Combining of *Chaetomium globosum* (Sordariales: Chaetomiaceae) and botanical insecticide, neem to control Bemisia tabaci (Hemiptera: Aleyrodidae) biotype B on cucumber

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Abstract

The potential for using the entomopathogenic fungi and neem to control *Bemisia tabaci* has been previously evaluated under laboratory and greenhouse conditions. However, no study has been conducted on the efficacy of *Chaetomium globosum* alone or in combination with neem against *B. tabaci*. This study, therefore, was aimed to determie the virulence of *C. globosum* against second instar nymphs of *B. tabaci* biotype B and it is interaction with neem to control *B. tabaci* in the laboratory conditions. Three concentrations of *C. globosum* (1×10^7, 1×10^6 or 1×10^5 conidia ml^{-1}) and three concentrations of neem were tested. The highest mortaliy (38.%) caused 7 days post-treatment with 1×10^7 conidia ml^{-1}. Simalirly, the results demonstrate that the foliar application with 1.0% neem caused the highest nymph mortality (82.3%). Thus, 1.0% neem was combined with 1×10^7 conidia ml^{-1} of *C. globosum* as an integrated pest management prgramme against *B. tabaci*. The combination of *C. globosum* and neem caused the highest *B. tabaci* nymph mortalities

(90.2%).Thus, these two biocontrol agents have the potential to be complementary and they have proven effective for the control of *B. tabaci* on cucumber.

1. Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), commonly known as sweet potato or cotton whitefly, is one of the most devastating and serious insect pests of protected vegetable crops worldwide (Brown, 1994). All feeding stages of *B. tabaci* damage plants both directly, through the direct removal of phloem sap and indirectly by secreting large amounts of honeydew, encouraging the growth of sooty mould (Denholm et al., 1996). However, the most significant damage caused by *B. tabaci* is transmission of several economically important plant viruses (Alegbejo, 2000).

Although chemical control is still the primary method against *B. tabaci* worldwide, *B. tabaci* populations have developed a resistance to different classes of insecticides, especially organophosphates, pyrethroids and cyclodienes (Ahmad et al., 2002). Neonicotinoid resistance has been also described for *B. tabaci* populations by Longhurst et al. (2013). Secondary, there are increasing awareness of environmental concerns related to the use of insecticides (Koureas et al., 2012). Both reasons have encouraged the development of alternative methods of *B. tabaci* control such as Biological control and IPM for use in the greenhouses.

Several entomopathogenic fungi including *Beauveria bassiana*, *Lecanicillium muscarium*, and *Metarhizium anisopliae* have been shown significant potential for the control of *B. tabaci* (Cuthbertson et al., 2009). More recently, an Iraqi isolate *Chaetomium globosum* (Sordariales: Chaetomiaceae) has been shown a significant against aphids under laboratory and greenhouse conditions (Kadhim, 2018). Neem and neem-based products are another

important biological control agent (botanical insecticides), which have a significant effect in controlling of *B. tabaci*. For example, azadirachtin is a steroid-like tetranortriterpenoid derived from the neem tree (Azadirachta indica Juss). It is a strong antifeedant and repellent, prevents moulting and causes high mortality in several phytophagous insects, including *B. tabaci* (Mitchell et al., 2004; Kumar et al., 2005). However, the major problem with neem-based products is the rapid photo-degradation by UV radiation (Caboni et al., 2002; Johnson et al., 2003).

As *B. tabaci* is a polyphagous insect with a high reproductive capacity, it can be difficult to achieve a good level of control under more reliable conditions using only a single biological control agent. The combined use of entomopathogenic fungi and azadirachtin for the biological control of sucking insect pests has been suggested previously. For example, Islam et al. (2011) reported that soil application of neem along with foliar application of *B. bassiana* might be useful for the control of *B. tabaci*. However, detail information on the optimal method and doses of these two bio-control agents to achieve a higher level of *B. tabaci* management are missing. Hence, it is difficult to predict the outcome of intraguild interactions between azadirachtin and entomopathogenic fungi on *B. tabaci* suppression.

