Efficacy of *Beauveria bassiana* in combination with NeemAzal-T/S® on the whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) and its natural enemies

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The cotton whitefly, *Bemisia tabaci,* is an important pest of cotton. Because of resistance development in *B. tabaci* populations to different classes of insecticides, application of alternative compounds is of high importance in IPM programmes. In the present study, effects of *Beauveria bassiana* (EUTP105 Isolate) alone or in combination with NeemAzal-T/S® and Tiacloprid (Calypso 480 SC) on the eggs and nymphs of *B. tabaci* and pupae of its parasitoid *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) were evaluated in field and laboratory conditions. The LC_{50} values of *B. bassiana,* NeemAzal-T/S and Calypso against eggs and nymphs of *B. tabaci* were 1.36×10^{9} conidia ml^{-1}, 6.83 mg L^{-1} AZA, 0.28 ml l^{-1} and 1.14×10^{7} conidia ml^{-1}, 6.00 mg L^{-1} AZA and 0.15 ml L^{-1}, respectively. The LC_{50} values of *B. bassiana,* NeemAzal-T/S and Calypso against pupae of *E. mundus* were 1.30×10^{10} conidia ml^{-1}, 36.90 mg L^{-1} AZA and 0.61 ml L^{-1}, respectively. In the laboratory conditions, efficacy of *B. bassiana* + NeemAzal-T/S on nymphs of the pest was higher than that of *B. bassiana,* and they caused the lowest mortality in the parasitoid pupae. In the field conditions at 3 days after treatment (DAT), the highest mortality of eggs (42.73%) and nymphs of the pest (89.57%) and pupae of the parasitoid (13.76%) occurred in Calypso. At 7 DAT, NeemAzal-T/S had the highest efficacy (86.52%) on nymph, which was not significantly different to Calypso (76.91%). At 14 DAT, the highest mortality on eggs (18.26%) and nymphs of the pest (46.39%) as well as pupae of the parasitoid (3.25%) were observed in NeemAzal-T/S. Based on the field results, NeemAzal-T/S and *B. bassiana* can be used as effective and environmentally safe alternatives for chemical insecticides in management of *B. tabaci* in cotton fields, either alone or in combination.

Keywords: Cotton, entomopathogenic fungus, non-chemical control, parasitoid, whitefly

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is an important wide host range pest that attacks many field and horticultural crops worldwide (Martins et al. 2012). The pest damage is caused by direct feeding, the transmission of plant viruses and honeydew production, which is associated with fungal growth (Khan and Wan 2015; Oliveira et al. 2001).

Chemical control is the main component of whitefly management programmes all over the world (Ellsworth and Martinez-Carrillo 2001). But continuous use of chemical insecticides causes development of resistance and negative environmental impacts. Therefore, to control this pest, alternative pest management strategies should be developed (Faria and Wraight 2001; Ahmad et al. 2002). *Eretmocerus mundus* Mercet (Hymenoptera: Aphelinidae) is an important solitary parasitoid of whitefly nymphs (Sohrabi et al. 2013) but use of this parasitoid is not enough to control the pest economically and the use of a different strategy is needed in field conditions (Stansly et al. 2004; 2005). One of the most important alternatives to chemical control methods is entomopathogenic fungi as a biological control agent which has a wide variety of control methods and the potential to reduce development of resistance. This method has been used to control many important pests including sucking-insects such as whiteflies (Faria and Wraight 2001).

Beauveria bassiana (Balsamo) vuillemin is a soil-borne fungi which is used as an entomopathogenic agent against whiteflies and other leaf-feeding insect pests (Lacey et al. 2008; Fuxa and Kunimi 1997; Shah and Goettel 1999).The main reasons for interest in this fungus are its entry mechanism (contact

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instead of ingestion) (Fuxa 1987), wide host range, replication in hosts (Ferron 1978; Roberts and Humber 1981), safety to non-target organisms (Hokkanen and Lynch 1995), *in vitro* mass-culture (Jackson et al. 2000) and the availability of various strains (St. Leger et al. 1992). Usually, these fungi take several days to kill the target pest (Lacey et al. 2008), so immature insects that molt frequently may shed conidia during ecdysis, which could reduce their infection (Vey and Fargues 1977). Thus, a combination of some compatible products with the fungi could increase efficiency and decrease the use of chemical insecticides, which will minimize the risks of environmental contamination and insecticide resistance (Quintela and McCoy 1998).

