

Microbial control of the invasive spiraling whitefly on cassava with entomopathogenic fungi

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Abstract

The entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii* and *Isaria fumosorosea* were tested for their efficacy in managing the exotic spiraling whitefly *Aleurodicus dispersus* (Hemiptera, Aleyrodidae) on cassava (*Manihot esculenta*) during 2 seasons (2011-2012 and 2012-2013). The fungi *I. fumosorosea* and *L. lecanii* exhibited promising levels of control (> 70% mortality of the *A. dispersus* population). The percent mortality increased over time in both seasons. Application of *I. fumosorosea* was highly pathogenic to *A. dispersus* in both seasons compared to the other entomopathogenic fungi. Analysis of the percent mortality in both seasons revealed differences in efficacy between 3 and 15 days after treatment. The season also influenced the effects of the fungi on the *A. dispersus* population. Thus, entomopathogenic fungi have the potential to manage *A. dispersus* infestation of cassava.

Key words: *Aleurodicus dispersus*, *Manihot esculenta*, biocontrol, entomopathogenic fungi, mortality.

Introduction

Cassava (*M. esculenta* Cranrz.) is the most important starchy root crop grown in the tropics (Sánchez *et al.*, 2009) and the main crop cultivated in the Southern Peninsular regions of India. Among the various insect pests of cassava, the exotic spiraling whitefly *A. dispersus* Russell (Hemiptera, Aleyrodidae) can cause losses that reach 53% (Geetha, 2000). *A. dispersus* is a polyphagous pest with an extensive host range covering 481 plant species belonging to 295 genera from 90 families of vegetables, fruits and ornamental trees (Srinivasa, 2000; Boopathi and Karuppuchamy, 2013; Boopathi *et al.*, 2012; Boopathi *et al.*, 2014c). Infestation causes premature leaf drop. Moreover, the production of copious amounts of honeydew serves as a substrate for sooty mold growth (Akinlosotu *et al.*, 1993; Boopathi *et al.*, 2013), which in turn reduces the photosynthetic activity and plant vigor (Kumashiro *et al.*,

1983; John *et al.*, 2007; Boopathi *et al.*, 2014a; Boopathi *et al.*, 2014b). The insect's natural enemies, especially the parasitoids *Encarsia guadeloupae* Viggiani and *Encarsia* sp. nr. *meritoria* Gahan, (Geetha, 2000) and predators, *Mallada astur* (Banks) and *Cybocephalus* spp., (Mani and Krishnamoorthy, 1999) have proven to be highly useful in suppressing the spiraling whitefly.

The entomopathogenic fungi have shown good epizootic potential against whiteflies, *i.e.*, *Bemisia* spp. and *Trialeurodes* spp., in both field environments and under greenhouse conditions (Fang *et al.*, 1983; Osborne and Landa, 1992; Carruthers *et al.*, 1993; Lacey *et al.*, 1996). *I. fumosorosea* (Wize) caused the highest mortality to nymphs of the silver leaf whitefly (*Bemisia argentifolii* Bellows and Perring) under laboratory conditions (Wraight *et al.*, 1998, 2000) and 100% mortality to *A. dispersus* nymphs 15 days after treatment (DAT) under laboratory conditions (Boopathi *et al.*, 2013). *L. lecanii* (Zimmerm.)

Zare and Gams produced 80-90% mortality in *A. dispersus* 15 days after application under *in vivo* conditions (Aiswariya *et al.*, 2007) and 97.8% mortality to *A. dispersus* nymphs 15 DAT under laboratory conditions (Boopathi *et al.*, 2013). The entomopathogenic fungus *B. bassiana* (Balsamo) Vuillemin produced higher mortality to the first instars and adults of the silver leaf whitefly (Nagasi *et al.*, 1998) and 52-98% mortality to *Bemisia* at concentrations of $1-4 \times 10^6$ conidia mL⁻¹ (Eyal *et al.*, 1992).

In India, substantial scientific research into crop pest management using entomopathogenic fungi against agricultural and horticultural insect pests began in the early 1990s. Many of the economically important vegetable insect pest species from Hemiptera, Lepidoptera, Coleoptera and Isoptera have been shown to be susceptible to various entomopathogenic fungal isolates (Geetha, 2000; Aiswariya *et al.*, 2007; Boopathi *et al.*, 2013; Boopathi *et al.*, 2015), including *A. dispersus*. The present study investigated the usefulness of the entomopathogenic fungi *B. bassiana*, *M. anisopliae* (Metschnikoff) Sorokin, *L. lecanii* and *I. fumosorosea* as effective biocontrol agents against the most destructive pest of cassava, *A. dispersus*.

