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Microbial control of the exotic spiralling whitefly, *Aleurodicus dispersus* (Hemiptera: Aleyrodidae) on eggplant using entomopathogenic fungi

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Biological control using entomopathogenic fungi is a promising alternative to chemical control. The entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Lecanicillium lecanii* (Zimmerm.) Zare and Gams and *Isaria fumosorosea* (Wize) were tested for their efficacy in controlling the spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae) on eggplant, *Solanum melongena* L. (cv. Pusa Purple Round) for two seasons in a randomized completely block design. Two applications of fungi were made at the rate of 2 × 10^9 conidia/mL. Observations on *A. dispersus* population were recorded on three leaves from the top, middle and bottom of 5 tagged plants per plot. *I. fumosorosea* and *L. lecanii* exhibited promising levels of control (>75% mortality) as compared to the other entomopathogenic fungi. *I. fumosorosea* was highly pathogenic to *A. dispersus* in both applications and seasons than other species of entomopathogenic fungi. Mortality from both seasons indicated differences in efficacy between days 3-15 after treatment. Application 2 produced the highest mortality in both seasons. Seasons had no influence on reduction of *A. dispersus* population. There is potential for use of entomopathogenic fungi to manage *A. dispersus* on eggplant.

Key words: *Aleurodicus dispersus*, *Solanum melongena*, biocontrol, entomopathogenic fungi, mortality.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is the most important vegetable crop grown in the tropics and the main crop cultivated in throughout India. Among various insect pests of eggplant, the spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae) is considered to be a major pest causing extensive damage to eggplant. *A. dispersus* is a polyphagous pest with an extensive host range including many crops and weed species

Table 1. Details of entomopathogenic fungi isolates.

Entomopathogenic fungi	Fungal strains	Sources
B. bassiana	B_2	Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India
M. anisopliae	M_2	Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India
L. lecanii	L_1	Sun Agro Biotech Research Centre, Madanantapuram, Porur, Chennai, Tamil Nadu, India
I. fumosorosea	P_1	Sun Agro Biotech Research Centre, Madanantapuram, Porur, Chennai, Tamil Nadu, India

(Srinivasa, 2000; Boopathi et al., 2014). Infestation of dense population causes premature leaf drop and production of copious amount of honeydew serves as a substrate for sooty mould growth (Akinlosotu et al., 1993). Sooty mould blackens the leaves, decreases photosynthetic activity and vigour of the host plants (Kumashiro et al., 1983; John et al., 2007). Insecticides such as triazophos and acephate have been proven to control this species, however, chemicals should be applied judiciously to minimize the development of resistance and not to interfere with the natural enemies that offer biological control of other members of the pest complex. Biological control agents, such as the predators *Mallada astur* (Banks) and *Cybocephalus* spp. (Mani and Krishnamoorthy, 1999) and parasitoids *Encarsia guadeloupae* Viggiani and *Encarsia* sp. nr. *meritoria* Gahan (Geetha, 2000), are the most commonly reported natural enemies in India in the fields.

There is a good epizootic potential of entomopathogenic fungi against *Bemisia* spp. and *Trialeurodes* spp. in field environments and greenhouse (Carruthers et al., 1993; Lacey et al., 1996). *Lecanicillium lecanii* (Zimmerm.) Zare and Gams at 3.6×10^9 spores·mL^{-1} produced ~90% mortality of nymphs and ~80% of adults of *A. dispersus* at 15 days after application (Aiswariaya et al., 2007). Wraight et al. (1998, 2000) observed that *Isaria fumosorosea* (Wize) and *Beauveria bassiana* (Balsamo) Vuillemin caused mortality in nymphs of silver leaf whitefly (*Bemisia argentifolii* Bellows and Perring) under laboratory conditions. Nagasi et al. (1998) reported that *B. bassiana* was most pathogenic to first instars and adults of the silver leaf whitefly. Boopathi et al. (2013) observed that *I. fumosorosea* and *L. lecanii* produced the highest pathogenic to *A. dispersus* under laboratory conditions than *Metarhizium anisopliae* (Metschnikoff) Sorokin. In India, serious scientific research in crop protection using entomopathogenic fungi against agricultural pests only started in the early 1990s. Many of the important vegetable pest species from Lepidoptera, Homoptera, Isoptera and Coleoptera have been tested to be susceptible to various fungal isolates (Geetha, 2000; Aiswariaya et al., 2007; Boopathi et al., 2013) including *A. dispersus*. The present investigation is to study the usefulness of entomopathogenic fungi, *B. bassiana*, *M. anisopliae*, *L. lecanii* and *I. fumosorosea* as effective biocontrol agents against the most destructive pest *A. dispersus* on eggplant.