Azadirachtin is a steroid-like triterpenoid isolated from neem tree seeds, *Azadirachta indica* (Hernandez et al. 2012). This compound has been used against more than 200 species of insect pests and has many attributes including repellent, antifeedant, growth regulation (ecdysone inhibitor) and oviposition deterrent (Hernandez et al. 2012; Mitchell et al. 2004; Kumar et al. 2005; Ascher 1993). Nowadays, it is used alone (Kumar et al. 2005; Kumar and Poehling 2006) or in combination with some entomopathogens to control different pests (Taheri Sarhozaki et al. 2020; Mohan et al. 2007; Depieri et al. 2005).

Neonicotinoid insecticides have a satisfactory efficiency against sucking hemipteran insects such as aphids and whiteflies (Iwasa et al. 2004). Calypso®, (Z)-3-(6-chloro-3-pyridylmethyl)-1, 3-thiazolidin-2-ylid enecyanamide, is a systemic insecticide that like other chloronicotinyl insecticides acts selectively on the insect nervous system as an agonist of the nicotinic acetylcholine receptor. It is a highly active novel insect control agent with broad spectrum efficacy against sucking and biting insects depending on crop, pest and application type (Elbert et al. 2008; Saimandir et al. 2009).

The study of the impact of insecticides on the natural enemies of a pest species is of high importance in integrated pest management programmes (Sohrabi et al. 2013). Thus, the present study was conducted to evaluate the efficacy, under laboratory and field conditions, of Calypso, *B. bassiana* (EUTP105 Isolate) and NeemAzal-T/S® alone, as well as the combination of *B. bassiana* and NeemAzal-T/S®, on eggs and second instar nymphs of *B. tabaci* and pupae of *E. mundus*.

Materials and methods

Biocontrol agent

Beauveria bassiana (EUTP105 Isolate) were obtained from the College of Agriculture, Tehran University, Iran. The fungus was cultured on sabouraud dextrose agar (SDA) (Bbl, USA) in petri dishes (9 cm diameter) and incubated at 24 ± 2 °C; 16: 8 h (L:D) photoperiod and 60 ± 5 % humidity for 10-14 days. Conidia were harvested by scraping the surface of the culture with an inoculation needle into 50 ml aq. The conidia were suspended in distilled water containing 0.02% Tween®-80. The suspension was then shaken and the mixture filtered through a sterile fine mesh muslin to remove the hyphal debris and obtain a concentration of conidia. The different concentrations of the conidia were prepared by a hemocytometer using serial dilutions.

Botanical insecticide

A commercial neem product, NeemAzal-T/S® EC 1% (10000 mg a.i./L containing azadirachtin-A; Trifolio-M GmbH, Lahnau, Germany) was used in the experiments.

Chemical insecticide

The insecticide used in this research was Thiacloprid (Calypso® 480 SC, Bayer Crop Science Limited, Germany).

Laboratory experiments

Plant culture and physical conditions

For the laboratory experiments, cotton seeds were grown in plastic pots (20 cm depth and 20 cm diameter) in a greenhouse at 16-25 °C, 40 - 50% humidity under a 14:10 h (L:D) photoperiod and used at the 4-5 leaf stage. Laboratory tests were carried out at 24 ± 2 °C, a 16: 8 h (L: D) photoperiod and 60 ± 5 % humidity.

Insect cultures

B. tabaci

A colony of *B. tabaci* was initiated by collecting adults using an aspirator from cotton fields in the research field of scientific staff of Cotton Research Center of East Iran, Kashmar in July 2019. The adults were transferred to a growth chamber (muslin-walled cage 120×60×60 cm) containing 40 potted cotton plants (cultivar *Varamin*) under the experimental laboratory conditions described above.

E. mundus

Cotton leaves bearing parasitized nymphs of *B. tabaci* were collected from cotton fields (free of insecticides) in the research field of scientific staff of Cotton Research Center of East Iran, Kashmar, in July 2019. The collected leaves were transferred to open petri dishes (9 cm diameter) and kept in a growth chamber (muslin-walled cage 120×60×60 cm) containing 40 potted cotton plants in the laboratory conditions described above. Emerged adults of the parasitoid were used to maintain the colony and obtain cohorts of the parasitoid. Adults were provided weekly with new whiteflies nymphs.

Using an aspirator, adult whiteflies were collected from the laboratory colony and transferred to plastic tubes (10 cm depth and 3 cm diameter). The tubes were incubated at 10 °C for 5 min in order for the adults to be handled more easily. Afterwards, groups of 30 adults were released into the clip cages confined to the lower surface of the potted cotton leaves and allowed to oviposit for 24 hours. Each clip cage was made from two plastic containers (5.5 cm depth and 8 cm diameter); one cage was placed in the upper surface of the cotton leaves and the other in the lower surface, both were fixed with an elastic string. Each container was ventilated by means of a fine mesh glued to an aperture cut in the bottom (5 cm diameter). After 24 hours, adults were removed from the cages and leaves (potted plants) bearing eggs were labelled and used in the bioassay or incubated under experimental conditions for another 10 days to obtain cohorts of whitefly nymphs. After this period, the second instar nymphs (the most susceptible stage to fungus) were used in the bioassays or offered to the parasitoid (the most suitable stage for the parasitoid) to obtain cohorts of the parasitoid (Cuthbertson et al. 2005; Kumar et al. 2005; Taheri et al. 2020).

