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Full Length Research Paper

Mycoinsecticide effects of *Beauveria bassiana, Metarhizium anisopliae,* and *Isaria fumosorosea* on the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) in different strata of bean

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***Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* are entomopathogenic fungi used as biological control agents for whitefly. They have been individually evaluated under laboratory, greenhouse and field conditions; however, there is little information on their comparative efficacy under greenhouse conditions particularly with respect to the height of the canopy in which they act. Mortality of eggs, first, second and third instar nymphs, pupae and adults were monitored on leaves located at three different heights in the canopy. The *Bemisia tabaci* population was homogeneously distributed in the tree strata of the plant and there was not any significant difference in mortality of the individuals for all life stages between the different strata. *M. anisopliae* was significant more effective against eggs, first, second and third instar nymphs and pupae, even under the adverse weather conditions in which the experiment was conducted; however, *B. bassiana* caused the greater mortality of adults. Our results indicate that mixed applications of *M. anisopliae* and *B. bassiana* could maximize the likelihood for control of all stages of *B. tabaci*.**

Key words: Entomopathogenic fungi, whitefly stages, mortality, vegetable stratum.

INTRODUCTION

The entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae* and *Isaria fumosorosea* (*Paecilomyces fumosoroseus* designated as *Isaria* clade, Luangsa-Ard et al., 2005) have been used as mycoinsecticides providing biological alternatives to chemical insecticides. Among their advantages are their reproductive potential and their persistence in the environment and they can play an important role in promoting integrated pest management (Castrillo et al., 2004; Faria and Wraight, 2001).

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Bemisia tabaci (Homoptera: Aleyrodidae) causes great damage due to its polyphagous feeding habits on over 500 plant species in 74 families (Cano et al., 2001; Chang et al., 2001; Gelman et al., 2001). *B. tabaci* is usually controlled with high concentrations of synthetic chemical insecticides, leading to the development of resistance to some of these products (Garcia et al., 1999; Cano et al., 2001).

Aggregated distributions of stages of *B. tabaci* differ between different strata of their host plants (Leite et al., 1998; Germano et al., 2005). Adult whiteflies stages are typically located in the upper parts of the plant, while nymphs are mainly concentrated in the lower stratum

while eggs are located in the middle stratum (Arnó et al., 2006). However Muñiz et al. (2002) found that the middle stratum had the greatest number of *B. tabaci* adults while Naranjo and Flint (1995), found adults were most abundant on younger leaves near the top of the plant.

The present study evaluates the efficacy of the entomopathogenic fungi *B. bassiana, M. anisopliae* and *I. fumosorosea* with respect to the egg, nymph, pupa and adult stages of the whitefly *B. tabaci* in different plant strata. There have been few head-to-head comparisons of these three common insect pathogens under greenhouse conditions, especially under very warm greenhouse conditions.

MATERIALS AND METHODS

Fungal entomopathogens

Commercial products formulated as clay based wettable powers containing 5×10^9conidia per gram of *B. bassiana* (Bea Sin), *M. anisopliae* (Meta Sin) or *I. fumosorosea* (Pae Sin) were used. They are produced by Agrobiologicos del Noroeste S.A. from Culiacan Sinaloa, Mexico and were deposited in CINVESTAV (Centro de Investigaciones y Estudios Avanzados del Instituto Politécnico Nacional, México).

Plants infestation

Bean seeds (*Phaseolus vulgaris* L.cv. Flor de Mayo) were grown in 7-inch-diameter pots and placed in the greenhouse until needed. When they had two leaves they were placed in an isolated area (2.50 × 0.70 × 0.70 m) with a fine mesh material and bean leaves that were principally infested with pupae and adults previously identified as *B. tabaci* (Martin et al., 2000) were placed at each of the four cardinal points. A new infestation was established 15 days later to ensure the presence of at least 10 pupae per plant.

Experimental design and procedures

It was used a completely randomized design in a greenhouse, where in four treatments were evaluated (*B. bassiana, M. anisopliae, I. fumosorosea* and a water control) with four replicates. Each replicate was located inside a 70 × 70 × 70 cm cage built with ¼ inch PVC tubing and covered with a fine mesh material (266 × 818 microns) so as to prevent insects from mixing between experimental units. The mycoinsecticide (5×10^9 conidias ha^{-1}) application was conducted outside of the greenhouse 63 days after seeding. The application was made using a 20 L portable sprayer (75 psi) by introducing the conic nozzle inside the PVC structure and spraying to the underside of the leaf to the point of runoff. Conidial concentrations for the sprayed suspension were confirmed using standard (improved Neubauer) hemacytometers. Viability of conidia was assessed by plating onto agar and after 24 h at 26±0.5°C germination (%) was determined. Conidia were considered viable when they formed a germ tube. When necessary the solution was adjusted to achieve the desired concentration.

