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Potential of the entomopathogenic fungus, *Metarhizium anisopliae s.l.* in controlling live-wood eating termite, *Microtermes obesi* (Holmgren) (Blattodea: Termitidae) infesting tea crop

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Abstract

Background: In recent years, *Microtermes obesi* (Holmgren) (Blattodea: Termitidae) has been recorded as a major pest of tea crop, causes significant losses in production. Managing termite pests in tea crops through an integrated approach has been suggested, and the use of microbial biocontrol agent is one of the economical methods. The present study evaluated the pathogenic efficacy of an entomopathogenic fungus *Metarhizium anisopliae s.l. (= M. anisopliae)* against *M. obesi* damaging tea plants under field conditions.

Results: *Metarhizium anisopliae s.l.* was formulated as 5% aqueous suspension (AS). Large-scale field trials with formulated entomopathogen revealed that 1000 and 1200 ml concentrations of *M. anisopliae s.l.* 5%AS (each concentration containing 2×10^7 conidia/ml) each in 400 l of water/ha significantly ($P < 0.05$) reduced the population of *M. obesi* in tea gardens at Dooars and Darjeeling regions, India. In the field study, *M. anisopliae s.l.* was more effective than the standard insecticide and was non-pathogenic on the beneficial insects present in the tea gardens. In addition, *M. anisopliae s.l.* 5%AS had no phytotoxic effect on the tea leaves, with acceptable organoleptic attributes.

Conclusion: *Metarhizium anisopliae s.l.* isolate can be commercialized as an alternative natural termiticide to reduce the load of synthetic insecticides in the tea crop.

Keywords: *Metarhizium anisopliae s.l.*, Tea crop, Pathogenic activity, Formulation, *Microtermes obesi*, Biopesticide

Background

Tea (*Camellia* sp.) is a perennial plantation crop which requires a warm, humid climate for adequate growth and production. The warm and humid conditions favor the attack of tea crop by many insect pests, mites, and diseases. The live wood-eating termite, *Microtermes obesi* (Holmgren) (Blattodea: Termitidae), is one of the major pests of tea crop in India, especially in West Bengal (Dooars, Terai and Darjeeling) and Assam (Biswa and Mukhopadhyay 2013), which hamper the tea production amounting to 30–50% of total production (Zhang et al. 2017). As per estimate, the live wood eating termites cause about 20–25% crop damage in northeast Indian tea plantations (Debnath et al. 2012). In certain areas of Barak Valley tea plantation, the damage caused by termite to mature and young tea areas have been found to be enormous ranging from 30 to 90% (Singha

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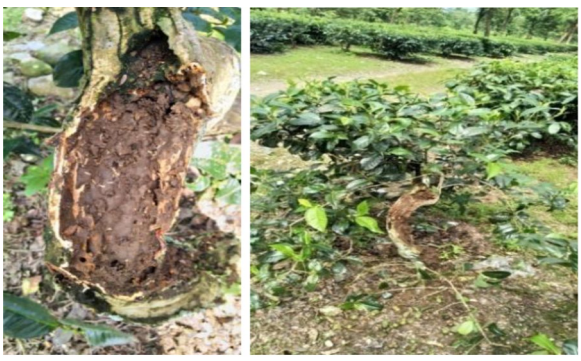
et al. 2012). This pest remains active throughout the year, and un-checked infestation would lead to 100% crop loss, if appropriate management practices are not adopted (Fig. 1A, B).

Earlier, tea growers used to use synthetic pesticides to manage this pest below the Economic Threshold Level (ETL) of 10% (Babu 2010). Due to their detrimental effects on soil-flora, -fauna and overall soil health and sensitive nature of the crop, their use in tea is now restricted. Currently, there are no recommended soil pesticides or termiticides in the PPC list (Plant Protection Code, Govt. of India) for controlling this notorious pest. Presently, conventional tea planters are using thiamethoxam 25WG @ 100 ml in 400 l of water/ha, a recommended foliar pesticide commonly used for the management of sucking tea pests, to get rid of termite's problem along with recommended cultural practices. However, continuous use of synthetic pesticides leads to certain undesirable issues like soil, water pollution, and degradation of beneficial soil microbes, the decline in natural enemy population, resurgence, and development of resistance in termite pest along with the residues in manufactured tea (Bora and Gurusubramanian 2007). To overcome the current situation, adopting non-chemical management strategies become essential. Application of microbial bio-control agents, especially entomopathogens, plays a significant role in the management of tea pests in an eco-friendly way (Pandey et al. 2021). The problems associated with residues of synthetic pesticides in made tea have encouraged the tea growers to look for such eco-friendly approaches to deal with different types of tea pests, which includes selective use of pesticides and incorporation of Biological Control Agents (BCAs) like predators and parasitoids and beneficial microorganisms (Babu 2010).