Bioassays

Bioassay of eggs and second instar nymphs of B. tabaci

Based on the method of Muniz and Nombela (2001), serial dilutions of NeemAzal-T/S and Calypso were prepared to cause 10-90% mortality in treated eggs and nymphs of the pest. Before use, the solutions were shaken for 10 minutes by a mechanical shaker. Then, the cotton leaves bearing eggs and nymphs of the pest were dipped in NeemAzal-T/S (5, 10, 15, 25 and 50 mg AZA l^{-1}) and Calypso (0.05, 0.1, 0.3,0.5, 0.6, 0.7 and 0.8 ml L^{-1}) for 5 seconds. The leaves were allowed to dry at room temperature for 10 minutes and then kept at experimental conditions. Counting of the dead eggs (non-hatched) and dead nymphs (shrunken and lost the normal yellow-green colour (Golce and Kubilay 2005) was

performed 3 days after treatment under a microscope. In the bioassay with fungi, serial dilutions of conidia (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 and 1×10^{11} conidia ml^{-1}) were prepared in distilled water containing 0.02% Tween®-80. Afterwards, the leaves bearing eggs and nymphs of *B. tabaci* were dipped in the different concentration of conidia as above. The control leaves were dipped in distilled water containing 0.02% Tween®-80. Counting of the dead eggs and nymphs was done 7 days after treatment. All the treatments had three replications and 20 eggs or 20 nymphs (on leaves of potted cotton) were used in each replication (n = 60 per treatment) (Taheri et al. 2020).

Bioassay of pupae of the parasitoid

Groups of 5-6 females and 2-3 males of the parasitoid were collected with an aspirator from the colony and released to the clip cages which were placed on labelled cotton leaves as described above and allowed to parasitize the second instar nymphs of the pest for 24 hours. Then the parasitoids were removed and leaves with parasitized nymphs were incubated for another 11 days under similar conditions as above, until pupation of the parasitoid (parasitoid pupae with the cherry coloured eyes became visible inside the whitefly cuticle). Afterwards, these leaves were dipped in the different concentrations of NeemAzal-T/S, Calypso and *B. bassiana*. In the control, the same procedure was performed but the leaves were dipped in distilled water plus 0.02% Tween®-80. The number of empty pupal and pupae that failed to emerge was counted at 3 and 7 days after treatment. Each treatment had three replications with 20 pupae in each replication (n = 60 pupae per treatment) (Taheri et al. 2020).

Treatment on the pest and parasitoid

After determining the lethal concentrations (using mortality data of bioassays, calculated by probit analysis), the same procedure was performed to evaluate the effects of Calypso, NeemAzal-T/S, and *B. bassiana* alone and the combination of NeemAzal-T/S, and *B. bassiana* on the eggs and nymphs of the pest as well as the pupae of the parasitoid. Eggs and second instar nymphs of *B. tabaci* and pupae of the parasitoid were exposed to different treatments as follow: NeemAzal-T/S (LC_{50}), *B. bassiana* (LC_{50}), Calypso (LC_{50}), NeemAzal-T/S (LC_{25}) + *B. bassiana* (LC_{25}), Control (distilled water + Tween 80, 0.02%). All treatments were performed with 3 replications (n = 60 per treatment). The mortality of the exposed eggs and nymphs was counted at 3 and 7 days after treatment (Taheri et al. 2020).

Field experiments

Field planting

By disking after deep plowing, experimental plots were prepared. Nitrogen (150 kg ha^{-1} NH_4NO_3), phosphate (100 kg ha^{-1} P_2O_5) and potassium (50 kg ha^{-1} K_2O) was used as the fertilizer. In August 2019, a susceptible cultivar of cotton *Gossypium hirsutum* L. to *B. tabaci* i.e. cv *Varamin*, was planted in the research field of scientific staff of Cotton Research Center of East Iran, Kashmar (35° 13′ N, 58° 26′ E), in a randomized complete block design (RCBD) with three replications. The experimental plots were 6 m^2 and 1.5 m apart. The distances between rows and plants (after thinning) were 70 and 20 cm, respectively. Each block was 2 m apart from the others. As they are very mobile, to reduce the possibility of adult whiteflies entering into the treated plots, the control plots were located 30 m apart from treated plots. Weeds were picked by hand twice; when the cotton was in the four-leaf stage and in the eight-leaf stage. Irrigation was done every 2 weeks. When the cotton was in two-leaf stage, an insecticide (Imidacloprid SC 35% EXIR, Iran; 250 ml ha^{-1}) was used to control aphids and thrips.

