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Research Article

Effect of insecticides on different developmental stages of *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae), a parasitoid of solenopsis mealybug, *Phenacoccus solenopsis* Tinsley

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ABSTRACT: Experiments carried to assess the effect of different insecticides, namely, profenophos 50EC (1.5g a.i./ha), thiodicarb 75WP (1.12g a.i./ha), quinalphos 25EC (1g a.i./ha), novaluron 10EC (0.1g a.i./ha), spinosad 45SC (0.18g a.i./ha), imidacloprid 200SL (0.6g a.i./ha) and nimbecidine EC (0.03% Azadirachtin) (5 ml/litre of water) which are recommended in the cotton crop for the control of various insect pests, on different stages of solenopsis mealybug parasitoid, *A. bambawalei* under laboratory conditions has revealed that profenophos, quinalphos and thiodicarb were found to be most toxic (100% mortality within 24h after exposure) to the adults of *A. bambawalei* followed by imidacloprid (54%), novaluron (42%), spinosad (36%) and nimbecidine (10%). Number of mummies formed was lowest (2) when larval stage (in two days old parasitised mealybugs) of the parasitoid was sprayed with quinalphos while it was highest (40.20) when sprayed with novaluron followed by imidacloprid (38.60) and nimbecidine (30). Very low adult emergence was recorded when pupal stage (in five days old mummies) was sprayed with profenophos (5.40%) and quinalphos (4.70%) while highest (86.06%) adult emergence was recorded when sprayed with novaluron followed by imidacloprid (82.14%), nimbecidine (76.60%) and thiodicarb (60.60%).

KEY WORDS: *Aenasius bambawalei,* Adults, Developmental Stages, Effect, Insecticides

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INTRODUCTION

Cotton, *Gossypium hirsutum* L. is one of the important cash crops providing livelihood to millions of people associated with its cultivation, textile and apparel industries (Sahito *et al.*, 2011). Cotton crop suffers from various insect-pests right from sowing to harvesting. Among these solenopsis mealybug, *Phenacoccus solenopsis* Tinsley, a polyphagous pest, is of major importance which causes considerable damage to this crop in India. Parasitoids have been a favorite subject for biological control programme because they tend to be highly specific to one or a few species of host, and therefore can be used to target specific pests. *Aenasius bambawalei* Hayat is the most important endoparasitoid of solenopsis mealybug, *P. solenopsis* (Ram *et al*., 2009; Kumar *et al*., 2009; Hayat, 2009). Surveys of cotton growing areas of Haryana have showed 23.7 to 76.6 per cent parasitisation of mealybugs by *A. bambawalei* on cotton (Ram *et al*., 2009). The parasitoid has successfully controlled the pest and no pest outbreak was reported on cotton since 2007-08 (Ram and Saini, 2010).

Aenasius bambawalei is a newly described and an important nymphal endoparasitoid of *P. solenopsis*. Inspite of the success of the biological control, chemical control is still being largely used as an important component of Integrated Pest Management (IPM) and is used in conjunction with the biological control. But pesticides when used along with natural enemies may limit the efficacy of these biocontrol agents and have deleterious effects on parasitoids such as *A. bambawalei*. Hence, keeping this in view the present study has been proposed to investigate the effect of different insecticides, recommended for insect-pests control in cotton crop, on solenopsis mealybug parasitoid, *A. bambawalei* and to identify safer insecticides that can be used when parasitoids are available in the field.

MATERIALS AND METHODS

Host and parasitoid culture

The culture of *P. solenopsis* was maintained on the potato sprouts placed in the plastic tubs. Third instar mealybugs were transfered to the single hole wooden cage where these were exposed to *A. bambawalei* adults for parasitisation for regular supply of different stages of the parasitoid in different experiments. Initially the parasitoid culture in single hole wooden cage was made by placing the field collected mummies (mealybugs transformed into dark red or brown coloured hard structure after 5-6 days of parasitisation) into single hole wooden cage. The present studies were conducted under laboratory conditions (26 ± 2°C temperature, 55 ± 5% relative humidity).

