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*Full Length Research Paper*

# The integrated use of entomopathogenic fungus, *Beauveria bassiana* with botanical insecticide, neem against *Bemisia tabaci* on eggplant

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**In a previous experiment, sweetpotato whitefly was found to be difficult to control with either neem or *Beauveria bassiana* alone. This earlier research also reported that the combined application of neem and *B. bassiana* increased mortality of whitefly nymphs when both of these products were applied to eggplant foliage; but a higher concentration of neem (more than 0.5%) was slightly toxic for young eggplant. Therefore, the present research was undertaken with these two products in which neem was applied as soil drench and *B. bassiana* was applied to foliage. Three concentrations of neem- 0.25, 0.5 and 1.0%; and three concentrations of *B. bassiana*- $10^6$, $10^7$ and $10^8$ conidia/ml were used to investigate the combined efficacy against sweet potato whitefly, *Bemisia tabaci*. The results demonstrate that maximum nymph mortality (92.3%) occurred when 1.0% neem was combined with $10^8$ conidia/ml of *B. bassiana*. The highest (14.3) mortality ratio (N/Bb; mortality caused by neem/mortality caused by *B. bassiana*) occurred when 1.0% neem was combined with $10^6$ conidia/ml of *B. bassiana*; and the lowest mortality ratio (5.7) occurred when 0.25% neem was combined with $10^8$ conidia/ml of *B. bassiana*. The results showed that neem was compatible with *B. bassiana*; and suggest that soil application of neem along with foliar application of *B. bassiana* might be useful for the control of *B. tabaci*.**

**Key words:** Botanical insecticide, compatibility, eggplant, entomopathogenic fungus, integrated pest management (IPM).

## INTRODUCTION

Sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is considered as one of the most destructive pests of eggplant. The reduction percentages of this whitefly on various growth parameters of eggplant, leaf area, leaf fresh weight, leaf dry weight, chlorophyll content, and rate of photosynthesis, were 26.6, 21.8, 19.3, 9.7, and 65.9%, respectively (Islam and Ren, 2009). Chemical control is an essential component of whitefly management programs worldwide (Ellsworth and Martinez-Carrillo, 2001). However, the use of chemicals has been compromised principally because of rapid emergence of resistance to various classes of insecticides, especially organophosphates, pyrethroids, and cyclodienes (Cahill et al., 1995). As potential alternatives, certain chemicals derived either from plants or from microorganisms, termed biopesticides, have been promoted in recent years. These include the entomopathogenic fungus *B. bassiana* as well as the botanical insecticide neem.

*B. bassiana* is exploited in greenhouse and outdoor crops as a tool for the control of many agricultural pest arthropods, including whiteflies, aphids, thrips, psyllids, weevils, and mealybugs (Shah and Goettel, 1999). Among many entomopathogenic fungi, *B. bassiana* is potentially the most useful in whitefly control. Neem, *Azadirachta indica* A. Juss (Meliaceae) is one of the general-purpose botanical pesticides used in organic agriculture. It can have many effects on susceptible insects, including repellency, moulting disruption, growth reduction, interference with development and oviposition,

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and high mortality, particularly in immature insects, as documented for a wide group of phytophagous insects including sweetpotato whitefly (Mitchell et al., 2004). It is widely used around the world today either as a stand-alone treatment (Kumar and Poehling, 2006; Kumar et al., 2005) or in conjunction with synthetic pesticides or entomopathogens (Islam et al., 2010a, 2010b; Mohan et al., 2007; Depieri et al., 2005).

In Integrated Pest Management (IPM), biological control with entomopathogens represents a potentially important reduction factor of pest population density. Entomopathogenic fungi are ideal for IPM programs because they are relatively safe to use and have a narrower spectrum of activity than chemical insecticides (Lacey and Goettel, 1995). Microbial organisms such as *B. bassiana* are sustainable in IPM programs through their dynamic relationship with insects. Modern-day crop protection is based on IPM, which includes all the available techniques in a compatible manner along with judicious use of different agrochemicals. Therefore, for successful establishment of *B. bassiana* and to reduce insecticidal load in IPM program, the role of this fungus is very important. Keeping this view, the present study was conducted to investigate the combined efficacy of *B. bassiana* with neem through combination of two application methods (soil and foliar) against *B. tabaci* on eggplant.

## MATERIALS AND METHODS

### Experimental condition

The experiment was conducted in the Laboratory of Insect Microbiology, Department of Entomology, South China Agricultural University, Guangzhou, P.R China. The present study was carried out under laboratory conditions at 25 ± 1°C temperature, 70 ± 10% relative humidity (r.h.) and 12 h light: 12 h dark photoperiod.