Pest population estimation

The pest population was estimated prior to the trials by counting eggs and nymphs of the pest and pupae of the parasitoid by randomly selecting five plants in each plot. From each selected plant, three leaves from top, middle and bottom, were excised and transferred to the laboratory inside plastic bags (altogether 45 leaves per treatment).

Two pieces, each1 cm^2, from each leaf were chosen so that the pieces included the main vein (altogether 90 pieces per treatment). This was repeated at 3, 7 and 14 days after treatment. Using a microscope the total live eggs and second instar nymphs of the pest on each piece of leaf were counted.

Estimating the parasitoid population

To estimate the population of the parasitoid, the same procedures were used to count the pupae of the parasitoid formed in the nymphs of the pest and incubating all of them at the experiment conditions for 5 days.

Efficacy of the treatments in field conditions

Using a knapsack sprayer (16 L capacity), a recommended dose of NeemAzal-T/S (15 mg L^{-1} AZA), Calypso (0.3 ml L^{-1}), a concentration of 1×10^{10} conidia ml^{-1} of *B. bassiana* and a dose of NeemAzal-T/S (7.5 mg L^{-1} AZA) plus a concentration of 1×10^{5} conidia ml^{-1} of *B. bassiana* were sprayed on upper and lower surfaces of the cotton plants at a volume of 300 ml per plot, equivalent to an application volume of 500 L ha^{-1}. The control treatment was sprayed with water. Using the Henderson and Tilton (1955) equation, mortality caused by each treatment on the eggs or nymphs of *B. tabaci* and pupal stage of *E. mundus* was calculated.

$$\text{Efficacy of treatment (\%)} = 1 - \left(\frac{T_a \times C_b}{C_a \times T_b}\right) \times 100$$

where

T= number of live eggs, nymphs or pupae per five plants in each treatment after (T_a) or before (T_b) application, and C= number of live eggs, nymphs or pupae per five plants in the control after (C_a) or before (C_b) application.

Analysis of data

The mortality data of eggs, nymphs and pupae was subjected to probit analysis to obtain LC_{50} and LC_{25} values. In the laboratory tests one-way ANOVA was used to analyze the mortality of the treatments on eggs and nymphs of whiteflies or pupae of parasitoids and means were compared by Tukey's test. In the field experiments, the Henderson and Tilton formula was used to calculate efficacy of treatments in the field experiments, and the General Linear Model-univariate and Tukey's HSD were used to analyze and separate means using SPSS statistical analysis software (SPSS v.18.0; SPSS, Chicago, IL, USA).

Results

Probit analysis

Table 1 shows the estimated LC_{25} and LC_{50} values of NeemAzal-T/S, *B. bassiana* EUTP105 Isolate and Calypso on eggs and nymphs of *B. tabaci* as well on the pupae of the parasitoid *E. mundus* using probit analysis.

Table 1: Probit analysis data for egg and second instar nymph of *B. tabaci* and pupae of *E. mundus* three days after treatment with NeemAzal-T/S® and Calypso and seven days after treatment with *B. bassiana*

Treatment	Stage	LC_{50} (95% CL)	LC_{25} (95% CL)	Intercept ± SE	Slope ± SE	χ^2
				B. tabaci		
NeemAzal-T/S (a.i.)	Egg	6.83 mg L^{-1} (5.43 - 8.15)	1.77 mg L^{-1} (1.07 - 2.51)	5.19 ± 0.04	1.14 ± 0.10	15.43
B. bassiana		1.36×10^9 conidia ml^{-1} (4.66×10^8 - 6.48×10^9)	1.37×10^6 conidia ml^{-1} (4.80×10^5 - 3.06×10^6)	2.94 ± 0.18	0.22 ± 0.02	13.09
Calypso		0.28 ml L^{-1} (0.25 - 0.30)	0.12 ml L^{-1} (0.10 - 0.14)	6.04 ± 0.09	1.89 ± 0.15	19.02
NeemAzal-T/S (a.i.)	Nymph	6.00 mg L^{-1} (4.50 - 7.40)	1.30 mg L^{-1} (0.70 - 2.00)	5.23 ± 0.04	1.04 ± 0.10	19.64
B. bassiana		1.14×10^7 conidia ml^{-1} (5.21×10^6 - 3.17×10^7)	1.03×10^4 conidia ml^{-1} (2.14×10^3 - 3.06×10^4)	3.43 ± 0.15	0.22 ± 0.02	8.44
Calypso		0.15 ml L^{-1} (0.13 - 0.17)	0.04 ml L^{-1} (0.03 - 0.05)	5.97 ± 0.09	1.21 ± 0.10	10.78
				E. mundus		
NeemAzal-T/S (a.i.)	Pupa	36.90 mg L^{-1} (31.11-44.28)	6.87 mg L^{-1} (4.51 - 9.25)	4.47 ± 0.05	0.92 ± 0.09	21.17
B. bassiana		1.30×10^{10} conidia ml^{-1} (4.38×10^9 - 6.55×10^{10})	1.05×10^7 conidia ml^{-1} (3.36×10^6 - 2.45×10^7)	2.79 ± 0.21	0.21 ± 0.02	8.80
Calypso		0.61 ml L^{-1} (0.59 - 0.63)	0.42 ml L^{-1} (0.39 - 0.45)	5.91 ± 0.08	4.30 ± 0.34	14.84