One day prior to the initial application of treatments and ninety-six hours after spraying, the population density was monitored in each experimental unit. Counts were made early in the morning before adults became active. The monitored plant was divided into three strata, the top, middle, and bottom thirds of all plants. A leaf was randomly taken from each stratum and a circular area of 1 cm^2 was marked with a cork borer close to the midvein. Using a stereo microscope, we recorded the number of live and dead eggs, nymphs pupae and adults (Byrne and Draeger, 1989). The greenhouse conditions were monitored using detectors (Hobo S-THB-M003 for temperature and humidity and Hobo PAR S-LIA-M003 for solar radiation) that were connected to a Hobo H21-001climatologic station.

Statistical analysis

A statistical analysis was conducted for each stratum and between different strata. Data were assessed for normality and homoscedasticity prior to analysis. An analysis of covariance was used followed by a comparison using the Tukey test (SAS, 2001). In some cases Kruskal-Wallis non-parametric analysis of variance was used when data violated these assumptions and could not be corrected using a transformation. Mortality was determined based on the total population (live and dead individuals).

RESULTS

Environmental conditions

The average temperature during the experiment was 22.53°C; the highest temperature was 49.0°C and the minimum temperature was 8.2°C (Table 1, Figure 1). The relative average humidity was 74.1%; with maximum humidity observed at sunset (after 1800 h) and sunrise (0600-0800). The minimum humidity occurred close to 1400, when the maximum average solar radiation (830.6 Wm^{-2}) also occurred.

Lower vegetal stratum

In this stratum (Table 2), was observed significantly more dead individuals of all stages for all three treatments compared to the control. *M. anisopliae* produced significantly greater mortality of all three nymphal stages (11.5 ± 0.51; 12.7±0.50; 8.5±0.38) and pupae (14.2±0.61) than the other treatments. *B. bassiana* and *M. anisopliae* showed significantly greater egg mortality. *B. bassiana* caused significantly greater mortality of adults (55.6% mortality) compared to the other treatments.

Middle vegetal stratum

In this stratum (Table 3) *M. anisopliae* showed the best efficacy (P≤0.01) against nymphal (28.7±1.1; 9±0.3; 10±0.4) and pupal (15.5±0.9) stages compared to the other treatments. There were no significant differences in numbers of dead nymphs for *B.* bassiana and *I. fumosorosea. M. anisopliae* was also as effective as *I. fumosorosea* (P≤0.01) against eggs. As in the lower stratum, *B. bassiana* was most effective in controlling adults (46.4% mortality).

Table 1. Average values of climatic conditions.

Variable	Average	Standard error
Mean solar radiation	723.6Wm^{-2}	±17.8
Mean temperature	22.53°C	±0 .34
Maximum temperature	47.25°C	±0.43
Minimum temperature	8.87°C	±0.25
Mean humidity	59.64%	±1.58
Maximum humidity	90.13%	±1.48
Minimum humidity	28.73%	±1.58