Toxicity of different insecticides to adults of *Aenasius bambawalei*

Effects of different insecticides at their recommended concentrations on adult parasitoids was tested by the dry film technique (Wilkinson *et al*., 1975). Solutions of insecticides were prepared by taking the recommended concentration of the insecticide using pipette and mixing it to 1000 ml of tap water. Quantity of water was measured by using a measuring cylinder. Filter paper strips (5.0×2.5cm) were made with the help of the scissor. These filter paper strips were dipped in the insecticidal solutions and air dried for approximately 20 minutes. Honey drops were put on these strips. Filter paper strips dipped in tap water were kept as control. A filter paper strip treated with insecticide solution was then introduced into the glass tube. Ten 1-2 days old honey fed adult parasitoids (5 females and 5 males) were collected into the glass vial from the single hole wooden cage and introduced into each glass tube and the open end of test tube was closed with cotton plug. Each treatment was replicated five times. Mortality of the adult parasitoids was recorded after 1, 3, 6, 12 and 24 h of exposure and per cent cummulative mortality was calculated. Insecticides were classified on the basis of adult mortality within 24h after exposure where: class 1 - harmless (<30%), class 2 - slightly harmful (30-79%), class 3 - moderately harmful (80-99%) and class 4 - harmful (>99%) (Sterk *et al*., 1999).

Effect of different insecticides on larval stage of *Aenasius bambawalei*

Fifty big sized mealybugs (3rd instar) on potato tuber sprouts were taken from the culture and placed in glass jar. Ten pairs of *A. bambawalei* were collected in the glass vial from the single hole wooden cage. The potato tuber sprouts in glass jars were exposed to these adult parasitoids for parasitisation for 24h. Open end of the glass jar was covered with the muslin cloth. Muslin cloth was tightened with the help of the rubber band. After 24h parasitoids were removed from the glass jar. These parasitized mealybugs were kept for two days to ensure there is larval stage of the parasitoid within the parasitized host body. Earlier studies on the biology of *A. bambawalei* had revealed that the egg and larval stages of the parasitoid are not visible being an internal feeder, but swelling and poor movement of the parasitized mealybugs were observed after 2 to 3 days of parasitisation Sangle (2013). Solutions of different insecticides were prepared as in the previous experiment. After two days these mealybugs were sprayed with insecticide solution with the help of an atomizer till surface run-off. These sprayed mealybugs were air dried for approximately 20 minutes. After drying these mealybugs were again kept in glass jar and covered by muslin cloth. Mealybugs sprayed with tap water were kept as control. Each treatment were replicated five times. Observations on number of mummies formed in different treatments were recorded 10 days after treatment. Per cent adult emergence from these mummies was also calculated.

Effect of different insecticides on pupal stage of *Aenasius bambawalei*

Two days old 25 mealybug mummies (containing pupal stage of the parasitoid inside) on potato tuber sprouts were taken from the culture. Previous work has shown that the mealybugs transformed into dark brown mummies (containing pupal stage of the parasitoid) within 5.8 days (Vijaya *et al*., 2011). Solution of different insecticides were prepared in tap water as in previous experiments. These mummies were sprayed with different insecticidal solutions with the help of an atomizer till surface run-off. Mummies sprayed with tap water were kept as control. The potato tuber sprouts were dried. These mummies were transfered and glued on to hard paper strips with the help of camel hair brush without damaging them. The hard paper strips were then introduced into glass tube. Open end of the glass tube was closed by cotton plug. There were five replications in each treatment. Observations were recorded on parasitoid adult emergence, parasitization potential, sex-ratio and adult longevity. Per cent adult emergence from these mummies was also calculated.

For recording parasitization potential five mated females from each treatment were provided healthy mealybugs for 24h. For observing adult longevity, emerged adults in each treatment were placed in glass tubes with a streak of honey as food on a piece of paper. Mortality of adults was recorded daily. The sex-ratio was calculated by counting the number of male and female adults after their death in each treatment.

Insecticides were classified into different categories on the basis of reduction in adult emergence over control where: class I - harmless (<30%), class II - slightly harmful (30-79%), class III - moderately harmful (80-99%) and class IV - harmful (>99%) Hassan (1986).

Statistical analysis

The data obtained in all the experiments was subjected to the analysis of variance using single factor Completely Randomized Design. Angular and square root transformations of the data were done when required and then analyzed using the statistical software, OPSTAT developed by CCS Haryana Agricultural University, Hisar.

