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Field evaluation of *Lecanicillium psalliotae* and development of an integrated pest management strategy against *Sciothrips cardamomi*

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HIGHLIGHTS

- *Sciothrips cardamomi* is a major pest of cardamom worldwide.
- An EPF, *Lecanicillium psalliotae* was evaluated against the pest in the field.
- Soil application of fungal granules was effective in reducing capsule damage.
- An IPM strategy using the fungus and an insecticide, spinosad was developed.
- The fungus holds promise for eco-friendly management of the pest.

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ABSTRACT

Cardamom thrips, *Sciothrips cardamomi* Ramk. (Thysanoptera: Thripidae) cause huge economic losses to cardamom, *Elettaria cardamomum* (L.) Maton., a high-value spice crop, in all growing regions worldwide. To date, the pest is managed by synthetic pesticides, which results in harmful residues in the produce and also pose a serious threat to the environment. In our studies, we evaluated the biocontrol potential of a recently isolated entomopathogenic fungus, *Lecanicillium psalliotae* (Treschew) Zare & W. Gams (Ascomycota: Hypocreales) under field conditions for two years in two major cardamom growing states, Kerala and Karnataka in India. The results indicated that four rounds of soil application of the fungus granules reduced capsule damage by thrips significantly compared to control, whereas spray application of the fungus was ineffective. The compatibility of the fungus with commonly used pesticides in cardamom was tested under laboratory conditions and the fungus was found compatible with the pesticides, spinosad, fipronil and copper oxychloride. Further, we evaluated the fungus along with other management options such as application of recommended insecticide (quinalphos), reduced-risk insecticide (spinosad) and soil application of fungal entomopathogen (*L. psalliotae*) and in combination with insecticides for developing an integrated pest management (IPM) module for the management of the pest. The trials indicated that initial sanitization of the crop with either quinalphos or spinosad sprays followed by three rounds of soil application of the fungus or spray application of spinosad and soil application of *L. psalliotae* twice alternatively, reduced capsule damage by thrips significantly. Our findings offer a scope for integrated management of cardamom thrips with reduced risk to the environment. This is the first IPM schedule developed against this major pest of cardamom with biological control as a component.

1. Introduction

Cardamom, *Elettaria cardamomum* (L.) Maton. (Zingiberaceae), a large perennial, herbaceous rhizomatous monocot is a high-value spice crop, ranked next to saffron and vanilla. It is mainly grown in India, Guatemala, Sri Lanka and other South East Asian and Central American countries. The spice is widely used for its flavour and medicinal value across the globe (Ravindran, 2002). Cardamom thrips, *Sciothrips cardamomi* Ramk. (Thysanoptera: Thripidae) is a major limiting factor in the production of cardamom. Adults and larvae of cardamom thrips suck the sap from shoots, panicles (inflorescences) and young capsules resulting in premature shedding of flowers and immature capsules and

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shrivelling and 'scab' formation on mature capsules (Fig. 1 A, B) with poorly developed seeds (Jacob et al., 2020). Under favourable conditions, the insect can damage 30–90 % of cardamom capsules resulting in 45–48 % crop loss in major cardamom producing countries (Dharmadasa et al., 2009; Gopakumar and Chandrasekar, 2002; Milian, 2014).

Current control measures targeting thrips management in cardamom are mainly based on synthetic chemical insecticides. Farmers often apply more than the recommended schedule of sprays to control the pest leading to environmental and pesticide residue issues, adversely affecting its domestic use and export (Beevi et al., 2014; Murugan et al., 2011b). Many of the chemicals used for thrips control in cardamom are also highly toxic to honey bees, the major pollinators of the crop (Murugan et al., 2011a). The rapid multiplication potential and the cryptic behaviour of the pest, feeding and sheltering within the leaf sheaths and flower bracts, lead to high population density during the crop-yielding period (Chandrasekar and Balu, 1993), making chemical control measures ineffective. Further, cardamom is grown in biodiversity hotspot regions of India, Guatemala, Sri Lanka and other countries and hence development of integrated pest management (IPM) strategies that would result in minimum damage to these ecologically fragile regions would be a prudent approach rather than relying exclusively on insecticides.

IPM schedules were not available earlier for cardamom thrips due to lack of information about potential natural enemies, especially microbial biocontrol agents, and reduced-risk insecticides. Microbial pesticides are gaining popularity in India and worldwide because they are less toxic to the environment, target specific and safe to non-target organisms and hence form an excellent component of IPM programmes (reviewed by Kumar et al., 2019). Among the microbial biocontrol agents, entomopathogenic fungi (EPF) are microorganisms that cause fatal diseases in insects (Gulzar et al., 2021; Skinner et al., 2014; Zhang et al., 2019). Isolation of native virulent strains of EPF against an insect pest is an essential step for including them in successful IPM programmes (Senthil Kumar et al., 2021). Until recently, no microbial biocontrol agent was available for incorporation in IPM schedules for the management of cardamom thrips. Recently, Senthil Kumar et al. (2015), reported natural infection of cardamom thrips by a native isolate of *Lecanicillium psalliotae* (Treschew) Zare & W. Gams (Ascomycota: Hypocreales) and demonstrated its pathogenicity to thrips in laboratory studies. The fungus was also found to possess multiple plant growth promoting traits (Senthil Kumar et al., 2018), making it an ideal component in IPM programme.

