Egyptian Journal of Biological Pest Control

RESEARCH Open Access

Lecanicillium lecanii (Zimmermann) Zare & Gams, as an efficient biocontrol agent of tea thrips, *Scirtothrips bispinosus* Bagnall (Thysanoptera: Thripidae)

Mariappan Sankara Rama Subramaniam[1], Azariah Babu[2*] and Bhabesh Deka[2]

Abstract

Background: Continuous and non-judicial application of synthetic insecticides to control the tea thrips, *Scirtothrips bispinosus* (Bagnall), one of the major tea pests in South India has led to certain undesirable issues in the ecosystem besides the presence of the pesticide residues in manufactured tea. Biological control agents are of immense importance in tea cultivation. The present study was designed to isolate *Lecanicillium lecanii* (Zimmermann) Zare & Gama from the field-collected cadavers of the insects/mites infected by fungi of tea growing areas of Anamallais (Tamil Nadu, South India), and to evaluate their field bio-efficacy against the tea thrips.

Results: *Lecanicillium lecanii* isolated from the tea ecosystem had been formulated into a wettable powder (WP) formulation and evaluated against tea thrips under both laboratory and field conditions. Among the several media evaluated, the PDAY (Potato Dextrose Agar + 1% Yeast powder) was found to be the best suitable medium for the growth and germination of spores. Optimum conditions for the growth of *L. lecanii* were found in PDAY medium at the pH 6-7, temperature 25-30°C and 90-95% RH. Exposure to UV light for more than 30 min significantly inhibited the growth of the fungus. *Lecanicillium lecanii* at (1×10^7 spore/ha) was found significantly effective against thrips. Fungal development index (FDI) of *L. lecanii* + jaggery significantly differed than other treatments. *Lecanicillium lecanii* at 1500g (1×10^7 conidia/ml) mixed in 400 l of water was effective against the tea thrips. Addition of equal amount of jaggery with *L. lecanii* wettable powder in the tank mixture could increase the efficacy of the mycopesticide against tea thrips.

Conclusion: The powder formulation of *L. lecanii* was found safer to natural enemies present in the tea ecosystem. After fulfilling the requirements for its registration and label claim on tea, this strain of *L. lecanii* could be commercialized for the benefit of the tea industry for the management of tea thrips in an eco-friendly manner.

Keywords: Tea, *Scirtothrips bispinosus*, *Lecanicillium lecanii*, Mycopesticide, Thrips

* Correspondence: azariah.babu@gmail.com
[2]Department of Entomology, Tea Research Association, North Bengal Regional R&D Centre, Nagrakata, Jalpaiguri, West Bengal 735225, India
Full list of author information is available at the end of the article

Background

The immense expanse of tea ecosystem provides an uninterrupted food supply, stable, suitable sites, and favorable microclimate for the reproduction and survival of several insect and mite pests. Over one thousand pest species have been already reported on tea plants. Among the different pests, the tea thrips, *Scirtothrips bispinosus* (Bagnall) (Thysanoptera: Thripidae) is an endemic species of peninsular India (Chakraborty et al. 2019). In general, thrips are phytophagous, and they attack leaves, buds, flowers, and even fruits of different plants. Nymphs of thrips do more damage than adults (Kawai 1990) because they are numerous and less mobile. It completes its life cycle within 14-20 days and duration of development mainly influenced by different weather factors, particularly temperature. Nymphs used to feed in groups, mostly along the midrib of the matured leaf, young leaves, and buds (Babu and Muraleedharan 2010). Feeding by thrips causes lacerations of tissue and makes it appear as streaks. Leaf surface becomes uneven, curled, and matty and parallel streaks appear on either side of the midrib of buds as a result of feeding, and the streaks become prominently visible when the leaf unfolds (Sanap and Nawale 1987). Yellowing of leaf margins is the typical symptom of thrips infestation. Appropriate control measures have to be adopted for reducing the economic loss due to thrips infestation when the thrips population is around three thrips per shoot (Babu 2009). Different synthetic chemicals are being used for the control of this pest in south India, resulting in pesticide residue problem in made tea (Babu and Muraleedharan 2010; Azad et al. 2020). Several species of natural enemies were reported to regulate the population of thrips in different cropping systems. Furthermore, the intensive use of pesticides will adversely affect the natural enemies in tea ecosystem. Because of these problems, sustainable yield production by adopting pest management techniques in an eco-friendly way is gaining momentum.

Entomopathogenic fungi (EPF) are considered as an efficient biological control agent, with their broad host range. These fungi have the attractive feature of infection by contact, and the penetration mediated control (Nadeau et al. 1996). Approximately with 750 species over 100 genera, these fungi include a heterogeneous group were reported from various insects. Many of them proffer an immense potential in the management of pests (as *Beauveria* spp., *Metarhizium* spp., *Verticillium* spp., *Nomuraea* spp., and *Hirsutella* spp.) (Annamalai et al. 2016). A thorough assessment of those valuable fungi may show the way to lucrative utilization in microbial control programs (Burges 1998).

Lecanicillium lecanii (Zimm.) (Hypocreales: Cordycipitaceae) synonym *Verticillium lecanii* (Zimm.) commonly known as "white halo fungus" is known to cause mycosisin insects. As far as, the use of biopesticides in tea crops is a concern, most of the bio-pesticides are developed from other than tea ecosystems (outsourced) and are substandard as well. The use of such bio-pesticides has not been serving the purpose up to the mark and increasing the production cost, as well.

Therefore, the present study was designed to isolate *L. lecanii* from the soil of tea growing areas of Southern India and to study its field efficacy against the tea thrips by using its liquid formulations.

Methods

Collection and laboratory culture of tea thrips

Adults and nymphs of thrips were collected from the tea fields of experimental plots of UPASI (United Planters' Association of Southern India) (25.5° 55′ 0″ N, 87.5° 54′ 0″ E longitude) in India. Insects were released into a rearing chamber (a glass chimney of (15×16 cm^2) provided with young tea shoots (each of 3 leaves and a bud) kept in small glass vials containing water. The culture of thrips was maintained in different wooden cages and glass chimneys in laboratory conditions (26 ± 3 °C; 80 ± 3% RH; 17L:7D photoperiod) on a susceptible tea clone, UPASI 17. Fresh tea leaves were provided on every alternate day as food. The susceptible cultures of insects maintained for more than 30 generations (from F1 to F30 without exposure to any pesticides) were utilized for different bio-efficacy evaluations.

Isolation and identification of entomopathogenic fungi

Extensive surveys were carried out in the tea gardens of the Anamallais (Tamil Nadu, Coimbatore District, South India) during 2015-2016. The field-collected cadavers of the insects/mites infected with fungi were used for isolation. For isolation of entomopathogens, PDA (potato dextrose agar) medium sterilized at 121 °C, 15 psi for 20 min was used. To isolate the fungi, the field-collected insects were sterilized by 5% NaClO and in 70% ethanol for 3 min and rinsed with sterile water for many times.

The diseased specimens were crushed in a Petri dish in a sterile condition, and a portion was transferred to a culture plate that contained the chosen medium and kept under constant observation for monitoring growth and development. The organisms were sub-cultured after 5 days for obtaining the pure culture. From the pure culture, slants of individual culture were prepared, and morphological characteristics of conidia and mycelium were observed under the microscope. Following Atlas of Entomopathogenic Fungi, the initial identification of fungi was made (Samson et al. 1988). Finally, the identified specimens were confirmed by molecular analysis carried out at Agharkar Research Institute, Pune, Maharashtra, India.

Biomass and conidial production

The biomass of the fungal isolate was determined using 100ml of sterile PDA amended with 1% yeast media. The liquid medium was inoculated using 1ml of fungal spore suspension (1×10^7 spores/ml) and incubated at 25+1°C, 75±2% RH and 14L: 8D photoperiod for 7 days in a growth chamber. To enumerate the biomass production, culture was agitated robustly and filtered, using Whatman filter paper after 7 days of growth. The same was dried at 40-45°C in an oven until it reached a constant weight. The biomass production observations were recorded in triplicate independent experiments.

The amount of conidia produced by the fungal isolate was evaluated on the 5th- and 8th-day-old cultures. A fungal culture of 1 cm diameter was suspended in sterile distilled water (5 ml) containing Tween-80(0.05% v/v). The conidia were harvested using a camel hairbrush and homogenized the suspension for 10 min on a magnetic shaker. Using the hemocytometer, the conidia were quantified, and the average number per milliliter was determined by following the formula of Lipa and Slizynski's (1973):

$$C = (Cc)\ (4 \times 1060Df)/80$$

where C=number of conidia/ml, Cc=number of conidia counted, and Df =dilution factor.

