DOI : 10.5958/0974-0112.2015.00072.9

Indian J. Hort. 72(3), September 2015: 370-375

Microbial control of exotic spiraling whitefly, *Aleurodicus dispersus* with entomopathogenic fungi on cassava under open field conditions

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ABSTRACT

The entomopathogenic fungi *Isaria fumosorosea*, *Lecanicillium lecanii*, *Beauveria bassiana* and *Metarhizium anisopliae* were tested for their efficacy for the management of invasive spiraling whitefly, *Aleurodicus dispersus* (Hemiptera, Aleyrodidae) during two seasons (2011-2012 and 2012-2013) on cassava (*Manihot esculenta*). The fungi, *I. fumosorosea* and *L. lecanii* exhibited promising levels of control (>70% mortality of *A. dispersus* population). Mortality of *A. dispersus* increased with increase in time in both seasons. Application of *I. fumosorosea* was highly pathogenic to *A. dispersus* in both the seasons compared to the other entomopathogenic fungi. Mortality of *A. dispersus* in both seasons indicated difference in efficacy between 3 and 15 days after treatment. Season influenced the effects of the fungi on *A. dispersus* population. There is potential for entomopathogenic fungi to manage *A. dispersus* on cassava.

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

INTRODUCTION

Cassava (*Manihot esculenta* Cranrz.) is the most important starchy root crop grown in the tropics and the main crop cultivated in Southern Peninsular regions of India. Among various insect pests of cassava, exotic spiraling whitefly, *Aleurodicus dispersus* Russell (Hemiptera, Aleyrodidae) can cause losses up to 53% (Geetha, 6), which is a polyphagous pest with an extensive host range including many crops and weed species (Boopathi *et al*., 4). Infestation causes premature leaf drop and the production of copious amount of honeydew serves as a substrate for sooty mould growth (Boopathi *et al*., 3), which reduces the photosynthetic activity and plant vigour. Biological control agents, such as the predators, *Mallada astur* (Banks) and *Cybocephalus* spp. and parasitoids, *Encarsia guadeloupae* Viggiani and *Encarsia* sp. nr. *meritoria* Gahan, are the most commonly reported natural enemies in India. Good epizootic potential of entomopathogenic fungi against *Bemisia* spp. and *Trialeurodes* spp. in field and greenhouse conditions makes them as potent candidates of biological control agent. *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*., 1). Wraight *et al*. (10) 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*. (9) reported that *B. bassiana* was most pathogenic to first instars and adults of the silver leaf whitefly. Eyal *et al*. (5) reported *B. bassiana* produced 52-98% mortality of whitefly with concentrations of 1-4 × 10^6 conidia·ml^{-1}. Boopathi *et al*. (3) observed that *I. fumosorosea* and *L. lecanii* produced the highest pathogenic to *A. dispersus* under laboratory conditions than *Metarhizium anisopliae* (Metschnikoff) Sorokin.

Many of the economically important vegetable insect pest species from Hemiptera, Lepidoptera, Coleoptera and Isoptera have been found to be susceptible to various entomopathogenic fungal isolates (Aiswariaya *et al*., 1; Boopathi *et al*., 3; Geetha, 6) including *A. dispersus*. The present investigation was carried out to determine 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 cassava.

MATERIALS AND METHODS

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

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Table 1. Isolates of entomopathogenic fungi.

Entomopathogenic fungi	Fungal strain	Host insect	Source
Beauveria bassiana	B_2	*Helicoverpa armigera*	Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore
Metarhizium anisopliae	M_2	*Bemisia tabaci*	Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore
Lecanicillium lecanii	L_1	*Bemisia tabaci*	Sun Agro Biotech Research Centre, Madanantapuram, Porur, Chennai, Tamil Nadu
Isaria fumosorosea	P_1	*Bemisia tabaci*	Sun Agro Biotech Research Centre, Madanantapuram, Porur, Chennai, Tamil Nadu

