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RESEARCH

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Assessment of bio-formulations of indigenous strains of *Bacillus thuringiensis*, *Metarhizium robertsii* and *Metarhizium majus* for management of the rhinoceros beetle, *Oryctes rhinoceros* L., in field

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Abstract

Background Among all the coconut pests, rhinoceros beetle causes acute and serious damage to coconut palm. Management of this pest is very difficult due to its nocturnal activity, and also, it damages the emerging leaf inside the bud. Management of rhinoceros beetle using entomopathogens will be of great importance as it is economical and ecofriendly. Studies were carried out to decipher the biocontrol potential of indigenous entomopathogenic bacterium (*Bacillus thuringiensis*) and entomopathogenic fungi (*Metarhizium robertsii* and *M. majus*) against *Oryctes rhinoceros* in the field, individually as well as in combination, by soil drench as well as by topical spray method.

Results The study showed that *B. thuringiensis* strain NBAIR-BTAN4 showed 24.8% mortality at 5th week in soil drench method and 24% mortality in topical spray method. *M. robertsii* showed 24% mortality at 5th week in soil drench method as well as topical spray method. Similarly, *M. majus* showed 24% mortality in soil drench method and 23.2% in topical spray method at 5th week. In combination, NBAIR-BTAN4 + *M. robertsii* showed 40.8 and 44% in soil drench and topical spray method at 5th week, respectively. Combination of NBAIR-BTAN4 + *M. majus* showed 44.8 and 40.8% in soil drench and topical spray method at 5th week, respectively. Combination of NBAIR-BTAN4 + *M. robertsii* + *M. majus* showed 52.8 and 57.6% mortality at 5th week in soil drench and topical spray method, respectively.

Conclusion The present study showed application *B. thuringiensis* in combination with *M. robertsii* and *M. majus* is effective in management rhinoceros beetle in coconut orchard. Study also indicated that soil drench method is more promising strategy than topical spray method in managing larval population of the beetles and also confirmed that *B. thuringiensis*, *M. robertsii* and *M. majus* are compatible and they seemed to have a synergistic effect in controlling the pest in coconut orchard.

Keywords Coconut, Biocontrol, Entomopathogenic fungi, Entomopathogenic bacteria, Biopesticides

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Background

Coconut is commonly known as "Kalpavruksha" in Sanskrit language, and to describe its importance, almost all the parts of the palm are economically important. Coconut oil finds extensive use in food and industrial sectors because of its unique characteristics. The saturated fats in coconut oil have antimicrobial properties. Coconut oil also helps in the absorption of vitamins, minerals and amino acids. In India, coconut is grown in an area of 1.89 million hectares covering 18 states/union territories; the southern states including Kerala, Tamil Nadu, Karnataka and Andhra Pradesh account for 89.7% of total area. The productivity of coconut in these four states vary between 5193 (Karnataka) to 13,771 nuts/ha (Tamil Nadu). Biotic stresses are the major constraints in coconut cultivation. Rhinoceros beetle is a serious pest in coconut causing severe economic losses (Chandran et al. 2017). Rhinoceros beetle causes economic loss of 299.3 million USD in coconut plantation, (Abidin et al. 2014) in tropical regions of the world. They are endemic in coconut-growing regions of South and South East Asia (Bedford 1980) and it has also been reported in Guan and Saipan (Moore 2007). Integrated pest management (IPM) is considered to be best management practice to manage rhinoceros beetle. Biocontrol agents are found to have significant role in IPM in the era of organic agriculture. Management of rhinoceros beetle by entomopathogenic bacteria (*Bacillus thuringiensis*) (*Bt*) and entomopathogenic fungi (EPF) (*Metarhizium robertsii* (Metchnikoff) Sorokin. and *Metarhizium majus* Johnst., Bisch., Rehner and Humber) will be of great significance looking upon advantages of adoption, availability, proven virulence and environment friendliness. Commercial products containing *Bt* acting against coleopteran pests are rare; however, certain *Bt* strains toxic to beetles have been characterized (Yamaguchi et al. 2008). The *Bt* isolate NBAIR-BTAN4 (KY451835.1) was originally isolated from Andaman and was found to be toxic to the beetles in initial laboratory studies. Among EPF, *Metarhizium* spp. are widely used against coleopteran pests. *M. anisopliae* is the most studied; however, studies on *M. robertsii* and *M. majus* are very limited, and hence, they were included in the present study. Also, very limited data are available on combined use of *Bt* and EPF in pest management and very few studies have shown that *Bt* and EPF are compatible (Bahmani et al. 2020). Hence, the present study was carried out to study the biocontrol potential of bio-formulations of *Bt* and EPF both individually and in combination against *Oryctes rhinoceros* L. under both potted and field conditions.