MATERIALS AND METHODS

Entomopathogenic fungi isolates

Strains of the entomopathogenic fungi *B. bassiana*, *M. anisopliae*, *L. lecanii* and *I. fumosorosea* and their sources are listed in Table 1. Isolates were maintained on potato dextrose agar (PDA) in universal bottles (30 mL) and stored at 4°C. Continuous cultures were maintained on slants with sub-cultures grown for 14 days at 25°C following lids which were tightly sealed and cultures stored at 4°C.

Production of spore solution and counting of spores

A sterile, aqueous solution was prepared with 0.5% Tween 80. Spores from the fungal isolates were suspended in the solution. To homogenize the suspension, the solution was blended with a vortex mixer for two minutes (Lacey, 1997). To establish the concentration of the conidia in the solution, they were counted by using a haemocytometer under a compound microscope. The conidial suspension was diluted with 0.5% Tween® 80 solution, until it reached a concentration with a countable number of spores. After having established the concentration of conidia, suspensions were diluted with distilled water to the concentration of 2×10^9 conidia/mL.

Spore harvesting and drying

The spores were harvested at 3 and 12 days after the inoculation to evaluate spore yield. The spores harvested following this procedure can be preserved for a long time without loss of germination or pathogenicity (Bateman et al., 1995). The cultures were allowed to air dry overnight in a room with a temperature of 25 ± 5°C and

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relative humidity of 50 ± 5%.

Formulation and application equipment

Wettable powder formulation was prepared by thoroughly mixing pure air-dried conidia of *B. bassiana*, *M. anisopliae*, *L. lecanii* and *I. fumosorosea* with commercial diluent clay at a ratio of 1:4 (20% w/w a.i.) in a sterile room. All treatments were sprayed using a hand-held, single-nozzle, atomizing (air-assist) sprayer (pneumatic knapsack sprayer, model: Masand's Mahashakti Kissan). The spray nozzle was carried near ground level and directed at a right angle to the row. Each row was treated twice, once on each side of the row. Spray volume was 500–700 L/ha. Spraying was done at late evening in order to minimize the possible suppressive effect of the sun's rays on the conidial germination.

Field evaluation

Field experiments were conducted in eggplant for two seasons, 2011-2012 (Season 1) and 2012-2013 (Season 2) at the Research farm (Eastern Block), TNAU, Coimbatore, Tamil Nadu, India. Eggplant, cv. Pusa Purple Round, seedlings were grown on raised beds, 1.5 × 1.0 m, in greenhouses. At 25-days after sowing, seedlings were transplanted to 5 × 5 m plots at a spacing of 60 × 60 cm. Treatments were applied to 5 replicates arranged in a Randomized completely block design (RCBD). Weeding, application of manure and fertilizers, and other cultural operations were followed as per crop production guidelines (TNAU, 2012). Furrow irrigation (approximately 175–200 L/plot) 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 control. Two applications of fungi were made 15 days apart due to heavy infestation of *A. dispersus* at the rate of 2 × 10^9 conidia/mL. The first application was at the reproductive stage of eggplant. The second application covered new leaves and shoots, and also infected, and had increased populations, in newly emerged nymphs and adults of *A. dispersus*. Both applications were made on the same plants of the same age. Pre-treatment observations on *A. dispersus* population were taken 24 h before each application of fungi, and post-treatment observations were at 3, 7, 10 and 15 days after each treatment (DAT). Observations on *A. dispersus* populations were recorded from three leaves, top, middle and bottom leaves of 5 tagged plants per plot after the first and second applications.