RESULTS AND DISCUSSION

Toxicity of different insecticides to adults of *Aenasius bambawalei*

After 1h of exposure, adult mortality was seen only in imidacloprid (16%) and profenophos (44%) (Table 1). No adult mortality was recorded in other treatments. After 3h of exposure, maximum adult mortality was recorded in profenophos (100%), followed by quinalphos (68%), thiodicarb (44%) and imidacloprid (28%). No adult mortality was recorded in novaluron, spinosad and nimbecidine upto 3h of exposure. After 6h of exposure, maximum adult mortality was recorded in profenophos (100%) and quinalphos (100%) followed by thiodicarb (84%), imidacloprid (42%), spinosad (6%) and nimbecidine (2%). There was no adult mortality recorded in novaluron upto 6h of exposure. Hence upto 6h of exposure novaluron, spinosad and nimbecidine were found to be safer for the parasitoid. After 12h of exposure 100 per cent adult mortality was recorded in profenophos, quinalphos and thiodicarb followed by imidacloprid (50%), spinosad (20%) and nimbecidine (6%). No adult motality was recorded in novaluron. Profenophos, quinalphos and thiodicarb caused 100 per cent adult mortality 24h after exposure, while mortality recorded in case of imidacloprid, novaluron, spinosad and nimbecidine was 54, 42, 36 and 10 per cent, respectively. On the basis of adult mortality within 24h after exposure imidacloprid, novaluron and spinosad were categorized as slightly harmful (30-79%). Profenophos, quinalphos and thiodicarb as harmful (>99%) while nimbecidine alone was categorized as harmless (<30%).

The present findings are in consistent with that of Nalini and Manickavasagam (2011) who reported that profenophos caused 100 per cent mortality of *A. bambawalei and A. advena* adults within one hour after exposure. Brunner *et al.* (2001) also reported that organophosphates and carbamate insecticides were highly toxic to *Colpoclypeus florus* adults. IGRs have been reported to show low contact toxicity to *Anagrus nilaparvatae* (Wang *et al*., 2008). The present findings are also in conformation with the results of Veire and Tirry (2003) who found that spinosad and imidacloprid were highly toxic and caused considerable mortality to adult parasitoid, *Encarsia formosa*.

Table 1. Toxicity of different insecticides to adults of *Aenasius bambawalei*

			Mean cummulative adult mortality (%) at different hours of exposure					
Treatments	Dose/litre of water	Dose (g a.i./ha)	1h	3h	6h	12h	24h	Toxicity category
Imidacloprid	0.3ml	0.6g	16.00 (23.00)	28.00 (31.14)	42.00 (40.31)	50.00 (44.98)	54.00 (47.51)	2
Novaluron	1ml	0.1g	0 (0.99)	0 (0.99)	0 (0.99)	0 (0.99)	42.00 (40.31)	2
Spinosad	0.4ml	0.18g	0 (0.99)	0 (0.99)	6.00 (11.47)	20.00 (26.25)	36.00 (36.58)	2
Nimbecidine	5ml	-	0 (0.99)	0 (0.99)	2.00 (4.48)	6.00 (11.47)	10.00 (16.58)	1
Profenophos	3ml	1.5g	44.00 (38.96)	100.00 (88.15)	100.00 (88.15)	100.00 (88.15)	100.00 (88.15)	4
Quinalphos	4ml	1g	0 (0.99)	68.00 (55.73)	100.00 (88.15)	100.00 (88.15)	100.00 (88.15)	4
Thiodicarb	1.5g	1.12g	0 (0.99)	44.00 (40.82)	84.00 (66.66)	100.00 (88.15)	100.00 (88.15)	4
Control	-	-	0 (0.99)	0 (0.99)	0 (0.99)	0 (0.99)	0 (0.99)	1
C.D. (p = 0.05)			(12.72)	(5.77)	(7.46)	(6.55)	(8.35)	

Figures in parentheses are means of angular transformed values.

Effect of different insecticides on larval stage (in two days old parasitised mealybugs) of *Aenasius bambawalei*

Highest number of mummies was observed in control (44.00) when the host carrying the larvae (in two days old parasitized mealybugs) of *A. bambawalei* were sprayed with different insecticides followed by novaluron (40.20), imidacloprid (38.60) and nimbecidine (30.00) (Table 2). Lowest number of mummies were formed in quinalphos (2.00), profenophos (2.40), thiodicarb (4.60) and spinosad (5.40).