The use of microbial bio-control agents are of immense importance in tea cultivation since they protect the crop from the attack by several pests including termites and could be considered as an important component of integrated management strategy. Besides, they are natural resources, relatively safer to the human being, environment friendly and have no residual problems. In microbial bio-control agents, the entomopathogenic fungi (EPF) are considered as important tools as bio-pesticides due to their potential efficacy against many arthropod pests in ecological farming as a secured substitute to toxic chemical insecticides (Lovett and Leger 2017). Nowadays, the application of such beneficial fungi has become worldwide popular and commercially developed for the management of pests (Anitha et al. 2019).

This EPF, *Metarhizium anisopliae* sensu lato *(s.l.)* Sorokın (=*M. anisopliae*), commonly known as "green muscardine fungus" is a sordariomycetes fungus of order hypocreales, has shown a potential efficacy against a wide range of insect pests in different crops, including tea crop (Anitha et al. 2019). Under field conditions, the formulations of *M. anisopliae s.l.* isolates have shown efficient activity against a wide range of pests of tea crop in Kenya (Cheramgoi et al. 2016). Few studies have been conducted to evaluate the efficacy of *M. anisopliae s.l.* against *M. obesi* infesting tea crop. Therefore, the present study was proposed to isolate indigenous *M. anisopliae s.l.* from the soil of tea growing areas and assess their field efficacy against *M. obesi* using liquid formulations.



A B

Fig. 1 Termite infested tea bushes

Methods

Isolation and identification of *M. anisopliae s.l.*

Soil samples were collected by the help of auger from the rhizospheres of the tea plantations of Dooars (26° 55′ 0″ N latitude, 88° 56′ 0″ E longitude and 228 m above *sea level*) and Darjeeling regions (26° 93′ 6″ N latitude, 88° 16′ 44″ E longitude and 3,000 m above sea level) in the pre-sterilized zip bags, separately during 2018 and were kept in a container with an ice bag (Ogunmwonyi et al. 2008). Collected samples were brought to the laboratory and *M. anisopliae s.l.* was isolated through the serial dilution method (Morris and Rideout 2005). One ml aliquot of the soil sample from the 10^{-4} dilution of the soil in the sterilized distilled water was placed on the Potato Dextrose Agar (PDA) plates in 3 replicates and the plates were incubated in a BOD (Biological Oxygen Demand) incubator at 28 °C for the isolation of the target entomopathogen. After 6 days of incubation, the colonies of *M. anisopliae s.l.* was isolated from the plates and purified by the single spore isolation method, and the culture of the entomopathogen was maintained on the agar slants at 4 °C for further use. ICAR-Indian Agricultural Research Institute (IARI), New Delhi, India identified the isolated *M. anisopliae s.l.* by studying cultural and morphological characteristics, as well as by using

a fungal key. The culture of the isolate was sent to the Indian Type Culture Collection (ITCC) and ICAR-IARI, India for long term preservation under accession number MET 5/1. The isolate, *M. anisopliae s.l.* was also identified by sequencing the ITS regions (ITS1, 5.8S and ITS2) of the nuclear rDNA. The gDNA (genomic DNA) from the isolate was extracted, following the CTAB method of Moller et al. (1992) and quantified with the help of NanoDrop1000 spectrophotometer (Thermo Scientific). The rDNA gene cluster was amplified by PCR, using universal primer pairs ITS1/ITS4 (White et al. 1990). The amplified PCR products of the isolate were separated by gel electrophoresis on a 2% agarose gel, and the obtained bands were excised and purified (UniPro Gel extraction kit) for sequencing (Macrogen, Inc., Korea.). BLASTn was used to match *M. anisopliae s.l.* sequencing results of the isolate with known sequences of *M. anisopliae s.l.* strains accessible at the public database GenBank.