The objectives of the present study were, therefore, to determine the optimal method and dose of neem application that will reduce competition between *C. globosum* and azadirachtin, and thus enhance their additive interactions against *B. tabaci*.

2. Materials and methods

2.1 Host plant

Cucumber seedlings, cultivar Sayff F1 (Bayer, Nunhems, Netherlands) was used for the *B. tabaci* cultures and for the experimental work. Seeds were germinated in 40 cell Styrofoam trays filled with potting compost (John Innes No.2: Roffey Brothers,

Bournemouth, UK). in a glasshouse with temperatures maintained between 18°C night minimum and 24–29°C during the day. Two weeks after emergence, the seedlings were transplanted individually into 15-cm-diameter plant pots. The plants were maintained in a glasshouse at the same conditions described above. The plants were selected for all experiments when they were 5 weeks old and 6 to 9 true leaves had developed.

2.2 The insect colony

The stock culture of *B. tabaci* biotype B originated from greenhouse populations infesting cucumber plants at the Faculty of Agriculture, University of Kufa, Iraq in 2017. This population was reared on cucumber in 45 × 45 × 45 cm cages at 23 ± 2°C and with 16:8 h daily photoperiod at 20 ± 1 C and L:D 16:8 photoperiod for several generations

2.3 Entomopathogenic fungi

C. globosum was obtained from the Plant Protection Department, University of Kufa, which was originally isolated from aphids. This isolate was chosen due to it is high efficacy against aphids (Kadhim, 2018). The fungus was cultivated on potato dextrose agar (PDA) or Sabouraud's agar at 25°C. Aerial conidia were harvested from 10-d-old cultures by adding 12 ml of 0.02% Tween 80 to culture agar plates and gently scraping the surface of the cultures with a sterile inoculating loop to dislodge the conidia from the surface of the agar plates. The conidial suspension was pipetted from the plate and filtered through three layers of cheesecloth. The number of conidia in the suspension was determined using a haemocytometer (Neubauer improved, Superior Marienfeld, Germany). The resulting suspension was diluted to the desired concentrations with 0.02% Tween 80 (BDH Chemicals Ltd., Poole, UK) as required. The viability of the conidia was determined by spraying 0.1 ml of 1×10^6 conidia ml^{-1} on a sterile Petri dish with 1.5% Sabouraud dextrose agar (SDA). The dishes were sealed with parafilm and incubated at 20 °C, 90 ± 2% RH and a photoperiod of

16:8 (L:D) h. After 24 h, the number of germinated spores per 100 spores of each plate was assessed under the microscope (400× magnification). Germination was considered positive when the length of the germ tube was at least half the spore length. The viability exceeded 90%.

2.4 Neem formulation

Azadirachtin EC (0.3%) was obtained from Russell IPM Ltd., (Deeside, Flintshire, UK). Three concentrations of this formulation were used in this study, including 0.25, 0.5 and 1.0%. To obtain these concentrations, 2.5, 5.0, and 10.0 ml of Azadirachtin EC was diluted in 1L of sterile water. The suspensions were then agitating for 30 minutes on a magnetic stirrer, and were shaken again before they were used in experiments.

2.5 Obtaining a uniform age of *B. tabaci*

In order to produce even-aged second instar nymphs of *B. tabaci* biotype B for use in all bioassays, adults were transferred from the stock cultures onto 4-week-old cucumber (25 both male and females per leaf) were put into 3-cm-diameter clip-cages on the two cucumber leaves per plant, one clip-cage per leaf. They were allowed to lay eggs for 1 day in a growth chamber at 20 °C, 75 ± 2% RH and a photoperiod of 16:8 (L:D) h. The adults were then removed and the eggs counted and allowed to develop to second instar nymphs on the plants for 11 additional days before the beginning of each experiment.