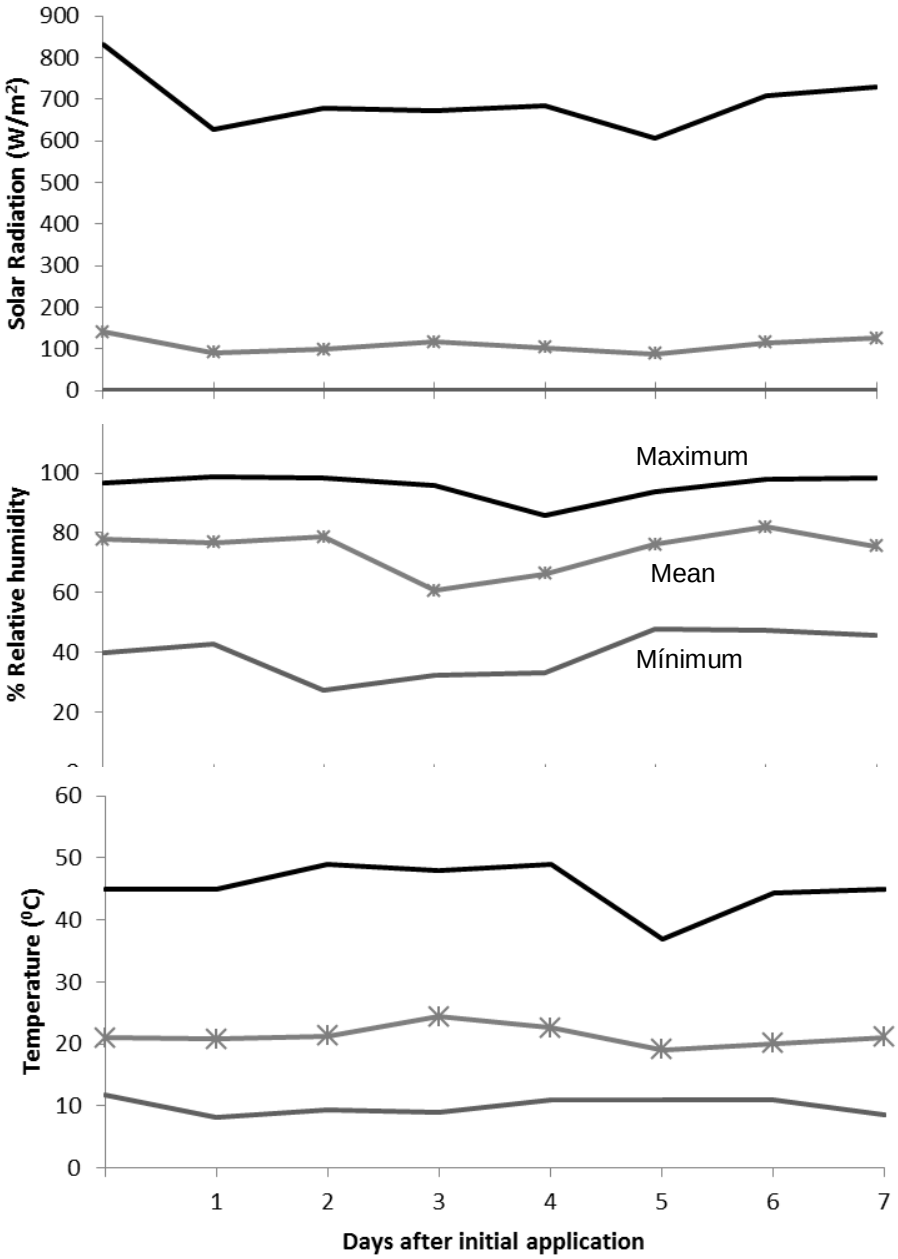

Figure 1. Climatic conditions registered daily after the initial application of the treatments (data are average daily).

Table 2. Dead individuals for different whitefly growth stages caused by *B. bassiana, M. anisopliae* and *I. fumosorosea* in the lower stratum.

Treatment	Number of dead individuals of whitefly (*B. tabaci*) cm^2					
	Egg	Nymph 1	Nymph 2	Nymph 3	Pupa	Adult
Control	0^c	0.2^c	0^c	0^c	0^c	0^c
B. bassiana	10.5^a	6.7^b	6^b	4.25^b	6^b	5.7^a
M. anisopliae	17.2^a	11.5^a	12.7^a	8.5^a	14.2^a	2.5^b
I. fumosorosea	3.7^b	5.7^b	4.2^b	3^b	8.7^b	2^b

Within a column, different letters indicate significantly different means according to Tukey´s test (P ≤ 0.01).

Table 3. Dead individuals for different whitefly growth stages caused by *B. bassiana, M. anisopliae* and *I. fumosorosea* in the middle stratum.

Treatment	Number of dead individuals of whitefly (*B. tabaci*) cm^2					
	Egg	Nymph 1	Nymph 2	Nymph 3	Pupa	Adult
Control	0^b	0^d	0^c	0^c	0^d	0^c
B. bassiana	7^b	4.2^b	5.5^b	5.5^b	5.7^c	6.7^a
M. anisopliae	21.5^a	28.7^a	9^a	10^a	15.5^a	2.3^b
I. fumosorosea	13.2^a	3^b	5.5^b	3^b	8.5^b	2^b

Within a column, different letters indicate significantly different means according to Tukey´s test (P≤0.01).

Table 4. Dead individuals for different whitefly growth stages caused by *B. bassiana, M. anisopliae* and *I. fumosorosea* in the upper stratum.