Preparation of the fungal inoculum

For the preparation of the suspensions of *M. anisopliae s.l.,* the isolate was grown on the Potato Dextrose Broth (PDB) in conical flasks (500 ml) for 15 days at 28 °C in a BOD incubator. After the stipulated incubation, the fungal mycelial mat (conidia plus mycelia) was harvested from the flask and ground in a grinder with 50 ml of double-distilled water under the aseptic conditions to prepare the spore suspension. The homogenized spore suspension was filtered through a muslin cloth to make the stock suspension, and spore density of stock suspension was adjusted to 2×10^7 conidia/ml using a hemocytometer (Singleton and Sainsbury 1981). Then, from the stock spore suspension of the isolate (MET 5/1), a stock solution of effective entomopathogen's spore suspension, i.e., isolate MET 5/1, was prepared and conidia were adjusted to 2×10^7 conidia/ml. From this stock solution, different amounts such as 600 ml, 800 ml, 1000 ml, and 1200 ml (each concentration containing 2×10^7 conidia/ml) were taken and mixed in the requisite amount of water for field applications to assess their bio-efficacy, separately.

Evaluation of *M. anisopliae s.l.* against *M. obesi* through large scale field trials

Experimental layout

Large-scale field trials were conducted to evaluate the efficacy of the *M. anisopliae s.l.* 5%AS for 2 consecutive seasons 2018 and 2019 in tea gardens, heavily infested with *M. obesi* representing 2 different geographical locations, namely Kumai Tea Garden, Darjeeling (26° 94′ 5″ N, 88° 17′ 43″ E longitude) and Bhatpara Tea Garden, Dooars (26° 71′ 2″ N, 89° 47′ 8″ E longitude). The tea estates were selected based on the basis of the previous history of termite problems.

Treatments

A liquid formulation of *M. anisopliae s.l.* containing 5%AS (aqueous spore suspension) was prepared, following the methodology of Godonou et al. (2000). The experiment consisted of 7 treatments [*M. anisopliae s.l.* 5%AS @ 600 (T1), 800 (T2), 1000 (T3), 1200 (T4) ml/400 l of water/ha (each treatment containing 2×10^7 conidia/ml), combination of *M. anisopliae s.l.* 5%AS @1000 ml /400 l of water/ha (containing 2×10^7 conidia/ml), along with Thiamethoxam 25WG @ 100 gm/400 l of water/ha (T5), recommended standard insecticide (Thiamethoxam 25WG @ 100 gm/400 l of water (T6) and untreated control (T7)] per replication, and each plot (75 m^2) contained 100 tea bushes. Each trial was conducted in a randomized complete block design (RCBD) with five replications.

Application schedule

Forking of the collar region of the tea bushes and watering was carried out prior to the spraying after pruning during winter (January). The spraying was applied after employing cold-weather practices as described in the Field Management manual of Tea Research Association.

Spraying method

Using a hand-operated calibrated knapsack sprayer, spraying was carried out and bushes were drenched properly for better coverage and control. Performance of each test substance against *M. obesi* was assessed by following the standard indirect sampling method adopted by Debnath et al. (2012) by recording the changes of number of earth-runs developing on collars and branches of affected tea plants. Count on fresh earth-runs was taken prior to application for comparison, which acted as pre-treatment count. Percentage reduction/increase in population was worked out based on the number of fresh earth-runs in each of the 30 bushes selected previously. After every observation, termite earth run was wiped off completely from the surface of the tea bushes so that fresh build-up of each run was detectable. Post-treatment observations on fresh earth runs were taken at 3 months interval up to 9 months. Percentage (%) of reduction in termite infestation was calculated following the formula,

$$\% \text{ reduction of termite population} = \frac{(A - B)}{A} \times 100$$

where A = pre-treatment population count and B = post treatment population count.

Effect of *M. anisopliae* on yield of harvestable shoots

During the trials, the crop yield was also recorded both from treated and control plots at each plucking round. The yield of harvestable shoots (kg/plot) was recorded for the first 6rounds of plucking by maintaining a standard plucking round of 7 days interval. The green leaf yield recorded at every plucking round was converted into processed tea for one hectare as described by Ponmurugan and Baby (2007) using the formula:

Green leaf yield (kg) × no. of bushes/ha × conversion factor (0.225).