Maintenance of fungal cultures and inoculum production

A loopful of inoculum from subcultured plates of *L. lecanii* was transferred to PDA and maintained as a pure culture. The fungus was cultured for 10 days at room temperature (26 ± 2°C) on sterilized PDA medium for laboratory studies. Using a small sterile metal spatula, conidia from the medium were harvested after complete sporulation and air-dried under laminar airflow, which was later stored in a small airtight screw cap vials (10 cm × 2.5 cm diameter) in the refrigerator at 4 °C for further studies. By plating technique, colony forming units (CFU) were estimated. After necessary serial dilutions, spore count was made under a phase-contrast microscope, using a double rolled Neubauer's hemocytometer. For further studies, the required dilutions were made to obtain the required concentrations.

Laboratory bioassays of *L. lecanii* against 2nd instar nymphs of thrips, *S. bispinosus*

A total of 10 individuals of thrips (2nd instar nymphs) were released onto the fresh tea shoots, and the cut ends of the shoots were wrapped together by a piece of wet cotton. These shoots were kept in Petri dish (9 cm dia.) containing a round strips of tissue paper at the bottom. Treatment details were T1—*L. lecanii* (3.75 g/l), T2—*L. lecanii* + jaggery (for enhancement of the germination of *L. lecanii*) (3.75 g/l each), T3—Biocatch (commercial formulation of *V. lecanii*) (3.75 g/l), T4—Biocatch (commercial formulation of *V. lecanii*) + jaggery (3.75 g/l each), T5—NKAE @ 5%, and T6—Quinalphos 25 EC (Organo-Phosphatic insecticide) (2.5 ml/l). Neem Kernel Aqueous Extract (NKAE at 5%) was prepared, and spraying was done as described by Babu et al. (2008a). Using a glass atomizer, the spray fluids (T1 to T6) were sprayed on the shoots. Tea shoots with the 10 individuals of thrips were sprayed by distilled water and kept as control, and the bioassays were conducted using 5 replications. The treated tea shoots were kept in the round plastic containers sealed with transparent tape. The experiments were monitored at 24 h interval for 4 days for the mortality, and the percentage of bio-efficacy was calculated, and the corrected mortality was obtained using Abbot's formula (Abbott 1925). Dead thrips were transferred to Petri dishes lined with moist filter paper for 5 days to observe mycosis for confirmation of their death by microscopic examination of mycelial growth on the surface of the thrips nymphs.

Assessment of the pathogenicity of *L. lecanii* conidial suspension

For assessment of the pathogenicity of *L. lecanii* strain against 2nd instar nymph of thrips, the prepared conidial suspensions of fungus were used by adopting the following different methodologies (direct spray, dipping, and leaf exposure methods).

Direct spray

A total of 10 individuals of 2nd instar nymphs of *S. bispinosus* were transferred on tea leaves kept in sterile Petri dish (9cm in diameter) and were sprayed by 3 ml conidial suspension of fungus (1×10^7 conidia ml^{-1}) using a fine atomizer.

Leaf exposure

A piece of tea leaf (4 cm^2) was placed in a Petri dish and was used for this experiment. Three milliliters of conidial suspension (1×10^7 conidia ml^{-1}) was sprayed on each side of the leaf. The treated leaf materials were dried for 20-30 min on a clean laboratory bench. After that these leaves were provided to thrips for feeding for 24 h.

Dipping

A leaf containing a group of thrips nymphs (10 numbers) was dipped in a 3 ml conidial suspension (1×10^7 conidia ml^{-1}) for 10 s and kept on a sterile Petri dish. After treatment, nymphs were maintained on a sterile Petri dish and were provided untreated leaves for feeding. Similarly, nymphs were provided by leaves treated with sterile distilled water containing a drop of Tween-80, served as control. The experiment was performed

with 5 independent replications. The treatments were prearranged in factorial design consisting of 4 conidial concentrations and 3 exposure methods, and all the treatments were maintained at 25±1 °C. Mortality of thrips was observed at 24 h interval for 4 days using stereomicroscope (Olympus 1220; ×10). Dead insects were transferred on a moist Petri dish to observe the fungal growth.

Multiple-concentration bioassays of *L. lecanii* against thrips, *S. bispinosus*

A liquid suspension of the fungal strain *L. lecanii* was prepared for bioassays. The conidial suspension was mixed in 20 ml of liquid culture (2% sucrose and 5% peptone) containing 1 mg of the talc powder in 50 ml of water taken in conical flasks and incubated for 48 h in a rotary shaker (100 rpm). Before the application, the produced conidia from the formulations were examined for viability by spreading 0.01 ml of the culture onto PDYA medium. A stock of 1×10^8 conidia/ml was prepared, and 5 different concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia/ml) of conidial suspension were prepared in an aqueous solution containing 0.05% (v/v) of Tween 80 as the method of Butt and Goettel (2000). These suspensions were sprayed on tea leaves containing 10 thrips. The leaves sprayed with Tween-80 (0.05%) solution alone served as control. The thrips mortality rates were recorded, normalized with the natural mortality as observed in control (Abbott 1925).

Fungal development index (FDI)

The level of growth for *L. lecanii* on the nymphs and adult thrips were assessed as FDI. All assays were rated daily either until sporulation of fungus on the insect host or eclosion of the adult thrips. Control was used to assess the quantity of nymphs, and adult thrips emerged from these assays. Each nymph was assessed separately, under a compound light microscope, and the stage of fungal development on the nymphs and adult was recorded using the FDI.

Micro-plot field study

To evaluate the efficacy of *L. lecanii* against tea thrips, a micro-plot field trial was conducted in Paralai Tea Estate, Valparai (Coimbatore District, Tamil Nadu, India), The Anamallais. During the study period, the meteorological data were recorded. Treatments included were (1) *L. lecanii* W.P. at 1500 g/ha, (2) *L. lecanii* + Jagerry at both 1500 g/ha, (3) Biocatch at 1500 g/ha, (4) Biocatch at 3000 g/ha, (5) NKAE at 5.0%, (6) the recommended standard insecticide (Quinalphos 25 EC) at 1000 ml/ha, and (7) untreated control. Plots with ETL above 3% were selected and labeled based on treatments and replications. A hand-operated knapsack sprayer was used for spraying. To completely drench the bushes, special care was taken for better coverage and control. Thrips populations were assessed at weekly interval by collecting 10 shoots (3 leaves and a bud) at random from 10 bushes at each plot. Data were analyzed and the corrected percentage efficacy calculated according to Handerson and Tilton (1955) formula.

$$\text{Corrected mortality}\% = 1 - [(\text{Ta}/\text{Cb}) \times (\text{Tb}/\text{Ca})] \times 100$$

where Tb and Cb represent the population density before treatment (pre-treatment) and Ta and Ca represent density after treatment in treated and control plots, respectively.

Large scale field study

Two large scale RBD field trials were carried out at estates in Paralai estate, Valparai (Coimbatore District, Tamil Nadu, India), The Anamallais (26° 54′ 0″ N, 88° 55′ 0″ E longitude), during two different seasons. The study was conducted in RBD (randomized block design) with 7 treatments, each replicated in 3 plots. Each plot contained 100 bushes. Spraying was carried out by maintaining a spray volume of 400 l/ha, as mentioned above in the micro-plot study. Both the pre- and post-treatment assessments were recorded as in a similar way described in the micro-plot study. Data were analyzed, and ANOVA was calculated.

Enhancement of virulence of *L. lecanii* with the addition of the additives

Seven commonly available vegetable oils were used to find out the impact on the condition and the growth of mycelia of *L. lecanii*. By following the poisoned food technique method, every 100 ml of sterilized PDA media was amended with 0.2% concentration (v/v) of selected vegetable oils, viz., groundnut oil (*Arachis hypogaea* L. Fabales: Fabaceae), sunflower (*Helianthus annuus* L. Asterales: Asteraceae) oil, coconut oil (*Cocos nucifera* L. Arecales: Arecaceae), paraffinic oil, neem oil (*Azadirachta indica* A. Juss. Sapindales: Meliaceae), and castor oil (*Ricinus communis* L. Malpighiales: Euphorbiaceae) under aseptic conditions. PDA medium without any oil was used as control. In a sterilized Petri dish, the media were poured for solidification. Using a cork borer, an isolate of *L. lecanii* grown on PDA for 2 weeks was cut into an 8-mm disk. The block was inverted and transferred onto the center of the vegetable oil + PDA-amended Petri dishes, placed gently on the surface of PDA, and then incubated at 25±1°C, 65 R.H. and a photocycle of 16 L: 8 D in a BOD incubator. Individual plant oil and the wetting agent were considered as a treatment, and each treatment was replicated 3 times.