Efficacy of the treatments in laboratory conditions

Effects on eggs of the pest

Figure 1 shows the mortalities of eggs of the pest in laboratory conditions at 3 days after treatment (DAT) ($F_{4,\ 10} = 280.79$, $P<0.001$) and at 7 DAT ($F_{4,\ 10} = 339.58$, $P<0.001$); at both 3 DAT and 7 DAT the highest and lowest mortalities were observed in Calypso and control, respectively, but there was no significant difference ($P>0.05$) between the Calypso and NeemAzal-T/S.

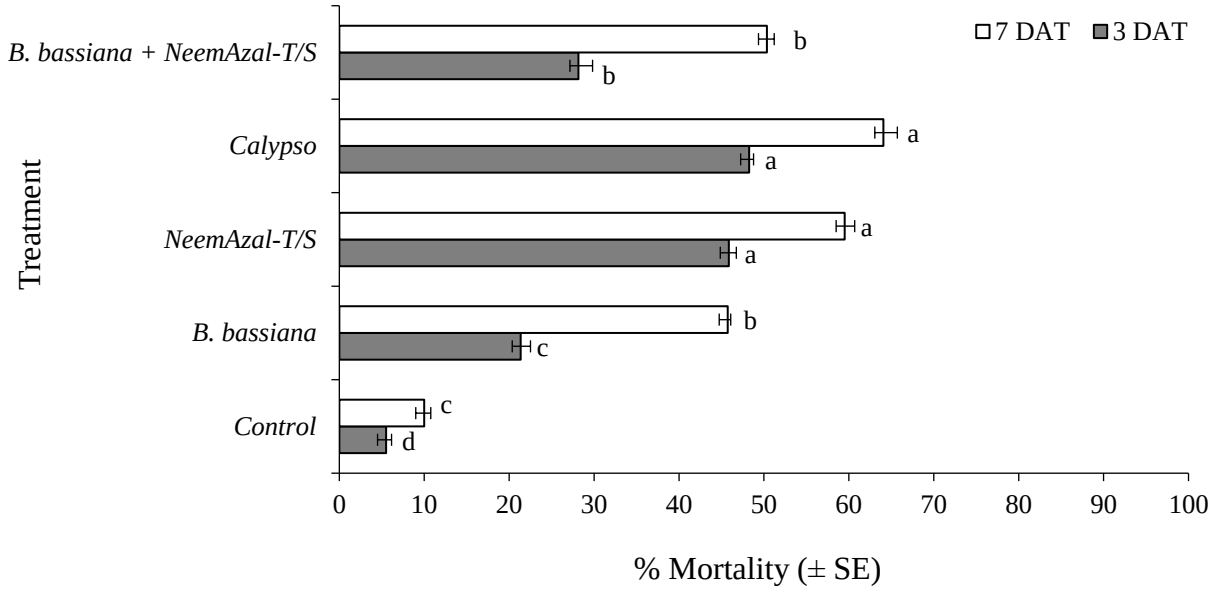

Figure 1: Mean (± SE) mortality (%) of different treatments in controlling eggs of *B. tabaci* on cotton applied either alone (LC_{50}) or in pairwise combination ($LC_{25} + LC_{25}$), measured 3 and 7 days after treatment (DAT). Means marked with different letters at the same DAT are significantly different ($P \leq 0.05$, Tukey).

Effects on nymphs of the pest

Figure 2 shows the mortalities of nymphs of the pest at 3 and 7 DAT ($F_{4, 10}$ = 284.80, P<0.001 and $F_{4, 10}$ = 304.66, P<0.001, respectively). The highest and lowest mortalities of nymphs at 3 DAT was observed in Calypso and control, respectively. At 7 DAT, the highest and lowest mortalities were observed in NeemAzal-T/S and control, respectively. At both 3 and 7 DAT there was no significant difference (P>0.05) between Calypso and NeemAzal-T/S.