2.6 Efficacy of *C. globosum* against the second instar of *B. tabaci*

Following the protocol of Insecticide Resistance Action Committee (2009), leaves with *B. tabaci* (50 second instar nymphs/leaf) were immersed into 100 ml of conidia suspension with 1×10^7, 1×10^6 or 1×10^5 conidia ml^{-1} for 5 s. The protocol was used because the leaf dip can provide a good coverage of the leaf underside, where the immature stages of *B. tabaci* are located (Cuthbertson et al., 2009). Control leaves were immersed into 0.02%

sterile aqueous Tween 80 for the same length of time. After treatment, leaves were placed on filer papers at room temperature for 30 min to dry and then they were individually kept inside plastic cups (18 x 9cm) with two screened windows in the growth chamber at 25°C, 85 ± 2% RH and a photoperiod of 16:8 (L:D) h. There were ten leaves in each treatment. Mortality was recorded 1, 3, 5 and 7 days post-treatment by checking the leaves under light microscope. Dead nymphs were surface sterilised by rinsing twice with 70% ethanol for 30 seconds and then with sterilised distilled water and thereafter placed on water agar (3 g of agar/L of water) in Petri dishes for five days, to confirm infection by *C. globosum*. A cadaver was regarded as having died from infection by this fungus if conidia of *C. globosum* were recovered from it (Mohammed and Hatcher, 2016).

2.7 Effect of neem application method on the mortality of *B. tabaci*

2.7.1 Foliar application with neem

Plant leaves each with 50 second instar nymphs of *B. tabaci* were immersed into 100ml of either 0.25, 0.5, or 1.0% of Azadirachtin EC (0.3%) for 5 s. Control leaves were immersed into sterile water for the same length of time. After treatment, leaves were individually kept inside plastic cups (18 x 9cm) with two screened windows in the growth chamber at 25°C, 70 ± 2% RH and a photoperiod of 16:8 (L:D) h. There were ten leaves in each treatment. Mortality was recorded 1, 3, 5 and 7 days post-treatment by checking the leaves under light microscope. Second instar nymphs were considered dead if they had lost their normal yellow-green color, smooth cuticle structure and turgidity (Islam et al., 2009).

2.7.2 Soil application with neem

Soil application experiment was conducted with the same neem formulation and dose-rates-mentioned in leaf-treatment experiment. Five-week-old cucumber plants each with 50 second-instar nymphs of *B. tabaci* were used in this experiment. Potted cucumber plants were

each treated with 50 ml of 2.5, 5.0 or 10.0 g/L. Ten replicates were carried out at each neem concentration. In addition, ten potted cucumber plants each with 50 second-instar nymphs were sprayed with sterile water only, as a control treatment. Mortality of *B. tabaci* was recorded 1, 3, 5 and 7 days post-treatment by examine the leaves under light microscope.

2.8 The combined effect of *C. globosum* with neem on the mortality of *B. tabaci*

Concentrations of *C. globosum* and Azadirachtin EC (0.3%) and application method of neem were selected from the individual experiments based on the levels of *B. tabaci* mortality. Five treatments, each with ten replicates, were tested. One treatment was immersed into 100 ml of conidia suspension (1×10^7 conidia ml^{-1}) for 5 s for each leaf. In the second treatment, *B. tabaci* infested leaves were immersed into 100ml of 1.0% of Azadirachtin EC (0.3%) for 5 s. Plant leaves in the third treatment were immersed into 100 ml of conidia suspension (10^7 conidia ml^{-1}) for 5 s and immersed into 100ml of 1.0% of Azadirachtin EC (0.3%) two days later. In the fourth treatment, plant leaves were immersed into 100 ml of 100ml of 1.0% of Azadirachtin EC (0.3%) for 5 s and immersed into 100 ml of conidia suspension (1×10^7 conidia ml^{-1}) two days later, and the fifth treatment was a control and *B. tabaci* infested leaves were immersed into sterile water only. Mortality of *B. tabaci* was recorded 1, 3, 5 and 7 days post-treatment by examine the leaves under light microscope.