Data analysis

Statistical analysis of data was performed following Gomez and Gomez (1984) using SAS Software Version 9.3 (SAS Institute Inc. 2011). Data were analyzed using three-way ANOVA. All ANOVA were performed on original values and the means were separated using least significant difference (LSD) test at $P \leq 0.05$ or $P \leq 0.01$. Percent mortality of *A. dispersus* populations was corrected using the method of Henderson and Tilton (1955):

$$\text{Corrected mortality} = 1 - \left[\frac{T_a - C_b}{T_b - C_a} \right] \times 100$$

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 check before spraying and C_a = number of insects in the untreated check after spraying.

RESULTS AND DISCUSSION

All treatments caused medium to high mortality on *A. dispersus*. Individual *A. dispersus* killed by fungi dried rapidly and remained attached to leaves. Mycelia and sporulation of fungi on cadaver occurred during extended periods of rain, or late in trials, after many nights of high-humidity. There were differences between fungi ($F = 4472.37$; $P < 0.0000$), applications ($F = 140.87$; $P < 0.0000$), observation dates ($F = 555.20$; $P < 0.0000$) and between the interactions: fungi × applications ($F = 9.722$; $P < 0.0000$), fungi × observation dates ($F = 35.22$; $P < 0.0000$), applications × observation dates ($F = 18.69$; $P < 0.0000$), applications × seasons ($F = 10.82$; $P < 0.0011$), fungi × applications × seasons ($F = 2.801$; $P < 0.0260$) and applications × observation dates × seasons ($F = 4.731$; $P < 0.0030$) on the mortality of *A. dispersus* (Table 2). There were no differences between seasons ($F = 0.661$; $P < 0.4167$) and between the interactions: fungi × seasons ($F = 0.427$; $P < 0.7891$), observation dates × seasons ($F = 2.458$; $P < 0.0629$), fungi × applications × observation dates ($F = 1.526$; $P < 0.1135$), fungi × observation dates × seasons ($F = 0.490$; $P < 0.9204$) and fungi × applications × observation dates × seasons ($F = 0.764$; $P < 0.6871$) on the mortality of *A. dispersus* on eggplant.

Percent mortality from both applications in season 1 indicated differences in efficacy between fungi (Table 3). Higher mortality occurred with *I. fumosorosea* in both application 1 (65.55%) and application 2 (71.92%) in season 1 as compared to the other fungi. Similarly, in season 2, *I. fumosorosea* produced higher mortality in both application 1 (66.34%) and application 2 (73.64%) as compared to the other fungi. Percent mortality from the season 2 indicated that *I. fumosorosea* had the highest mortality due to both applications than season 1. Season influenced effect of fungi on reduction of *A. dispersus*. Season 2 had the highest mortality in eggplant than season 1.

A. dispersus individuals infected by *B. bassiana* were red to red-brown. Pathogenicity of the fungus *B. bassiana* on mortality of *A. dispersus* on eggplant indicated differences in efficacy between days 3-15 after treatment (Figure 1A and B). *Beauveria bassiana* caused the highest mortality due to application 2 than application 1 in both seasons. Per cent mortality of *A. dispersus* by *B. bassiana* increased with increase in time in both applications and seasons. The highest mortality was at 15 DAT due to both application 1 (69.78%) and application 2 (72.15%) in season 1 (Figure 1A). Similarly, in season 2, *B. bassiana* had the highest mortality at 15 DAT in both applications 1 (73.81%) and 2 (71.72%) (Figure 1B). The lowest mortality was at 3 DAT due to both application 1 (44.36 and 43.40%, respectively) and application 2 (49.54 and 48.37%, respectively) in seasons 1 and 2. Earlier, Eyal et al. (1992) reported 52-98% mortality of *Bemisia tabaci* (Gennadius) by *B. bassiana*. Studies by Wright and Chandler (1992) also

Table 2. Three-way analysis of variance (ANOVA) for percent corrected mortality of *A. dispersus* on eggplant.