each fungal isolate was prepared in 0.5% aqueous Tween80® and homogenized with a vortex mixer for two minutes (Lacey, 8) and spores were counted using a haemocytometer. The conidial suspension was further diluted with 0.5% aqueous Tween 80® solution to obtain concentrations of 2×10^9 conidia.ml^{-1}. Maize (150 g) with 60 ml of sterile water was autoclaved in polypropylene bags (10 cm × 25 cm) at 121°C and a pressure of 1.05 kg cm^{-3} for 15 min. and cooled at room temperature for 24 h. One millilitre of the 2×10^9 conidia·ml^{-1} suspension was introduced into each polypropylene bag and incubated for 2 weeks at 26 ± 3°C. The two-week-old fermented cultures were then harvested. The conidia/spores were sieved through a particle size of 125 µm. The cultures were allowed to air dry overnight in a room with a temperature of 25 ± 5°C and relative humidity of 50 ± 5%. Freshly harvested conidia were used for field applications and conidia were remained viable and active up to 8 months without any loss in the efficacy.

Wettable powder formulations were prepared by thoroughly mixing air-dried conidia with commercial diluent clay, kaolin at a ratio of 1:4 (20% w/w a.i.) in a sterile room. Conidial formulation was sprayed using a single-nozzle, atomizing (air-assist) sprayer (pneumatic knapsack sprayer) in all the treatments. The spray nozzle was carried near ground level in each spray 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^{-1}. Spraying was done at late evening in order to reduce the possible oppressive effect of the solar radiation on the conidial/spore germination.

Field experiments were conducted in cassava for two seasons, 2011-2012 (Season 1) and 2012-2013 (Season 2) at Pollachi, Coimbatore, Tamil Nadu for cassava. Rooted setts of cassava (cv. Co 2) were planted in 10 m × 10 m plots at a spacing of 90 cm × 90 cm. Treatments were applied to five replicates arranged in a completely randomized block design. Weeding, application of manures and fertilizers, and other cultural operations were followed as per crop production guidelines. Furrow irrigation (approximately 700-800 l·plot^{-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 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^{-1}. Both application 1 and application 2 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 taken at 3, 7, 10 and 15 days after each treatment (DAT). Observations on *A. dispersus* population were recorded on leaves from the top, middle and bottom of 5 tagged plants per plot after the first and second applications.

Statistical analysis of data was done using SAS Software Version 9.3. 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 $P \leq 0.01$. Per cent mortality of *A. dispersus* populations was determined and corrected with that in the control using the method of Henderson and Tilton (7).

RESULTS AND DISCUSSION

All entomopathogenic fungi treatments caused medium to high mortality on *A. dispersus*. Individual *A. dispersus* killed by these entomopathogenic fungi dried rapidly on the cassava leaves. The cadaver was remained attached to cassava leaves. *Aleurodicus dispersus* population prior to first spraying during first season was 88.76 to 90.40 per leaf and second season 53.72 to 56.20 per leaf, respectively (Table 2). Pathogenicity of *B. bassiana* to *A. dispersus* on cassava indicated differences in efficacy between days 3-15 after treatment (Fig. 1). *Beauveria bassiana* produced the highest mortality after application 2 in

Table 2. *Aleurodicus dispersus* population (No. per leaf) in pretreatment count (PTC) on cassava during 2011-2012 (season 1) and 2012-2013 (season 2).

Treatment	*Aleurodicus dispersus* population per leaf	
	Season 1 (Mean ± SE)	Season 2 (Mean ± SE)
Beauveria bassiana	89.80 ± 2.1494	55.36 ± 2.1046
Metarhizium anisopliae	90.00 ± 2.1649	56.20 ± 2.5443
Lecanicillium lecanii	88.76 ± 0.6802	55.24 ± 0.5125
Isaria fumosorosea	89.96 ± 1.6236	55.52 ± 1.6137
Control	90.40 ± 1.4071	53.72 ± 0.3706