Materials and Methods

Fungal isolates

Strains of the entomopathogenic fungi and their sources are listed in Table 1. Isolates were maintained on potato dextrose agar (PDA) in test tubes and stored at 4 °C. Continuous cultures were maintained on slants; sub-cultures were grown for 14 days at 25 °C and then stored at 4 °C.

Production and enumeration of spores

Spore suspensions of each fungal isolate were prepared in 0.5% aqueous Tween[®] 80 and homogenized with a vortex mixer for two minutes (Lacey, 1997). Then, the spores were counted using a hemocytometer. The conidial suspension was further diluted with 0.5% aqueous Tween[®] 80 solution in test tubes to obtain concentrations of 2×10^9 conidia.mL⁻¹.

Spore production

Air-dried conidia were produced as follows. Maize (150 g) suspended in 60 mL of sterile water was autoclaved in polythene bags (10 x 25 cm) at 121 °C and a pressure of 1.05 kg cm⁻³ for 15 min. and cooled to room temperature for 24 h. One milliliter of the 2×10^9 conidia mL⁻¹ suspension was introduced to each polythene bag and incubated for 2 weeks at 26 ± 3 °C. The two-week-old cultures were harvested, and the air-dried conidia/spores were filtered through a sieve with a particle size of 125 µm.

Spore harvesting and drying

The cultures were allowed to air dry overnight in a room with a temperature of 25 ± 5 °C and a relative humidity of $50 \pm 5\%$. Air-dried conidia remained viable and active for up to 8 months without any loss in efficacy for the management of insect pests.

Formulation and application equipment

A wettable powder formulation was prepared by mixing air-dried conidia with commercial diluent clay (Kaolin, Ashapura Group of Industries, Chennai, Tamil Nadu, India) at a ratio of 1:4 (20% w/w a.i., active ingredient) in a sterile room. All treatments were sprayed using a single-nozzle atomizing (air-assist) sprayer (pneumatic knapsack sprayer). The spray nozzle was carried near ground level for each spray and directed at a right angle to the row. Each row was treated twice, once on each side of the row. The spray volume was 500-700 Lha⁻¹. Spraying was performed in the late evening to reduce the possible oppressive effect of solar radiation on conidial/spore germination.

Field evaluation

Field experiments were conducted in cassava for two seasons at Pollachi, Coimbatore, Tamil Nadu, India, in 2011-2012 (Season 1) and 2012-2013 (Season 2). Rooted sets of cassava (cv. CO-2) were planted in 10 x 10 m plots at a spacing of 90 x 90 cm. Treatments were applied to 5 replicates arranged in a randomized complete block design (RCBD). Weeding, application of manures and fertilizers, and other cultural operations were performed as per crop production guidelines (TNAU, 2012). Furrow irrigation

Table 1 - Details of entomopathogenic fungal isolates.

Entomopathogenic fungi	Fungal strains	Host insects	Sources
<i>B. bassiana</i>	B ₂	<i>Helicoverpa armigera</i>	Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India
<i>M. anisopliae</i>	M ₂	<i>Bemisia tabaci</i>	Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India
<i>L. lecanii</i>	L ₁	<i>Bemisia tabaci</i>	Sun Agro Biotech Research Centre, Madanantapuram, Porur, Chennai, Tamil Nadu, India
<i>I. fumosorosea</i>	P ₁	<i>Bemisia tabaci</i>	Sun Agro Biotech Research Centre, Madanantapuram, Porur, Chennai, Tamil Nadu, India

(approximately 700-800 Lplot⁻¹) was applied every 2-3 weeks in the absence of rain. The respective wettable powder formulated entomopathogenic fungi were suspended in 1.0% Teepol[®] excluding the control. Two applications of fungi were applied 15 days apart due to heavy infestation (> 200 *A. dispersus* per leaf) of *A. dispersus* (more than the economic threshold level, ETL) at the rate of 2 x 10⁹ conidiamL⁻¹. The first application was applied during the vegetative/reproductive stage of the cassava. The second application was applied to new leaves and shoots that had high populations of newly emerged nymphs and adults of *A. dispersus*. Both applications were applied to the same plants. Pre-treatment observations of the *A. dispersus* population (no. of nymphs and adults of *A. dispersus* per leaf) were taken 24 h. before each application of fungi, and post-treatment observations were taken at 3, 7, 10 and 15 days after each treatment (DAT). Observations of the *A. dispersus* populations (no. of nymphs and adults of *A. dispersus* per leaf) were recorded on leaves from the top, middle and bottom of 5 tagged plants per plot after the first and second applications.