The effectiveness of *L. lecanii* was determined by measuring the radial growth of *L. lecanii* on the 5th, 10th, and 15th days after inoculation. Colony growth, spore germination, and conidial production were studied.

Effect of *L. lecanii* on natural enemies of thrips

Laboratory bioassays were conducted to study the infectivity of the fungal isolate, *L. lecanii* on the selected natural enemies present in the tea ecosystem, viz., *Oligota pygmaea* S. (Coleoptera: Staphylinidae), *Stethorus gilvifrons* M. (Coleoptera: Coccinellidae), *Mallada boninensis* O. (Neuroptera: Chrysopidae), and *Neoseiulus longispinosus* E. (Mesostigmata: Phytoseiidae). The predators were collected from the tea gardens at The Anamallais and were reared in the laboratory. Larvae of the respective predators were used for the study. Experiments were carried out by using a spore concentration of (1×10^7 spores/ml) (1×10^7 CFU/g in case of talc powder). All the experiments were replicated 3 times using 10 insects per treatment. Test insects sprayed with 0.05% Tween-80 solution served as control. Observation on mortality, pupation, adult emergence, and mycosis were recorded. Different methodologies were adopted in respect to each experiment, viz., (1) dipping of larvae in talc formulation (dip larva method), (2) dipping of larvae in spore suspension, (3) allowing of larvae to crawl on dried spore suspension (dry film method), (4) spraying *L. lecanii* talc powder on the larvae, and (5) dipping of larvae in only 0.05% Tween-80 solution as a control.

Results

Isolation and identification of entomopathogenic fungi

Frequent surveys on the incidence of the insect mycopathogens were carried out in The Anamallais, Coimbatore District, Tamil Nadu. The cadavers of tea thrips collected throughout the survey, which was brought to the laboratory and isolated the fungi in PDA medium. A total of 6 strains were isolated, and among them, *L. lecanii* was found to be the most predominant and infecting tea thrips, *S. bispinosus*. Based on the microscopic observation and colony characteristics were identified and later their taxonomic determinations were confirmed at Agarkar Research Institute, Pune, India.

Biomass, conidial production, maintenance of fungal cultures, and inoculum production

Lecanicillium lecanii exhibited a significant variation concerning the time taken for spore yield, colony diameter, sporulation, and time taken to cover the given diet for mass production (Table 1). Colonies incubated for 10 days on PDA were white to cream, and thin cottony with reverse colorless to pale or deep yellow. The conidiophores of *L. lecanii* were erect and verticillate with loose whorls of phialides conidiogenous cells, which mostly awl-shaped and sometimes to some extent inflated at the base. Conidia were hyaline, single-celled, produced in slimy heads, and smooth-walled (Fig. 1). The results revealed, more than 90% of spore germination on the surface culture after 24 h cultivation, but the spore germination ratio estimated was only about 50-60% in the submerged culture, with a mean germination percentage of 3.6% (spore 96.4 ±3.5%, n=3) (Table 1).

Mycelial growth became noticeable on the nymphs 24 h after death. Quick augment of spore production occurred between the 3rd and 7th days. The highest spore production occurred after the 7th day. Sporulation on *S. bispinosus* was directly proportional to RH, and the lowest limit was 75%. However, *L. lecanii* isolated from thrips recorded a maximum spore yield (3.2×10^7) and took the minimum time (8.21±0.42 days) to cover the entire medium. Germination rate (GT_{50}) of *L. lecanii* was 9.5 h (Table 1).

Laboratory bioassays of *L. lecanii* against 2nd instar nymphs of thrips *S. bispinosus*

The present study highlighted the fact that the entomopathogenic fungus, *L. lecanii* (UPASI TRF strain) was capable of causing considerable mortality of thrips. The 2nd instar nymphs of the thrips were highly susceptible to the fungal pathogen (57.5% mortality). The mortality increased to 70% when jaggery was added (Fig. 2).

Assessment of the pathogenicity of *L. lecanii* conidial suspension

In this context, 3 different bioassays, namely, direct spray, dipping, and leaf exposure methods, were carried out to evaluate the efficacy of *L. lecanii* against 2nd instar nymphs of tea thrips. The cumulative percent mortality significantly varied with different bioassays with direct spray yielding high mortality (66% at 1×10^8 conidia/ml), followed by a dipping method (62% at 1×10^8 conidia/ml) and leaf exposure method (58% at 1×10^8 conidia/ml) (Table 2).

Table 1 Morphological and cultural characters of *Lecanicillium lecanii*

Parameters	Remarks
Color of conidia	White
Colony diameter on 10th day (mm)	19.0
Sporulation (1×107 conidia/plate)	1.5
Spore yield (g/10^7)	3.2
No. of days for sporulation	8.21±0.42
GT_{50}	9.5 h

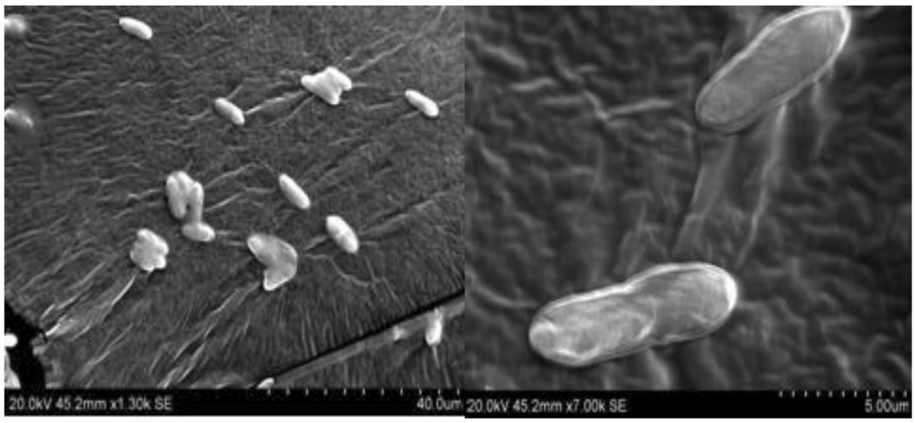

Fig. 1 Scanning electron micrographs of *Lecanicillium lecanii spores*. **a** Low magnification. **b** High magnifications

Multiple-concentration bioassays of *L. lecanii* against thrips, *S. bispinosus*

To find out the optimum dose of *L. lecanii* for the control of thrips, the multiple-dose bioassays were carried out against the 2nd instar nymphs and adult of *S. bispinosus*. The results showed that *L. lecanii* caused varying degrees of mortality of nymphs and adults (62 and 60) and (44 and 42) percent at the concentrations of (1×10^8 and 1×10^7 spore/ml), respectively. However, low concentration (1×10^5 spore/ml) caused only 46% mortality (nymphs) and 26% (adults) (Fig. 3).

Fungal development index (FDI)

Fungal development index (FDI) significantly differed with the treatments (DF 2, 27 F= 6.778 at p=5%) (Table 3 and Fig. 4). Among the 3 treatments, conidiogenesis occurred only in the treatment of *L. lecanii* with jaggery (FDI value: 2.6 on the 8th day), followed by *L. lecanii* with Tween-80 (FDI value 2.2). However, there was no marked difference between these two treatments. *Lecanicillium lecanii* alone showed the minimum FDI value of 1.7. The nymphs of *S. bispinosus* began succumbing to the fungus 2 days after spraying in all the treatments. Nymphal mortality was 100% (FDI value 1.5) in T1, followed by T2 and T3 (FDI value 1.7).

Micro-plot field study

Findings of the micro-plot experiment also revealed that the application of *L. lecanii* WP at 1500 g/ha and NKAE at 5.0% significantly reduced the population density of thrips than the untreated control (Fig. 5). Post-treatment assessment indicated that the density of thrips was significantly reduced, in plots treated with quinalphos and *L. lecanii* when compared to that of the untreated control. Between the treatments, the local strain was found to be more effective than the commercial formulation (Bio catch).