Effect of *M. anisopliae s.l.* 5%AS on the non-target organisms

The formulation of *M. anisopliae* s.l. 5%AS was also evaluated against commonly available predators to examine any negative/pathogenic impact on them at each location, following the methodology of Leatemia and Isman (2004). The treatments included were similar to the RCBD trials in 3replications. The population of the non-target insects were recorded on 0 day (pre-spray), and 14th day (7th day after 2nd spray) (total 2 rounds of spraying were carried out: 1st spray at day-0 and 2nd spray at day-7). The number of adult predatory insect populations in each plot was recorded from 50 randomly selected bushes on the visual basis as described by Safarzoda et al. (2014). Similarly, randomly 50 tea leaves were collected from each treatment plots and were observed under the binocular microscope to count the larval population of predators.

Phytotoxic effect and organoleptic evaluation

To evaluate the phytotoxicity effect (yellowing, stunting, necrosis, epinasty, hyponasty, etc.) of the *M. anisopliae s.l.* 5%AS (at 'X' '2X' and '4X' concentration) on tea leaves, field experiments were carried out in 3 replicates at 84 m^2 per replication in an RCBD design at the experimental plot of North Bengal Regional Research and Development Centre (NBRR&DC), Nagrakata, Dooars (26° 54′ 0″ N, 88° 55′ 0″ E longitude), Nagrakata, West Bengal. There were in total of 4 treatments such as 2.5 ml/l (T1), 5 ml/l (T2), 10 ml/l of water (T3), and untreated control (T3). The conidial density of each treatment solution, i.e., T1, T2 and T3 was 2×10^7 conidia/ml. Spraying was applied by a hand-operated knapsack sprayer using a spray volume of 400 l of water/ha. Observations were recorded on 0 day (pre-treatment) and day 3, 7 and 14 (post-treatment) on yellowing, stunting, necrosis, epinasty, hyponasty, etc., and the injury levels were graded, using the Phytotoxicity Rating Scale (PRS) as follows: no crop response/crop injury = 0.1–10% crop injury = 1, 10.1–20% crop injury = 2, 20.1–30% crop injury = 3, 30.1–40% crop injury = 4, 40.1–50%crop injury = 5, 50.1–60% crop injury = 6, 60.1–70% crop injury = 7, 70.1–80% crop injury = 8, 80.1–90% crop injury = 9, 90.1–100% crop injury = 10 (Babu 2010).

Statistical analysis

In the present study, field experiments were carried out in 5replicates in a RCBD. All the data were statistically analyzed using SPSS17. Statistical analysis was performed using the General Linear Model procedure (Friedman et al. 2010). The differences among the treatments in pre-spray percent and percent reduction of termite infestation were assessed. After significant effects were identified, differences between means were considered significant at 95% confidence interval based on Tukey's post-hoc Honestly Significant Difference (HSD) test to separate the means. Arcsine transformation was performed on mortality data before statistical analyses. For the green-leaf yield, the treatments were compared to untreated control using Tukey's post-hoc HSD test to separate the means at the 95% confidence interval. Student's t-test was carried out to compare the mean number of each non-target beneficial insects before and after the spraying in a tea plantation with *M. anisopliae s.l.* 5%AS.

Results

Evaluation of *M. anisopliae s.l.*against *M. obesi* through large scale field trials

Results of the field trials conducted using *M. anisopliae s.l.* 5%AS against *M. obesi* at 2 different locations; Darjeeling and Dooars regions, are presented in Table 1.

In Darjeeling region, after a period of one year of spraying, all the treatments were found to be significantly superior over un-treated check in minimizing the population of *M. obesi* (Table 1). The percentage of control of termite was significantly superior at the concentrations of 1000 and 1200 ml/ha of *M. anisopliae s.l.* 5%AS (each concentrations containing 2×10^7 conidia/ml) and Thiamethoxam 25WG 100 gm/400 l of water/ha. The percent reduction of termite population *M. obesi* was slightly inferior in plots treated with lower concentrations of *M. anisopliae s.l.* 5%AS at 600 and 800 ml/ha (each concentrations containing 2×10^7 conidia/ml), Thiamethoxam 25WG at 100 g/ha as compared to plots treated with combination of *M. anisopliae s.l.* 5%AS @1000 ml/ha and Thiamethoxam 25WG at 100 g/ha and high concentrations *M. anisopliae s.l.* 5%AS, *i.e.*1000 and 1200 ml/ha ($F_{2,126} = 19.75$, $P < 0.001$). Similarly after second year, *M. anisopliae s.l.* 5%AS at 1000 and 1200 ml/ha gave better control of *M. obesi,* similar to the combination of *M. anisopliae s.l.* 5%AS @1000 ml/ha and Thiamethoxam