The identification of a potential biological control agent in laboratory studies prompted us to study the field performance of the fungus as spray and granular formulations against thrips. We also studied the compatibility of the fungus with commonly used insecticides and fungicides in cardamom for utilisation of the fungus along with other pest management options. An IPM package integrating the entomopathogen (*L. psalliotae*), and a reduced-risk insecticide (spinosad) that was found to be effective earlier (Jacob et al., 2015b) and their combinations along with the existing chemical and cultural (phytosanitation) methods as components were field tested for cardamom thrips management. This paper reports the first ever development of an IPM module for cardamom thrips management incorporating biological control as one of its components.

2. Materials and methods

2.1. Fungus source

Lecanicillium psalliotae (Treschew) Zare & W. Gams (Ascomycota: Hypocreales) strain IISR-EPF-02 (MTCC 25169) isolated from infected cardamom thrips during a survey in Wayanad District of Kerala, India in 2012 (Senthil Kumar et al., 2015), maintained at the Entomopathogenic Fungal Repository of Indian Council of Agricultural Research-Indian Institute of Spices Research (ICAR-IISR), Kozhikode, was used in the study. The molecular data of the conserved regions of the strain are available in the National Centre for Biotechnology Information (NCBI) database with accession numbers for internal transcribed spacer (ITS) region (KF358373), beta tubulin (TUB) (KF358374) and translation elongation factor (TEF) (KF358375) (Senthil Kumar et al., 2015).

2.1.1. Multiplication of fungus spores for field applications

For field applications, the fungus was mass multiplied on paddy grains (variety: *Kerala red rice*) purchased from the local market. Briefly, 75 g of paddy grains were soaked overnight in water, which gave a final weight of approximately 150 g through imbibition of water. The grains were washed in tap water, cooked for 10 min and crushed in a 750 W kitchen blender (MEENUMIX®, India), and then transferred to a 500 ml conical flask (BOROSIL®, India) and sterilized in a vertical autoclave (EQUITRON®, India) at 121 °C, 15 psi for 40 min. After cooling, 3 ml of fungal spore culture drawn after vortexing from 7 days old actively growing culture of *L. psalliotae* on potato dextrose broth (PDB) having a spore concentration of about 1×10^6 conidia/ml was added to the paddy media, mixed well and incubated in a Biochemical Oxygen Demand (BOD) incubator (REMI, India) at 25 ± 1 °C for 2 weeks, which yielded on an average of 1×10^8 cfu (colony forming units)/g.

2.1.2. Preparation of spore suspension and granular formulation

For spray purpose, fungal spore suspension was prepared by washing 2-week old fungal culture grown on paddy grains as described above. The paddy grain clumps were crushed with a sterile glass rod and 0.05%

Fig. 1. Healthy cardamom capsules (A) and capsules damaged by thrips with scab formation on the pericarp (B).

sterile aqueous Triton-X 100 (HIMEDIA®, India) was added, vortexed and the resulting spore suspension was filtered through a double layered muslin cloth. Spore concentration of the suspension was enumerated using a Neubauer improved haemocytometer (FEINOPTIK, Germany). Spore viability was checked prior to all the experiments and it was always higher than 95%. For soil application, 150 g of 2-week old fungus granules grown on paddy grains (1×10^8 cfu/g) was hand-mixed, wearing sterile gloves, with 3 kg of sterilized cow manure (sterilized twice at 121 °C, 15 psi for 1 h each time in a vertical autoclave (EQUITRON®, India)) and used for the experiments at the rate of 1 kg/plant.

2.2. Biocontrol trials

2.2.1. Study locations

The trials were conducted in two locations at two main cardamom producing states, Kerala and Karnataka in India: (i) Experimental Farm of the Regional Station of ICAR- IISR, Appangala (12°26′N, 75°45′E; 920 m MSL), Kodagu District, Karnataka, and (ii) M/s Chulika AVT Estate, Meppadi (11°50′N, 76°08′E, 855 m MSL), Wayanad District, Kerala during 2014 and 2015. The monthly rainfall received during the study period (March, April, May and August), ranged from 11.2 to 682.8 mm and 13.5 to 350.9 mm at the first location and 0 to 570.7 mm and 24.8 to 239.8 mm at the second location in 2014 and 2015, respectively. The monthly average minimum and maximum temperatures ranged from 16.2 to 19.1 °C and 24.6 to 33.1 °C in 2014 and 16.9 to 18.5 °C and 26.2 to 31.7 °C in 2015 in the first location. In the second location, the monthly average minimum and maximum temperatures ranged from 18.9 to 20.7 °C and 25.4 to 32.7 °C in 2014 and 19.7 to 21.1 °C and 26.6 to 31.7 °C in 2015 (Supplementary Table S1). The plants were irrigated with sprinklers during summer months (March to May) at fortnightly intervals.