Source	Corrected mortality of *A. dispersus* (%)			
	F value	SEd	CD (P = 0.01)	Probability
Fungi (F)	4472.366	0.5908	1.5311	0.0000**
Application (A)	140.870	0.3737	0.9683	0.0000**
Day after treatment (D)	555.203	0.5284	1.3695	0.0000**
Season (S)	0.661	0.3737	0.9683	0.4167NS
Interaction				
F × A	9.722	0.8355	2.1653	0.0000**
F × D	35.218	1.1816	3.0622	0.0000**
F × S	0.427	0.8355	2.1653	0.7891NS
A × D	18.692	0.7473	1.9367	0.0000**
A × S	10.823	0.5284	1.3695	0.0011**
D × S	2.458	0.7473	1.9367	0.0629NS
F × A × D	1.526	1.6710	4.3306	0.1135NS
F × A × S	2.801	1.1816	3.0622	0.0260*
F × D × S	0.490	1.6710	4.3306	0.9204NS
A × D × S	4.731	1.0569	2.7389	0.0030**
F × A × D × S	0.764	2.3632	6.1244	0.6871NS

*, $P < 0.05$; **, $P < 0.01$; NS, not significant; ANOVA.

Table 3. Percent corrected mortality of *A. dispersus* due to both applications on eggplant in both seasons 1 and 2.

Season	×	Application	×	Fungi	Corrected mortality of *A. dispersus* (%)	*A. dispersus* population per leaf
1		1		Control	0.00^{e}	103.45
				B. bassiana	54.89^{c}	45.60
				M. anisopilae	48.99^{d}	51.74
				L. lecanii	59.38^{b}	40.54
				I. fumosorosea	65.55^{a}	34.67
		2		Control	0.00^{e}	113.73
				B. bassiana	62.23^{c}	12.49
				M. anisopilae	56.13^{d}	17.81
				L. lecanii	66.85^{b}	9.11
				I. fumosorosea	71.92^{a}	6.07
2		1		Control	0.00^{e}	64.70
				B. bassiana	57.48^{c}	27.28
				M. anisopilae	50.63^{d}	32.33
				L. lecanii	62.03^{b}	24.24
				I. fumosorosea	66.34^{a}	21.37
		2		Control	0.00^{d}	93.96
				B. bassiana	59.57^{bc}	28.46
				M. anisopilae	54.55^{c}	33.96
				L. lecanii	64.73^{b}	24.63
				I. fumosorosea	73.64^{a}	20.70

Data analyzed with least squares means and means separated using LSD ($P < 0.01$; Henderson and Tilton, 1955).

reported that *B. bassiana*, could suppress the *Anthonomus grandis* Boheman (boll weevil) in the field but comparatively slower than the insecticide dicrotophos. However, Wraight and Knaf (1994) reported