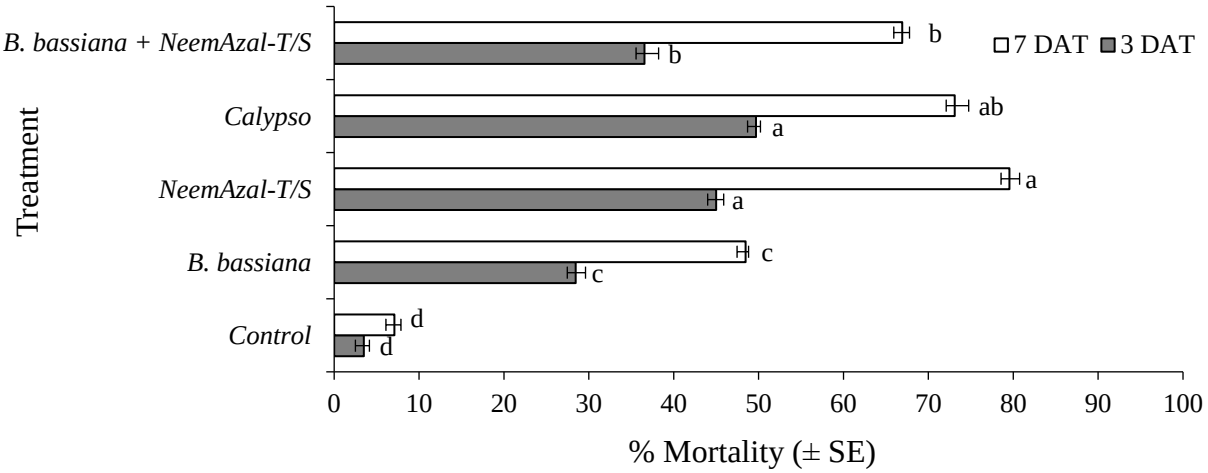

Figure 2: Mean (± SE) mortality (%) of different treatments in controlling nymphs of *B. tabaci* on cotton applied either alone (LC_{50}) or in pairwise combination (LC_{25} + LC_{25}), measured 3 and 7 days after treatment (DAT). Means marked with different letters at the same DAT are significantly different (P ≤ 0.05, Tukey).

Effects on pupae of the parasitoid

Figure 3 shows the mortalities of the pupae of the parasitoid at 3 and 7 DAT ($F_{4, 10}$ = 312.24, P<0.001 and $F_{4, 10}$ = 131.16, P<0.001, respectively. At both 3 and 7 DAT the highest and lowest mortalities were observed in Calypso and control, respectively. At both 3 and 7 DAT there was no significant difference (P>0.05) between Calypso and NeemAzal-T/S.

Figure 3: Mean (±SE) effect mortality (%) of different treatments on pupae of the parasitoid *E. mundus* parasitized nymphs of *B. tabaci* on cotton applied either alone (LC_{50}) or in pairwise combination (LC_{25}+LC_{25}), measured 3 and 7 days after treatment (DAT). Means marked with different letters at the same DAT are significantly different (P ≤ 0.05, Tukey).

Efficacy of treatments in field conditions

The efficacies of different treatments on eggs at 3, 7 and 14 DAT are shown in Table 2. At 3 DAT ($F_{3,\ 6}$ = 93.26, P<0.001), Calypso and *B. bassiana* had the highest and lowest efficacies, respectively. At 7 DAT ($F_{3,\ 6}$ = 20.61, P = 0.001), the highest efficacy was observed in Calypso, however, there was no significant difference (P > 0.05) between the *B. bassiana* and *B. bassiana* + NeemAzal-T/S. At 14 DAT ($F_{3,\ 6}$ = 15.53, P = 0.003) the highest and lowest efficacies were observed in NeemAzal-T/S and *B. bassiana*, respectively

Table 2: Mean (±SE %) efficacy of different treatments in controlling eggs of *B. tabaci* on cotton at 3, 7 and 14 days after treatment (DAT) in field

Treatment	Applied concentration	Mean (±SE)			Mean
		3 DAT	7 DAT	14 DAT	
B. bassiana	1×10^{10} conidia ml^{-1}	$7.25^{d} \pm 0.07$	$19.22^{c} \pm 0.10$	$1.60^{c} \pm 0.06$	9.35
NeemAzal-T/S	15 mg L^{-1}	$24.44^{b} \pm 0.05$	$40.93^{b} \pm 0.12$	$18.26^{a} \pm 0.04$	27.87
Calypso	0.3 ml L^{-1}	$42.73^{a} \pm 0.07$	$65.71^{a} \pm 0.13$	$11.98^{ab} \pm 0.08$	40.14
B. bassiana + NeemAzal-T/S	1×10^{5} conidia ml^{-1}+ 7.5 mg L^{-1}	$15.17^{c} \pm 0.04$	$23.41^{c} \pm 0.05$	$8.98^{bc} \pm 0.06$	15.85

Means marked with different letters within the same column are significantly different (GLM univariate followed by Tukey's test: P ≤ 0.05).