2.2.2. Field evaluation

Field experiments were laid in a randomized complete block design (RCBD) with cardamom varieties, “Appangala-1” (3–4 years old) in Kodagu and “Green Gold” (4–5 years old) in Wayanad. A plant to plant spacing of 3 × 3 m was maintained in a plot of size 9 × 3 m. There were three plants (15–20 tillers/plant) per treatment and each treatment was replicated four times. A blank row of plants without treatment was maintained between treatments to avoid contamination. All other agronomic practices were followed (Ankegowda et al., 2015) except application of insecticides.

The treatments evaluated were: T1 (foliar spray of *L. psalliotae* fungus @ 1×10^5 spores/ml), T2 (soil application of fungus @ 50 g *L. psalliotae* granules with 1×10^8 CFU/g per plant), T3 (foliar spray of fungus and soil application of fungus), T4 (foliar spray of quinalphos 25% EC, 2.0 ml/L) and T5 (untreated control). For spray application treatments (T1 and T4), 500 ml of spray fluid was used per plant. The spray fluid for fungal application had 0.05% v/v Triton-X 100 as a surfactant. The plants were treated to the point of run off using a knapsack sprayer (ASPEE®, India). Treatments T2 and T3 received 1 kg of cow manure fortified with 50 g of *L. psalliotae* granules as described under Section 2.1.2, and this mixture was broadcasted in the root zone at the rate of 1 kg/plant, whereas all other treatments received 1 kg of well decomposed, powdered cow manure without the fungus. The treatments were applied during March, April, May and August each year. The plant basins were irrigated in summer and mulched with dry leaves to maintain soil moisture levels. All the treatments were applied in the morning before 10:30 a.m.

2.3. Compatibility of L. psalliotae with pesticides

Prior to developing an IPM strategy for management of thrips, the compatibility of *L. psalliotae* with commonly used insecticides and fungicides to manage insect pests and diseases in cardamom were evaluated under *in-vitro* conditions at their field recommended doses. The insecticides tested were: imidacloprid 17.8% SL (0.5 ml/L), fipronil 5% SC (1.0 ml/L), quinalphos 25% EC (2.0 ml/L) and spinosad 45% SC (0.3 ml/L) and the fungicides tested were: mancozeb 75% WP (2.0 g/L), copper oxychloride 50% WP (2.0 g/L), carbendazim 50% WP (1.0 g/L), metalaxyl 8% + mancozeb 64% WP (1.25 g/L) and carbendazim 12% + mancozeb 63% WP (1.0 g/L). The chemicals were added individually to 100 ml of autoclaved potato dextrose agar (PDA) media before solidification, vortexed and poured into petri dishes (100 × 17 mm) (BOROSIL®, India). Six mm disc of *L. psalliotae* obtained from 2-week old actively growing colony was inoculated at the centre of each plate and incubated in a BOD incubator at 25 ± 1 °C. The radial growth of the fungus was measured on 5, 7 and 10 days after inoculation (DAI). There were four replications per treatment and the experiments were conducted separately for insecticides and fungicides. Medium without pesticide inoculated with the fungus served as control. The experiment was repeated once to confirm the results.

2.4. IPM trials

An IPM strategy involving chemical insecticides and the biocontrol agent, *L. psalliotae* was tested under field conditions in 2016 and 2017 at M/s Chulika AVT Estate, Meppadi, Wayanad District, Kerala. During the study period, the total monthly rainfall received ranged from 2.5 to 230.2 mm and 164.3 to 500.9 mm in 2016 (March, April, May and August) and 2017 (May, June, August and September), respectively. The monthly average minimum and maximum temperatures ranged from 20.6 to 21.8 °C and 26.1 to 33.9 °C in 2016 and 20.7 to 21.2 °C and 26.2 to 30.6 °C in 2017, in the study location (Supplementary Table S1). Cardamom plants were irrigated with sprinklers during March to May at fortnightly intervals. The following treatments were tested in four replications : T1 (soil application of fungus alone, four times), T2 (single spray of quinalphos 25% EC, 2 ml/L followed by soil application of fungus, thrice), T3 (single spray of spinosad 45% SC, 0.3 ml/L followed by soil application of fungus alone, thrice), T4 (two sprays of spinosad 45% SC, 0.3 ml/L and soil application of fungus twice, alternatively), T5 (four sprays of quinalphos 25% EC, 2 ml/L), T6 (four sprays of spinosad 45% SC, 0.3 ml/L) and T7 (untreated control). The treatment, T1 received 1 kg of cow manure per plant fortified with the fungus in all the four applications, whereas the treatments T2 – T4 received 1 kg of cow manure per plant without the fungus during insecticide sprays alone, followed by fungus fortified cow manure during the subsequent applications. The treatments T5 – T7 uniformly received 1 kg of cow manure per plant without the fungus throughout the applications. The insecticides spinosad and quinalphos were selected for the study because spinosad was found effective in controlling thrips damage in our earlier studies (Jacob et al., 2015b) and quinalphos is a chemical approved by Central Insecticides Board & Registration Committee (CIBRC, 2021), Government of India, for the management of cardamom thrips. However, quinalphos was excluded in a combination treatment like T4 because it was found incompatible with *L. psalliotae* under *in-vitro* conditions. The treatments were applied during March, April, May and August in 2016 and during May, June, August and September in 2017 due to unexpected rainfall during March and April in 2017 leading to practical difficulties in conducting the trial. Nevertheless, all treatments were made in a total of four applications for consistency. All other experimental conditions like plot size and application parameters were the same as described under Section 2.2.2, unless otherwise specifically mentioned.