Table 2. Effect of insecticides on larval stage (in two days old parasitised mealybugs) of *Aenasius bambawalei*

Treatments	Dose per litre of water	Dose (g a.i./ha)	Number of mummies formed	Adult emergence (%)	Reduction in adult emergence over control	Toxicity category
Imidacloprid	0.3ml	0.6g	38.60 (6.29)*	78.49 (62.59)**	14.95	I
Novaluron	1ml	0.1g	40.20 (6.41)	84.14 (66.82)	8.83	I
Spinosad	0.4ml	0.18g	5.40 (2.49)	11.01 (18.98)	88.07	III
Nimbecidine	5ml	-	30.00 (5.56)	64.11 (53.35)	30.53	II
Profenophos	3ml	1.5g	2.40 (1.82)	0 (0.99)	100	IV
Quinalphos	4ml	1g	2.00 (1.71)	0 (0.99)	100	IV
Thiodicarb	1.5g	1.12g	4.60 (2.31)	31.61 (33.65)	65.73	II
Control	-	-	44.00 (6.70)	92.29 (73.96)	-	-
C.D. (p = 0.05)			(0.41)	(6.68)		

*Figures in parentheses are means of square root transformed values, **figures in parentheses are means of angular transformed values

The data on adult emergence revealed that highest adult emergence took place in control (92.29%). Among insecticidal treatments highest adult emergence was recorded in novaluron (84.14%) which was at par with imidacloprid (78.49%) followed by nimbecidine (64.11%), thiodicarb (31.61%) and spinosad (11.01%). There was no adult emergence in profenophos and quinalphos. Based on reduction in adult emergence over control imidacloprid (14.95%) and novaluron (8.83%) were categorised as harmless (<30%), nimbecidine (30.53%) and thiodicarb (65.73%) were categorised as slightly harmful (30-79%), spinosad (88.07%) was categorized as moderately harmful (80-99%) and profenophos (100%) and quinalphos (100%) were categorized as harmful (>99%).

The present studies are in confirmations with the findings of Hill and Foster (2000) who reported that spinosad caused significantly higher larval mortality of *Diadegma insulare* at field rates as compared to imidacloprid. Similarly, Osman and Bradley (1993) explained high mortality of *Cotesia glomerata* larvae and morphogenetic defects of larvae developed in neem-treated hosts mainly as effects of azadirachtin on the metamorphosis of the parasitoids.

Effect of different insecticides on pupal stage (in two days old mummies) of *Aenasius bambawalei*

Adult emergence

When pupal stage was sprayed with different insecticidal solutions highest adult emergence was observed in control (95.42%) while all the insecticides resulted in significantly low adult emergence. Among the insecticides, highest adult emergence was observed in novaluron (86.06%) which was on par with imidacloprid (82.14%) followed by nimbecidine (76.60%) and thiodicarb (60.60%). Lowest adult emergence was observed in quinalphos (4.70%), profenophos (5.40%) and spinosad (6.02%). Based on reduction in adult emergence over control imidacloprid (13.91%), novaluron (9.81%) and nimbecidine (19.72%) were categorized as harmless (<30%), thiodicarb (36.49%) as slightly harmful (30-79%) and spinosad (93.69%), profenophos (94.34%) and quinalphos (95.07%) were categorised as moderately harmful (80-99%).

The present findings are supported by (Suh *et al*., 2000) who reported spinosad to be very toxic resulting in only 25.8 per cent adult emergence of *T. exiguum* whereas thiodicarb was safe resulting in 95.4 per cent emergence. Feldhege and Schmutterer (1993) revealed that azadirachtin showed little effect on emergence of the parasitoid, *E. formosa*.

Proportion of female progeny

There was no significant difference in the percentage of females in the progeny of the parasitoid in different treatments (Table 3). The per cent females in the progeny of the parasitoid in different treatments ranged from 73.98 to 77.38.

The present findings are in line with those of Ramesh and Manickavasagam (2006) who reported that the per cent females of *T. chilonis* emerging from parasitized eggs treated with different insecticides were on par with control.