Methods

Isolation and mass multiplication of *Bacillus thuringiensis* strain NBAIR-BTAN4

Bacillus thuringiensis strain NBAIR-BTAN4 (KY451835) was isolated from soils obtained from Andaman Islands as per protocols described by Travers et al. (1987) at ICAR-NBAIR, Bengaluru. The culture was stored and maintained on Luria–Bertani agar medium (HiMedia), and T_3 sporulation medium was used for mass multiplication. The BTAN4 strain was inoculated to T3 medium and grown at 37 °C for 72–96 h @ 150 rpm. After complete sporulation, the broth was centrifuged at 8000 rpm for 10 min and the pellet obtained was dissolved in half of the supernatant. This solution was further mixed with surfactants, dispersants and UV protectants to get a liquid-based formulation (Varshney et al. 2020).

Isolation and mass multiplication of *Metarhizium robertsii* and *M. majus*

Two promising indigenous EPF, viz. *M. robertsii* (ArMz6W) and *M. majus* (VjMz1W), were originally isolated as per the protocol described by Velavan et al. (2021). These efficient strains of *M. robertsii* (KU983797) and *M. majus* (KU983771) were chosen for field studies based on earlier research (Velavan et al. 2017). The selected EPF were grown on potato dextrose agar (PDA) media and incubated at 25 ± 1 °C for 10 days to confirm their viability before being used (Bischoff et al. 2009). For mass multiplication, the strains of EPF were inoculated to the Sabauraud's broth medium and kept for shaking at 150 rpm for 7–8 days at 27 °C. Initial count (colony-forming unit) of *M. robertsii* and *M. majus* in talc formulation was calculated and maintained as described by Easwaramoorthy (2002). The fully grown fungal culture was mixed with talc at 1:2 ratio and kept for drying to obtain up to 12% moisture content. The moisture of the talc was analyzed using moisture analyzer (Denver). The talc-based formulation was mixed with water @ 5 g/l for field trials. For each palm, in both the methods of application, individual and combination, the total volume of formulation was kept constant as one liter.

Collection, culturing and maintenance of *Oryctes rhinoceros* larvae

For field studies, rhinoceros beetle larvae were collected from a single coconut farm situated at Thanjavur District, Tamil Nadu. Collected rhinoceros larvae were sorted based on size and body mass, and larvae were cultured and mass-multiplied in large containers having organically decomposed FYM, decayed coconut wood and root debris for at least one week before used in the experiment for acclimatization.

Soil drench application of bio-formulations in the field

Soil drench experiments were conducted for three bio-formulations (NBAIR-BTAN4-liquid formulation and powder-based formulations of *M. robertsii* and *M. majus*) in seven different treatments including control comprising of T1: NBAIR-BTAN4 (liquid formulation), T2: *M. robertsii* (powder-based formulation), T3: *M. majus* (powder-based formulation), T4: NBAIR-BTAN4 + *M. robertsii* (liquid + powder), T5: NBAIR-BTAN4 + *M. majus* (liquid + powder), T6: NBAIR-BTAN4 + *M. robertsii* + *M. majus* (liquid + powder + powder), and T7: control (water + binding solution). Coconut orchards having one- to two-year-old plants were chosen for soil drench experiment. Optimum drainage and moisture were maintained during entire period of experiment. For each plant pit, 5 larvae of similar size and body weight were released, and similar experiment conditions maintained for all the treatments. Five replications (five seedlings) were maintained for each treatment. Twenty-five larvae were used for feeding in soil drench experiments per treatment in each replication. Larval mortality was recorded during 1st to 5th week in soil drench method. Dead larva was gently collected, washed with water, surface-sterilized with sodium hypochloride (0.1%) and 70% alcohol, further washed with sterile water and incubated in Petri plate for spore-forming EPF or bacterial growth to prove the Koch postulates.