Statistical analysis

Statistical analyses were carried out using GenStat (version 16; VSN International, Hemel Hempstead, UK). Normality of data distribution was estimated using a Shapiro–Wilk test (W-test). The data were transformed using arcsine square root when it was necessary to meet the assumption of normality. For the effect of conidial concentration of *C. globosum*, mortality data of *B. tabaci* were corrected for natural death in the control using Abbott's formula (Abbott 1925), then analysed repeated measurement analysis ANOVA. The effect of

concentration and application method of neem the mortality of *B. tabaci* were analysed using Two-factor repeated measurement analysis ANOVA. One-factor repeat-measurements ANOVA was again used to determine the combined effect of *C. globosum* and neem on the mortality of *B. tabaci*, compared to those only exposed to single agent or untreated plants. Mean comparisons were performed using LSD test at 5% level of significance ($P < .05$).

3. Results and Discussion

3.1 Efficacy of *C. globosum* against the second instar of *B. tabaci*

There was a significant effect of the fungal treatments, with *B. tabaci* mortality ranged between 10% and 38%, compared with mortality rate in the control treatment which was 3% (P <0.001). The results also showed that conidial concentration of *C. globosum* significantly affected the mortality of *B. tabaci* 7 days post-treatment ($F_{(2,119)} = 59.13$; P <0.001). The control values for *B. tabaci* treated with 1×10^7 conidia ml^{-1} were much higher than those of 1×10^6 and 1×10^5 conidia ml^{-1} (Fig. 1). Time after fungal treatment significantly affected the mortality of *B. tabaci* ($F_{(3,119)} = 57.34$; P <0.001). The interactions between fungus concentration and time after application were significantly different ($P \leq .0001$).

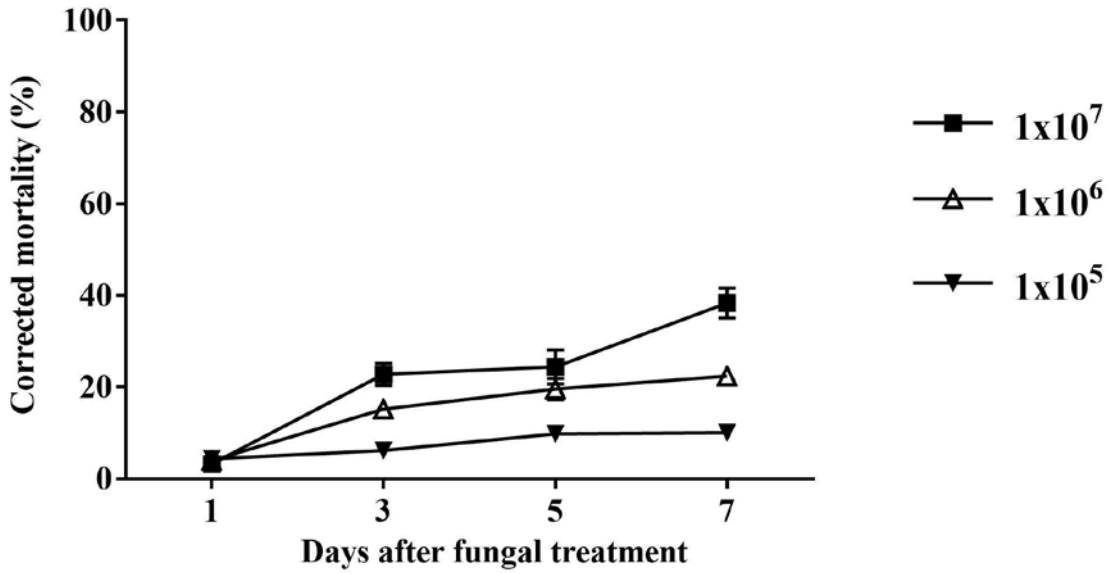

Fig.1: Effect of conidial concentration (conidia ml^{-1}) of *C. globosum* and days after fungal application on the corrected mortality (mean ± SE) of *B. tabaci* at 25°C and 85% RH.