### Insect

*B. tabaci* (Gennadius) (Hemiptera: Aleyrodidae) was originally collected from hibiscus, *Hibiscus rosa-sinensis* L. (Malvaceae) in Guangzhou, PR China. It was identified as the B biotype based on Random Amplified Polymorphic DNA/Polymerase Chain Reaction (RAPD-PCR) (De Barro and Driver, 1997) and the mitochondrial cytochrome oxidase I (mt COI) gene (Qiu et al., 2007). The whitefly population was maintained on eggplant for at least two successive generations before being used in experiments.

### Host plant

Seeds of eggplant, *Solanum melongena* L., var. 'Baiyu' (Solanaceae), were obtained from Guangdong Agricultural Institute in Guangzhou. The variety was chosen due to its susceptibility to *B. tabaci* (Islam et al., 2010c). The plants were grown individually in 15 cm-diameter plastic pots containing 1 kg autoclaved fertile soil and used in the experiment at the 4-5 leaf stage. These pots were placed into cages (60 × 60 × 60 cm). The plants were fertilized with mixed fertilizer (N:P:K = 13:7:15; Shenzhen Batian Ecotypic Engineering, Shenzhen, China) at the rate of 1 g per pot.

### Biocontrol agent

*B. bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) (isolate Bb62) was obtained from the Institute of Guangdong Forestry, Guangzhou, PR China which was originally isolated from pine moth, *Dendrolimus punctatus* (Walker) (Lepidoptera: Lasiocampidae). The isolate was chosen due to its activity against *B. tabaci* nymphs (Olleka et al., 2009). The fungus was cultured on PDA medium. Conidia of *B. bassiana* were collected from 15-day-old cultures (maintained at 25 ± 1°C temperature, 70 ± 10% r.h., and 12 h light:12 h dark photoperiod) and were suspended in water with 0.02% Tween 80. The conidia were quantified using a hemo-cytometer and a light microscope. The required fungal suspension (conidia/ml) was adjusted with regards to conidial viability (Goettel and Inglish, 1997).

### Botanical insecticide

Azadirachtin EC (0.3%) was obtained from Yunnan Zhongke Bio-industry (Kunming, PR China). Three concentrations of 0.3% Azadirachtin EC were used: 0.25, 0.5 and 1.0%. To obtain these concentrations, 2.5, 5.0, and 10.0 ml of 0.3% Azadirachtin EC was diluted in 1 l of tap water. The suspensions were then shaken for 30 min on a mechanical shaker, and were shaken again before they were used in experiments.

### Bioassay protocol

When plants were 6 weeks old (4-5 true leaves), approximately 50 adult whiteflies (2 days old) were released onto each plant using an aspirator. After 48 h, which was sufficient time for the adult whiteflies to oviposit, the adult whiteflies were removed manually from the plants by using an aspirator (Islam et al., 2010c). The plants were then incubated for a 6 days to allow eggs to hatch and the drenching application with 50 ml of neem was used to give sufficient time for persistence. Thereafter, the plants were incubated for a further 6 days to reach the second nymphal instar. Afterwards, infested leaves were labeled and the foliage was sprayed with *B. bassiana* on both leaf surfaces until runoff. Tap water alone served as control. There were 6 plants for each treatment. Numbers of living and dead nymphs were recorded 7 days after applying spray treatments to the second instar. Mortality caused by *B. bassiana* was distinguished from that caused by neem based on their morphological structure. A mortality ratio (N/Bb; mortality caused by neem/mortality caused by *B. bassiana*) was calculated.

### Statistical analysis

The experiment was repeated three times with freshly prepared fungal and neem suspension. Abbott's formula was used to correct for control mortality (Abbott, 1925) before subjecting mortality data to analysis of variance (ANOVA). Data regarding mortality percentage and mortality ratio of *B. tabaci* were subjected to one-way ANOVA. The statistical analyses were performed using the PROC GLM procedure (SAS Institute, 2001). Means were separated using the Least Significant Difference (LSD) test at 5% level of significance.

## RESULTS AND DISCUSSION

Nymph mortality was significantly different among the treatments when neem was combined with $10^6$ ($F$ =

**Table 1.** Mean ±SE nymph mortality (%) of *Bemisia tabaci* on eggplant treated by combined treatment of neem (N) with $10^6$ conidia/ml of *Beauveria bassiana* (Bb) compared with control. n = the number of whitefly nymph.

| Treatment | n | Nymph mortality (%) |
|---|---|---|
| Control | 98 | 3.58±0.17[d] |
| N-0.25+Bb-$10^6$ | 105 | 52.25±2.81[c] |
| N-0.5+Bb-$10^6$ | 108 | 77.59±4.57[b] |
| N-1.0+Bb-$10^6$ | 101 | 86.87±2.47[a] |

Means with same letters are not significantly different (one-way ANOVA, LSD test, P < *0*.05).