2.5. Assessment of thrips damage

Thrips damage in different treatments was assessed based on the percentage of capsules that developed scabs on the pericarp subsequent to thrips feeding following the procedure of Jacob et al. (2020) with modifications. Briefly, mature capsules were harvested treatment-wise

during July, September and December and cured following standard procedures (Ankegowda et al., 2015). The capsules were stored in polythene bags at ambient room temperature until final harvest for further observations. From each treatment, five random capsule samples of 50 g each (~250 capsules) were drawn after pooling the capsules from all the harvests. The total number of capsules and the number of capsules with scabs were visually recorded replication-wise to calculate mean percent capsule damage in a treatment.

2.6. Yield estimation

Yield was recorded treatment-wise during each harvest at Wayanad and mean dry weight yield per plant was calculated as it is impractical to cure the capsules in a mechanical dryer at low volumes. Assessment of yield in the trials carried out in Kodagu was not done because the plants had not reached their potential yielding capacity.

2.7. Statistical analyses

Per cent capsule damage data were normalized by arc sine $[(x + 0.5)/100]^{1/2}$ or square root transformation prior to analysis based on the range of percent values (Gomez and Gomez, 1984). Field trial data were subjected to one-way analysis of variance (ANOVA) using the general linear model (GLM) and the data on colony diameter were analysed by two-way ANOVA and mean comparisons were done by Tukey's honest significant difference (HSD) test ($\alpha = 0.05$). All analyses were carried out in GraphPad Prism® Version 7.0 for Windows, GraphPad Software, La Jolla, California USA and RStudio Version 1.4.1106.

3. Results

3.1. Biocontrol trials

Results of the field trials conducted in 2014 indicated significant differences among treatments compared to control at Kodagu (location 1) ($F = 14.27$; $df = 4, 16$; $P < 0.001$) and Wayanad (location 2) ($F = 16.72$; $df = 4, 16$; $P < 0.001$). However, there were no differences between the test locations ($t = 1.52$; $df = 4$; $P = 0.2025$). At both locations, soil application of the fungus alone (T2) reduced the capsule damage significantly compared to control but the efficacy was not profound as the insecticide (T4) in the first location, whereas T2 was comparable with T4 in the second location (Fig. 2 A, B).

In 2015, there were also significant differences between the treatments at Kodagu ($F = 20.287$; $df = 4, 16$; $p < 0.001$) and Wayanad ($F = 20.695$; $df = 4, 16$; $p < 0.001$), with slight difference in efficacy of treatments between the two locations ($t = 3.71$; $df = 4$; $P = 0.0206$). The treatments, T2 and T3 were equally effective to T4 in both locations (Fig. 2 C, D). Across locations and years, the fungus spray alone (T1) did