Table 1 Field evaluation of *Metarhizium anisopliae s.l.* 5% AS against termite

Treatment details	Concentration/ha (400 l spray fluid)	Darjeeling Region		Dooars Region	
		Mean % reduction of termite			
		After year I	After year II	After year I	After year II
Metarhizium anisopliae s.l. 5% AS	600 ml[$]	52.40[b]	53.11[b]	51.85[b]	54.97[b]
Metarhizium anisopliae s.l. 5% AS	800 ml[$]	59.24[b]	59.11[b]	60.40[b]	56.48[b]
Metarhizium anisopliae s.l. 5% AS	1000 ml[$]	78.85[c]	76.75[c]	77.77[c]	75.93[c]
Metarhizium anisopliae s.l. 5% AS	1200 ml[$]	82.85[c]	84.75[c]	83.77[c]	86.93[c]
Metarhizium anisopliae s.l. 5% AS ± Thiamethoxam 25WG	1000 ml[$] ± 100 gm	76.95[c]	78.77[c]	76.46[c]	81.17[c]
Thiamethoxam 25WG	100 gm	62.16[b]	63.48[b]	67.92[b]	63.84[b]
Control	–	0.00[a]	0.00[a]	0.00[a]	0.00[a]
CD	–	5.23	4.47	4.38	4.19

Mean of five replications, differences among the tested doses are grouped in same column with small letters and the identical letters do not differ significantly at $P = 0.05$ according to Tukey's multiple comparison test. [$]Containing 2×10^7 conidia/ml

25WG at 100 g/ha ($P = 0.56$) and significantly superior than *M. anisopliae s.l.* 5%AS at 600, 800 ml/ha and Thiamethoxam 25WG at 100 g/ha ($F_{4,72} = 38.19$, $P < 0.001$).

In Dooars region, after one year of spraying, the incidence of *M. obesi* infestation was significantly reduced in plots treated with both bio- and synthetic insecticide than the un-treated control (Table 1). Among different treatments, *M. anisopliae s.l.* 5%AS, when sprayed at 1200 ml/ha was found to be more effective in reducing the population of *M. obesi* ($F_{2,54} = 45.3$, $P < 0.001$) and the pathogenicity decreased with decrease in the concentrations of *M. anisopliae* 5%AS (Table 3). However, there were non-significant differences between the concentrations of 1200 and 1000 ml/ha of *M. anisopliae* 5%AS and combination of *M. anisopliae s.l.* 5%AS @1000 and Thiamethoxam 25WG at 100 g/ha, in terms of percent reduction of termite population ($F_{13,106} = 1.35$, $P = 0.22$) was found. Both concentrations were effective than the control achieved in 600 and 800 ml/ha of *M. anisopliae* 5%AS, and Thiamethoxam 25WGat 100 g/ha ($F_{2,54} = 45.3$, $P < 0.001$). After the second year, the plots treated with 1200 and 1000 ml/ha concentrations of *M. anisopliae s.l.* 5%AS were effective treatments for the management of *M. obesi*. While comparing the overall mean data recorded during the period under study, the mean infestation incidence was significantly on par in plots treated *M. anisopliae s.l.* 5%AS at 1200 & 1000 ml/ha and combination of *M. anisopliae s.l.* 5%AS @1000 ml/ha and Thiamethoxam 25WG @ 100 g/ha. The lower concentrations of *M. anisopliae s.l.* 5%AS, 600 ml/ha and 800 ml/ha, were found lesser potential than the higher concentrations, i.e., 1000 and 1200 ml/ha of *M. anisopliae* 5%AS, and synthetic insecticide Thiamethoxam 25WG@ 100 g/ha ($F_{12,108} = 2.92$, $P = 0.002$) used in this study.