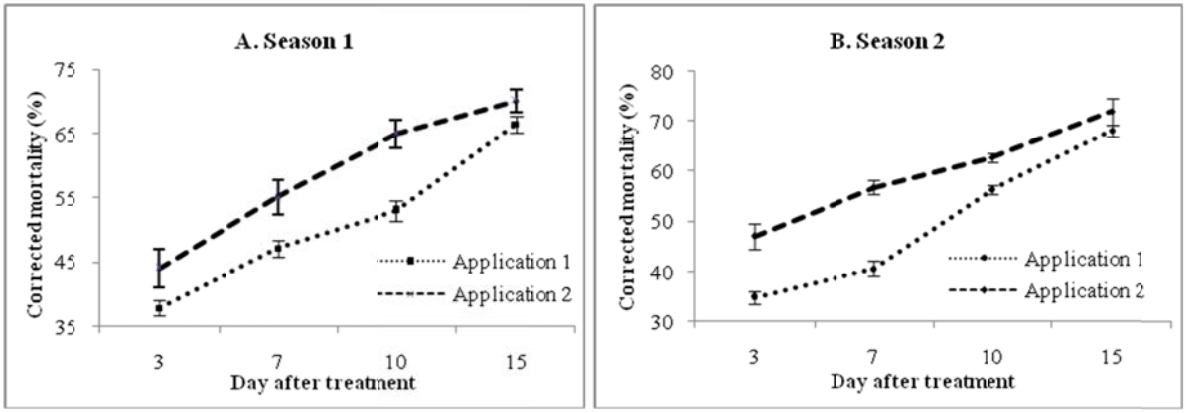

Figure 1. Efficacy of *B. bassiana* on the mortality of *A. dispersus* on eggplant at different DAT in both season 1 and season 2.

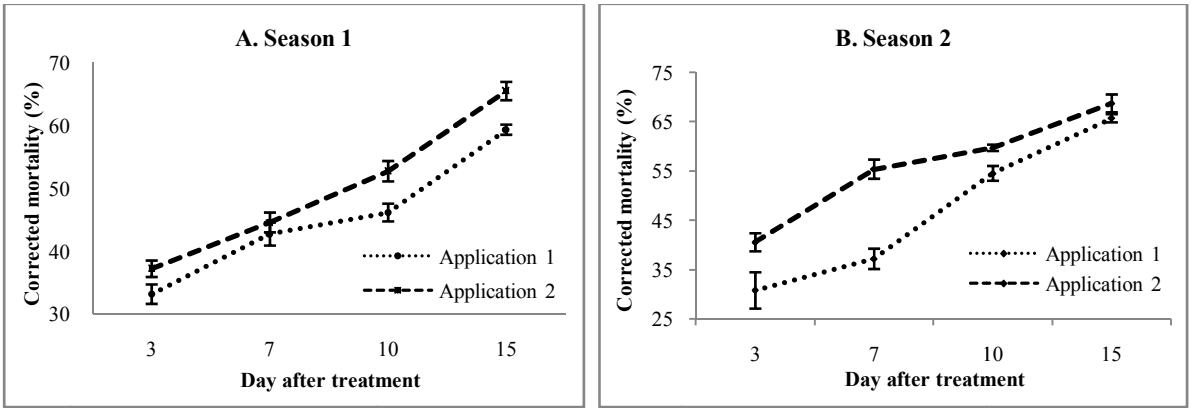

Figure 2. Efficacy of *M. anisopilae* on the mortality of *A. dispersus* on eggplant at different DAT in both seasons 1 and 2.

that higher dose of 5×10^{13} conidia (2.5 conidia/mL) and achieved 90% control of *B. tabaci* nymphs on 7 DAT. Nagasi et al. (1998) reported that *B. bassiana* was most pathogenic to first instars and adults of the silver leaf whitefly. Wraight et al. (1998, 2000) observed pathogenicity of *B. bassiana* against nymphs of *B. argentifolii* under laboratory conditions. Studies by Boopathi et al. (2013) who reported that *B. bassiana* had comparatively more pathogenicity against *A. dispersus* under laboratory conditions.

M. anisopliae produces green conidia (phialospores) from closely packed and parallely oriented conidiogenous cells born upon a sporodochium–like mass of hyphae. Percent mortality of *A. dispersus* by *M. anisopliae* increased with increase in time in both applications and seasons. The highest mortality was at 15 DAT due to both application 1 (63.32 and 67.18%, respectively) and application 2 (66.60 and 66.94%, respectively) in both seasons 1 and 2 (Figure 2A and B). The lowest mortality was due to both applications 1 (38.84%) and 2 (41.35%) in season 1 at 3 DAT (Figure 2A). Similar trends were also observed in season 2 which caused 39.27% mortality in application 1 and 44.22% mortality in application 2 at 3 DAT (Figure 2B).