Table 3 shows the efficacies of different treatments on controlling nymphs of the pest. The differences were significant at 3, 7 and 14 DAT (respectively $F_{3,\ 6}$ = 78.47, P<0.001; $F_{3,\ 6}$ = 30.29, P=0.001 and $F_{3,\ 6}$ = 40.44, P<0.001). At 3 DAT, Calypso and *B. bassiana* had the highest and lowest efficacies, respectively. At 7 and 14 DAT, the highest and lowest efficacies were observed in NeemAzal-T/S and *B. bassiana*, respectively.

Table 3: Mean (±SE) efficacy (%) of different treatments in controlling nymphs of *B. tabaci* on cotton applied either alone or in pairwise combination, measured 3, 7 and 14 days after treatment (DAT) in field

Treatment	Applied concentration	Mean (±SE)			Mean
		3 DAT	7 DAT	14 DAT	
B. bassiana	1×10^{10} conidia ml^{-1}	$13.47^{c} \pm 0.05$	$32.28^{b} \pm 0.05$	$2.18^{c} \pm 0.05$	15.97
NeemAzal-T/S	15 mg L^{-1}	$45.45^{b} \pm 0.09$	$86.52^{a} \pm 0.11$	$46.39^{a} \pm 0.10$	59.45
Calypso	0.3 ml L^{-1}	$89.57^{a} \pm 0.07$	$76.91^{a} \pm 0.18$	$24.16^{b} \pm 0.04$	63.54
B. bassiana + NeemAzal-T/S	1×10^{5} conidia ml^{-1} + 7.5 mg L^{-1}	$35.10^{b} \pm 0.06$	$45.76^{b} \pm 0.09$	18.49^{b} ±0 .04	33.11

Means marked with different letters within the same column are significantly different (GLM univariate followed by Tukey's test: P ≤ 0.05).

Mortality effect of different treatments on the parasitoid pupae in field conditions

Table 4 shows the mortalities of the pupae caused by the different treatments. The differences were significant at 3, 7 and 14 DAT (respectively $F_{3,6}$ = 19.67, P = 0.002; $F_{3,6}$ = 14.00, P = 0.004 and $F_{3,\ 6}$ = 15.04, P= 0.0031). At 3 DAT, the highest and the lowest mortalities on parasitoid pupae were caused by Calypso and *B. bassiana*, respectively. At 7 DAT the highest and lowest mortalities were caused by NeemAzal-T/S and *B. bassiana*, respectively. At 14 DAT, the highest and the lowest mortalities were caused by NeemAzal-T/S and *B. bassiana* + NeemAzal-T/S, respectively.

Table 4: Mean (±SE %) mortality of different treatments on pupae of the parasitoid *E. mundus* parasitized nymphs of *B. tabaci* on cotton, at 3, 7, and 14 days after treatment (DAT) in field conditions

Treatment	Applied concentration	Mean (±SE)			Mean
		3 DAT	7 DAT	14 DAT	
B. bassiana	1×10^{10} conidia ml^{-1}	$2.53^{c} \pm 0.05$	$6.04^{c} \pm 0.05$	$0.35^{bc} \pm 0.00$	2.97
NeemAzal-T/S	15 mg L^{-1}	$10.50^{ab} \pm 0.06$	$15.94^{a} \pm 0.08$	$3.25^{a} \pm 0.05$	9.89
Calypso	0.3 ml L^{-1}	$13.76^{a} \pm 0.07$	$13.46^{ab} \pm 0.06$	$0.72^{ab} \pm 0.09$	7.96
B. bassiana + NeemAzal-T/S	1×10^{5} conidia ml^{-1} + 7.5 mg L^{-1}	$4.34^{bc} \pm 0.07$	$7.24^{bc} \pm 0.05$	$0.00^{c} \pm 0.00$	3.86

Means marked with different letters within the same column are significantly different (GLM univariate followed by Tukey's test: $P \leq 0.05$).