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 (34.32 *A. dispersus* per leaf) and application 2 (10.28 *A. dispersus* per leaf) in season 1 (Fig. 1a). Similar trends were also observed in season 2, which had 20.72 *A. dispersus* per leaf in application 1 and 7.32 *A. dispersus* per leaf in application 2 (Fig. 1b). The lowest mortality was at 3 DAT due to both application 1 (57.56 *A. dispersus* per leaf) and application 2 (19.20 *A. dispersus* per leaf) in season 1 and also in season 2 (application 1, 37.12 *A. dispersus* per leaf and application 2, 12.44 *A. dispersus* per leaf). Earlier, Eyal *et al*. (5) reported 52-98% mortality of *Bemisia tabaci* (Gennadius) by *B. bassiana*. Nagasi *et al*. (9) reported that *B. bassiana* was highly pathogenic to first instars and adults of the silver leaf whitefly. Wraight *et al*. (10) observed *B. bassiana* caused the highest mortality to nymphs of *B. argentifolii* under laboratory conditions. Studies by Boopathi *et al*. (3) reported that *B. bassiana* had comparatively more pathogenicity against *A. dispersus* under laboratory conditions compared to *M. anisopliae*.

Metarhizium anisopilae produced the highest mortality following application 2 in both seasons (Fig. 2). Per cent mortality increased with increase in time in both applications and seasons. The highest mortality was at 15 DAT due to both application 1 (41.68 *A. dispersus* per leaf and 26.40 *A. dispersus* per leaf, respectively) and application 2 (15.08 *A. dispersus* per leaf and 11.00 *A. dispersus* per leaf, respectively) in both season 1 and season 2 (Fig. 2a, 2b). *Metarhizium anisopilae* produced the least mortality compared to *B. bassiana*, *L. lecanii* and *I. fumosorosea* in both seasons.

The fungus *L. lecanii* caused the highest pathogenic to *A. dispersus* due to application 2 than application 1 in both seasons (Fig. 3). Per cent mortality of *A. dispersus* by *L. lecanii* increased with increase in time in both applications and seasons. *Lecanicillium lecanii* produced the highest mortality at 15 DAT due to both application 1 (28.40 *A. dispersus* per leaf) and application 2 (7.56 *A. dispersus* per leaf) in season 1 (Fig. 3a). Similarly, in season 2 *L. lecanii* caused the highest mortality due to application 1 (16.72 *A. dispersus* per leaf) and application 2 (4.68 *A. dispersus* per leaf) at 15 DAT (Fig. 3b). Similar results were reported by Aiswariaya *et al*. (1) with *L. lecanii* at 3.6×10^9 spores·ml^{-1} with ~90% mortality of nymphs and ~80% of adults of *A. dispersus* at

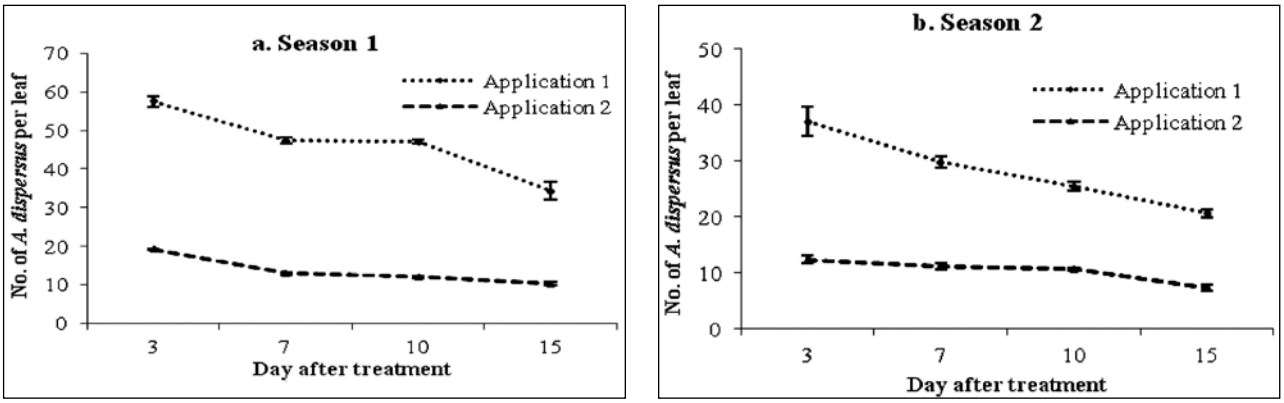

Fig. 1. Efficacy of *Beauveria bassiana* on the mortality of *Aleurodicus dispersus* on cassava during 2011-2012 (season 1) and 2012-2013 (season 2) between 3 and 15 days after treatment.