Data analysis

Statistical analysis of the data was performed using the methods of Gomez and Gomez (1984) with SAS Software Version 9.3 (SAS Institute Inc., 2011). The data were analyzed using four-way ANOVA. All ANOVA analyses were performed on original values, and the means were separated using the least significant difference (LSD) test at $p < 0.05$ or $p < 0.01$. The percent mortality of *A. dispersus* populations was determined and corrected using the control method of Henderson and Tilton (1955) as follows:

$$\text{Correct percent reduction} = 1 - \left[\frac{T_a - C_b}{T_b - C_a} \right] \times 100$$

where T_a = number of insects in the treatment after spraying, T_b = number of insects in the treatment before spraying, C_b = number of insects in the untreated control before spraying and C_a = number of insects in the untreated control after spraying.

Results and Discussion

All entomopathogenic fungal treatments caused medium to high mortality of *A. dispersus*. Individual *A. dispersus* killed by these entomopathogenic fungi dried rapidly on the cassava leaves, and the cadavers remained attached to the cassava leaves. *A. dispersus* individuals infected by the white muscardine fungus *B. bassiana* were red to red-brown in color. Mycelia and sporulation of entomopathogenic fungi on the cadaver occurred during extended periods of rainfall, higher relative humidity over many nights, or late in the experimental trials. *M. anisopliae* produced green conidia/spores from tightly packed and parallel-oriented conidiogenous cells. The

amounts of *I. fumosorosea* hyphal growth and sporulation were obviously greater 3-5 days after spraying than the other entomopathogenic fungi; however, the postmortem development of the entomopathogenic fungi was not monitored and quantified (Figure 1).

All entomopathogenic fungi caused substantial reductions in *A. dispersus* populations on cassava following both applications in both seasons. The percent mortality of all entomopathogenic fungi increased over time in both applications and seasons, whereas the highest *A. dispersus* population (> 180 per leaf) was observed in the control populations in both seasons 1 and 2. There were differences between the effects of fungi ($F = 8284.393$; $p < 0.0000$), applications ($F = 706.327$; $p < 0.0000$), observation dates ($F = 1489.155$; $p < 0.0000$), seasons ($F = 40.523$; $p < 0.0000$) and the interactions on the mortality of *A. dispersus*: fungi x applications ($F = 47.459$; $p < 0.0000$), fungi x observation dates ($F = 93.773$; $p < 0.0000$), fungi x seasons ($F = 8.416$; $p < 0.0000$), applications x observation dates ($F = 19.655$; $p < 0.0000$), applications x seasons ($F = 15.256$; $p < 0.0000$), observation dates x seasons ($F = 11.249$; $p < 0.0000$), fungi x applications x observation dates ($F = 1.998$; $p < 0.0240$), fungi x observation dates x seasons ($F = 2.287$; $p < 0.0085$), applications x observation dates x seasons ($F = 12.252$; $p < 0.0000$), and fungi x applications x observation dates x seasons ($F = 3.056$; $p < 0.0004$) (Table 2). However, there was not a significant difference between the fungi x applications x seasons ($F = 2.329$; $p < 0.0560$) interaction on the mortality of *A. dispersus*.

