Action Committee) MOA classification (www.irac-online.org)

emerged *A. arizonensis* adults were released in each cage to ensure sufficient parasitization. The selected insecticidal solutions were sprayed with the help of knapsack sprayer on these potted cotton plants having parasitized mealybugs (mummies). After spray, all mummies were collected from each plant and brought to the laboratory. They were kept in separate glass jars covered with muslin to record per cent adult emergence. The per cent reduction in adult emergence over control was also worked out. The insecticides were classified into different toxicity categories on the basis of reduction in adult emergence under semi-field conditions as per Sterk *et al*. (1999).

Toxicity	Reduction in adult emergence (%)	Categories
Harmless	<25 %	Class 1
Slightly harmful	25-50 %	Class 2
Moderately harmful	51-75 %	Class 3
Harmful	>75 %	Class 4

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using complete randomized block design (CRD). The significance of differences were tested by F-tests, and the significance of differences between treatment means were compared using least significant difference (LSD) at 5 per cent probability level. The data were transformed using arc sine transformation and the values of 0 % and 100 % were substituted by 1/4n and 100-1/4n, respectively prior to statistical analysis (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The data on the adult emergence of *Aenasius arizonensis* that emerged from mummies after exposure to different insecticides under semi-field conditions are presented in table 2. There were significant differences with respect to adult emergence in different treatments. Among different insecticides, significantly higher (88.00 %) adult emergence was recorded in buprofezin 25 EC and spiromesifen 240 SC, which were statistically at par with flonicamid 50 WG (87.00 %). The mean adult emergence in imidacloprid 17.8 SL, diafenthiuron 50 WP and thiodicarb 75 WP was 71.00, 44.00 and 29.00 per cent, respectively. No adults emerged from mummies sprayed with profenophos 50 EC and sulfoxaflor 24 SC. However, the adult emergence was highest (99.00 %) in untreated control.

Among the insecticidal treatments, the per cent reduction in adult emergence over control was relatively low (11.11 %) in insecticides like spiromesifen 240 SC and buprofezin 25 EC. They were followed by flonicamid 50 WG (12.12 %) imidacloprid 17.8 SL (28.28) diafenthiuron 50 WP (55.56 %) and thiodicarb 75 WP (70.70 %). However, the reduction in adult emergence was cent per cent in profenophos 50 EC and sulfoxaflor 24 SC. Based on per cent reduction in adult emergence over control, spiromesifen 240 SC, buprofezin 25 EC and flonicamid 50 WG were categorized as harmless; imidacloprid 17.8 SL as slightly harmful; diafenthiuron 50 WP and thiodicarb 75 WP as moderately harmful, whereas, profenophos 50 EC and sulfoxaflor 24 SC were categorized as harmful insecticides to development adults of *A. arizonensis* within mummies (Fig. 1).

Table 2. Adult emergence of *Aenasius arizonensis* from mummies of *Phenacoccus solenopsis* treated with different insecticides under semi-field conditions

Treatments	Dose (g a.i. ha^{-1})	*Mean adult emergence (%)
Profenophos 50 EC	625	0.00 (0.64)
Thiodicarb 75 WP	468.75	29.00 (32.53)
Buprofezin 25 EC	312.50	88.00 (69.80)
Imidacloprid 17.8 SL	17.8	71.00 (57.51)
Flonicamid 50 WG	100	87.00 (68.93)
Spiromesifen 240 SC	120	88.00 (70.03)
Diafenthiuron 50 WP	250	44.00 (41.52)
Sulfoxaflor 24 SC	90	0.00 (0.64)
Untreated control	-	99.00 (85.31)
CD (*p*=0.05)		(3.80)

*Mean of 5 replications; Figures in parentheses are means of arc sine $\sqrt{\text{percentage}}$ transformed values.