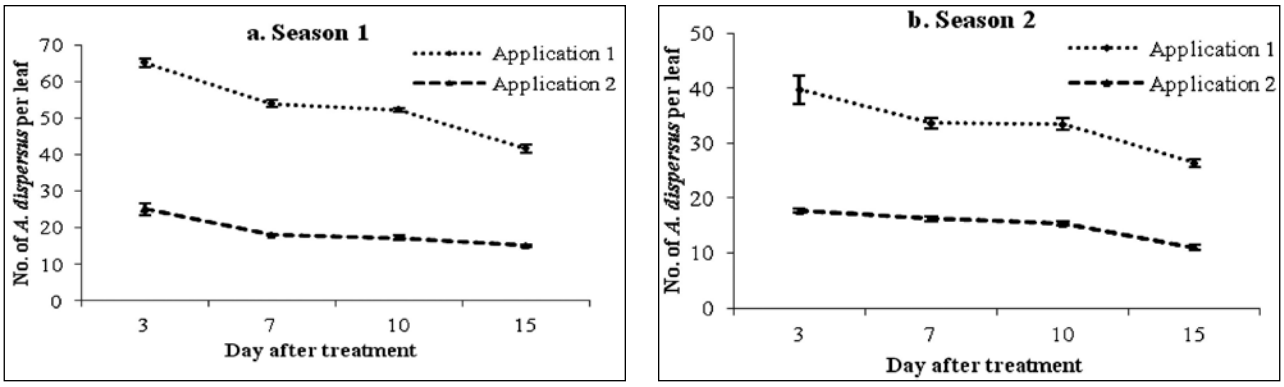

Fig. 2. Efficacy of *Metarhizium anisopliae* on the mortality of *Aleurodicus dispersus* on cassava during 2011-2012 (season 1) and 2012-2013 (season 2) between 3 and 15 days after treatment.

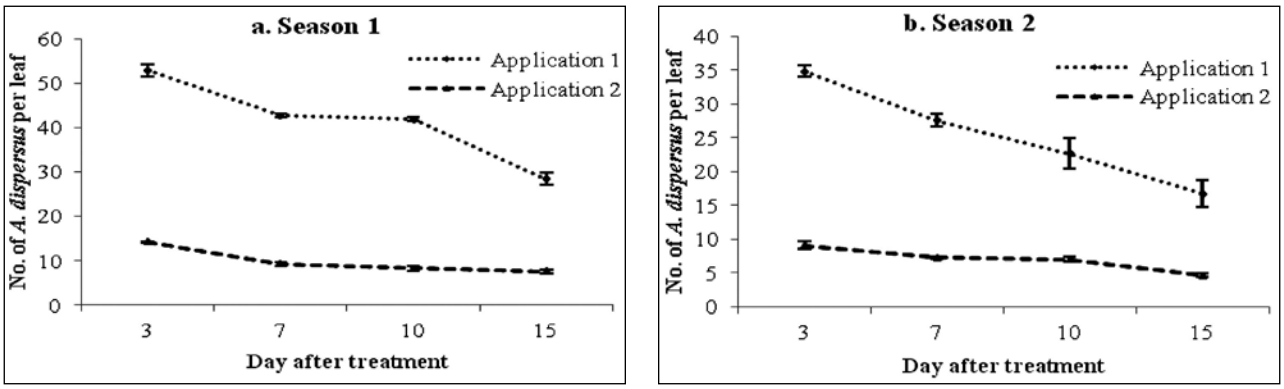

Fig. 3. Efficacy of *Lecanicillium lecanii* on the mortality of *Aleurodicus dispersus* on cassava during 2011-2012 (season 1) and 2012-2013 (season 2) between 3 and 15 days after treatment.