The percent mortality resulting from both applications in season 1 demonstrated differences in efficacy between pathogens (Table 3). Higher mortality occurred with *I. fumosorosea* in both application 1 (59.04%, 100.35 *A. dispersus* per leaf) and application 2 (68.34%, 22.55 *A. dispersus* per leaf) in season 1 than that of the other fungi, whereas in the control treatment, the highest *A. dispersus* populations, 228.30 per leaf and 237.30 per leaf, were observed in season 1 following application 1 and application 2, respectively. Similarly, in season 2 *I. fumosorosea* produced higher mortality following both application 1 (60.70%, 80.66 *A. dispersus* per leaf) and application 2 (70.63%, 15.55 *A. dispersus* per leaf) than that of the other fungi. However, the highest *A. dispersus* population in the control treatment was registered for both application 1 (187.34 per leaf) and application 2 (208.14 per leaf) in season 2. Therefore, the season influenced the effect of the fungi on the reduction of *A. dispersus*. Furthermore, a higher rate of mortality was observed in cassava in season 2 than in season 1.

The differences in the mortality of *A. dispersus* induced by *B. bassiana* on cassava indicated that there were differences in efficacy between days 3-15 post-treatment (Figure 2a, 2b). *B. bassiana* produced the highest mortality after application 2 in both seasons. The percent mortality of



Figure 1 - Cadavers of *A. dispersus* infected with entomopathogenic fungi.

Table 2 - Analysis of variance (ANOVA) of percent corrected mortality of *A. dispersus* on cassava.

Source	% corrected mortality of <i>A. dispersus</i>			
	F value	SEd	CD (p = 0.01)	Probability
Fungi (F)	8284.393	0.4065	1.0534	0.0000**
Application (A)	706.327	0.2571	0.6663	0.0000**
Day after treatment (D)	1489.155	0.3636	0.9422	0.0000**
Season (S)	40.523	0.2571	0.6663	0.0000**
Interaction				
F x A	47.459	0.5749	1.4898	0.0000**
F x D	93.773	0.8130	2.1069	0.0000**
F x S	8.416	0.5749	1.4898	0.0000**
A x D	19.655	0.5142	1.3325	0.0000**
A x S	15.256	0.3636	0.9422	0.0001**
D x S	11.249	0.5142	1.3325	0.0000**
F x A x D	1.998	1.1497	2.9796	0.0240*
F x A x S	2.329	0.8130	2.1069	0.0560ns
F x D x S	2.287	1.1497	2.9796	0.0085**
A x D x S	12.252	0.7272	1.8845	0.0000**
F x A x D x S	3.056	1.6260	4.2138	0.0004**

ns, *, ** non-significant or significant at $p \leq 0.05$ or $p \leq 0.01$, ANOVA.

Table 3 - Percent corrected mortality of *A. dispersus* following both applications on cassava in season 1 and season 2.

Season	x	Application	x	Fungi	% corrected mortality of <i>A. dispersus</i>	<i>A. dispersus</i> population per leaf	
1	1	1		Control	0.00e	228.30e	
				<i>B. bassiana</i>	51.14c	121.08c	
				<i>M. anisopliae</i>	45.36d	130.40d	
				<i>L. lecanii</i>	54.43b	111.98b	
				<i>I. fumosorosea</i>	59.04a	100.35a	
	2	2			Control	0.00e	237.30e
					<i>B. bassiana</i>	58.59c	36.58c
					<i>M. anisopliae</i>	50.01d	50.73d
					<i>L. lecanii</i>	62.17b	29.81b
					<i>I. fumosorosea</i>	68.34a	22.55a
2	1	1		Control	0.00e	187.34e	
				<i>B. bassiana</i>	49.99c	101.49c	
				<i>M. anisopliae</i>	47.03d	105.77d	
				<i>L. lecanii</i>	55.40b	91.38b	
				<i>I. fumosorosea</i>	60.70a	80.66a	
	2	2			Control	0.00d	208.14d
					<i>B. bassiana</i>	59.65c	29.53c
					<i>M. anisopliae</i>	56.07c	34.51c
					<i>L. lecanii</i>	65.96b	21.75b
					<i>I. fumosorosea</i>	70.63a	15.55a

Data analyzed with least squares means; means separated using LSD ($p \leq 0.01$).