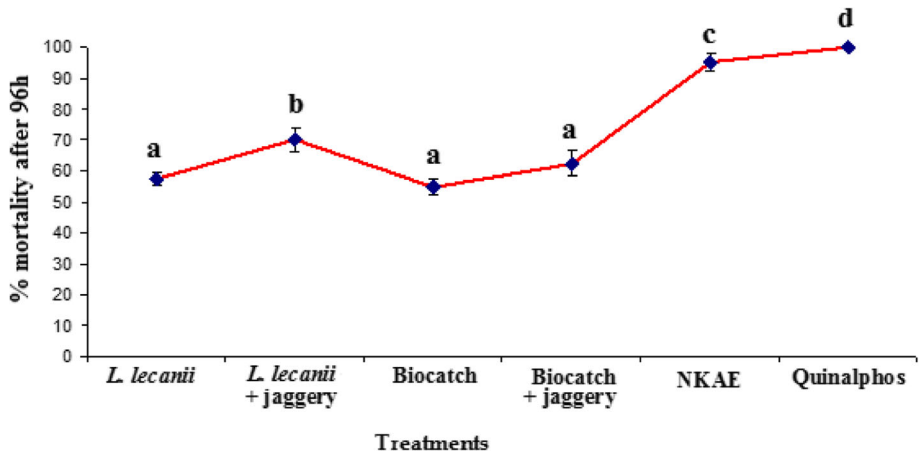

Fig. 2 Laboratory bio-efficacy of different treatments against tea thrips. Values represent cumulative percent mortality, means followed by the similar letter(s) in the columns are not significantly different at 5% by DMRT

Table 2 Different laboratory bioassays adopted against tea thrips nymphs

Bioassay type	Cumulative percent mortality after 96 h of infected thrips by *Lecanicillium lecanii* at different concentrations (conidia/ml)			
	1×10^5	1×10^6	1×10^7	1×10^8
Direct spray	48^b	54^a	62^a	66^a
Leaf exposure	32^a	48^a	56^a	58^a
Dipping	46^b	52^a	60^a	62^a
CD at 5%	3.10	2.74	1.83	2.74
SE (±)	7.14	6.32	4.21	6.32
CV (%)	14.74	10.67	6.15	8.83

Figures followed by the same alphabets in a vertical column are not significantly different at 5% level (DMRT *F* test, $p>0.05$)

Large-scale field study (season I)

This field study revealed that application of both *L. lecanii* W.P. and NKAE could significantly reduce thrips population. Pre-treatment population of thrips ranged from 251 to 260 individuals/75 shoots (Table 4). After 3 sprays at fortnight intervals, thrips population came down from 260 to 21 individuals in *L. lecanii* W.P. at 1500 g/ha; 258 to 13 individuals in *L. lecanii* W.P. + Jaggery at 1500 g; 251 to 20 individuals in *L. lecanii* W.P. at 1500 g/ha + Tween 80; 260 to 32 individuals in NKAE at 5%; 253 to 20 individuals in NKAE at 7.5%, and 250 to 11 individuals in Quinalphos at 1000 ml/ha.

Large-scale field study (season II)

In this study, before spraying, the thrips population was counted which ranged between 376 and 384/75 shoots (Table 5). But their densities declined after the spraying, in chemical insecticides, biopesticide NKAE, and *L. lecanii* treatments than the untreated control. However, there was a significant difference in the efficacy among all the treatments. A decrease in the thrips population was higher in *L. lecanii* + Jaggery, *L. lecanii* + Tween-80, and quinalphos treatments. Addition of jaggery to the *L. lecanii* W.P. yielded better results.

Enhancement of virulence of *L. lecanii* with the addition of the additives

During the first 5 days, maximum mycelial colony diameter of 11.33 mm was recorded in neem oil-amended PDA, followed by 10 mm in coconut oil (Table 6). The lowest results were obtained by groundnut oil and paraffinic oil, which were not compatible in comparison with the control. Similarly, during the 5th and 10th days, the maximum mycelial growth in terms of cumulative growth of colony diameter of 20.33 mm was observed in neem oil, indicating high compatibility with the fungus, followed by coconut oil and castor oil, which were significantly different from each other. Still, the cumulative mycelia growth was significantly more than the control. Sesame oil was non-compatible with the fungus, showing slow growth rate. At the 15th day, neem oil was found to enhance mycelial growth to the maximum: 22 mm, followed by 21.33 mm in coconut oil and 21 mm in groundnut oil vs. 18 mm in control. The spore germination was high in neem oil (93%) and coconut oil (92.33%) and control (91%). The spore germination was

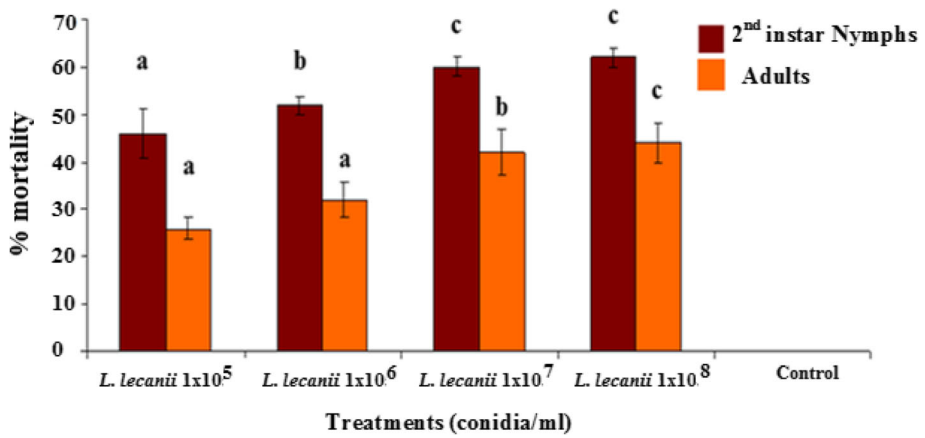

Fig. 3 Laboratory bio-efficacy of different concentrations of *Lecanicillium lecanii* against tea thrips *Stethorus bispinosus* (after 96 h). Values represent cumulative percent mortality, Means followed by the similar letter(s) in the columns are not significantly different at 5% by DMRT

Table 3 Comparison of fungal development index (FDI) of conidial treatments *Lecanicillium lecanii* infecting the 2nd instar nymphs of *Scirtothrips bispinosus*

T. no.	Treatments	FDI value*							
		1	2	3	4	5	6	7	8
T1	*L. lecanii*	0±0.0^{a}	0.0±0.00^{a}	0.3±0.08^{a}	0.6±0.12^{a}	0.8±0.15^{a}	1.2±0.21^{a}	1.5±0.22^{a}	1.7±0.26^{a}
T2	*L. lecanii* + Jaggery	0±0.0^{a}	0.1±0.05^{a}	0.6±0.12^{b}	1.0±0.07^{b}	1.3±0.13^{b}	1.7±0.11^{b}	2.1±0.10^{b}	2.6±0.10^{b}
T3	*L .lecanii* + Tween 80	0±0.0^{a}	0.1±0.05^{a}	0.6±0.10^{b}	1.0±0.05^{b}	1.4±0.08^{b}	1.7±0.08^{b}	2.0±0.11^{b}	2.2±0.11^{b}

*Means followed by the same letter in a column are not significantly different (DMRT *F* test, $p>0.05$), $n=10$ nymphs assessed for each fungal strain at each daily period under a 16:8 L: D photoperiod

low in paraffinic oil (79.33%). An observation on the conidial spore showed the highest of 94.33×10^{7} ml^{-1} in coconut oil. The lowest spore count of 76×10^{7} ml^{-1} was recorded in groundnut oil amended PDA.

Effect of *L. lecanii* on natural enemies of thrips

Effect on O. pygmaea

Larval mortality (0 to 6.67%), larval period (6.00 to 6.67), pupal period (15.00 to 15.33 days), and adult emergence (80.00 to 83.33%) were non-significant among the treatments (p=0.05) (Table 7). Although larval mortality was observed in all treatments (except T3), none of them was due to mycosis.

Effect on S. gilvifrons

In *S. gilvifrons*, no larval mortality was observed (Table 8). There was non-significant variation on the larval period, pupal period, and adult emergence after *L. lecanii* treatment and untreated control, which revealed that *L. lecanii* was harmless to *S. gilvifrons*.