Effect of *M. anisopliae on* yield

The average yield recorded from the first six harvestings (at 7 days interval) from the experimental trials is presented in Table 2. The yield was significantly low in untreated plots as high level of infestation by the termite was reported, confirming the best efficacy of the different tested formulations ($F_{2,654} = 46.2$, $P < 0.001$). Among all the treatments, tea crop yield was significantly high in plots treated with high concentrations of *M. anisopliae s.l.* 5%AS (@ 1000 and 1200 ml/ha, (each containing 2×10^7 conidia/ml) and combination of *M. anisopliae* 5%AS @1000 ml/ha with Thiamethoxam 25WG at 100 gm/ha than plots treated with the Thiamethoxam 25WG at 100 gm/ha and 600, 800 ml/ha concentrations of *M. anisopliae s.l.* 5%AS (Table 2).

Effect of *M. anisopliae* 5%AS on the non-target organisms

In tea ecosystem, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), *Oxyopes javanus* Thorell (Araneae: Oxyopidae), and *Stethorus gilvifrons* Mulstant (Coleoptera: Coccinellidae) were collected and identified as main predatory species. The population level of the non-target predators was recorded at the 0-day (pre-treatment) and 14th day (post-treatment) (Table 3). The population of each species was not affected after the spraying of *M. anisopliae s.l.* 5%AS, as evidenced from the abundance of predators that were non-significantly different at 95% of confidence interval ($F_{12,108} = 1.24$, $P = 0.27$) (Table 3).

Phytotoxic effect

The phytotoxic study of *M. anisopliae s.l.* 5%AS was carried out separately at 2.5, 5.0, and 10 ml/l of water (2×10^7 conidia/ml). Observations recorded on the phytotoxicity symptoms indicated that none of the concentrations showed any type of phytotoxic effect on the tea

Table 2 Effect of *Metarhizium anisopliae* 5% AS on tea yield

Treatments	Concentration/ha	*Green leaf yield (kg/plot) (mean* ± SE)	Processed tea (kg/ha/year) (mean* ± SE)
Metarhizium anisopliae s.l. 5%AS	600 ml[$]	$2.50^{de} \pm 0.29$	388 ± 4.19
	800 ml[$]	$2.51^{cd} \pm 0.19$	390.5 ± 4.25
	1000 ml[$]	$2.61^{ab} \pm 0.16$	405 ± 4.45
	1200 ml[$]	$2.65^{a} \pm 0.22$	411 ± 4.37
Metarhizium anisopliae s.l. 5%AS + Thiamethoxam 25WG	1000 ml[$] + 100 gm	$2.63^{a} \pm 0.23$	408.5 ± 4.21
Thiamethoxam 25WG	100 gm	$2.55^{bc} \pm 0.18$	396 ± 4.36
Untreated control	–	$2.43^{e} \pm 0.14$	378 ± 4.25

*Values represent the mean of three replications ± standard error, for green leaf yield (obtained from six rounds of leaf plucking after treatments at weekly interval for two consecutive seasons)

[$] Containing 2×10^7 conidia/ml. Values in the same column followed by the same letter are not significantly different from each other at the 95% confidence interval ($P < 0.05$) using Tukey's post-hoc Honestly Significant Difference (HSD) test

Table 3 Effect of *Metarhizium anisopliae* s.l. 5%AS on non-target beneficial organisms in tea gardens