**Table 2.** Mean ±SE nymph mortality (%) of *Bemisia tabaci* on eggplant treated by combined treatment of neem (N) with $10^7$ conidia/ml of *Beauveria bassiana* (Bb) compared with control.

| Treatment | n | Nymph mortality (%) |
|---|---|---|
| Control | 104 | 4.09±0.53[d] |
| N-0.25+Bb-$10^7$ | 101 | 57.84±3.92[c] |
| N-0.5+Bb-$10^7$ | 96 | 79.31±2.84[b] |
| N-1.0+Bb-$10^7$ | 97 | 89.79±1.91[a] |

Means with same letters are not significantly different (one-way ANOVA, LSD test, P < *0*.05).

**Table 3.** Mean ±SE nymph mortality (%) of *Bemisia tabaci* on eggplant treated by combined treatment of neem (N) with $10^8$ conidia/ml of *Beauveria bassiana* (Bb) compared with control.

| Treatment | n | Nymph mortality (%) |
|---|---|---|
| Control | 99 | 4.65±0.15[d] |
| N-0.25+Bb-$10^8$ | 102 | 58.95±1.64[c] |
| N-0.5+Bb-$10^8$ | 94 | 83.84±2.38[b] |
| N-1.0+Bb-$10^8$ | 103 | 92.25±3.04[a] |

Means with same letters are not significantly different (one-way ANOVA, LSD test, P < 0.05)

159.20; df = 3, 23; P < 0.0001; Table 1); $10^7$ (*F* = 212.83; df = 3, 23; P < 0.0001; Table 2); and $10^8$ conidia/ml of *B. bassiana* (*F* = 353.55; df = 3, 23; P < 0.0001; Table 3) compared with their respective controls. The result demonstrates that maximum nymph mortality (92.3%) occurred when 1.0% neem was combined with $10^8$ conidia/ml of *B. bassiana* (Table 3). One-way ANOVA results of mortality ratio of nymph of *B. tabaci* also showed significant difference among the treatments when neem was combined with $10^6$ (*F* = 19.31; df = 2, 17; *P* < 0.0001; Figure 1A); $10^7$ (*F* = 23.34; df = 2, 17; P < 0.0001; Figure 1B); and $10^8$ conidia/ml of *B. bassiana* (*F* = 22.08; df = 2, 17; P < 0.0001; Figure 1C). The highest mortality ratio (14.3) occurred when 1.0% neem was combined with $10^6$ conidia/ml of *B. bassiana* (Figure 1A); while the lowest mortality ratio (5.7) investigated when 0.25% neem was combined with $10^8$ conidia/ml of *B. bassiana* (Figure 1C).