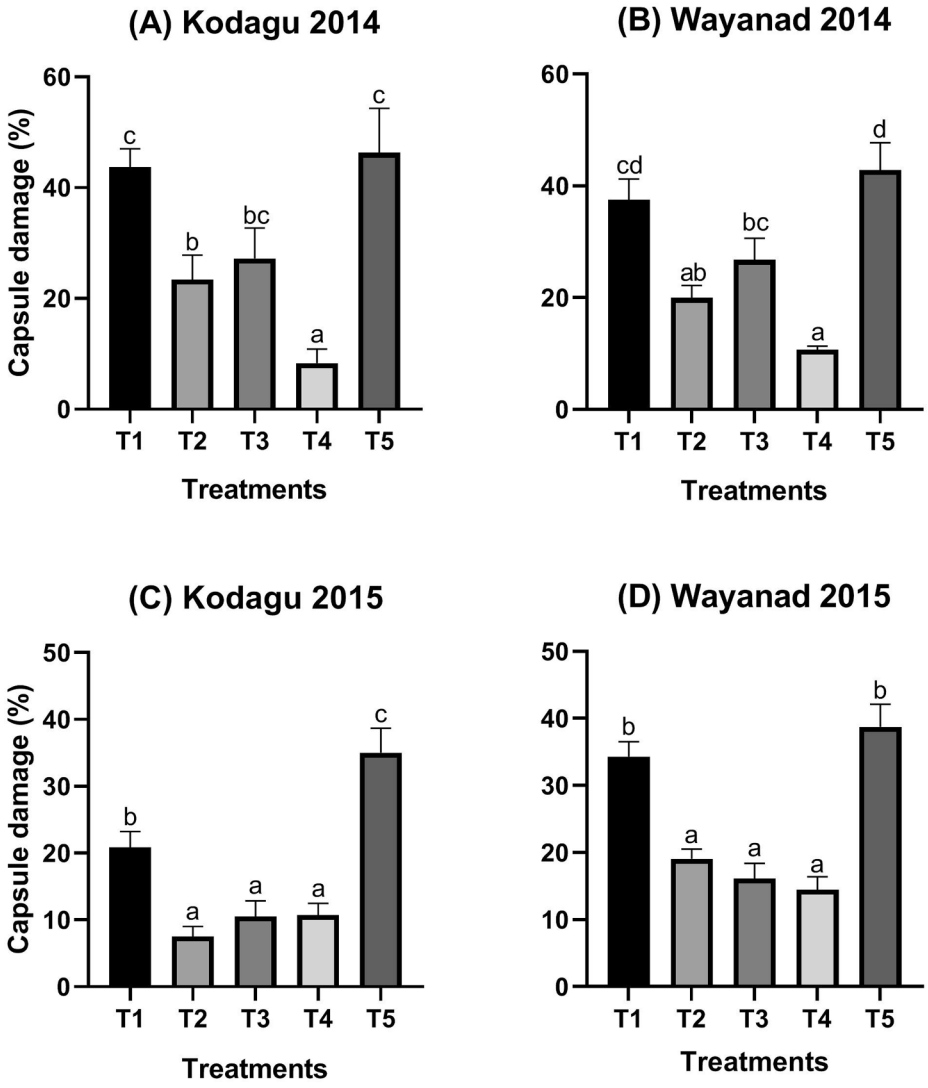

Fig. 2. Field evaluation of *Lecanicillium psalliotae* for its efficacy against *Sciothrips cardamomi* at Kodagu and Wayanad in 2014 (A & B) and 2015 (C & D). T1 = Foliar spray of fungus (1×10^5 spores/ml); T2 = Soil application of fungus (50 g *L. psalliotae* granules @ 1×10^8 cfu/g); T3 = Foliar spray and soil application of fungus; T4 = Foliar spray of quinalphos 25% EC (2 ml/L); T5 = Control. All applications were made four times. Bars represent original data. Data were analyzed after arc sine $[(x + 0.5)/100]^{1/2}$ transformation. Bars represented by the same letter are not significantly different by Tukey's HSD ($\alpha = 0.05$). Error bar represents standard error (SE) of five replicates.

not reduce damage except at Kodagu in 2015 (Fig. 2 C); there was no difference between T2 and T3, suggesting the foliar spray was not effective.

3.2. Compatibility of L. psalliotae with pesticides

In-vitro studies conducted to assess the compatibility of *L. psalliotae* with insecticides indicated that the mean colony diameter of the fungus differed significantly between the treatments at 5 DAI ($F = 17.85$, $df = 4, 15$; $P < 0.0001$), 7 DAI ($F = 50.68$, $df = 4, 15$; $P < 0.0001$) and 10 DAI ($F = 36.44$, $df = 4, 15$; $P < 0.0001$). At 5 DAI, quinalphos (T3) significantly reduced the growth of the fungus. However, none of the other insecticides tested significantly inhibited the growth of the fungus (Fig. 3 A). At 7 DAI, the growth of the fungus was significantly inhibited by quinalphos (T3), whereas maximum growth was observed in spinosad (T4) treated plates followed by other treatments, fipronil (T2), imidacloprid (T1) and control (T5) that were on par (Fig. 3A). At 10 DAI, other than quinalphos (T3), imidacloprid (T1) also reduced fungal growth and fipronil (T2) had no difference compared to control (T5) and the fungal growth was highest in spinosad (T4) treated plates (Fig. 3 A).

Fungicides were also found to significantly influence the growth of the fungus ($F = 311$, $df = 5, 18$, $P < 0.0001$, $F = 236.60$, $df = 5, 18$, $P < 0.0001$ and $F = 138.40$, $df = 5, 18$, $P < 0.0001$ at 5, 7 and 10 DAI, respectively). At all observation times, except copper oxychloride (T2) that had increased fungal growth, all other fungicides inhibited the growth of the fungus, with carbendazim + mancozeb (T5), mancozeb (T1) and metalaxyl + mancozeb (T4) having the highest inhibition followed by carbendazim (T3) (Fig. 3 B).