Effect on M. boninensis

Larval mortality (10 to 13.33%), larval period (13.33 to 14.33), pupal period (13.33 to 14.33 days), and adult emergence (93.33 to 97.67%) were non-significant among the treatments (p=0.05) (Table 9). In none of the cases, larval mortality, mycosis occurred. Thus, *L. lecanii,* either as formulation or unformulated spore suspension, did not have any harmful effect of *M. boninensis*.

Effect on N. longispinosus

Lecanicillium lecanii did not cause any nymphal mortality on *N. longispinosus*, and non-significant variation observed in larval period, and adult emergence (Table 10).

Discussion

Lecanicillium (*Verticillium*) *lecanii*, a ubiquitous fungal species and was described by Zimmermann (Zimmermann 1898), on coffee scale insects. This species was competent in infecting a wide variety of insect hosts from wide-ranging agro-climatic conditions and geographical locations. Similar to all microorganisms, entomopathogenic fungi have explicit biological distinctiveness that influences their action in the environment (Parker et al. 2003). To opt for the fungal pathogen for management of thrips, it is indispensable to study simple essential characteristics that are requisite to exterminate the target insects under both laboratories as well as in field conditions (Moore and Prior 1993).

Verticillium lecanii, a most often isolated species which forms cylindrical conidia of white colonies in awl-shaped phialides. It refers to a collective of minute species (Gams 1971). It is a facultative parasite but does not act as a pathogen of mammals (Samson and Rombach

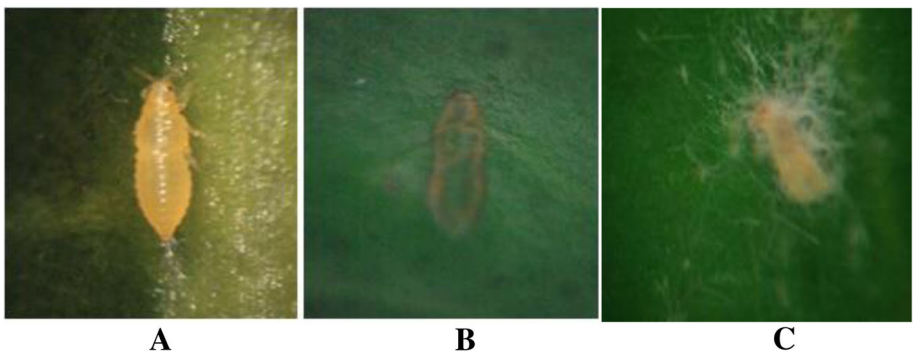

Fig. 4 **a** Live nymph (day 1). **b** Mummified nymph (day 3). **c** Sporulation on nymph (day 7)

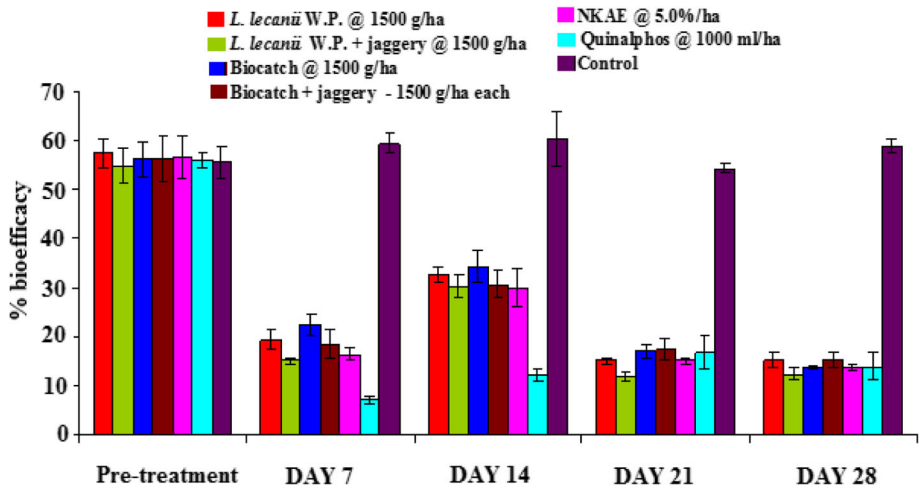

Fig. 5 Micro plot field trial on *Lecanicillium lecanii* against tea thrips *Stethorus bispinosus*

1985). To initiate infection, fungus requires temperatures between 15 and 25°C and RH of a minimum of 85% for 10-12 h per day (Hall and Burges 1979). It can grow by pre-entering the hosts inter segmentally, possibly on ex-natural orifices (Samson and Rombach 1985).

Cortez-Madrigal et al. (2003) studied morphological, physiological comparison of 28 isolates of *L. lecanii* to identify the most effective strain, and the isolates with a high conidial germination rate has been considered for utilization in biocontrol. The rapid growth and a high sporulation rate are necessary for successful and cost-effective production of entomopathogens for biological control (Jenkins et al. 1998). These results showed the viability of this fungus to be used for the development of a suitable bio-inoculant for mass production. Culture conditions can very much influence the longevity, virulence, and optimum ecology of the resultant propagules; hence, they can be manipulated to enhance myco-insecticide efficacy. Vilas-Bôas et al. (1998) noticed variations in sporulation and growth of a variety of entomopathogens on various solid culture media.

During laboratory bioassays of *L. lecanii* against *S. bispinosus*, the mycelial growth was noticed on the nymphs within 3-4 days after application. Similar results were reported by Annamalai et al. (2016) on *Thrips tabaci* L.

Table 4 Large scale field bio-efficacy trial (RBD) of *Lecanicillium lecanii* against thrips (season I)

T. no.	Treatments	No. of thrips/75 shoots							
		Pre-treatment	After I spray		After II Spray		After III spray		
			Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 21	Day 28
T1	*L. lecanii* @ 1500 g	260 (28.07)	72 (14.97)^{c}	156 (21.82)^{c}	76 (15.39)^{a}	156 (21.82)^{a}	28 (9.64)^{b}	32 (10.17)^{a}	30 (9.83)^{a}	21 (8.44)^{ab}
T2	*L. lecanii* + Jagerry @ 1500 g	258 (27.97)	46 (12.10)^{b}	131 (20.04)^{abc}	53 (12.88)^{a}	135 (20.34)^{a}	12 (6.63)^{a}	29 (9.60)^{a}	22 (8.49)^{a}	13 (6.88)^{ab}
T3	*L. lecanii* + Tween 80 @1500 g	251 (27.51)	57 (13.34)^{bc}	143 (20.87)^{bc}	55 (13.12)^{a}	142 (20.80)^{a}	17 (7.41)^{a}	26 (9.00)^{a}	24 (8.70)^{a}	20 (8.28)^{ab}
T4	NKAE @ 5%	260 (28.08)	53 (12.93)^{bc}	128 (19.71)^{abc}	63 (13.90)^{a}	151 (21.30)^{a}	29 (9.76)^{b}	33 (10.39)^{a}	35 (10.60)^{a}	32 (10.16)^{b}
T5	NKAE @ 7.5%	253 (27.71)	50 (12.60)^{b}	107 (18.14)^{ab}	128 (19.81)^{b}	180 (23.43)^{a}	20 (8.11)^{ab}	26 (9.26)^{a}	27 (9.43)^{a}	20 (8.28)^{ab}
T6	Quinalphos 25 EC @ 1000 ml	250 (27.54)	29 (9.70)^{a}	95 (17.01)^{a}	118 (18.78)^{b}	168 (22.58)^{a}	13 (6.90)^{ab}	14 (7.06)^{a}	16 (6.84)^{a}	11 (6.31)^{a}
T7	Control	258 (27.97)	243 (27.13)^{d}	226 (26.18)^{d}	246 (27.31)^{c}	238 (26.89)^{b}	258 (27.97)^{c}	226 (26.18)^{b}	239 (26.94)^{b}	211 (25.32)^{c}
CD at p=0.05		NS	2.29	3.20	4.02	3.15	2.94	3.43	4.27	2.16
SE (±)			1.05	1.47	1.85	1.44	1.35	1.57	5.99	0.99

Figures in parentheses are transformed values of $(x+1)^{0.5}$; figures followed by the same alphabets in a vertical column are not significantly different at five percent level

Table 5 Large scale field bio-efficacy trial (RBD) of *Lecanicillium lecanii* against thrips (season II)