3.2 Effect of neem application method on the mortality of *B. tabaci*

Nymph mortalities of *B. tabaci* were affected significantly by the application method and the dose rate of neem (Foliar application: $F_{(2,119)} = 84.61$; $P < 0.001$); Soil application: $F_{(2,119)} = 20.35$; $P < 0.001$). The results showed that the mortality level of *B. tabaci* immeresed into neem suspenision casued a high level of corrected mortalities at all three dose rates, compared with soil application of neem (Fig. 2). In both foliar and soil application methods, the highest level of corrected mortalities caused by a high dosage (1.00%) seven days post-treatment (82.47 and 19.82%, respectively). The interactions between application method, dosage and time after neem treatment were significantly different ($P \leq .0001$). Cumulative mortality in control treatments ranged between 3% and 4.6%.

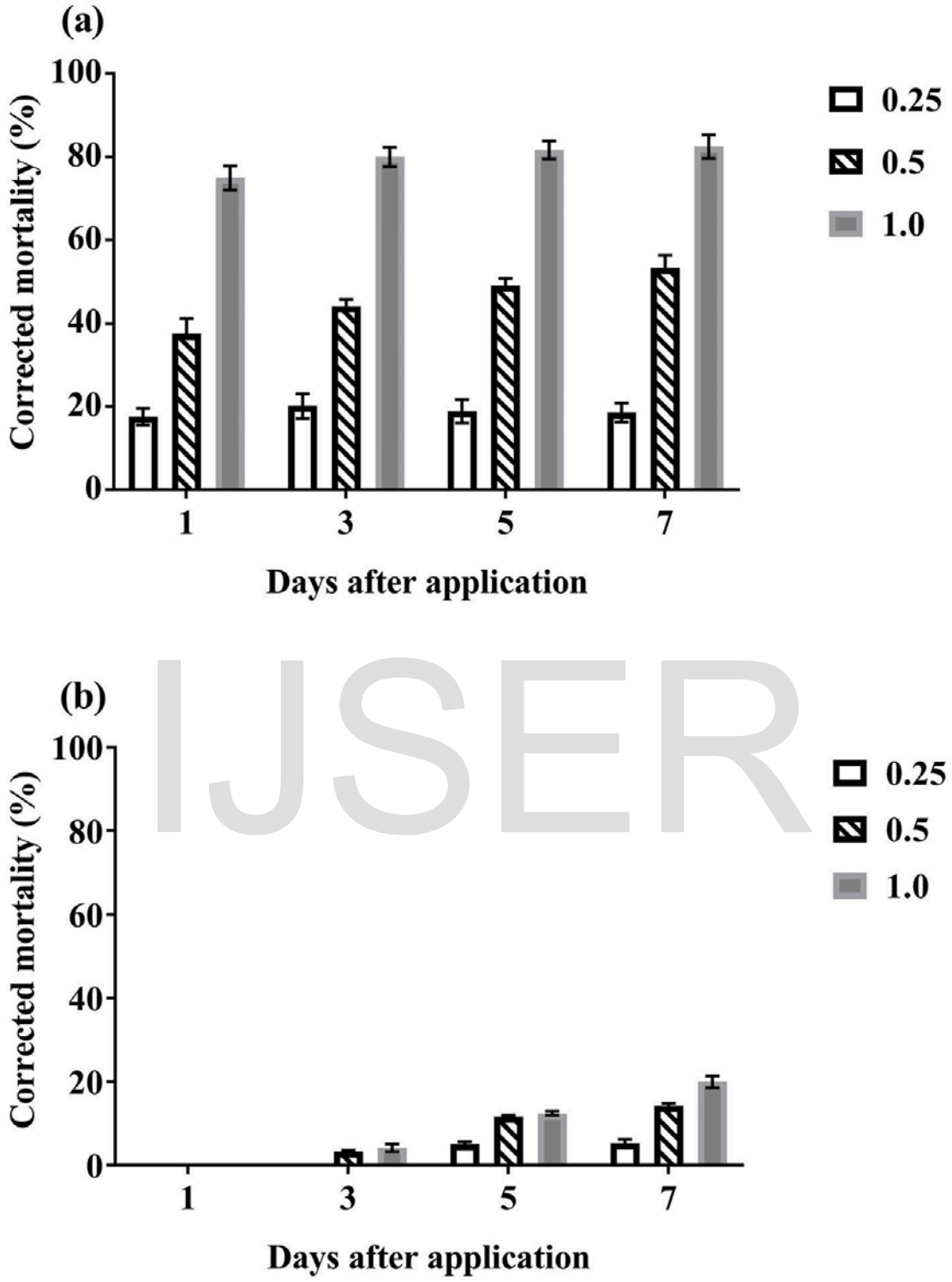

Fig.2: Corrected mortality of nymph mortality of *B. tabaci* (± SE) on cucumber caused with various concentrations of neem using (a) foliar application method, (b) soil application.

3.3 The combined effect of *C. globosum* with neem on the mortality of *B. tabaci*