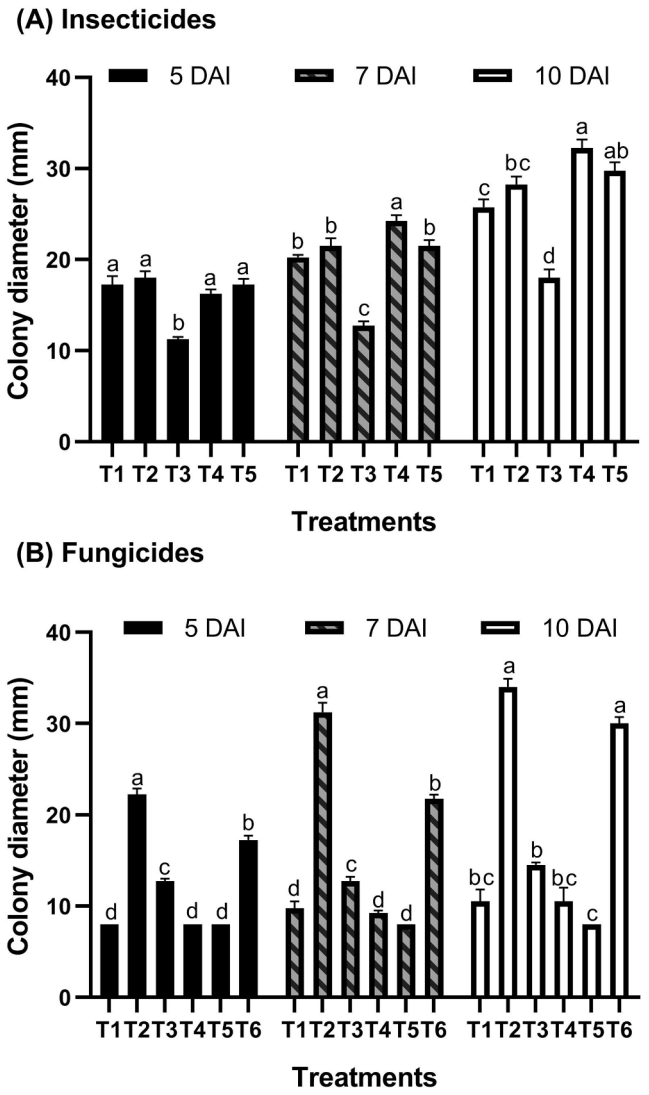

Fig. 3. Effect of (A) insecticides (T1 = Imidacloprid 17.8% SL (0.5 ml/L); T2 = Fipronil 5% SC (1.0 ml/L); T3 = Quinalphos 25% EC (2.0 ml/L); T4 = Spinosad 45% SC (0.3 ml/L); T5 = Control) and (B) fungicides (T1 = Mancozeb 75% WP (2.0 g/L); T2 = Copper oxychloride 50% WP (2.0 g/L); T3 = Carbendazim 50% WP (1.0 g/L); T4 = Metalaxyl 8% + Mancozeb 64% WP (1.25 g/L); T5 = Carbendazim 12% + Mancozeb 63% WP (1.0 g/L); T6 = Control) on the *in-vitro* growth (Mean ± SE) of *Lecanicillium psalliotae* at 5, 7 & 10 days after inoculation (DAI). Error bars represent standard error (SE) of four replicates. Bars represented by the same letter are not significantly different by Tukey's HSD ($\alpha = 0.05$).

3.3. IPM trials

In the IPM trials conducted at Wayanad, all the treatments significantly reduced thrips damage compared to control ($F = 61.14$; $df = 6, 24$; $P < 0.001$ and $F = 121.32$; $df = 6, 24$; $P < 0.001$) in 2016 and 2017, respectively. In 2016, all the fungus-insecticide combination treatments (T2-T4) recorded the lowest capsule damage by thrips to and was comparable to spinosad sprays (T6). This was followed by soil application of fungus alone (T1), which was comparable with the spray application of quinalphos (T5) (Fig. 4 A). However, in the second-year trial (2017), all the treatments (T1-T6) were on par with each other in reducing the thrips damage on capsules compared to the untreated control (T7) (Fig. 4 B).

3.4. Yield

During the trials conducted at Wayanad in 2014 and 2015, highest mean capsule dry weight yield per plant was recorded in plants treated with soil application of the fungus (T2) (463 and 583 g/plant, respectively), which was followed by the combination treatment (T3) (spray and soil application of the fungus). All the other treatments recorded higher yield than control during both the seasons (Supplementary Fig. S1 A & B). In IPM trials, all the treatments resulted in higher yields compared to control. In 2016, the yield was higher in plants treated with single spray of quinalphos followed by three rounds of soil application of the fungus (T2) (570 g/plant) and soil application of the fungus alone (T1) (487 g/plant) (Supplementary Fig. S1 C). In 2017, the yields were lower in all the treatments compared to previous year. Plants treated with soil application of the fungus alone (T1), recorded the highest yield (333 g/plant), followed by other treatments, which were higher compared to control (Supplementary Fig. S1 D).

4. Discussion

In our field trials, foliar application of the fungus was not effective in controlling the thrips damage to capsules as compared to soil application of fungus granules. Though the fungal spore concentration at 1×10^5 conidia/ml was effective under laboratory conditions (Senthil Kumar et al., 2015), it did not deliver the same results in the field since laboratory results may not always translate into field conditions (Zhao et al., 2021). Moreover, in field or glasshouse trials, inundative application of EPF involves application of at least 10^{13}–10^{14} propagules/ha (Wraight and Carruthers, 1999) for the insect to acquire sufficient spores for pathogenesis. Many of the earlier works (reviewed by Jaronski, 2010) suggest that the planar spore concentration need to be increased many folds than the LD_{50} values for higher fungal efficacy. In comparison to the earlier works, we have used a lower spore concentration (1.0×10^{11} spores/ha) for foliar treatment. Another plausible explanation would be that thrips surviving within the leaf sheath and flower bracts might have been protected from coming in direct contact with fungal spores.