The entomopathogenic fungus *L. lecanii* caused the highest pathogenic to *A. dispersus* due to application 2 than application 1 in both seasons (Figure 3A and B). Percent mortality of *A. dispersus* by *L. lecanii* increased with increase in time in both applications and seasons. *L. lecanii* produced the highest mortality at 15 DAT due to both applications 1 (74.66%) and 2 (75.49%) in season 1 (Figure 3A). Similarly, in season 2, *L. lecanii* caused the highest mortality due to applications 1 (78.89%) and 2 (76.49%) at 15 DAT (Figure 3B). The lowest mortality was at 3 DAT due to both applications 1 (48.41% and 48.65%, respectively) and 2 (52.94 and 50.73%, respectively) in both seasons 1 and 2 (Figure 3A and B). Similar results were reported by Aiswariaya et al. (2007) with *L. lecanii* at 3.6×10^9 spores/mL with ~90% mortality of nymphs and ~80% of adults of *A. dispersus* at 15 days

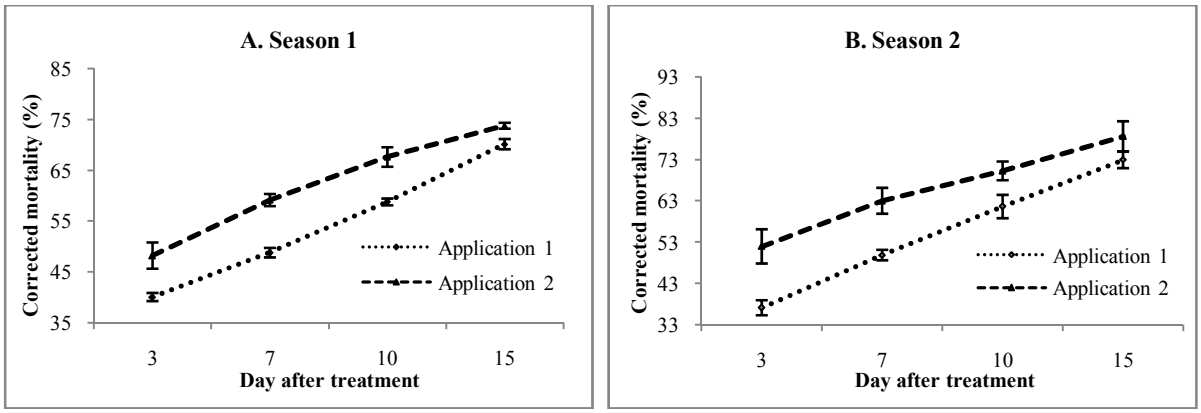

Figure 3. Efficacy of *L. lecanii* on the mortality of *A. dispersus* on eggplant at different DAT in both seasons 1 and 2.

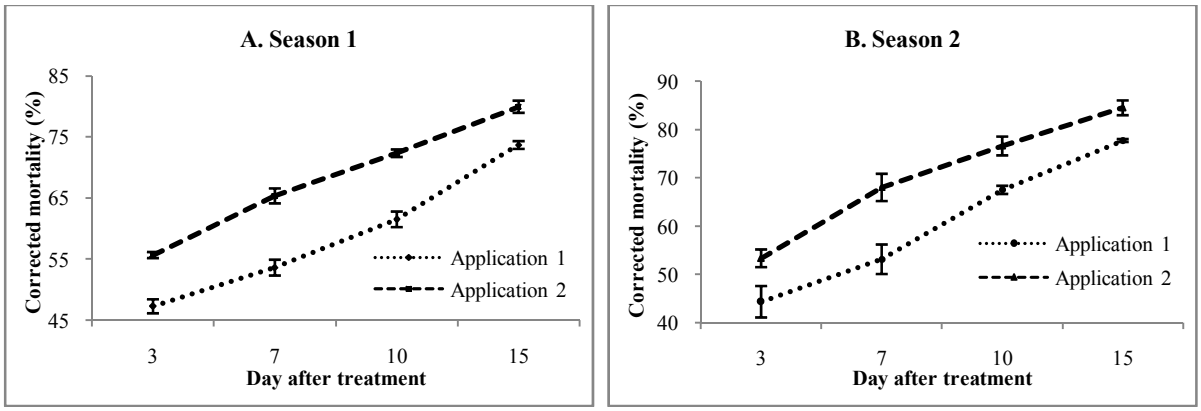

Figure 4. Efficacy of *I. fumosorosea* on the mortality of *A. dispersus* on eggplant at different DAT in both seasons 1 and 2.