Nymph mortality of *B. tabaci* was significantly different among the treatments ($F_{(3,279)} = 667.11$; $P < 0.001$). Infested leaves that treated with *C. globosum* only showed about 32% nymph mortality seven days post-treatment, compared with their respective controls. The nymph mortality of *B. tabaci* exposed to neem at concentration 1.0% only was significantly higher every day post-treatment, compared to the control or those nymphs treating with *C. globosum* only ($P < 0.001$). Furthermore, infested leaves that treated with *C. globosum* and later received neem significantly produced a more rapid mortality response in *B. tabaci* nymphs, compared with *C. globosum* or neem. However, the cumultive mortality of *B. tabaci* nymphs treated with neem and later exposed to *C. globosum* was not significantly different than those nymphs treating with neem only (Fig. 3).

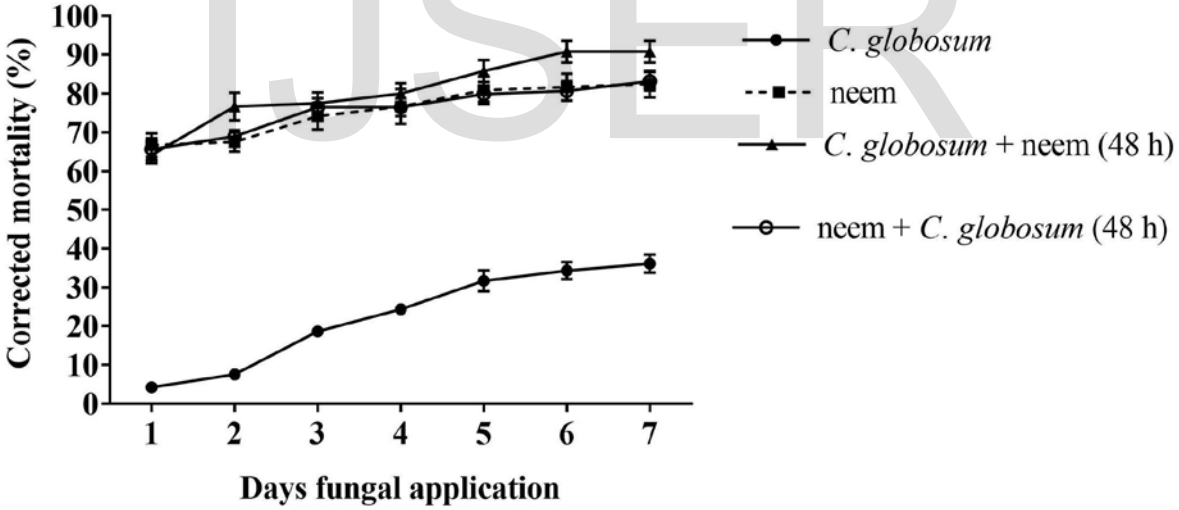

Fig. 3: Individual and combined effects of *C. globosum* (1×10^7 conidia ml^{-1}) and neem (1.0%) on the corrected mortality in the second instar nymphs of *B. tabaci* on cucumber (mean ± SE).