T. no.	Treatments	No. of thrips/75 shoots							
		Pre-treatment	After I spray		After II spray		After III spray		
			Day 7	Day 14	Day 7	Day 14	Day 7	Day 14	Day 21	Day 28
T1	*L. lecanii* @ 1500 g	384 (33.92)^{a}	184 (23.65)^{c}	254 (27.76)^{e}	87 (16.42)^{b}	84 (16.15)^{c}	198 (24.52)^{b}	44 (11.87)^{bc}	31 (9.99)^{bc}	29 (9.70)^{c}
T2	*L. lecanii* + Jagerry @ 1500 g	378 (33.65)^{a}	160 (22.11)^{bc}	233 (26.54)^{d}	76 (15.38)^{ab}	70 (14.76)^{b}	170 (22.76)^{a}	40 (11.33)^{b}	23 (8.70)^{a}	19 (8.10)^{b}
T3	*L. lecanii* + Tween 80 @1500 g	376 (33.71)^{a}	175 (23.04)^{c}	245 (27.27)^{de}	65 (14.24)^{a}	60 (13.73)^{a}	179 (23.36)^{a}	42 (11.59)^{b}	25 (8.94)^{ab}	22 (8.59)^{b}
T4	NKAE @ 5%	377 (33.72)^{a}	165 (22.43)^{bc}	211 (25.28)^{c}	169 (22.70)^{d}	220 (25.85)^{f}	248 (27.41)^{e}	49 (12.48)^{c}	36 (10.75)^{c}	35 (10.60)^{c}
T5	NKAE @ 7.5%	382 (33.94)^{a}	154 (21.69)^{b}	183 (23.62)^{b}	200 (24.57)^{e}	180 (23.43)^{e}	230 (26.43)^{d}	44 (11.80)^{b}	34 (10.53)^{c}	31.00 (10.02)^{c}
T6	Quinalphos 25 EC @ 1000 ml	379 (33.84)^{a}	120 (19.19)^{a}	98 (17.28)^{a}	118 (18.78)^{c}	168 (22.58)^{d}	208 (25.15)^{c}	28 (9.52)^{a}	19 (7.84)^{a}	15 (7.13)^{a}
T7	Control	381 (33.94)^{a}	398 (34.68)^{d}	406 (35.03)^{f}	411 (35.24)^{f}	424 (35.79)^{g}	396 (34.59)^{f}	387 (34.20)^{d}	364 (33.18)^{d}	350 (32.54)^{d}
CD at p=0.05		NS	0.78	0.89	1.24	0.68	0.62	0.64	1.12	0.94
SE (±)			0.36	0.41	0.57	0.31	0.28	0.30	0.51	0.43

Figures in parentheses are transformed values of $(x+1)^{0.5}$; figures followed by the same alphabets in a vertical column are not significantly different at five percent level

(Thysanoptera: Thripidae) when treated with isolates of *V. lecanii*, *Beauveria bassiana* (Bals.) Vuill. (Hypocreales: Cordycipitaceae), *Metarhizium anisopliae* M. (Hypocreales: Clavicipitaceae), and *Paecilomyces fumosoroseus* W. (Hypocreales: Clavicipitaceae). They also reported better control of *T. tabaci* populations on cucumber in greenhouses with the fungus *V. lecanii* (Zimm.) Viegas.

An array of beneficial fungal species have been studied for western flower thrips (WFT) *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), management, and formulations based on *B. bassiana*, *M. anisopliae*, and *Lecanicillium muscarium* Zare & Gams (Hypocreales: Cordycipitaceae) controlled WFT populations on vegetable and flower crops (Shipp et al. 2003; Ugine et al. 2007).

The direct spray of *L. lecanii* (1×10^{8} conidia/ml) showed a significant difference in mortality in 2nd instar nymphs of *S. bispinosus* after 96-h exposure. In the leaf exposure method, low conidial concentrations (1×10^{5} conidia/ml) resulted in a low mortality rate. Multiple-concentration bioassays of *L. lecanii* against *S. bispinosus* indicated that the enhanced mortality of thrips with the increase in the concentration of inoculum recorded in the present study was found in agreement with the findings of Sajad Mohi-uddin et al. (2006) on *Spodoptera litura* Fab. (Lepidoptera: Noctuidae) on castor and white

Table 6 Compatibility of different vegetable oils on the growth of *Lecanicillium lecanii*

T. no.	Treatments	Colony diameter (cm)			Spore germination (%)	No. of conidia ($\times10^{7}$ ml^{-1})
		Day 5	Day 10	Day 15		
T1	Sun flower oil	9.33±2.12^{d}	16.00±3.11^{cd}	18.33±3.23^{b}	87.33±5.12^{bc}	84.67±5.34^{c}
T2	Ground nut oil	5.33±2.01^{a}	15.67±3.12^{bc}	21.00±3.36^{d}	88.33±5.45^{c}	76.00±4.57^{a}
T3	Coconut oil	10.00±2.34^{e}	15.00±3.14^{b}	21.33±3.26^{de}	92.33±5.56^{de}	94.33±5.78^{d}
T4	Paraffinic oil	5.67±1.87^{a}	10.67±2.16^{a}	15.67±2.14^{a}	79.33±4.36^{a}	79.33±5.39^{b}
T5	Castor oil	8.67±2.12^{c}	15.33±3.19^{bc}	19.67±3.19^{c}	85.67±5.35^{b}	85.67±5.47^{c}
T6	Neem oil	11.33±3.12^{f}	16.67±3.14^{d}	22.00±3.56^{e}	93.00±5.78^{e}	92.33±5.57^{d}
T7	Control	7.67±2.19^{b}	15.67±3.17^{bc}	18.00±3.43^{b}	91.00±5.67^{d}	93.00±5.69^{d}
CD=0.05		5.33^{a}	15.67^{bc}	21.00^{d}	1.85	2.47
CV (%)		10.00^{e}	15.00^{b}	23.67^{e}	1.43	1.96

Means followed by similar letters in a column are not significantly different at 5% (DMRT *F* test, p>0.05)

Table 7 Effect of *Lecanicillium lecanii* on some biological parameters of *Oligotapygmaea*

T. no.	Treatment	Larval mortality (%)	Larval period (days)	Pupal period (days)	Adult emergence (%)
T1	Dip talc powder	3.33±0.12[a]	6.67±1.17[a]	14.33±3.12[a]	83.33±5.23[a]
T2	Larval dip in spore suspension	3.33±0.12[a]	6.33±1.13[a]	15.00±3.16[a]	83.33±5.23[a]
T3	Larval crawl on dried spore suspension	0.00±0.00[a]	6.00±1.11[a]	15.33±3.14[a]	83.33±5.23[a]
T4	Spraying of spore suspension	6.67±1.17[a]	6.33±1.13[a]	15.33±3.13[a]	80.00±5.11[a]
T5	Control	3.33±0.13[a]	6.67±1.17[a]	15.33±3.13[a]	83.33±5.23[a]
	CD (5%)	9.60	1.36	1.25	10.65
	CV (%)	124.90	9.20	3.59	5.59

Means followed by similar letters in a column are not significantly different at 5% (DMRT *F* test, $p>0.05$)

grubs, respectively. The virulence, development, and subsequent colonization of entomopathogens on different insects might be affected by the type of plant species (Throne and Lord 2003). The knowledge of transmission is an important factor in understanding the survival of the pathogen in epizootiology; hence, the continuous presence of a pathogen in any ecosystem is dependent on its transmission ability. The transmission mechanism is a crucial factor for determining changes in population and expression of host disease (Aruthurs and Thomas 1999).

To find out the infectivity rate of blastospores and conidia of *L. lecanii* and its transmissions against nymphs of *S. bispinosus* between two generations, a glass-slide bioassay and a fungal development index (FDI) were used. Even though mortality occurred in the tested treatments, there was no growth of mycelium on the cadavers. On the 6th day after inoculation, all the nymphs were found dead in all the treatments.

Large-scale field study revealed that application of *L. lecanii* W.P. could significantly reduce thrips population. With the fungus, *V. lecanii* Gillespie (1986) achieved excellent control of *T. tabaci* population on cucumber in greenhouses. Between the trials, there was an enormous variation in thrips populations. Densities of thrips population and their damage to the tea plants were considerably declined by application of *L. lecanii* in all the trials. Hence, it will be a promising option to use this EPF against tea thrips under field conditions. Similarly, EPF were being developed for the management of *F. occidentalis*, in ornamental plants (Maniania et al. 2003), and the legume flower thrips, *M. sjostedti*, in cowpea crops (Ekesi et al. 1999).