after application. Earlier, Boopathi et al. (2013) reported that *L. lecanii* had more pathogenicity against *A. dispersus* under laboratory conditions.

Amounts of *I. fumosorosea* hyphal growth and sporulation were visibly greater, and faster than the other entomopathogenic fungi. *I. fumosorosea* produced the highest mortality due to application 2 than application 1 in both seasons (Figure 4A and B). Like other fungi, *I. fumosorosea* also observed that percent mortality increase with increase in time in both applications and seasons. The highest mortality was due to both applications 1 (80.21 and 83.52%, respectively) and 2 (82.43 and 83.71%, respectively) in both seasons 1 and 2 at 15 DAT (Figure 4A and B). The next highest mortality was with 10 DAT and the least mortality produced at 3 DAT due to both applications 1 and 2 in both seasons. Earlier, Avery et al. (2008) reported *I. fumosorosea* produced 99.5% mortality to greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) and this is in conformity with the present findings. *Isaria* isolates produced over 70% mortality to *T. vaporariorum* reported by Ayhan and Kubilay (2005). Wraight et al. (1998, 2000) observed equal pathogenicity of *I. fumosorosea* against nymphs of *B. argentifolii* under laboratory conditions. Studies by Boopathi et al. (2013) reported that *I. fumosorosea* had more pathogenicity against *A. dispersus* under laboratory conditions.

Temperature and relative humidity are important microclimatic factors in improving the pathogenicity of entomopathogenic fungi under field conditions. Total rainfall of 14.0 mm and 85.0% RH, and temperatures between 22.0-32.3°C were conducive for fungal growth. This study suggests that *I. fumosorosea* was the most effective as compared to *B. bassiana* or *M. anisopliae* or *L. lecanii* and consistent with the lower LC_{50} value for *I. fumosorosea* obtained in a pathogenicity test conducted earlier (Boopathi et al., 2013). *M. anisopliae* did not give added advantage since it could not suppress the *A. dispersus* population rapidly in the field, hence effective control was not achieved as compared to *I. fumosorosea*. This could be due to the fact that *M. anisopliae* was the least effective and this contained lesser density conidia.

Thus, the infection was slow as the speed of kill is related to the number of conidia received by the individual pest (Bateman et al., 1993). Studies by Boopathi et al. (2013) also observed that *M. anisopliae* caused the lowest pathogenic to *A. dispersus* under laboratory conditions. Two repeated sprays spaced fifteen days apart were needed before *I. fumosorosea* could completely suppress the *A. dispersus* population, an indication that its virulence was maintained throughout the duration of the experiment. In a permanent cropping system, *I. fumosorosea* could give permanent suppression of the *A. dispersus* if it could spread from the release site in subsequent seasons.

Among four enthomopathogenic fungi evaluated, *I. fumosorosea* and *L. lecanii* showed promising levels of virulence to *A. dispersus* in both applications and seasons than *M. anisopliae* or *B. bassiana*. Similar results were also reported by Boopathi et al. (2013) who observed that *I. fumosorosea* and *L. lecanii* caused the highest pathogenicity to *A. dispersus* under laboratory conditions. There is potential for use of these fungi to control *A. dispersus* on eggplant. Microbial pesticides can be used as an alternative control method in combating the pest. Its wide application as a biological pesticide could be taken up after exploring its toxicity and field trials.

Conflict of interest

The authors have not declared any conflict of interest.

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