The results thus showed that spiromesifen, buprofezin and flonicamid were comparatively safer to *A. arizonensis,* while, profenophos and sulfoxaflor were highly toxic to the parasitoid when potted cotton plants having parasitized mealybugs (mummies) were sprayed with different insecticides under semi-field conditions. Earlier workers have reported the toxicity of insecticides to mummies and adults of *A. bambawalei* under laboratory conditions only. The present findings corroborate with the studies of Meenu and Ram (2014) who have also reported profenophos as harmful insecticide to 2 and 5 day old mummies when these were exposed to different insecticides under laboratory conditions. Similar findings were reported by Suh *et al*. (2000), who found that the insecticide profenophos adversely affected the adult emergence of *Trichogramma* spp. from

Helicoverpa zea (Boddie) host eggs. High toxicity of profenophos to adults after direct exposure has also been documented by Nalini and Manickavasagam (2011). The studies by Fernandez *et al*. (2015) also showed that sulfoxaflor was the most harmful insecticide due to its non-selective nature, while spiromesifen and flonicamid were non-toxic to whitefly parasitoid, *Eretmocerus mundus* (Mercet) which are in agreement with the present findings. The safety of buprofezin corroborates with findings of Mgocheki and Addison (2009) who also reported that it was not toxic to the mummies of Vine mealybug parasitoids, *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutes* (Timberlake). Further, buprofezin has also been found safer to predatory complex, i.e. coccinellids, chrysopids and spiders in cotton (Dhawan *et al*., 2012).

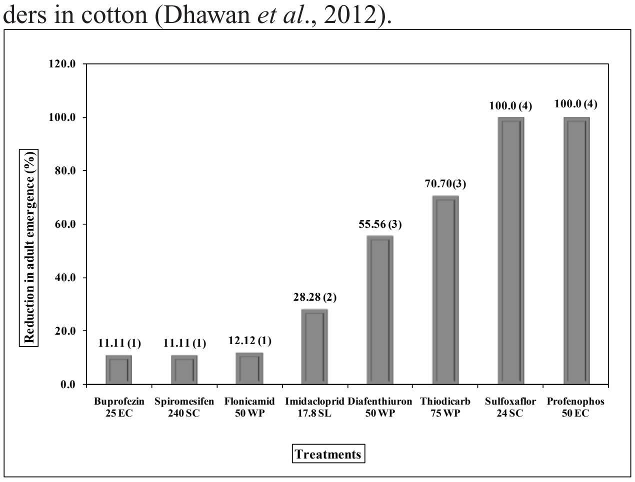

Fig. 1. Toxicity categories of different insecticides to *Aenasius arizonensis* under semi-field conditions. Bars followed by numbers in parentheses represents toxicity category as per Sterk *et al* (1999) - class 1 - harmless (<25 %), class 2 - slightly harm¬ful (25-50 %), class 3 - moderately harmful (51-75 %) and class 4 - harmful (>75 %).

Conclusively, buprofezin insecticide may be preferred as first spray to other recommended insecticides for the control of mealybug to conserve the parasitoid in cotton ecosystem. The use of spiromesifen and flonicamid in IPM strategies for other sucking pests like whitefly and leafhopper could further facilitate biologically based pest management in cotton production system.

ACKNOWLEDGEMENT

Authors are thankful to the Professor and Head, Department of Entomology, Punjab Agricultural University for providing the necessary facilities in accomplishing the research work.

REFERENCES

Anon. 2015. *Package of Practices for Kharif Crops*. Punjab Agricultural University, Ludhiana. 41–65 pp.

Ben-Dov Y, Miller DR. 2016. ScaleNet: Systematic Database of the Scale Insects of the World. Available from: *http://www.catalogueoflife.org/annual-checklist/2016.*

Dhawan AK, Shera PS, Aneja AK, Kumar V, Bhullar HS. 2012. Effectiveness of buprofezin against mealybug (*Phenacoccus solenopsis* Tinsley) and predatory complex in Bt cotton. *Indian J Agric Sci*. **82**(11): 1000–1002.

Dhawan AK, Singh K, Saini S, Aneja A, Singh J. 2011. Parasitizing potential of parasitoid (*Aenasius bambawalei*) on mealybug (*Phenacoccus solenopsis*) in cotton (*Gossypium* spp.) and weed plants. *Indian J Agric Sci*. **81**(1): 97–99.