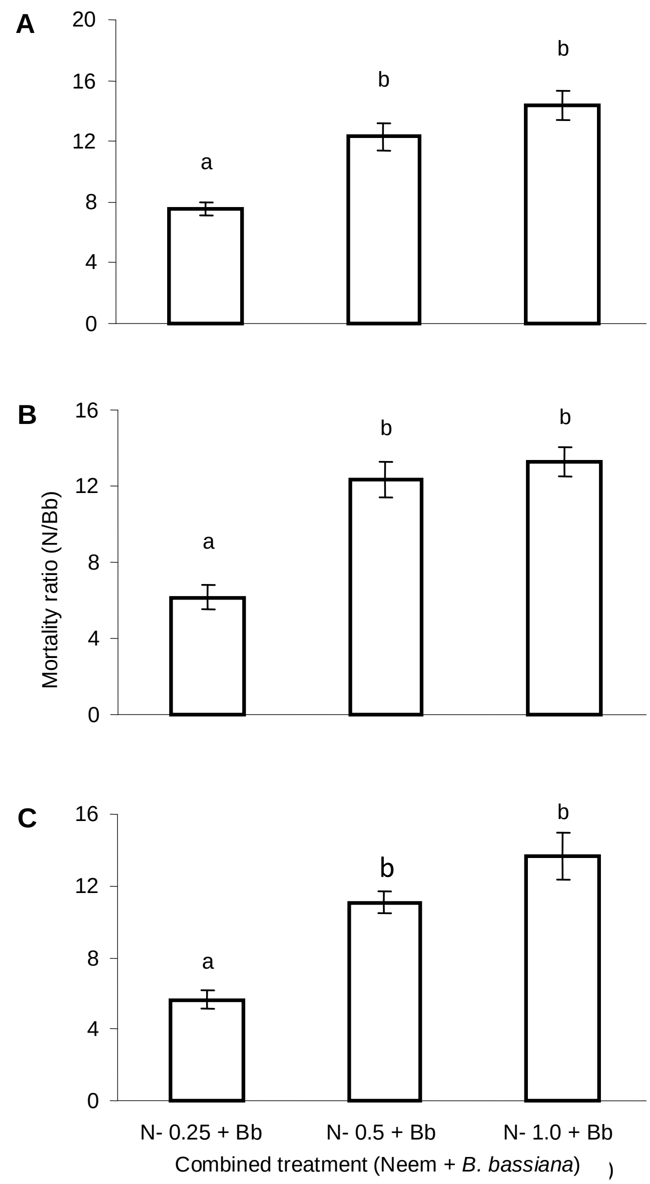


**Figure 1.** Mean ±SE mortality ratio (N/Bb) of *Bemisia tabaci* on eggplant treated by combined treatment of neem (N) with (A) $10^6$, (B) $10^7$ and (C) $10^8$ conidia/ml of *Beauveria bassiana* (Bb). Means with same letters under same fungal concentration are not significantly different (one-way ANOVA, LSD test, P < 0.05).

The foliar application of neem with *B. bassiana* yielded the highest nymph mortality (97.2%) 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). Systemic translocation of neem after treatment of plant roots greatly affects sucking insects (Kumar and Poehling, 2006; Kumar et al., 2005). Soil application of neem are efficiently absorbed through roots and transported via stems to the leaves, or absorbed by the leaves and distributed translaminar fashion (Islam et al., 2010b; Hossain et al., 2008; Sato et al., 2007; Thoeming et al., 2006). At the same time, the foliar application of *B. bassiana* increased the toxicity to *B. tabaci* nymphs on eggplant (Islam et al., 2010b). The mortality data indicates that the combined treatment significantly reduced the number of whitefly nymph on eggplants. The different effects of foliar vs. soil application may be related to the presence of different amounts of neem residues in or on the leaves.

The optimum combined use of fungi and chemicals for pest control may require sequential rather than simultaneous applications. The information regarding combined efficacy by two application approaches was investigated by Islam et al. (2010b) in relation to deterrence index of *B. tabaci*. They investigated that the highest adult (80.2) and oviposition (88.3) deterrence index of *B. tabaci* occurred when *B. bassiana* ($10^8$ conidia/ml) was applied topically and neem (1.0%) was drenched to the eggplants. A similar phenomenon was reported by Al-mazra'awi et al. (2009) that the topical of *B. bassiana* and drenching application of neem tree extract, the treated *Thrips tabaci* exhibited mortalities higher than using each control agent alone and the two control agents interacted synergistically at sub-lethal doses of the neem tree extract. The application method affected the interaction between *B. bassiana* and the neem tree extract because drenching neem tree extract while applying *B. bassiana* topically enhanced the efficacy of the entomopathogen (Al-mazra'awi et al., 2009).

A combined formulation or application of fungal candidates with chemical pesticides may enhance efficacies of both products and has proven effective for the control of sucking insects (Feng et al., 2004). The combination of *B. bassiana* with neem resulted in 27.6 and 20.5% more nymphal mortality of *B. tabaci* for 7 days post application than individual treatments of *B. bassiana* and neem, respectively, when both the products were applied to eggplant foliage (Islam et al., 2010a). A similar phenomenon was reported by Purwar and Sachan (2006) that the combination of *B. bassiana* with endosulfan was 4.9 times more toxic and resulted in higher mortality against *Spilarctia obliqua* than endosulfan alone. Up to 90% nymphal mortality of *B. argentifolii* was obtained when *Paecilomyces fumosoroseus* (Wize) Brown & Smith and azadirachtin were combined (James, 2003). Neemix 4.5 (4.5% azadirachtin) delayed pupation and did not reduce the germination rate of *B. bassiana* conidia, but it significantly reduced the mortality of *Tribolium castaneum* Herbst (Akbar et al., 2005).

The sweetpotato whitefly is difficult to control with the individual treatment of either neem or *B. bassiana* (Islam et al., 2010a); but the combined of these two factors under different application approaches has proven successful to control this insect in both the present and previous (Islam et al., 2010a; Islam et al., 2010b) studies. In this experiment, mortality ratio data also indicate that the products are compatible each other. Therefore, we can conclude that the soil application of neem along with foliar application of *B. bassiana* may contribute to the successful control of *B. tabaci* concerning environmental friendly strategy.

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