Topical spray of entomopathogenic fungi and bacteria bio-formulations against rhinoceros beetles in field

For the topical spray experiment, treatments were similar to soil drench method. Coconut orchard having one- to two-year-old plants was chosen for spaying. For each coconut seedling, five larvae of similar size and body mass were released at different young whorls, after spraying of bio-formulations. For each treatment, five replications were maintained, per each replication five seedlings were taken, and hence, total larvae used per treatment were 25 larvae for one treatment. Mortality of larvae was recorded every week; dead larvae were brought to laboratory to test the Koch postulates.

Statistical analysis

Data recorded were analyzed by three-factor analysis using SAS 9.3 version considering three factors, viz. microbial agents, method of application and duration by post hoc analysis. Significance of mortality of rhinoceros beetles was calculated using Tukey HSD.

Results

Liquid-based formulation of the *Bt* NBAIR-BTAN4 and powder-based formulations of *Metarhizium robertsii* and *M. majus* were tested by soil drench application and topical spray method against rhinoceros beetles in coconut orchards.

Effect of soil drench application of bio-formulations of entomopathogenic bacteria and fungi on mortality of rhinoceros beetle larvae in coconut orchards

Application of *Bt* strain NBAIR-BTAN4 liquid formulation individually by soil drench method showed 5.6% mortality in the first week, 16% in 3rd week and 24.8% in 5th week. Application of *M. robertsii* powder formulation individually by soil drench method showed 1.6, 12 and 23.2% mortality in 1st, 3rd and 5th week, respectively. The application of *M. majus* showed 3.2, 16 and 24% mortality in 1st, 3rd and 5th week, respectively. Combined application of NBAIR-BTAN4 liquid formulation + *M. robertsii* incited 8.8, 24 and 40.8% mortality in 1st, 3rd and 5th week, respectively. In the other combined application of NBAIR-BTAN4 + *M. majus*, % mortality observed was 7.2, 25.6 and 44.8 during 1st, 3rd and 5th week, respectively. However, when all three biocontrol agents were applied in combination, the larval mortality recorded was 12, 32 and 52.8% in 1st, 3rd and 5th week, respectively. In control, mortality recorded was 0, 2.4 and 4.8% in 1st, 3rd and 5th week, respectively. Hence, the results indicated that combined soil drench application of all three biocontrol agents was the most effective with more than 50% larval deaths in 5th week.

Effect of topical spray method on larval mortality

Application of NBAIR-BTAN4 liquid formulation individually by topical spray method caused 1.6, 12.8 and 24% mortality in 1st, 3rd and 5th week, respectively. Application of *M. robertsii* powder formulation individually by topical spray incited 1.6, 13.6 and 23.2% mortality in 1st, 3rd and 5th week, respectively. Application of *M. majus* showed 2.4, 12.2 and 20.8% larval death in 1st, 3rd and 5th week, respectively. Combined application in topical spray was also found to be effective as was observed in soil application. Combination of NBAIR-BTAN4 + *M. robertsii* recorded 5.6, 24.8 and 44% larval death in 1st, 3rd and 5th week, respectively. When NBAIR-BTAN4 was combined with *M. majus*, the larval mortality recorded was 4, 20.8 and 40.8% in 1st, 3rd and 5th week, respectively. Again, the combination of all three bio-agents was highly effective with recorded mortality of 9.6, 32.8 and 57.6% in 1st, 3rd and 5th week, respectively. By analyzing mortality data from all the treatments in topical spray, combination of NBAIR- BTAN4 + *M. robertsii* + *M. majus* showed maximum mortality of 57.6% by 5th week and could indicate a synergistic effect.

Results of means of the three-factor analysis (microbials, method of application and duration) for topical showed that T6 (combination of all three bio-agents) gave the highest mortality of 31.52 (18.61) % at 5 weeks which was significantly different to all other treatments (Table 1A). Combination of *Bt* with *Metarhizium* (T4 and T5) was on par recording 24 to 25% mortality. Individual treatments of NBAIR-BTAN4-liquid formulation (T1), powder-based formulation (PBF) of *M. robertsii* (T2) and PBF of *M. majus* (T3) did not vary significantly to each other and recorded 12 to 15% mortality (Table 1A). However, all treatments with biocontrol agents (T1 to T6) recorded mortality that was significantly higher than control. Three-factor analyses for soil application method gave similar results (Table 1B). The results indicated that combining *Bt* with *Metarhizium* was effective in controlling the coconut rhinoceros beetle larvae.