In a spray application for fungal inoculums, water is generally used as a carrier. Tween-80 was usually added for suspending them in water due to hydrophobic nature of the conidia of *L. lecanii*. Improvement in the formulation of fungal inoculums may improve their efficacy in plant protection (Bateman et al. 1993). Oils enhance the efficacy of entomopathogens used in augmentative biological control agents. Conidia of *L. lecanii* were formulated by different oils to examine the efficacy of the formulation on the antagonist and its effectiveness at different processes of application and production stages. Castor, coconut, and neem oils improved the mycelial growth of *V. lecanii*, whereas superior spore yield was observed with Pongamia, neem, and sunflower oils (Ganga Visalakshy et al. 2005).

As biological control agents, the achievement of EPF depends not only on high effectiveness against insect pests but also on low virulence against non-target insects. In this study, the impact of *L. lecanii* on the predators present in the tea ecosystem, viz., *O. pygmaea, S. gilvifrons*, *M. boninensis*, and *N. longispinosus* under laboratory conditions which did not show any harmful effect on these natural enemies.

Table 8 Effect of *Lecanicillium lecanii* on some biological parameters of *Stethorus gilvifrons*

T. no.	Treatment	Larval mortality (%)	Larval period (days)	Pupal period (days)	Adult emergence (%)
T1	Dip talc powder	0.00[a]	8.33±2.12[a]	4.67±0.13[a]	90.00[a]
T2	Larval dip in spore suspension	0.00[a]	8.33±2.12[a]	4.67±0.13[a]	93.33[a]
T3	Larval crawl on dried spore suspension	0.00[a]	8.33±2.12[a]	4.33±0.11[a]	90.00[a]
T4	Spraying of spore suspension	0.00[a]	9.33±2.34	4.67±0.13[a]	100.00[a]
T5	Control	0.00[a]	9.00±2.23[a]	4.33±0.11[a]	100.00[a]
	CD (5%)		1.16	1.53	14.09
	CV (%)		5.81	14.63	6.45

Means followed by similar letters in a column are not significantly different at 5% (DMRT *F* test, $p>0.05$)

Table 9 Effect of *Lecanicillium lecanii* on some biological parameters of *Mallada boninensis*

T. no.	Treatment	Larval mortality (%)	Larval period (days)	Pupal period (days)	Adult emergence (%)
T1	Dip talc powder	10.00[a]	14.33±3.13[a]	13.00±2.95[a]	96.67[a]
T2	Larval dip in spore suspension	10.00[a]	13.33±3.01[a]	13.33±3.01[a]	93.33[a]
T3	Larval crawl on dried spore suspension	10.00[a]	14.00±3.02[a]	13.33±3.01[a]	93.33[a]
T4	Spraying of spore suspension	13.33[a]	14.33±3.13[a]	14.00±3.02[a]	96.67[a]
T5	Control	10.00[a]	13.67±3.01[a]	13.00±2.95[a]	96.67[a]
	CD (5%)	15.53	2.28	2.16	15.30
	CV (%)	63.12	7.08	7.04	6.96

Means followed by similar letters in a column are not significantly different at 5% (DMRT *F* test, $p>0.05$)

In laboratory bioassays, *T. tabaci* has proved to be susceptible to isolates of *B. bassiana*, *M. anisopliae*, *V. lecanii*, and *P. fumosoroseus* (Gillespie 1986; Annamalai et al. 2016). The most pathogenic isolates were *M. anisopliae* and *B. bassiana* that cause mortality of the treated insects within 4 days, while *V. lecanii* isolate showed a maximum of 85% mortality. The efficacy of the EPF, *M. anisopliae*, *V. lecanii*, and *B. bassiana* was investigated in the laboratory, pot, greenhouse experiments, and fields against the western flower thrips (*F. occidentalis*). Field trials with *V. lecanii* indicated its efficacy against *F. occidentalis* on bush beans (*Phaselus vulgaris*).

Derakhshan et al. (2007) reported that *V. lecanii* is not pathogenic to the coccinellids but also had no significant adverse effect on its biological parameters. Pavlyushin and Smits (1996) reported that *V. lecanii, B. bassiana*, and *P. fumosoroseus* had an entomopathogenic effect on larvae of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Camponotus nipponensis* Santschi (Hymenoptera: Formicidae). *Verticillium lecanii* either as formulations or as unformulated spore suspension did not have any harmful effect on *C. carnea* (Derakhshan et al. 2007).

Obtained results suggested that *L. lecanii* is compatible with the predators of tea thrips and red spider mite of tea. Moreover, *L. lecanii* is known to have broad host range, and use of such biological control agents to control insect pests might affect the beneficial insects, such as the natural enemies of insect pests (Sankara Rama Subramaniam et al. 2011). Additionally, the most guidelines for the registration of biopesticides require laboratory testing for infectivity to the non-target organism, and it is essential to study their effects on non-target insects before their release (Babu et al. 2008b).

Conclusion

The present study has been attempted to isolate a local strain of *L. lecanii* from the tea ecosystem, formulate this strain as a mycopesticide in a wettable powder form, and to establish the potential of this strain against one of the major tea pests, *S. bispinosus*. Laboratory bioassay of *L. lecanii* against the thrips, *S. bispinosus* indicated that entomopathogenic fungus, *L. lecanii* (UPASI TRF strain) was capable of causing considerable mortality of thrips. Fungal development index study confirmed that *L. lecanii* mixed with Jaggery showed conidiogenesis within 8th days. Findings of the micro-plot and large scale field study revealed that the application of *L. lecanii* WP significantly reduced the population density of thrips compared to the commercial formulation. The WP formulation was found to be safer to natural enemies of tea ecosystem. After fulfilling the requirements for its registration and label claim on tea, this strain of *L. lecanii* could be commercialized for the benefit of the tea industry for the management of tea thrips in an eco-friendly manner.

Table 10 Effect of *Lecanicillium lecanii* on some biological parameters of *Neoseiulus longispinosus*

T. no.	Treatment	Larval mortality (%)	Larval period (days)	Adult emergence (%)
T1	Dip talc powder	0.0[a]	4.67±0.13[a]	100.00[a]
T2	Larval dip in spore suspension	0.0[a]	4.33±0.12[a]	100.00[a]
T3	Larval crawl on dried spore suspension	0.0[a]	4.00±0.11[a]	100.00[a]
T4	Spraying of spore suspension	0.0[a]	4.67±0.13[a]	96.67[a]
T5	Control	0.0[a]	4.67±0.13[a]	100.00[a]
	CD (5%)		1.75	5.33
	CV (%)		16.95	2.32

Means followed by similar letters in a column are not significantly different at 5% (DMRT *F* test, $p>0.05$)

Abbreviations

WP: Wettable powder; PDAY: Potato dextrose agar + 1% yeast powder; FDI: Fungal development index; UPASI: United Planters' Association of Southern India; PDA: Potato dextrose agar; C: Centigrade; Df: Dilution factor; CFU: Colony-forming units; NKAE: Neem kernel aqueous extract; ETL: Economic threshold level; RBD: Randomized block design; ANOVA: Analysis of variance; RH: Relative humidity; GT_{50}: Germination rate; TRF: Tea research foundation; WFT: Western flower thrips; FDI: Fungal development index

Acknowledgements

The authors are thankful to Dr. Sanjay K. Singh, Scientist, Mycology and Plant Pathology Group, Maharashtra Association for the Cultivation of Science Agharkar Research Institute (*An Autonomous Grant-in-aid Institute under the Department of Science and Technology, Govt. of India*) for identification of the fungal strain.

Authors' contributions

MSRS isolated the *Lecanicillium lecanii* from the tea ecosystem and conducted in vitro experiments. AB guided during bio-efficacy tests under both lab and field conditions and revised the draft manuscript. BD assisted in conduction of field trials of these fungal species, analyzed the data, and prepared the manuscript. The authors read and approved the final manuscript.

Funding

This study was performed as a part of in-house project of the organization. It was not financially funded by any external funding agency.

Availability of data and materials

All data generated and analyzed for this study are presented in the manuscript, and the corresponding author has no objection to the availability of data and materials.

Ethics approval and consent to participate

Not applicable. The study was conducted using different microbial species; those are abundant in the ecosystem hence does not require ethical approval.

Consent for publication

Not applicable.

Competing interests

It is declared that the authors have no competing interests.