Treatment	Number of dead individuals of whitefly (*B. tabaci*) cm^2					
	Egg	Nymph 1	Nymph 2	Nymph 3	Pupa	Adult
Control	0.2^c	0^c	0^c	0^c	0^c	0^b
B. bassiana	19^{ab}	5.7^b	3.7^b	7^{ab}	4.5^b	9.5^a
M. anisopliae	29^a	11.5^a	13^a	9.2^a	12.5^a	4.5^b
I. fumosorosea	13^b	6.5^b	4.5^b	3.5^{ab}	4.5^b	3^b

Within a column, different letters indicate significantly different means according to Tukey´s test (P≤0.01).

Upper vegetal stratum

The *M. anisopliae* mycoinsecticide showed the greatest overall efficacy. However its activity was superior only against the nymph 1 (11.5±.51), nymph 2 (13±0.46) and pupal (12.5±0.47) stages. Against adults, *B. bassiana* was again the best mycoinsecticide (Table 4), causing 51.3% mortality. As in the middle stratum, there were no significant differences among numbers of dead nymphs with *B. bassiana* and *I. fumosorosea.*

All stratums and stages

The total dead individuals of all stages varied significantly between treatments (F=58.7, df = 3, P<0.0001). However there was no significant difference in total mycoinsecticide activity between *B. bassiana* and *I. fumosorosea*. Application of any of the three fungi resulted in significant increases in numbers of dead individuals of all stages relative to controls in all three strata. *M. anisopliae* resulted in the highest number of dead individuals (233.3±5.2) for all stages (P ≤ 0.01) in all three strata (Figure 2) and maintained this superiority in the total mortality effect (43.9%).

DISCUSSION

During the assessments, it was frequently observed that the temperatures, which should have suppressed mycoinsecticide activity, were above 45°C (Figure 1).

Temperatures higher than 32-35°C inhibit most *B. bassiana* and *I. fumosorosea* isolates (Fargues and Luz, 2000; Devi et al., 2005). The average humidity was 59.6%, with a minimum humidity that was 20% lower than the level reported as being inadequate to promote *B. bassiana* infection (He et al., 2005; Vassilakos et al.,

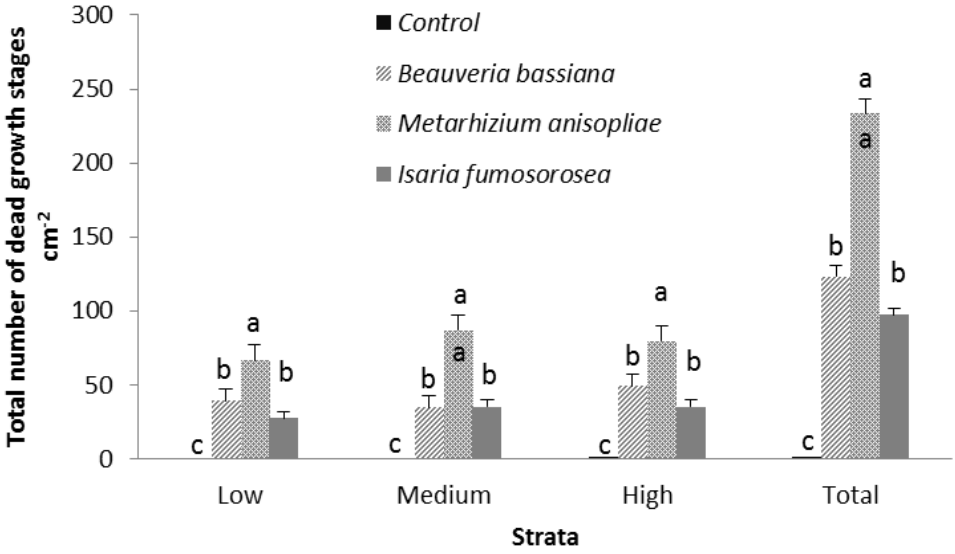

Figure 2. Efficacy of *B. bassiana, M. anisopliae* and *I. fumosorosea* against *B. tabaci* on each stratum and total strata. Within a stratum, different letters indicate significance differences according to Tukey´s test (P≤0.01).

2006).

Temperatures between 23 and 26°C and humidity >85% have been reported as optimal conditions to promote *B. bassiana* infection (He et al., 2005; Vassilakos et al., 2006); however, it has also been reported that *B. bassiana* efficacy enhanced at low humidity (43%) and temperatures ranging between 26 and 34°C (Lord, 2005). In another study, infection by *B. bassiana* and *M. anisopliae* increased as temperature moved from 20 to 35°C (Dimbi et al., 2004; Bugeme et al., 2008). The thermal death point (0% germination) for some isolates of *B. bassiana* has been reported to be 46°C for 6 h (Fernandes et al., 2008). In the present experiment, the highest temperatures peaked above 45°C, but they were only observed near noon and for a short period of time and it was not apparently a source that affected the results in the experiment.