15 days after application. Earlier, Boopathi *et al*. (3) reported that *L. lecanii* had more pathogenicity against *A. dispersus* under laboratory conditions.

Isaria fumosorosea produced the highest mortality due to application 2 than application 1 in both seasons (Fig. 4). Like other fungi, *I. fumosorosea* also observed that per cent mortality increased with increase in time in both applications and seasons. The highest mortality was due to both application 1 (22.44 *A. dispersus* per leaf and 13.12 *A. dispersus* per leaf, respectively) and application 2 (4.28 *A. dispersus* per leaf and 2.72 *A. dispersus* per leaf, respectively) in both season 1 and season 2 at 15 DAT (Fig. 4a, b). The next highest mortality was with 10 DAT and the least mortality produced at 3 DAT due to both application 1 and application 2 in both seasons. However, the control of the *A. dispersus* population was the higher in both season 1 (>90 *A. dispersus* per leaf) (Fig. 5a) and season 2 (>50 *A. dispersus* per leaf) (Fig. 5b) than for any other entomopathogenic fungi. Similar results were reported by Boopathi *et al*. (3) with *I. fumosorosea* at 2 × 10^9 conidia·ml^1 produced 100% mortality to *A. dispersus* at 15 DAT under laboratory conditions and this is in conformity with the present findings. Similarly, Wraight *et al*. (10) observed that *I. fumosorosea* was highly pathogenic to nymphs of *B. argentifolii* under laboratory conditions.

Temperature and relative humidity are important microclimatic factors in improving the pathogenicity of entomopathogenic fungi under field conditions. Rainfall during January to March 2012 and relative humidity (14.0 mm and 85.0% RH), and temperature (22.0-32.3°C) favoured the entomopathogenic fungal infection and growth. This is evident with *I. fumosorosea* (Boopathi *et al*., 3). This study proposes that *I. fumosorosea* was the most effective in suppressing of the exotic *A. dispersus* in the field 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*., 3). *Metarhizium anisopliae* did not produce added advantage since it could not suppress the *A. dispersus* population rapidly in the field, hence, effective control of *A. dispersus* was

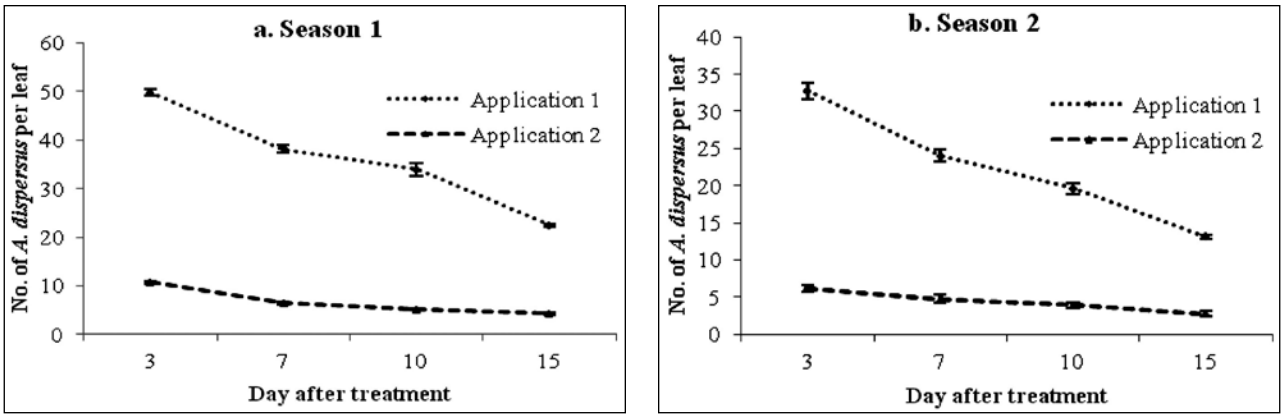

Fig. 4. Efficacy of *Isaria fumosorosea* on the mortality of *Aleurodicus dispersus* on cassava during 2011-2012 (season 1) and 2012-2013 (season 2) between 3 and 15 days after treatment.