Adult longevity

Among all the treatments highest adult longevity was observed in control (21.00 days) (Table 3). Among the insecticides, highest adult longevity was observed in novaluron (14.20 days) which was at par with nimbecidine (12.20 days) followed by imidacloprid (10.60 days) and thiodicarb (8.80 days). While it was lowest in spinosad (4.20 days), quinalphos (5.20 days) and profenophos (5.60 days). Based on the longevity of adult parasitoids nimbecidine, novaluron, imidacloprid and thiodicarb were found to be safe to the parasitoid.

Sublethal doses of the insecticides could limit the development, survival and growth of the parasitoid, *Apanteles galleriae* (Ergin *et al*., 2007).

Parasitisation

Among all the treatments highest number of mealybugs were parasitized by adults of *A. bambawalei* emerged from sprayed pupae (in two days old mummies) in control (43.20) (Table 4) followed by novaluron (33.80), nimbecidine (26.40), imidacloprid (21.16), thiodicarb (8.80), spinosad (5.60), quinalphos (1.80) and profenophos (0.80). Among all the treatments highest parasitization was observed in control (86.40%). Among insecticides, highest parasitization was observed in novaluron (67.60%) followed by nimbecidine (52.80%), imidacloprid (42.00%), thiodicarb (17.60%), spinosad (11.20%), quinalphos (3.40%) and profenophos (1.60%).

The present findings are in confirmation with the results of Srinivasan *et al.* (2001) who reported that adult emergence of *T. chilonis*, *T. japonicum* and *T. brasiliensise* and their subsequent parasitization was greatly influenced by the insecticide treatments.

Table 3. Effect of insecticides on pupal stage (in two days old mummies) of *Aenasius bambawalei*

Treatments	Dose/lt of water	Dose (g a.i./ha)	Adult emergence (%)	Reduction in adult emergence over control (%)	Toxicity category	Females in progeny (%)	Longevity (days)
Imidacloprid	0.3ml	0.6g	82.14 (65.01)	13.91	I	76.76 (61.18)	10.60
Novaluron	1ml	0.1g	86.06 (68.08)	9.81	I	75.22 (60.13)	14.20
Spinosad	0.4ml	0.18g	6.02 (13.82)	93.69	III	75.88 (63.43)	4.20
Nimbecidine	5ml	-	76.60 (61.14)	19.72	I	74.84 (60.02)	12.20
Profenophos	3ml	1.5g	5.40 (13.17)	94.34	III	75.10 (60.07)	5.60
Quinalphos	4ml	1g	4.70 (12.09)	95.07	III	74.10 (59.40)	5.20
Thiodicarb	1.5g	1.12g	60.60 (51.18)	36.49	II	73.98 (59.34)	8.80
Control	-	-	95.42 (79.16)	-	-	77.38 (61.60)	21.00
C.D. (p = 0.05)			(5.18)			(NS)	2.65

Figures in parentheses are means of angular transformed values.

Table 4. Parasitization potential of emerged adults of *Aenasius bambawalei* from the sprayed pupal stage (in five days old mummies of mealybugs)

Treatments	Dose/lt of water	Dose (g a.i./ha)	Number of mummies formed	Parasitisation (%)	Reduction in parasitisation over control	Toxicity category
Imidacloprid	0.3ml	0.6g	21.16 (4.69)*	42.00(40.53)**	51.18	II
Novaluron	1ml	0.1g	33.80 (5.89)	67.60 (55.45)	21.1	I
Spinosad	0.4ml	0.18g	5.60 (2.51)	11.20 (19.00)	86.98	III
Nimbecidine	5ml	-	26.40 (5.22)	52.80 (46.62)	38.63	II
Profenophos	3ml	1.5g	0.80 (1.34)	1.60 (6.88)	96.98	III
Quinalphos	4ml	1g	1.80 (1.63)	3.40 (9.93)	96.04	III
Thiodicarb	1.5g	1.12g	8.80 (3.11)	17.60 (24.63)	79.54	II
Control	-	-	43.20 (6.64)	86.40 (68.57)	-	-
C.D. (p = 0.05)			(0.48)	(5.79)		

*Figures in parentheses in the column are means of square root transformed values.

**Figures in parentheses in the column are means of angular transformed values.

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