Increase in control efficiency of soil applications compared to spray application of the fungus can be attributed to the fact that cardamom thrips pupate in soil and hence targeting the pupal stages of the pest

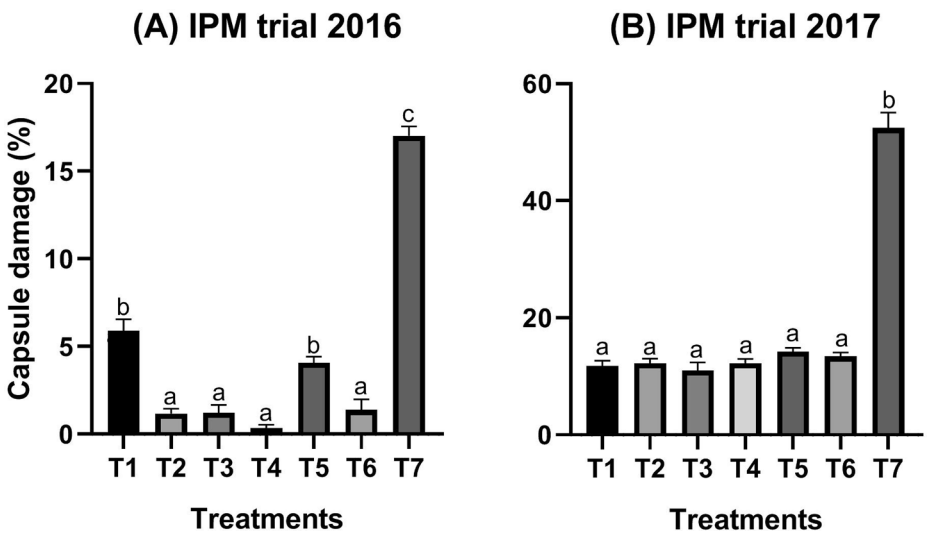

Fig. 4. Field evaluation of IPM treatments for the management of *Sciothrips cardamomi* at Wayanad during (A) 2016 and (B) 2017. T1 = Soil application of fungus alone four times; T2 = Single spray of quinalphos 25% EC (2 ml/L) followed by soil application of fungus thrice; T3 = Single spray of spinosad 45% SC (0.3 ml/L) followed by soil application of fungus alone thrice; T4 = Two sprays of spinosad 45% SC (0.3 ml/L) and soil application of fungus twice alternatively; T5 = Four sprays of quinalphos 25% EC (2 ml/L); T6 = Four sprays of spinosad 45% SC (0.3 ml/L) and T7 = Control. Bars represent original data. Data were analyzed after (A) square root or (B) arc sine $[(x + 0.5)/100]^{1/2}$ transformation. Bars represented by the same letter are not significantly different by Tukey's HSD ($\alpha = 0.05$). Error bar represents standard error (SE) of five replicates.

could have substantially reduced the damages due to larval and adult stages as evidenced in other thrips species (Zhang et al., 2019). Further, in the case of cardamom plants with prostrate panicles, even the adult stage of the thrips could be exposed to EPF infection because the panicles lie in close contact with the soil surface (Jacob et al., 2020). Earlier studies using granular formulations of EPF targeting the soil stage of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) had shown promising results (Skinner et al., 2012; Zhang et al., 2019). Though spray applications are generally targeted towards the aerial stages of the insects, soil application of EPF would be an ideal application strategy for insects with susceptible soil phase (Ansari et al., 2008; Lee et al., 2017; Skinner et al., 2014).

In general, EPF survive as weak saprophytes in soil and their population tend to decline in the absence of a susceptible host (reviewed by Lacey et al., 2015). During its saprophytic phase, the fungi proliferate as hyphae on organic matter in the soil (Boomsma et al., 2014), which provides additional nutrition for fungal growth (Clifton et al., 2015). We mixed *L. psalliotae* granules in cow manure prior to its field application since cow manure has been reported as a favourable growth media for this fungus (Domsch et al., 1980). Further, paddy grains act as a source of nutrients promoting conidial germination and growth ensuring the longevity and persistence of EPF in the soil (Zhao et al., 2021). Hence, soil application of fungus through well decomposed cow manure and the organic matter rich cardamom soils might have provided a nutritive medium for the fungus to germinate, grow and re-sporulate in the soil providing a better protection than foliar application. This supports the earlier reports that application of granular formulations in soil could be a more economical, practical and environmentally friendly with higher persistence than spray application (Jaronski, 2010; Skinner et al., 2014; Zhao et al., 2021).

Prior to including an EPF in any IPM strategy, it is necessary to understand the compatibility of other IPM components, especially insecticides and fungicides with the EPF, to schedule their operations (Skinner et al., 2014). Studies on compatibility of pesticides with EPF had given varying results under *in-vitro* and field conditions (Sardrood and Goltapeh, 2018). In our laboratory tests, some insecticides were found to influence the colony growth of *L. psalliotae*. Among the insecticides tested, only quinalphos significantly inhibited the fungal growth, and imidacloprid showed a negative effect only at 10 DAI; fipronil and spinosad were compatible with the fungus. Earlier studies reported that quinalphos (Raj et al., 2011), fipronil and imidacloprid were incompatible with *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin (Ascomycota: Hypocreales) (Sain et al., 2019). However, the negative effects seen in the laboratory studies could be less pronounced in the field as evident from our IPM trials. In the present study, the highest colony growth was observed in spinosad. Earlier reports also indicated that spinosad was compatible with *M. anisopliae* (reviewed by Sardrood and Goltapeh, 2018). The increase in growth of the fungus in the presence of an insecticide could be due to the direct uptake of nutrients from the formulation or by uptake of secondary nutrients by metabolizing the insecticide (Moino and Alves, 1998; Sain et al., 2019).