The increase of nymph mortality of *B. tabaci* in relation to increase concentration and time of exposure to *C. globosum* detected in our results fits well with previous studies on the

impact of entomopathogenic fungi on whiteflies. Islam et al. (2010a) found that the egg (25.2%) and nymph (72.9%) mortalities of *B. tabaci* treated with 1×10^{8} conidia ml^{-1} of *B. bassiana* were significantly higher than those of 1×10^{7}, and 1×10^{6} conidia ml^{-1}. Similarly, Kapongo et al. (2008) reported that the efficacy of *B. bassiana* against sweetpotato whitefly populations depends on the conidial concentrations. Where they observed that the corrected mortalities were 11, 34 and 35%, respectively. The results obtained in the present study are consistent with those of Vu et al. (2007) who reported that the control values for *M. persicae* treated with 1×10^{8} conidia ml^{-1} of *L. lecanii* 41185 were much higher than those of 1×10^{6}, 1×10^{5}, and 1×10^{4} conidia ml^{-1}.

A relatively long period of incubation is needed to enhance the efficacy of the entomopathogenic fungi for killing insect pests because of process of conidial germination and germ tube development and penetration through the host cuticle (Shi et al., 2008). Although there were some studies reported that the entomopathogenic fungi killing nymphs of *B. tabaci* after 3 days of incubation, the number of dead nymphs continued to increase until the 7th day after inoculation. Our results suggest that field and greenhouse experiments should employ a similarly long period of incubation before assessing their outcomes. This is in agreement Wraight et al. (2000), who demonstrated that the inoculation period was 8 days before recording the mortality of *B. argentifolii*.

Neem treatments increased mortality of *B. tabaci* on cucumber. Nymph mortality was highest with neem in foliar treatments (1.0%), reaching 82.3%. The high efficacy of neem against *B. tabaci* can be related to bitter compounds of neem that often have an antifeedant effect and can interfere with hormonal processes in insects (Schmutterer, 1990; Ascher, 1993). Efficacy of neem against different stages of *B. tabaci* has been reported in several studies (Nardo et al., 1997; Hammad et al., 2000). Islam et al. (2010a) found that nymph mortality of *B. tabaci* was highest with neem in foliar treatments (1.0%), reaching 75.5% for

7 days post-application. Similarly, Kumar and Poehling (2006) reported that nymph mortality of *B. tabaci* exposed to fresh neem residue in foliar treatments (10.0 ml l), reaching 100%. In our study, the foliar application of neem caused highest nymph mortality (97.2%) of *B. tabaci*, compared with soil application of neem. The different effects between foliar and soil application may be related to the presence of different amounts of neem residues in or on the leaves.

In the present study, the results showed the combined effects of neem with *C. globosum* resulted in a nymph mortality significantly higher than produced by one agent. One of the most important reasons which explain the highest mortality caused by combining neem with *C. globosum* is that the germination and vegetative growth and sporulation of entomopathogenic fungi are slightly by various neem concentrations (Mohan et al., 2007; Islam et al., 2010b). This confirms the findings of Islam et al. 2010 who found that the foliar application of neem with *B. bassiana* produced 97.2% mortality of *B. tabaci*, while this mortality was 77.3 and 70.4% for individual treatment of neem (0.5%) and *B. bassiana* (10^7 conidia/ml), respectively (Islam et al., 2010a). In addition, James (2003) found that up to 90% nymphal mortality of *B. argentifolii* was obtained when *Paecilomyces fumosoroseus* and azadirachtin were combined.

Conclusions

The results concluded that foliar application of neem alone was effective against nymphs of *B. tabaci*, but using neem along with *C. globosum* `significantly increased mortality of *B. tabaci* 7 days post-treatment. Thus, both biocontrol agents may contribute to the successful control of *B. tabaci* concerning environmental friendly strategy.

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