All fungal treatments caused higher rates of mortality (averaged=38.3%) than the control. The generally low mortality observed could be attributed to high temperatures and low humidity conditions during the trials (Figure 2). However humidity inside cages may have been much higher due to limited air circulation. Fargues et al. (2003) and Wraight et al. (2000) suggested that leaf transpiration could increase humidity micro conditions, which may increase mycoinsecticide action; nevertheless these variables were not monitored.

M. anisopliae and *I. fumosorosea* were equally effective against whitefly adults. Wraight et al. (2000) reported that *B. bassiana* minimally controlled *Bemisia argentifolii* adults, while in the present research *B. bassiana* caused 51.5% mortality of *B. tabaci* adults, significantly greater than for *M. anisopliae* and *I. fumosorosea*. In the previous study, *B. bassiana* and *I. fumosorosea* caused substantial reduction in nymphal whitefly populations (Wraight et al., 2000). In the present work, these fungi were efficacious against *B. tabaci* 3rd instar nymphs; however, they caused less total mortality than *M. anisopliae* (43.5%). In accord with previous observations (Wraight et al. 1998) *M. anisopliae* and *I. fumosorosea* were equatoxic against nymphs of *B. tabaci*.

Whitefly stages are not typically located randomly within the plant canopy (Naranjo and Flint, 1995; Leite et al., 1998; Muñiz et al., 2002; Germano et al., 2005; Arnó et al., 2006). However in the study we did not observe significant differences (P≤0.05) in distribution for any of the six *B. tabaci* stages among the three strata. This could be related to homogeneity in microclimatic conditions considering that the bean is a compact plant. Taller plants could present different microclimatic conditions due to different heights.

Comparisons of the numbers of dead individuals for all life stages between the different strata did not show any clear trends. This suggests that bean leaf microclimatic conditions did not influence the activities of the three mycoinsecticides, as suggested by Fargues et al. (2003). The bean plants were exposed to high levels of solar radiation strongly associated with unfavorable conditions (low humidity and high temperature) for stomatic transpiration and the creation of a high humidity gradient in the area close to the stoma.

Osborne et al. (1990) reported that all stages of *B. argentifolli* are highly susceptible to *I. fumosorosea*, with the highest mortality rate (90%) occurring within 72 h of a

106 conidia mm^2 application. In contrast with these results, and considering the different species of whitefly investigated, in the present study, *I. fumosorosea* produced an average mortality of only 34.6%. Vidal et al. (1997) observed that *I. fumosorosea* caused 68 to 94% mortality for the nymph 2nd and 3rd nymphal stages of *B. argentifolli*. In the present study, *M. anisopliae* was superior to *B. bassiana* and *I. fumosorosea*, causing higher mortality in both stages.

The relative lower efficacy of the three mycoinsecticide in this study is most probably associated with the temperature and humidity; however the treatments were only applied one time in this trial. It has been reported that multiple applications can provided 90% control (Wraight et al., 2000).

Conclusion

The *B. tabaci* population in this study was homogeneously distributed in the three strata of the host plant canopy. The insecticidal actions of each fungus were studied individually, and they showed no differences in their entomopathogenic effects between the different bean plant strata. *M. anisopliae, B. bassiana* and *I. fumosorosea* showed significantly greater levels of control across all life stages of *B. tabaci* than the control treatment. The total number of dead individuals for all stages due to *M. anisopliae* treatment was significantly greater than the other treatments in all plant strata. *M. anisopliae* can thus be considered the best control agent among the mycoinsecticides evaluated under the conditions of the study. However, as *B. bassiana* showed significantly greater insecticide activity against the adult stage, it is the mycoinsecticide with the best potential to combat whitefly adults. Weather conditions and the limited number of applications apparently limited the mortality caused by the entomopathogenic fungi.

Our results indicates that the three fungi, *B. bassiana, M. anisopliae and I. fumosorosea*, have considerable potential to control all stages of the whitefly *B. tabaci* in cultivated beans in the greenhouse. However, a mixed application of *B. bassiana and M. anisopliae* could maximize the likelihood for control of *B. tabaci*.

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