Author details

[1]Coromandel International Ltd., 10, Shri Ganesh Illam, SBO Colony, Cantonment, Lawsons Road, Trichy, Tamil Nadu 620001, India. [2]Department of Entomology, Tea Research Association, North Bengal Regional R&D Centre, Nagrakata, Jalpaiguri, West Bengal 735225, India.

Received: 26 August 2020 Accepted: 12 February 2021
Published online: 19 February 2021

References

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J Econ Entomol 18:265–267

Annamalai M, Kaushik H, Selvaraj K (2016) Bioefficacy of *Beauveria bassiana* (Balsamo) Vuillemin and *Lecanicillium lecanii* Zimmerman against *Thrips tabaci* Lindeman. Proc Natl Acad Sci USA India Sect B Biol Sci 86:505–511

Aruthurs SP, Thomas MB (1999) Factors affecting horizontal transmission of entomopathogenic fungi in locusts and grasshoppers. In: Thomas MB, Kewards T (eds) Challenges in applied population biology, vol 53, pp 89–97

Azad M, Ahmad I, Mainuddin A (2020) Application of plant extracts for pest management in tea: a study on tea leaf's thrips, *Scirtothrips bispinosus*. J Biosci 28:87–93

Babu A (2009) Eco-friendly insect pest management in tea in South India. Ins Pest Magmt Envl Safety 4(II):49–56

Babu A, Muraleedharan N (2010) A note on the use of pesticides for the control of insect and mite pests of tea in South India. J Plant Crops 23:1–15

Babu A, Perumalsamy K, Sankara Rama Subramaniam M, Muraleedharan N (2008a) Use of neem kernel aqueous extract for the management of red spider mite infesting tea in South India. J Plant Crops 36(3):393–397

Babu A, Sachin PJ, Sankara Rama Subramaniam M, Shanmugapriyan R, Achuthan R, Siby M (2008b) Enhanced efficacy of *Verticillium lecanii* against tea thrips by the addition of jaggery. Newsl UPASI Tea Res Found 18(2):3

Bateman RP, Carey M, Moore D, Prior C (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Ann ApplBiol 122(1):145–152

Burges HD (1998) Formulation of mycopesticides. In: Burges HD (ed) Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatment. Kluwer Academic Publishers, Dordrecht., pp 131–186

Butt TM, Goettel M (2000) Bioassays of entomopathogenic fungi. In: Navon A, Ascher KRS (eds) Bioassays of Entomopathogenic microbes and nematodes. CAB International, WallingfordChapter 4, pp 141–195

Chakraborty R, Singha D, Kumar V, Pakrashi A, Kundu S, Chandra K, Patnaik S, Tyagi K (2019) DNA barcoding of selected *Scirtothrips* species (Thysanoptera) from India. Mitochondrial DNA Part B 4(2):2710–2714. https://doi.org/10.1080/23802359.2019.1644547

Cortez-Madrigal H, Allatorre-Rosas R, Mora-Aguilera G, BravoMojica H, Ortiz-Garcia CF, Aceves-Navarro LA (2003) Characterization of multisporic and monosporic isolates of *Lecanicillium* (=*Verticillium*) *lecanii* for the management of *Toxoptera aurantii* in cocoa. Biocontrol 48:321–334

Derakhshan A, Rabindra RJ, Ramanujam B (2007) Efficacy of different isolates of entomopathogenic fungi against *Brevicoryne brassicae* (L.) at different temperatures and humidities. J Biol Control 21:65–72

Ekesi S, Maniania NK, Ampong-Nyarko K, Onyu I (1999) Effect of intercropping cowpea with maize on the performance *Metarhizium anisopliae* against *Megalurothrips sjostedti* (Thysanoptera: Thripidae) and predators. Environ Entomol 28:1154–1161

Gams W (1971) Molds and other fungi in indoor environments. *Cephalosporium artigeschimmelpilze (*Hophomycetes*)*. Gustav Fischer Verlag, Stuttagart 7:161–169

Ganga Visalakshy PN, Krishnamoorthy A, Kumar M (2005) Effects of plant oils and adhesive stickers on the mycelia growth and conidiation of *Verticillium lecanii*, a potential entomopathogen. Phytoparasitica 33(4):367–369

Gillespie AT (1986) The potential of entomogenous fungi as control agents from onion thrips, *Thrips tabaci*. Proceedings of Brighton Crop Protection Conference: Biotechnology and Crop Improvement and protection, Brighton, pp 237–243

Hall RA, Burges HD (1979) Control of aphids in glasshouses with the fungus *Verticillium lecanii*. Ann Appl Biol 93:235–246

Handerson CF, Tilton EW (1955) Tests with acaricides against the brown mite. J Econ Entomol 48:157–161

Jenkins NE, Hrviefo G, Langewald J, Cherry AJ, Lomer CJ (1998) Development of mass production technology for aerial conidia for use as mycopesticides. Bicontrol News Inform 19(1):21–31

Kawai A (1990) Life cycle and population dynamics of *Thrips palmi* Karny. Jap Agr Res Quart 23:282–288

Lipa JJ, Slizynski K (1973) Wskazówkimetodycznel. Terminologia do wyznaczaniasrednejdawkismiertelnej (LD50) W PatologiaOwadow I TksykologiaPraceNavkomeInstytutuochrony Roslin Tom. XV, Seszyti, pp 59–83

Maniania NK, Sithanatham S, Ekesi S, Ampong-Nyarko K, Baumgartner J (2003) A field trial of the entomogenousfungus, *Metarhizium anisopliae* forcontrolof onion thrips. Thrips *tabaci*. Crop Prot 22:553–559

Moore D, Prior C (1993) The potential of mycoinsecticides. Biocon News Inform 14:31–40

Nadeau MP, Dunphy GB, Boisvert JL (1996) Development of *Erynia conica* (Zygomycetes: Entomophthorales) on the cuticle of the adult black flies *Simulium rostratum* and *Simulium decorum* (Diptera: Simuliidae). J Invertebr Pathol 68:50–58

Parker BL, Skinner M, Costa SD, Gouli S, Reid W, El-Bouhssini M (2003) Entomopathogenic fungi of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae): collection and characterization for development. Biol Control 27:260–272

Pavlyushin VA, Smits PH (1996) Effect of entomopathogenic fungi on entomophagous arthropods: insect pathogens and insect parasitic nematodes. Proc First Joint Meeting Bull OILB SROP 19:247–249

Sajad Mohi-uddin FA, Arif Jan Z, Bhat FA (2006) Dose optimization of promising entomopathogenic fungi against white grub (*Holotrichia* sp.). J Biol Sci 6: 958–960

Samson RA, Evans HC, Latg JP (1988) Atlas of entomopathogenic fungi. Springer, Berlin Heidelberg New York

Samson RA, Rombach MC (1985) Biology of the fungi *Verticillium* and *Archersonia*. In: Hussey NW, Scopes N (eds) Biological pest control. The Glasshouse Experience. Cornell Universoty Press, New York, pp 34–42

Sanap MM, Nawale RN (1987) Chemical control of chilli thrips, *Scirtothrips dorsalis*. Vegetable Science 14:195–199

Sankara Rama Subramaniam M, Babu A, Roobak Kumar A, Vasantha Kumar D (2011) Utilization of an entomopathogenic fungus, *Lecanicillium lecanii* (Zimmermann) and neem kernel aqueous extract for the management of *Scirtothrips bispinosus* (Bagnall) infesting tea. J Plant Crop 39(1):220–223

Shipp JL, Zhang Y, Hunt DWA, Ferguson G (2003) Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. Environ Entomol 32(5):1154–1163

Throne JE, Lord JC (2003) Tritrophic interactions and storage pest control: interaction of the fungus *Beauveria bassiana* with resistant oat varieties for control of *Oryzaephilus surinamensis*. The 2003 ESA Annual Meeting and Exhibition. USDA-ARS Grain Marketing and Production Research Center, Manhattan

Ugine TA, Wraight SP, Sanderson JP (2007) A tritrophic effect of host plant on susceptibility of western flower thrips to the entomopathogenic fungus *Beauveria bassiana*. J Invertebr Pathol 96(2):162–172

Vilas-Bôas GFLT, Vilas-Bôas LA, Lereclus D, Arantes OMN (1998) *Bacillus thuringiensis* conjugation under environmental conditions. FEMS Microbiol Ecol 25:369–374

Zimmermann A (1898) A fungus disease of *Lecanium viride*. Over eeneschimmelepidemie der groeneLuizen. Korte BerichtenUit's Lands Plantentuin 9(240):243

Publisher's Note