Though we did not include fungicides as a treatment in our field trials, we tested their compatibility with *L. psalliotae* in the laboratory since they are generally applied in the cardamom field before and after the rainy season as a prophylactic measure to prevent infection by various diseases. In the present study, except copper oxychloride, all other fungicides were found to inhibit fungal growth under *in-vitro* conditions. Earlier studies also reported that copper oxychloride was compatible with *A. lecanii* (Khalil et al., 1985; Olmert and Kenneth, 1974) and *B. bassiana* (Durán et al., 2004). It was also reported that copper oxychloride promotes mycelial growth of *B. bassiana* (Challa and Sanivada, 2014), which is also evident in our studies. A possible reason might be due to the siderophore producing ability of *L. psalliotae* (Senthil Kumar et al., 2018), which helps in assimilation of copper and other metals (Carrillo-Castañeda et al., 2005). All other tested fungicides, carbendazim, mancozeb, carbendazim + mancozeb and metalaxyl + mancozeb were found to inhibit the growth of *L. psalliotae*, which was also reported in other entomopathogenic fungi (reviewed by Sardrood and Goltapeh, 2018).

An important factor that needs to be considered before developing an IPM strategy in cardamom is that the crop is highly cross pollinated and honey bees (*Apis cerana* Fab., and *A. dorsata* Fab. (Hymenoptera: Apidae) are its principal pollinators. The crop continuously flowers from mid-March to November, having its peak flowering season from mid-May to August coinciding with the monsoon season (Kuriakose et al., 2009). Hence chemical control measures aimed at targeting the thrips population should be carried out with utmost care after the onset of the flowering season. It was evident from our IPM trials that initial sanitization of thrips population with an insecticide like spinosad or quinalphos followed by three rounds of soil application of the fungus or alternate application of insecticide (spinosad) and fungus can result in effective control of thrips. Warm weather during the pre-monsoon period (February–April) aid in rapid multiplication of thrips population (Chandrasekar and Balu, 1993; Singh et al., 1999). Hence the strategy of using insecticides initially, when the thrips population starts to build up will help to bring down the population drastically, thereby increasing the efficiency of the EPF leading to lower levels of capsule

damage by the insect, further safeguarding the bees and the environment from synthetic insecticides. Fungal application preceded with spinosad was reported to improve the performance of the EPF against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (Rivero-Borja et al., 2018). Further, synergistic activity of spinsosad, a biorational insecticide with entomopathogenic fungi such as *M. anisopliae* and *B.bassiana* have also been reported (Ericsson et al., 2007; Rivero-Borja et al., 2018). Therefore, spinosad is preferred over quinalphos for incorporation in IPM programmes against cardamom thrips.

There was an increase in yield in our biocontrol trials in treatments where the fungus was applied in the root zone of cardamom plants. This fungus has been reported to possess multifarious plant growth promoting traits (Senthil Kumar et al., 2018), that might have contributed to the increased yield in treated plants. Our IPM trials also registered higher yields in plants that received soil application of the fungus. However, there were fluctuations in yield during the study period. For instance, in the second year of the IPM trial, the yield was low in all the treatments compared to the previous year, probably because of heavy rains during the harvesting season, which led to rotting of capsules as they lie in close contact with the soil adversely affecting the yield. Cardamom crop yield is known to be influenced by several biotic as well as abiotic factors including rainfall (Murugan et al., 2012).

Our findings offer scope for integrated management of cardamom thrips with reduced risk to the environment integrating the existing cultural method (phytosanitation) with chemical (spinosad or quinalphos) and biological control (soil application of *L. psalliotae*). This is the first IPM strategy developed against this major pest of cardamom with biological control as a component. With increase in global concern about the harmful effects of pesticides, adopting an IPM package with fewer rounds of insecticide application will lead to clean production of cardamom. Further studies in this direction will aim at studying the long-term persistence of the EPF applied in soil and also establishing this pathogen as an endophyte in cardamom to induce systemic resistance against the pest (Senthil Kumar et al., 2018). Development of agronomically superior varieties from identified cardamom thrips resistant lines (Jacob et al., 2020) and exploitation of W*olbachia* endosymbiont in thrips (Jacob et al., 2015a) will further strengthen the strategy for managing this persistent menace effectively.

CRediT authorship contribution statement

C.M. Senthil Kumar: Conceptualization, Funding acquisition, Methodology, Data curation, Writing – original draft, Writing – review & editing, Validation. **T.K. Jacob:** Funding acquisition, Writing – original draft, Validation. **S. Devasahayam:** Writing – review & editing, Validation, Supervision, Resources. **Sharon D'Silva:** Investigation. **C. Geethu:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2021.104822.

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