A. dispersus induced by *B. bassiana* increased over time when comparing both applications and seasons. The highest mortality was observed at 15 DAT for both application 1 (66.45%, 81.00 *A. dispersus* per leaf) and application 2 (70.13%, 24.80 *A. dispersus* per leaf) in season 1 (Figure 2a, 2b). Similar trends were also observed in season 2, with 68.13% mortality following application 1 (66.36 *A. dispersus* per leaf) and 71.95% mortality following application 2 (19.60 *A. dispersus* per leaf) (Figure 2b). The lowest mortality was observed 3 DAT for both application 1 (37.90%, 141.80 *A. dispersus* per leaf) and application 2 (44.09%, 44.88 *A. dispersus* per leaf) in season 1. Similarly, in season 2 the lowest mortality was observed following application 1 (34.84%, 116.72 *A. dispersus* per leaf) and application 2 (47.07%, 34.36 *A. dispersus* per leaf) at 3 DAT. Weather parameters such as relative humidity, temperature and rainfall were 75-85.0%, 22.0-32.3 °C and 14.0 mm in season 1 and 72-81%, 20.8-31.1 °C and 9.0 mm in season 2, respectively. Previously, Eyal *et al.* (1992) reported that 52-98% mortality of *Bemisia tabaci* (Gennadius) was induced by *B. bassiana*. Wright and Chandler (1992) also reported that *B. bassiana* caused comparatively lesser mortality than *Anthonomus grandis* Boheman (boll weevil) in the field. Nagasi *et al.* (1998) reported that *B. bassiana* produced higher mortality of the first instars and adults of the silver leaf whitefly. Wraight *et al.* (1998, 2000) observed that *B. bassiana* caused the high-

est mortality to nymphs of *B. argentifolii* under laboratory conditions. Furthermore, Wraight and Knaf (1994) used a higher dose of 5×10^{13} conidia ($2.5 \text{ conidia.mL}^{-1}$) and achieved 90% control of *B. tabaci* nymphs 7 DAT, and Boopathi *et al.* (2013) reported that *B. bassiana* had a comparatively higher efficacy against *A. dispersus* under laboratory conditions than against *M. anisopliae*.

Metarhizium anisopliae produced the highest mortality following application 2 in both seasons (Figure 2c, 2d). The percent mortality increased over time for both applications and seasons. The highest mortality was observed at 15 DAT following both application 1 (59.36%, 95.92 *A. dispersus* per leaf) and application 2 (65.51%, 33.72 *A. dispersus* per leaf) in season 1 (Figure 2c). Similar results were also observed in season 2, with 65.70% mortality following application 1 (70.48 *A. dispersus* per leaf) and 68.71% mortality following application 2 (23.32 *A. dispersus* per leaf) (Figure 2d). The next highest mortality rate was observed at 10 DAT, whereas the lowest mortality rate was produced at 3 DAT of both application 1 and application 2 in both seasons.

The fungus *L. lecanii* caused a higher mortality to *A. dispersus* following application 2 than following application 1 in both seasons (Figure 3a, 3b). The percent mortality of *A. dispersus* induced by *L. lecanii* increased over time for both applications and seasons. *L. lecanii* produced the highest mortality at 15 DAT following both application 1