Discussion

The use of chemical pesticides is the main strategy adopted by growers in cotton fields to control *B. tabaci*; however, a biological control strategy involving the parasitoids *Eretmocerus mundus* decreased the whitefly abundance in cotton crops. Biological control by pathogen and plant compounds such as *B. bassiana* and NeemAzal-T/S are the best strategy to control pests and conserve parasitoids in IPM programs. Fungi are the only entomopathogens that are able to infect the host through the cuticle, an advantage for the management of piercing-sucking insects such as whiteflies (Faria and Wraight 2001). The mortalities of eggs, nymphs and pupae of the pest were higher when *B. bassiana* and NeemAzal-T/S were applied in combination compared to when applied alone. At 14 DAT, *B. bassiana* caused the lowest mortalities on eggs and second instar nymphs of *B. tabaci*. The laboratory data indicate an ability of *B. bassiana* to exploit the moist conditions of the incubated, leaf or insect boundary layer for germination and host infection; however, in the field environment the efficacy of this agent was affected by constantly changing temperature, humidity, wind speed, and even soil moisture levels on conditions at the leaf cuticle-air interface. The highest mortality on eggs of the pest was observed in the Calypso, followed by NeemAzal-T/S. Kumar and Poehling (2006; 2007) suggested that egg hatching failure is due to the penetration of azadirachtin solution sprayed onto the leaves. Additionally, ingestion of azadirachtin from plant tissues by the females could inhibit embryonic development or the hatching process and cause an intrinsic deficit of the eggs laid by the females. Antifeedant and deterrent activity of neem resulting in decreasing egg laying have also been reported in studies on *B. tabaci* (Kumar et al. 2005). Eggs are usually regarded less susceptible to insecticides compared to other stages. The apparent reduction in nymphs for NeemAzal-T/S could be due to the effect of NeemAzal-T/S on crawlers after eclosion from viable eggs when they came into contact with the residues on leaves. Similarly, Safavi and Bakhshaei (2017) observed that nymphs of *Trialeurodes vaporariorum* were the most susceptible individuals to Calypso. Moreover, pupal stage is usually regarded more resistant to insecticides and other fatal factors. This could be due to the presence of thick cuticular layers which protect the pupa from any contact to toxicant materials. Kumar et al. (2008) reported that using the recommended dose rates of NeemAzal-T/S (50 mg a.i./L) on *Eretmocerus warrae*, a parasitoid of *B. tabaci*, caused an emergence rate of 55% at pupal stage. Because the *E. mundus* larva penetrates its host by chewing a hole in the host cuticle it opens a path for the topically applied biopesticide solution to enter the body. Hence the whitefly as well as the parasitoid can be contaminated directly with the active ingredient, which is a kind of worst-case

situation. For that reason, these ecto-endo aphelinid parasitoid species are more susceptible to the foliar application of neem than exclusively endoparasitic species (Hoelmer et al. 1990). Neonicotinoids have proved relatively resilient to the development of resistance. However, resistance has been confirmed in some populations of *B. tabaci* (Nauen and Denholm 2005). In present study, the overall control of whitefly with NeemAzal-T/S was satisfactory. Similarly, Touhidul Islam et al. (2010a) reported that neem is compatible with *B. bassiana* for the control of *B. tabaci*, though *B. bassiana* was slightly affected by neem. Additionally, Touhidul Islam et al. (2010b) reported that an integration of *B. bassiana* with neem caused much more nymphal mortality than individual treatments of *B. bassiana* and neem respectively, 7 days post-application. Our result agrees with the results of Hernandez et al. (2012) who reported that the combination of the *B. bassiana* and azadirachtin had an additive effect on *Tetranychus urticae* larvae resulting in nearly 80% mortality, compared to the separate use of *B. bassiana* (31%) and azadirachtin (59%). Lower efficacy of NeemAzal-T/S on eggs and nymphs, 14 days after treatment, compared with 7 DAT maybe due to exposure to sunlight and environmental conditions, resulting in faster degradation of NeemAzal-T/S in the field conditions. The major problem with neem-based products, which have triterpenoids as the active ingredient, is the photo-degradation by UV radiation (Caboni et al. 2002; Johnson et al. 2003). This reduction in bio-efficacy of neem seems to be related to the amount entering into the plant system, exposure to sunlight and climate conditions (Johnson et al. 2003). In conclusion, due to the satisfactory efficacies of *B. bassiana* +NeemAzal-T/S and NeemAzal-T/S, on eggs and nymphs of the *B. tabaci* in field conditions, they can be recommended in the use of IPM of this cotton pest cotton. When applied alone, *B. bassiana* showed a low level of control on eggs and nymphs of the pest, however it also had a low level of negative effects on the parasitoid. Further studies need to be carried out on the effects of temperature and humidity factors that could limit the practical use of *B. bassiana* in the field conditions, Studies should also focus on methods which increase stability of entomopathogenic fungi in field conditions by protecting their conidia from dehydration.

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