In order to confirm the above observations, two-way analysis of means of mortality recorded in the two treatment methods (soil application and topical spray) with duration (weeks) was carried out. The results again showed that T6 (combination of *Bt* with two *Metarhizium* spp.) gave maximum a mortality of 32.4 (19.23) %, which was significantly high to all other treatments (Table 2). Treatments of *Bt* combined with either *M. robertsii* or *M. majus* (T4 and T5) recorded mortality of 24 to 25% and were on par, but were significantly high to individual treatments (T1, T2 and T3). Mortality of *O. rhinoceros* larvae with *Bt* alone (T2) was significantly higher than individual treatments of *Metarhizium.* All biocontrol interventions (T1 to T6) gave significantly higher mortality when compared to control. The results establish that combination of *Bt* with *Metarhizium* can be successfully used in the biological control of *O. rhinoceros*.

Discussion

Coconut is one of the most important plantation crops in South and South East Asia. Biotic stresses are known to cause significant economic loss to coconut orchards. The present emphasis is on the reduction in the use of chemical pesticides. Studies on the effect of combinations of biological control agents in pest control are very

Table 1 (A) Mortality of *Oryctes rhinoceros* in treatments as topical spray. (B) Mortality of *Oryctes rhinoceros* in treatments as soil drench method

Treatments	***F*1**					**Mean of *F*1**	
	***W*1**	***W*2**	***W*3**	***W*4**	***W*5**		
(A)							
T1	5.6 (3.21) ^{klmn}	10.4 (5.97)^{mknsolpqtru}	16.0 (9.20)^{mknjolphi}	20.0 (11.53)^{kjlhi}	24.8 (14.35)^{fegh}	15.36 (8.85)^{d}	
T2	1.6 (0.91)^{stu}	6.4 (3.67)^{sopqtru}	12.0 (6.89)^{mknsolpqr}	18.4 (10.60)^{mkjlhi}	23.2 (13.41)^{fjghi}	12.32 (7.09)^{ed}	
T3	3.2 (1.83)^{sqtru}	10.4 (5.97)^{mknsolpqtru}	16.0 (9.20)^{mknjolphi}	20.0 (11.53)^{kjlhi}	24.0 (13.88)^{feghi}	14.72 (8.48)^{ed}	
T4	8.8 (5.05)^{mnsopqtru}	17.6 (10.13)^{mknjlhi}	24.0 (13.88)^{feghi}	32.8 (19.15)^{fed}	40.8 (24.16)^{cd}	24.80 (14.47)^{cb}	
T5	7.2 (4.13)^{nsopqtru}	16.8 (9.67)^{mknjolhi}	25.6 (14.85)^{fegh}	33.6 (19.63)^{ed}	44.8 (26.69)^{cb}	25.60 (14.99)^{b}	
T6	12.0 (6.89)^{mknsolpqr}	20.8 (12.0)^{kjhi}	32.0 (18.67)^{fed}	40.0 (23.63)^{cd}	52.8 (31.87)^{ab}	31.52 (18.61)^{a}	
T7	0.0 (-0.0)^{u}	0.0 (-0.0)^{u}	2.4 (1.37)^{stru}	3.2 (1.83)^{sqtru}	4.8 (2.75)^{u}	2.08 (1.19)^{f}	
Mean	5.48 (3.14)	11.77 (6.77)	18.28 (10.58)	24.00 (13.98)	24.05 (14.04)^{b}	18.05 (10.5)^{a}	
Treatments	***F*2**					**Mean of *F*2**	**Mean of *F*1 and *F*2**
	***W*1**	***W*2**	***W*3**	***W*4**	***W*5**		
(B)							
T1	1.6 (0.91)^{uts}	7.2 (4.13)^{urtqposn}	12.8 (7.35)^{rqplojnkm}	20.0 (11.53)^{ihljk}	24.0 (13.88)^{ihgef}	13.12 (7.56)^{ed}	14.24 (8.21)^{c}
T2	1.6 (0.91)^{uts}	7.2 (4.13)^{urtqposn}	13.6 (7.82)^{iqplojnkm}	19.2 (11.06)^{ihljkm}	23.2 (13.41)^{ihgjf}	12.96 (7.47)^{ed}	12.64 (7.28)^{c}
T3	2.4 (1.37)^{urts}	5.6 (3.21)^{urtqps}	11.2 (6.43)^{rtqplosnkm}	16.0 (9.20)^{ihplojnkm}	20.8 (12.00)^{ihgjk}	11.20 (6.44)^{e}	12.96 (7.46)^{c}
T4	5.6 (3.21)^{urtqps}	13.6 (7.81)^{iqplojnkm}	24.8 (14.37)^{hgef}	32.8 (19.15)^{def}	44.0 (26.16)^{bc}	24.16 (14.14)^{cb}	24.48 (14.31)^{b}
T5	4.0 (2.29)^{urtqs}	12.0 (6.89)^{rqplosnkm}	20.8 (12.00)^{ihgjk}	31.2 (18.18)^{dgef}	40.8 (24.16)^{dc}	21.76 (12.70)^{c}	23.68 (13.85)^{b}
T6	9.6 (5.51)^{urtqplosnm}	19.2 (11.07)^{ihljkm}	32.8 (19.16)^{def}	47.2 (28.21)^{bc}	57.6 (35.28)^{a}	33.28 (19.84)^{a}	32.40 (19.23)^{a}
T7	0.0 (-0.0)^{u}	0.0 (-0.0)^{u}	0.8 (0.45)^{ut}	2.4 (1.37)^{urts}	4.8 (2.75)^{urtqs}	1.60 (0.91)^{f}	1.84 (1.05)^{d}
Mean	3.54 (2.03)	9.25 (5.32)	16.68 (9.65)	24.11 (14.10)	30.74 (18.23)	16.86 (9.870)b	