Dhawan AK, Singh K, Saini S, Mohindru B, Kaur A, Singh G, Singh S. 2007. Incidence and damage potential of mealybug, *Phenacoccus solenopsis* Tinsley on cotton in Punjab. *Indian J Ecol.* **34**(2): 110–116.

Fallahzadeh M, Abdimaleki R, Saghaei N. 2014. New records of Tetracneminae (Hymenoptera, Chalcidoidea, Encyrtidae) from Iran. *Turk J Zool*. **38**(4): 515–518.

Fernandez MDM, Medina P, Fereres A, Smagghe G, Vinuela E. 2015. Are mummies and adults of *Eretmocerus mundus* (Hymenoptera: Aphelinidae) compatible with modern insecticides? *J Econ Entomol.* **108**(5): 2268–2277.

Fuchs TW, Stewart JW, Minzenmayer R, Rose M. 1991. First record of *Phenacoccus solenopsis* Tinsley in cultivated cotton in the United States. *Southwest Entomol.* **16**(3): 215–221.

Gomez KA, Gomez AA. 1984. *Statistical Procedure for Agricultural Research*. Wiley Inter-Science Publications, New York.

Hayat M. 2009. Description of a new species of *Aenasius* Walker (Hymenoptera: Encyrtidae), India. *Biosystematica*. **3**(1): 21–26.

Kumar R, Kranthi KR, Monga D, Jat SL. 2009. Natural parasitization of *Phenacoccus solenopsis* Tinsley

(Hemiptera: Pseudococcidae) on cotton by *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae). *J Biol Control.* **23**(4): 457–460.

Mahmood R. 2008. Breakthrough in biological control of mealybug in Pakistan. *Biocontr News Infor.* **29**(3): 38–39.

Meenu, Ram P. 2014. Effect of insecticides on different developmental stages of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae), a parasitoid of solenopsis mealybug, *Phenacoccus solenopsis* Tinsley. *J Biol Control.* **28**(4): 204–209.

Mgocheki N, Addison P. 2009. Effect of contact pesticides on vine mealybug parasitoids, *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae). *S Afr J Enol Vitic.* **30**(2): 180–185.

Nagrare V, Kranthi S, Biradar VK, Zade NN, Sangode V, Kakde G, Shukla RM, Shivare D, Khadi BM, Kranthi KR. 2009. Widespread infestation of the exotic mealybug species, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) on cotton in India. *Bull Entomol Res.* **99**(5): 537–541.

Nalini T, Manickavasagam M. 2011. Toxicity of selected insecticides to mealybug parasitoids, *Aenasius bambawalei* Hayat and *Aenasius advena* Compere (Hymenoptera: Encyrtidae). *J Biol Control.* **25**(1): 14–17.

Noyes JS, Hayat M. 1994. *Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae).* CAB International, Abingdon, UK.

Ram P, Saini RK. 2010. Biological control of solenopsis mealybug *Phenacoccus solenopsis* Tinsley on cotton: a typical example of fortuitous biological control. *J Biol Control.* **24**(2): 104–109.

Ram P, Saini RK, Vijaya. 2009. Preliminary studies on field parasitization and biology of solenopsis mealybug parasitoid, *Aenasius bambawalei* Hayat (Encyrtidae: Hymenoptera). *J Cotton Res Dev.* **23**(2): 313–315.

Sterk G, Hassan SA, Baillod M, Bakker F, Bigler F, Blumel S, Bogenschutz H, Boller E, Bromand B, Brun J, Calis JNM, Pelseneer JC, Duso C, Garrido A, Grove A, Heimbach U, Hokkanen H, Jacas J, Lewis G, Moreth L, Polgar L, Roversti L, Petersen LS, Sauphanor B, Schaub L, Staubli A, Tuset JJ, Vainio A, Veire MVD, Viggiani G, Vinuela E, Vogt H. 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. *BioControl*. **44**(1): 99–117.

Suh CPC, Orr DB, Duyn JWV. 2000. Effect of insecticides on *Trichogramma exiguum* (Trichogrammatidae: Hymenoptera) pre-imaginal development and adult survival. *J Econ Entomol.* **93**(3): 577–583.