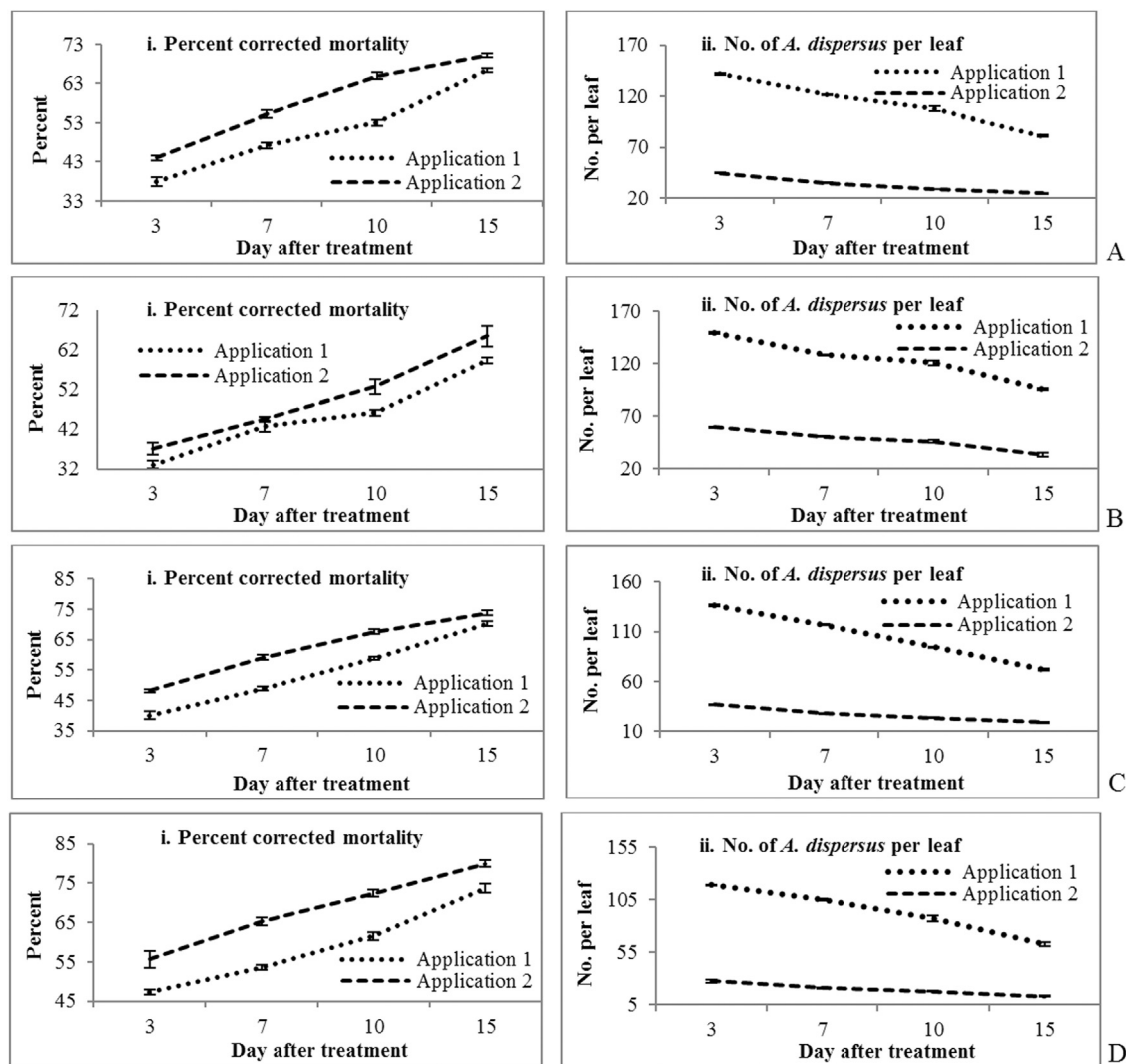


Figure 2 - Efficacy of entomopathogenic fungi on the mortality of *A. dispersus* on cassava in season 1 between 3 and 15 days after treatment (A) *B. bassiana* (B) *M. anisopliae* (C) *L. lecanii* (D) *I. fumosorosea*.

(70.13%, 71.68 *A. dispersus* per leaf) and application 2 (73.75%, 19.24 *A. dispersus* per leaf) in season 1 (Figure 3a). Similarly, in season 2 *L. lecanii* caused the highest mortality following application 1 (72.94%, 56.48 *A. dispersus* per leaf) and application 2 (78.59%, 12.76 *A. dispersus* per leaf) at 15 DAT (Figure 3b). Similar results were reported by Aiswariya *et al.* (2007), with 3.6×10^9 spores mL⁻¹ of *L. lecanii* inducing ~90% mortality of nymphs and ~80% mortality of adults of *A. dispersus* 15 days after application. However, Boopathi *et al.* (2013) reported that *L. lecanii* had higher mortality against *A. dispersus* under laboratory conditions.

Isaria fumosorosea produced higher mortality following application 2 than following application 1 in both seasons (Figure 2d). Similar to the other fungi, the percent mortality induced by *I. fumosorosea* increased over time for both applications and seasons. The highest mortality was due to both application 1 (73.70%, 62.68 *A. dispersus*

per leaf) and application 2 (79.96%, 12.80 *A. dispersus* per leaf) at 15 DAT in season 1 (Figure 3c). Similar trends were also observed in season 2, with 77.73% mortality following application 1 (45.88 *A. dispersus* per leaf) and 84.50% mortality following application 2 (7.48 *A. dispersus* per leaf) (Figure 3d). The next highest mortality was observed at 10 DAT, whereas the lowest mortality was produced at 3 DAT following both application 1 and application 2 in both seasons. However, the control of the *A. dispersus* population was the higher in both season 1 (> 200 *A. dispersus* per leaf) (Figure 4) and season 2 (> 150 *A. dispersus* per leaf) than for any other entomopathogenic fungi (Figure 4). Similar results were reported by Boopathi *et al.* (2013), who reported that 2×10^9 conidia mL⁻¹ of *I. fumosorosea* produced 100% mortality in *A. dispersus* 15 DAT under laboratory conditions. This result is in agreement with the present findings. Similarly, Wraight *et al.* (1998, 2000) observed that *I. fumosorosea* caused higher mortality to nymphs of *B.*