T1—NBAIR-BTAN4-liquid formulation, T2—powder-based formulation (PBF) in *Metarhizium robertsii;* T3—powder-based formulation (PBF) in *Metarhizium majus,* T4—*M. robertsii* + NBAIR-BTAN4; T5—*M. majus* + NBAIR-BTAN4, T6—*M. majus* + *M. robertsii* + NBAIR-BTAN4 and T7—Control + Triton X-100 and Glycerol

The means of same letters indicates non-significant

Table 2 Combined mortality of *Oryctes rhinoceros* in field

Treatments	*W1*	*W2*	*W3*	*W4*	*W5*	Mean
T1	3.6 (2.06)^{nlm}	8.8 (5.050)^{jilhk}	14.4 (8.27)^{ighf}	20.0 (11.53)^{edf}	24.4 (14.12)^{d}	14.24 (8.21)^{c}
T2	1.6 (0.91)^{nm}	6.8 (3.90)^{jnlmk}	12.8 (7.36)^{jigh}	18.8 (10.83)^{egdf}	23.2 (13.41)^{d}	12.64 (7.28)^{c}
T3	2.8 (1.60)^{nlm}	8.0 (4.59)^{jilmk}	13.6 (7.81)^{jighf}	18.0 (10.36)^{egdf}	22.4 (12.94)^{ed}	12.96 (7.46)^{c}
T4	7.2 (4.13)^{jlmk}	15.6 (8.97)^{eghf}	24.4 (14.12)^{d}	32.8 (19.15)^{c}	42.4 (25.16)^{b}	24.48 (14.31)^{b}
T5	5.6 (3.21)^{nlmk}	14.4 (8.28)^{ighf}	23.2 (13.43)^{d}	32.4 (18.90)^{c}	42.8 (25.43)^{b}	23.68 (13.85)^{b}
T6	10.8 (6.20)^{jihk}	20.0 (11.53)^{edf}	32.4 (18.91)^{c}	43.6 (25.92)^{b}	55.2 (33.58)^{a}	32.40 (19.23)^{a}
T7	0.0 (-0.0)^{n}	0.0 (-0.0)^{n}	1.6 (0.91)^{nm}	2.8 (1.60)^{nlm}	4.8 (2.75)^{nlmk}	1.84 (1.05)^{d}
Mean	4.51 (2.58)^{e}	10.51 (6.047)^{d}	17.48 (10.12)^{c}	24.05 (14.04)^{b}	30.74 (18.20)^{a}	