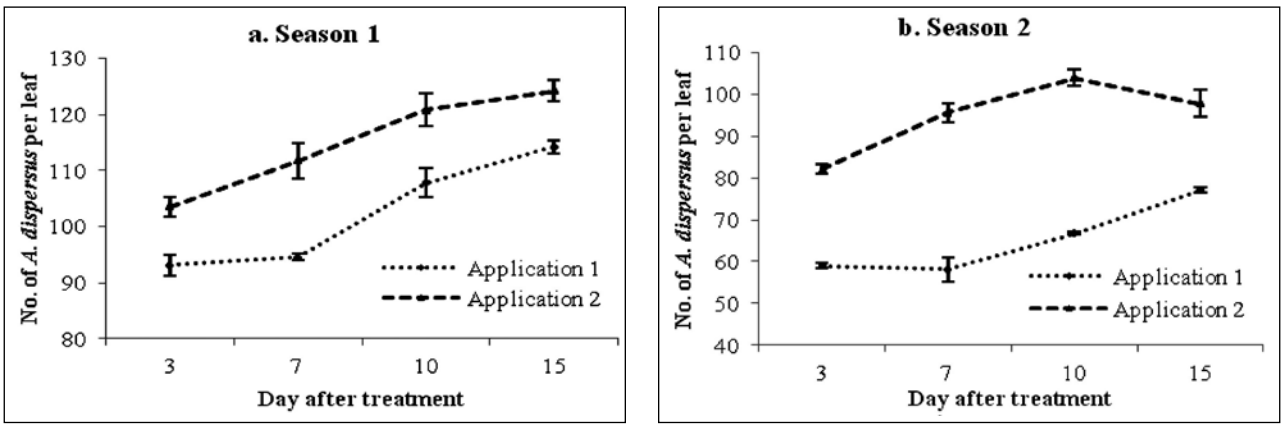

Fig. 5. *Aleurodicus dispersus* population (No. per leaf) in control treatment on cassava during 2011-2012 (season 1) and 2012-2013 (season 2) between 3 and 15 days after treatment.

not achieved compared to *I. fumosorosea*. This could be due to the fact that *M. anisopliae* was the least effective against *A. dispersus* and this contained lower density of conidia. Thus, the pathogenicity was slow as the speed of kill is narrated to the number of conidia obtained by the individual insect pest (Bateman *et al*., 2). Two repeated sprays gapped fifteen day apart were needed before *I. fumosorosea* could totally suppress the *A. dispersus* population. It is an indication that *I. fumosorosea* virulence was maintained throughout the duration of the field experiment. In a continuous cropping system *I. fumosorosea* could give permanent suppression of the *A. dispersus* if it could spread from the release site to other places in subsequent seasons.

Among four entomopathogenic 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*. (3) who observed that *I. fumosorosea* and *L. lecanii* caused the highest pathogenic to *A. dispersus* under laboratory conditions. There is potential for use of these fungi to control exotic *A. dispersus* on cassava. Microbial biopesticides can be used as an alternate pest control method in combating the pest insect, *A. dispersus*. Its wide application as mycoinsecticides could be taken up after exploring its pathogenicity and field trials. Additional testing with yield assessments and economic analyses must be conducted before ultimate conclusions are drawn.

ACKNOWLEDGEMENTS

The authors are grateful to the Professor and Head, Department of Agricultural Entomology and the Director, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore for providing the facilities and support. The assistance given by Mr. Muthumariappan, Mr. Mani and Mr. Arulnayagam in recording the observations is acknowledged.

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Received : December, 2014; Revised : July, 2015;
Accepted : August, 2015