argentifolii under laboratory conditions, while Ayhan and Kubilay (2005) and Avery *et al.* (2008) reported that *I. fumosorosea* produced the highest mortality of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood).

Temperature and relative humidity are important microclimatic factors that improve the pathogenicity of entomopathogenic fungi under field conditions. Rainfall (9.0-14.0 mm), relative humidity (72-85.0%), and tempera-

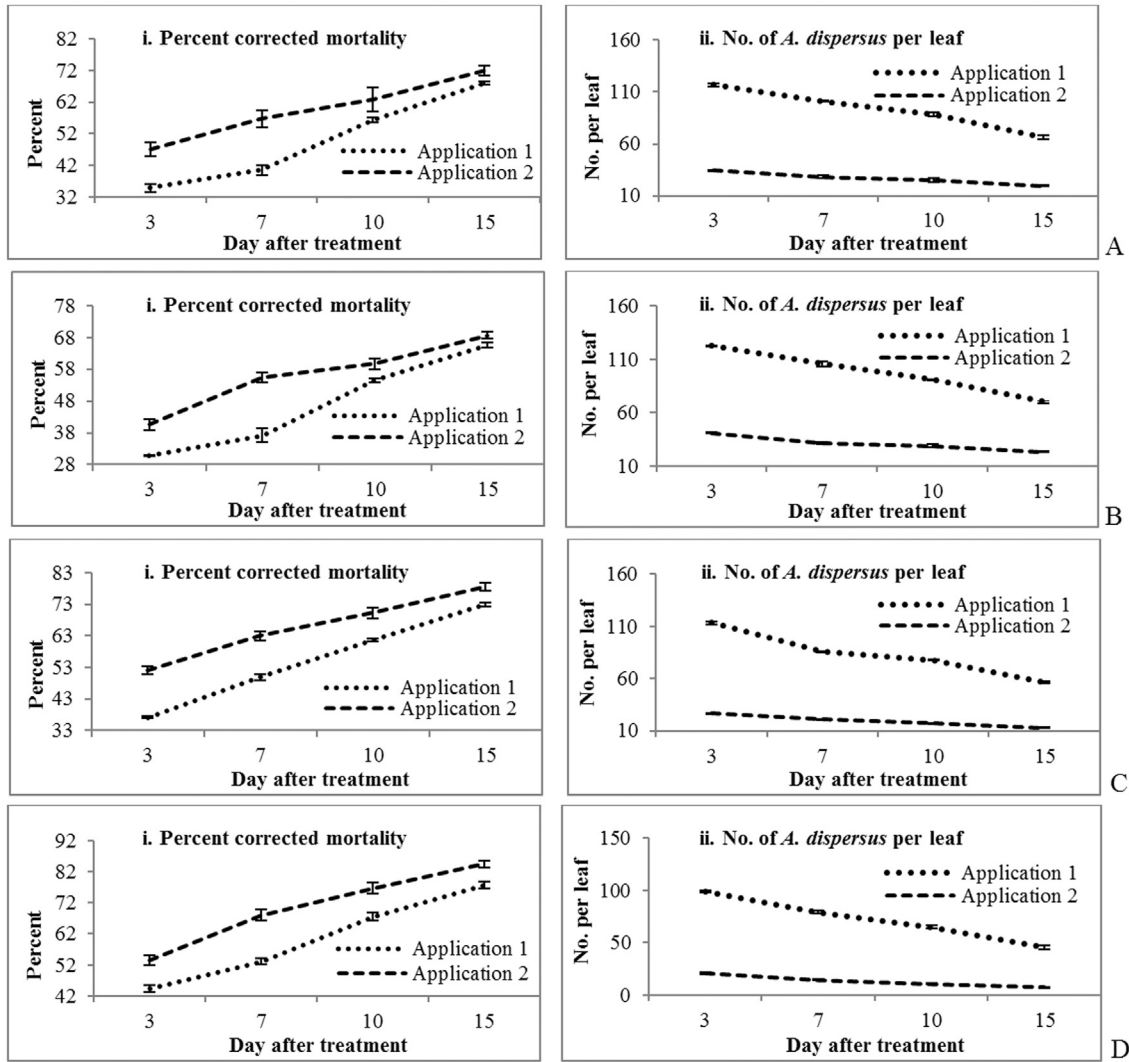


Figure 3 - Efficacy of entomopathogenic fungi on the mortality of *A. dispersus* on cassava in season 2 between 3 and 15 days after treatment (A) *B. bassiana* (B) *M. anisopliae* (C) *L. lecanii* (D) *I. fumosorosea*.

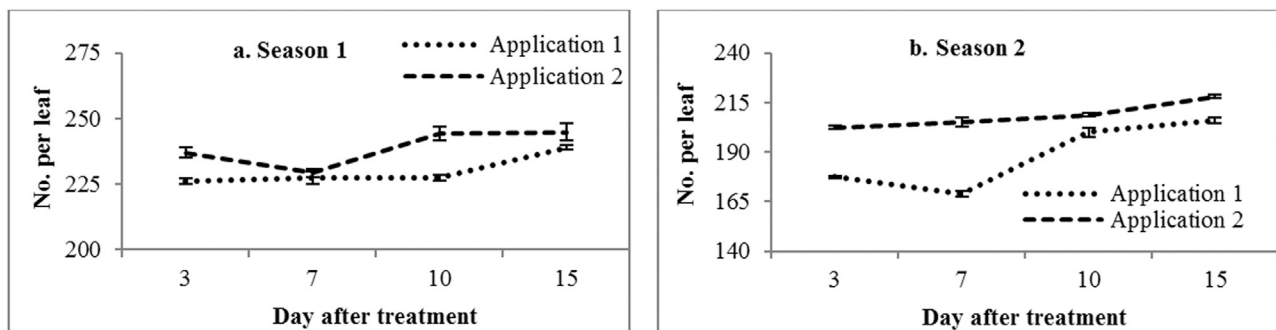


Figure 4 - *A. dispersus* population (no. per leaf) in control treatment on cassava in both season 1 and season 2 between 3 and 15 days after treatment.

ture (20.8-32.3 °C) in both season 1 and season 2 favored entomopathogenic fungal infection and growth. This finding is in agreement with previous studies with *I. fumosorosea* (Boopathi *et al.*, 2013; Boopathi *et al.*, 2015). Our results suggest that *I. fumosorosea* was more effective in suppressing the exotic *A. dispersus* in the field than in suppressing *B. bassiana*, *M. anisopliae* or *L. lecanii*; this finding is consistent with the lower LC₅₀ value reported for *I. fumosorosea* in a previously conducted pathogenicity test (Boopathi *et al.