The means of same letters indicate non-significant

limited, especially on interactions between *Bt* and EPF (Mwamburi et al. 2009). Hence, in the present study formulations of *Bt* and *Metarhizium* spp. were selected and evaluated against *O. rhinoceros* individually as well as in combination based on their proven efficacy against coleopteran pests. This study clearly indicates that there was improved pest control with possible synergistic interaction between *Bt* and *Metarhizium* as significant differences in mortality of larvae were observed. Individual treatments did record larval mortality, but there were twofold increases when *Bt* and *Metarhizium* were combined. The two-way analysis for topical spray and soil drench showed that individual treatments did not vary significantly, but combined treatments gave significantly high mortality rates. Mixed infections lead to diverse interactions among natural enemies (Pozo et al. 2021) and microbial consortia can provide diverse modes of action, thereby achieving better pest or disease control than single microorganisms (Sarma et al. 2015). In studies targeting other insect pests, synergistic interactions have been observed from certain combinations of *B. bassiana* and *Bt* (Wraight and Ramos 2005), independent (Lewis and Bing 1991) or antagonistic (Ma et al. 2008). Combining *Bt* with the two *Metarhizium* spp. gave significantly higher mortality rates when compared to all other treatments. Application of mixed formulations of biocontrol agents shows better pest control (Oestergaard et al. 2006) or may be used as a pesticide resistance management strategy (Corbel et al. 2002). It was carried out comprehensive three-way and two-way analyses of the present results and all results indicated that combination of *Bt* with *Metarhizium* spp. was highly efficient.

The *Bt* (NBAIR-BTAN4) expresses Cry1IA and Cry2Ab (as per genome analysis, results not shown) which could be playing a role in coleopteran toxicity. The two strains of *Metarhizium*, viz. *M. robertsii* and *M. majus*, were previously studied against banana stem weevil and found effective (Velavan et al. 2022). Presently *Bt* is mainly utilized for biological control of lepidopteran pests and very limited reports are available on coleopteran toxic *Bt* (Palma et al. 2014). In the present study, we could isolate coleopteran toxic *Bt* against *O. rhinoceros*, and when applied in combination with *Metarhizium* spp., there was an increased larval death indicating synergistic effect.

Conclusion

In the present study, biocontrol potential of coleopteran toxic *Bt, Metarhizium robertsii* and *M. majus* individually and in combination against rhinoceros beetle was demonstrated. The results indicated that combined application of *Bt* with *Metarhizium* spp. significantly increased the mortality rate of larvae. It was also observed that soil drench application was a more promising strategy than the topical spray method. Thus, synergistic effect of EPF and bacterial consortium in controlling *O. rhinoceros* in coconut orchards was confirmed by this study. Development of efficient formulations using both *Bt* and fungi can be an effective and long-term solution for rhinoceros beetle management in coconut orchards. Therefore, in bio-intensive management of rhinoceros beetle, application of *Bt* and *M. robertsii* and *M. majus* consortium is recommended.

Abbreviations

NBAIR	National Bureau of Agriculture Insect Resources
Bt	*Bacillus thuringiensis*
EPB	Entomopathogenic bacterium
EPF	Entomopathogenic fungi
PDA	Potato dextrose agar
FYM	Farm yard manure

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Author contributions

RR and CM gave the concept. VV and CM conducted the experiment and wrote the manuscripts with statistical analysis. MM, KA, SG, ANS and RHK helped in data recording statistical analysis of data. SM, PMK, PD and SSN gave their valuable suggestions during experiments and reviewed the manuscript. All authors have read and reviewed the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All procedures performed in studies are in accordance with the ethical standards of the institutional and/or national research committee. We further declare that no animal was harmed during this study.

Consent for publication
Informed consent was obtained from all individual participants included in the study.

Competing interests
The authors declare that there is no conflict of interest.