Treatments	Concentration/ha	Non-target beneficial insect populations (mean* ± SE)					
		Chrysoperla carnea		*Oxyopes javanus*		*Stethorus gilvifrons*	
		Day-0	Day-14	Day-0	Day-14	Day-0	Day-14
Metarhizium anisopliae s.l. 5%AS	600 ml[$]	0.11 ± 0.002	0.12 ± 0.002	0.08 ± 0.001	0.11 ± 0.002	0.14 ± 0.003	0.16 ± 0.004
	800 ml[$]	0.12 ± 0.002	0.13 ± 0.002	0.09 ± 0.001	0.10 ± 0.002	0.16 ± 0.004	0.14 ± 0.004
	1000 ml[$]	0.10 ± 0.002	0.11 ± 0.002	0.07 ± 0.001	0.10 ± 0.002	0.17 ± 0.004	0.17 ± 0.005
	1200 ml[$]	0.11 ± 0.002	0.12 ± 0.002	0.08 ± 0.001	0.09 ± 0.001	0.16 ± 0.004	0.16 ± 0.005
Metarhizium anisopliae s.l. 5%AS + Thiamethoxam 25WG	1000 ml[$] + 100 gm	0.10 ± 0.002	0.11 ± 0.002	0.08 ± 0.001	0.10 ± 0.002	0.17 ± 0.005	0.14 ± 0.004
Thiamethoxam 25WG	100 gm	0.12 ± 0.002	0.11 ± 0.002	0.09 ± 0.001	0.09 ± 0.001	0.14 ± 0.004	0.13 ± 0.002
Untreated control	–	0.12 ± 0.002	0.12 ± 0.002	0.11 ± 0.002	0.11 ± 0.002	0.16 ± 0.004	0.16 ± 0.005

Values represent the mean number of non-target beneficial insect populations before treatment (0 day) and after 14 days (7 days after 2nd spray). No significant differences were recorded for each species of test insects population, between the 0 day and after 14 days ($P > 0.05$; independent t-test)

[$] Containing 2×10^7 conidia/ml

*Values represent the mean of three replications ± Standard Error

leaves and harvestable shoots. There was no visible injury on the leaf tip, leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty (Additional file 1: Table S4).

Organoleptic evaluation

Similarly, tea shoots harvested on 1, 3, 5, 7, 10 and 14 days after the application of *M. anisopliae s.l.* 5%AS were processed in the miniature CTC unit at NBRR&DC, Nagrakata and tested by the professional tea tasters. The report from professional tea tasters revealed that the processed tea samples had no taint (taste and odor foreign to the tea) and acceptable organoleptic attributes .

Discussion

The tea production is hampered by many insect pests, and termite is an important pest of tea crop worldwide (Gnanapragasam 2018). In recent years, natural insecticides based on botanical or microbial bio-control agents have been attracted the attention of researchers to manage the pests of tea plants (Cheramgoi et al. 2016). In this regard, entomopathogens are no exception. Although some commercial bio-insecticides based on *M. anisopliae s.l.* are available in the market, they have some limitations, for instance, some of them are region-specific and having a narrow range of pesticidal activity as well as their short shelf life problems necessitates the search for new strains.

In the present study, *M. anisopliae s.l.* isolated from Dooars region has been used against *M. obesi*. The fungal strains/isolates, tested as a microbial biological control agent, are found to be more effective in managing pest population when it is isolated from the same ecosystem than the commercial formulations (Babu and Kumhar 2014). Laboratory study revealed that *M. anisopliae s.l.,*

as a potential agent similar to the synthetic insecticides; hence it was used for further field trials. The laboratory study revealed that *M. anisopliae s.l.* was pathogenic to *M. obesi* and field evaluation with liquid formulation also evidenced significant efficacy in the reduction of termite population.

Earlier, the effectiveness of *M. anisopliae s.l.* was reported against termite pests of tea such as *Odontotermes obesus* Rambur (Blattodea: Termitidae) (Hazarika et al.2009), *M. obesi* (Singha et al. 2011), live-wood eating termite (Debnath et al. 2012). During in vitro screening of *M. anisopliae* isolates against termite Singha et al. (2011) reported 78–100% mortality after 8th day of application. Hazarika et al. (2021), while studying on the bio-efficacy of *M. anisopliae* against termite reported that 1×10^9 conidia per ml concentrations of *M. anisopliae* could cause 92–100% mortality in both worker and soldier caste of termites. Field application of *M. anisopliae* (10^9 spores/ml) against termites damaging tea plants in Assam, India revealed that, the effectiveness was concentration dependent showing more effectiveness in reducing the insect populations than the lowest concentrations (Singha et al. 2011). In contrast, in the present study, low concentrations were also found to be effective in the reduction of termite population in tea crop.