*, 2013). *M. anisopliae* did not produce an added advantage because it could not suppress the rapidly growing *A. dispersus* population in the field. Thus, effective control of *A. dispersus* was not achieved compared to that of *I. fumosorosea*. This result could be because *M. anisopliae* was the least effective against *A. dispersus* and contained a lower density of conidia. Similarly, Bateman *et al.* (1993) reported that *M. anisopliae* was ineffective against the desert locust at low humidity. Two repeated sprays fifteen days apart were required before *I. fumosorosea* could totally suppress the *A. dispersus* population, indicating that *I. fumosorosea* virulence was maintained throughout the duration of the field experiment.

Among the four entomopathogenic fungi evaluated, *I. fumosorosea* and *L. lecanii* showed promising levels of virulence to *A. dispersus* in both applications and seasons compared to that of *M. anisopliae* and *B. bassiana*. Similarly, Boopathi *et al.* (2015) reported that *I. fumosorosea* and *L. lecanii* caused the highest mortality to *A. dispersus* on eggplants. Thus, these fungi (*I. fumosorosea* and *L. lecanii*) can be potentially used as an alternate pest control method to combat the pest insect *A. dispersus* on cassava. Their wide application as mycoinsecticides could be utilized after exploring their pathogenicity in field trials. However, additional testing with yield assessments and economic analyses must be conducted before ultimate conclusions are drawn.

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