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References

Abidin C, Ahmad A, Salim H, Hamid N (2014) Population dynamics of *Oryctes rhinoceros* in decomposing oil palm trunks in areas practicing zero burning and partial burning. J Oil Palm Res 26:140–145
Bahmani N, Latifian M, Ostovan H, Hesami S (2020) Pathogenic effects of *Beauveria bassiana* and *Bacillus thuringiensis* on the population dynamics of *Ephestia Kuehniella*. Egypt J Biol Pest Control 30:94. https://doi.org/10.1186/s41938-020-00285-2
Bedford GO (1980) Biology, ecology, a control of palm rhinoceros beetles. Annu Rev Entomol 25:309–339
Bischoff JF, Rehner SA, Humber RA (2009) A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101:512–530
Chandran KP, Thamban C, Prathibha VH, Prathibha PS (2017) Assessing status of pest and diseases with cluster approach-A case of coconut in Kasaragod district in northern Kerala. J Plant Crops 45(1):33–42
Corbel V, Darriet F, Chandre F, Hougard JM (2002) Insecticide mixtures for mosquito net impregnation against malaria vectors. Parasite 9:255–259
Easwaramoorthy S (2002) Granulovirus formulation in pest management in India. In: Proceedings of the ICAR-CABI workshop on biopesticide formulations and applications. PDBC, Bangalore, pp 41–48
Lewis L, Bing L (1991) *Bacillus thuringiensis* Berliner and *Beauveria bassiana* (Balsamo) Vuillimen for European corn borer control: program for immediate and season-long suppression. Can Entomol 123(2):387–393. https://doi.org/10.4039/ent123387-2
Ma XM, Liu XX, Ning X, Zhang B, Han F, Guan XM, Tan YF, Zhang QW (2008) Effects of *Bacillus thuringiensis* toxin Cry1Ac and *Beauveria bassiana* on Asiatic corn borer (Lepidoptera: Crambidae). J Invertebr Pathol 99(2):123–128
Moore A (2007) Rhinoceros beetle pest found in Guam and Saipan. Pest Alert. Plant Protection Service Secretariat of the Pacific Community, Suva, Fiji Islands
Mwamburi LA, Laing MD, Miller R (2009) Interaction between *Beauveria bassiana* and *Bacillus thuringiensis* var. israelensis for the control of house fly larvae and adults in poultry houses. Poult Sci 88(11):2307–2314
Oestergaard J, Belau C, Strauch O, Ester A, van Rozen K, Ehlers R-U (2006) Biological control of *Tipula paludosa* (Diptera: Nematocera) using entomopathogenic nematodes (*Steinernema* spp.) and *Bacillus thuringiensis* subsp. *israelensis*. Biol Control 39:525–531
Palma L, Muñoz D, Berry C, Murillo J, Caballero P (2014) *Bacillus thuringiensis* Toxins: an overview of their biocidal activity. Toxins 6:3296–3325
Pozo MJ, Zabalgogeazcoa I, Vazquez de Aldana BR, Martinez-Medina A (2021) Untapping the potential of plant mycobiomes for applications in agriculture. Curr Opin Plant Biol 60:102034
Sarma BK, Yadav SK, Singh S, Singh HB (2015) Microbial consortium-mediated plant defense against phytopathogens: readdressing for enhancing efficacy. Soil Biol Biochem 87:25–33. https://doi.org/10.1016/j.soilbio.2015.04.001
Travers RS, Martin PA, Reichelderfer CF (1987) Selective process for efficient isolation of soil *Bacillus* spp. Appl Environ Microbiol 53:1263–12666. https://doi.org/10.1128/aem.53.6.1263-1266.1987
Varshney R, Poornesha B, Raghavendra A, Lalitha Y, Apoorva V, Ramanujam B, Rangeshwaran R, Subaharan K, Shylesha AN, Bakthavatsalam N, Chaudhary M, Pandit V (2020) Biocontrol-based management of fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae) on Indian Maize. J Plant Dis Prot 128:87–95
Velavan V, Sivakumar G, Rangeshwaran R, Sundararaj R, Sasidharan TO (2017) *Metarhizium majus* and *Metarhizium robertsii* show enhanced activity against the coleopteran pests *Holotricha serrata* and *Oryctes rhinoceros*. J Biol Control 31:35–145
Velavan V, Rangeshwaran R, Sivakumar G, Sasidharan TO, Sundararaj R, Kandan A (2021) Occurrence of *Metarhizium* spp. isolated from forest soils in South India and their potential in biological control of banana stem weevil *Odoiporus longicollis* Oliver. Egypt J Biol Pest Control 31:131–143
Velavan V, Dhanapal R, Ramkumar G, Karthi S, Senthil-Nathan S, Ndomba OA, Kweka EJ (2022) Characterization and evaluation of *Metarhizium* spp. (Metsch.) Sorokin isolates for their temperature tolerance. J Fungi 8:68. https://doi.org/10.3390/jof8010068
Wraight SP, Ramos ME (2005) Synergistic interaction between *Beauveria bassiana* - and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against Weld populations of Colorado potato beetle larvae. J Invertebr Pathol 90:139–150
Yamaguchi T, Sahara K, Bando H, Asano S (2008) Discovery of a novel *Bacillus thuringiensis* Cry8D protein and the unique toxicity of the Cry8D-class proteins against scarab beetles. J Invertebr Pathol 99:257–262. https://doi.org/10.1016/j.jip.2008.05.009

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