Singha et al. (2011) reported that the formulation of *M. anisopliae s.l.* (1×10^9 spores/ml) reduced termite populations up to 50% and Hazarika et al. (2021), *reported M. anisopliae s.l.* (1×10^9 spores/ml) reduced termite populations up to 78% in different tea gardens in Assam states, India, however, in the present investigation more efficacy was reported, the concentration 1000 ml/ ha (2×10^7conidial/ml) concentration was capable to reduce 70 to 85% populations of *M. obesi*. Under the period of study, the yield was significantly low in untreated plots, confirming the best efficacy of the different tested formulations. Among the treatments, tea leaf yield was significantly high in plots sprayed with high concentrations of *M. anisopliae s.l.* 5%AS than plots sprayed with the Thiamethoxam 25WG.

In tea ecosystem, *C. carnea*, *O. javanus*, and *S. gilvifrons* were reported as major predators (Pandey et al. 2021). As far as the impact of entomopathogens on the natural enemies is concerned, Thungrabeab and Tongma (2007) found that *M. anisopliae s.l.* (1×10^8 conidia/ml) was nonpathogenic to the non-target insects such as: *C. carnea*, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and *Dicyphus tamaninii* Wagner (Heteroptera: Miridae) as well as *Heteromurus nitidus* Templeton (Collembola: Entomobryidae), a beneficial soil insect. Similarly, in the present study, the population level of the recorded predators; viz., *C. carnea*, *O. javanus* and *S. gilvifrons* in sprayed plots indicated that, the test bio-product, *M. anisopliae s.l.* 5%AS had no pathogenic effects on the 3 organisms. These results also supported the observations of earlier researchers (Dromph and Vestergaard 2002), who reported that some entomopathogens such as, *M. anisopliae s.l.*, *B. bassiana s.l.* and *Hirsutella* spp. did not affect the population of natural enemies. This shows that EPF could be a quite specific and might infect only a certain type of host.

Observations on the phytotoxicity on tea leaves revealed that *M. anisopliae s.l.* 5%AS was non-phytotoxic to tea, and the teas made from the leaves tested by professional tasters reported that the made tea samples had no taint. Based on the percentage reduction of mite's population, phytotoxicity, nonpathogenic on non-target organisms' population and green leaf yield recorded during the experimental periods, showed that *M. anisopliae s.l.* 5%AS can be commercialized as natural eco-friendly insecticide for the management of *M. obesi* in the tea gardens and can be an important component of integrated pest management strategies for future.

Conclusions

The present study revealed that, the formulation of *M. anisopliae s.l.* 5%AS efficiently controlled *M. obesi,* with a significant reduction the infestation by the termite besides increasing the crop production in multi-season and 2 location trials. The *M. anisopliae s.l.* 5%AS was equally effective like synthetic insecticide. Besides, the formulated *M. anisopliae s.l.* 5%AS did not show any phytotoxic effect on the harvestable shoots and had acceptable organoleptic attributes among the selected consumers. Therefore, the *M. anisopliae s.l.* 5%AS can be commercialized as potential eco-friendly mycopesticide for the mitigation of termite*, M. obesi* in the tea gardens.

Abbreviations

AS: Aqueous suspension; PPC: Plant Protection Code; BCA: Biological Control Agents; PDB: Potato Dextrose Broth; NBRR&DC: North Bengal Regional Research and Development Centre; ITCC: Indian Type Culture Collection; ICAR : Indian Council of Agricultural Research; IARI: Indian Agricultural Research Institute; M: Metre; L: Litre; ml: Milliliter.

Supplementary Information

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Additional file 1. Evaluation of phytotoxicity of *M. anisopliae* 5% AS on tea plants.

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Authors' contributions
BD: Performed laboratory and field trials, analyzed and interpreted the data of the work and prepared the original manuscript. AB: designed and guided the laboratory as well as the field trials, reviewed and edited the writing, KCK: collected soil samples and isolated *M. anisopliae s.l.*. AJP: finalized the protocol for the formulation of *M. anisopliae s.l.*. SS, HR and PD: assisted in conduction of field trials of *M. anisopliae s.l.*. ELA and VRT: prepared *M. anisopliae s.l.* 5% AS liquid formulation. The authors read and approved the final manuscript.

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

Declarations

Ethics approval and consent to participate
Not applicable. The study was conducted using local isolates of fungal antagonists that are abundant in the ecosystem hence does not need ethical approval.

Consent for publication
The authors agreed to publish this paper. The data have not been published partially or completely in any other journal.

Competing interests
The authors declare that they have no competing interests.

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