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Research Article

In Vitro evaluation of native isolate of *Metarhizium anisopliae* (Metchinkoff) sorokin and its oil in water formulations against *Odoiporus longicollis* olivier

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ABSTRACT: Banana pseudostem borer *Odoiporus longicollis* Olivier is one of the serious monophagous pest threatening the production and productivity of banana in south India. The native isolate of entomopathogenic fungi *Metarhizium anisopliae* (Metchinkoff) Sorokin was identified and evaluated for its virulence to *O. longicollis* compared with other entomopathogenic fungal isolates. The native isolate *M. anisopliae* (Ma-BW) showed higher virulence to adults with the minimum LC_{50} value ~1.0 x 10^7spores ml^{-1} and faster lethal effect on adult *O. longicollis* with the shortest LT_{50} value of 62.54 hours. Among different oil in water formulations of Ma-BW evaluated against adult *O. longicollis* in laboratory at 2% showed that the formulation of Ma-BW in ground nut oil performed superior with adult mortality of 83.33 per cent at 120 HAT followed by oil formulations of Ma-BW in sunflower oil, gingelly oil and neem oil with76.67 per cent adult mortality at the same concentration and time period.

KEY WORDS: Attract-and-kill, Native Isolate, Oil-In-Water Formulation, LC_{50}, LT_{50}

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INTRODUCTION

Banana pseudostem borer *Odoiporus longicollis*, Olivier (Coleoptera: Curculionoidea:Rhynchophoridae) is an important monophagous pest of banana causing economic damage in banana and plantains southern India. On the basis of plant stage attacked and management action undertaken the yield loss may vary upto 10-90 per cent. The grubs of this pest remain inside the pseudostem and feed by boring the pseudostem, whereas adults can be seen outside. At present, pseudostem injections with broad spectrum insecticides have been proven as the best management practice. Due to environmental concerns and adverse effects of broad spectrum insecticides, banana cultivation is shifting towards organic cultivation. Thus, it is imperative to find alternative eco-friendly management of banana pseudostem borer. One of the possible ways for managing the pest is the use of mass trapping and attract-and-kill strategy using banana pseudostem traps and biocontrol agents such as entomopathogenic fungi.

The entomopathogenic fungi are important natural regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture (Ambethgar, 2009). Entomopathogenic fungi are constantly present in populations of insect hosts and can cause epizootics among insect population (Fuxa and Tanada, 1987). Due to their ability of horizontal transmission, entomopathogenic fungi can be effectively used in banana ecosystem against borer pests (Lopes *et al.,* 2011). *Metarhizium anisopliae* (Metchinkoff) Sorokin, a hyphomycete entomopathogenic fungi belonging to the subdivision Deuteromycotina is a soil inhabiting fungus occurring worldwide and has been found to control several agricultural pests (Lingappa *et al.,* 2005). The successful development of entomopathogenic fungi as microbial insecticides requires careful and appropriate selection of the most efficacious strains (Soper and Ward, 1981). It is a general consensus that strains of entomopathogenic fungi are most pathogenic to the species of insect from which they are iso-

lated. Identification of such type of isolates will pave way for the development of biopesticide with high virulence. Furthermore, the field stability and performance of the myco-insecticides can be improved by finding alternatives to the conventional formulating technology, i.e., wettable powder containing conidia. Though, the talc based formulation containing beneficial microbes was found to be effective and cheaper for pest and diseases of different crops (Rajendran *et al*. 2007), oil formulations increases their effectiveness (Prior *et al*. 1988). Formulation technology also plays a role in "bait and kill" or "lure and kill" applications as summarized by Vega *et al*. (2007) and Baverstock *et al*. (2009). Keeping this in view, the native isolate of *M. anisopliae* (Ma-Bw) isolated from *O. longicollis*, was tested for its virulence against banana pseudostem borer *O. longicollis* under laboratory condition. Further, oil in water formulations of *M. anisopliae* (Ma-BW) with different oils were also tested for their efficacy against *O. longicollis* under laboratory conditions.

Source of fungal isolates

Native isolate of *Metarhizium anisopliae* (Ma-BW) was isolated from naturally infected banana pseudostem weevil, *O. longicollis* from Dinampalayam village (Block: Thondamuthur) of Coimbatore District, Tamil Nadu, India during 2015. The fungus was cultured on Sabouraud Maltose Agar with Yeast Extract (SMAY) medium for 7-10 days at 25°C. Pathogenicity of the fungus was proved by Koch's postulates by the method described by Goettel and Inglis (1997). Identity of the fungus was confirmed by the experts at the Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. Other entomopathogenic fungal isolate used for comparison were *Beauveria bassiana* (Bb-0367), provided by National Research Centre for Banana (Tiruchirapalli) and two isolates of *M. anisopliae* procured from Sugarcane breeding institute (*Ma*-SBI culture derived from Insect pathology lab, Department of Agricultural Entomology, TNAU) and Indian Institute of Horticulture Research (Ma-IIHR culture from biocontrol laboratory). The cultures were maintained at 25±2℃ in an incubator on SMAY media.

Virulence assay

Five different spore concentrations (~$1x10^9$ to $1x10^5$spores ml^{-1}) from the stock suspension were prepared in each isolate for the assay of concentration mortality response. Ten pre-starved laboratory reared adult weevils were dipped in ten ml of respective concentrations for 15 seconds and were released in plastic container (Diameter 10 cm and height 5 cm) containing fresh piece of banana leaf sheath. Weevils were dipped in 0.05 per cent Tween 80® solution as control. Treatments were replicated five times. The post treatment mortality counts were taken at 24 hours interval for 5 days.

Development of oil in water based formulations

Oil-in-water formulations of native isolate of *Metarhizium anisopliae* (Ma-BW) was prepared by mixing the surfactant mixed oil phase with the spore suspension in aqueous phase. *M. anisopliae* strain Ma-BW was cultured on SMAY for 15 days at 25± 2 °C. Spores were harvested using 0.01 % Tween-80 and spore suspension was prepared by centrifuging the conidia in 0.02 % Tween-80 and decanting the supernatant in the centrifuge tubes. The suspension was vortexed after adding sterile distilled water. Centrifugation and decanting procedure was repeated three times to eliminate Tween-80. Conidial pellet was re-suspended in water to prepare 200 µl conidial stock, which was then added to 9.8 ml distilled water. Conidial concentration was adjusted to ~1×10^8 spores/ml. Sunflower, gingelly, ground nut, castor, neem and mineral oil at 2% concentration were used to prepare the oil-in-water formulations. Oil phase was prepared by adding 2% sterilized oil, 2% Triton-X-100 (Non-ionic surfactant), 2% Na_2CO_3 (Sodium Carbonate- stabilizer) and 1% Paraffin oil (anti-foaming agent) to 93% sterile distilled water. Both phases, *i.e.,* oil and water phases were added and homogenized using magnetic stirrer for one hour to get a stable formulation (Ummidi and Vadlamani, 2014). The talc based formulation was prepared as per the procedure described by Jayarajan *et al*. (1994).

Efficacy oil in water based formulations against Odoiporus longicollis

Oil formulations of *Metarhizium anisopliae* (Ma-BW**)** with sunflower, gingelly, ground nut, castor and neem and mineral oil at 2% were compared with talc formulation of Ma-BW, crude extracts of Ma-BW and Bb-0367 and standard insecticide. Banana leaf sheath piece (5 × 5 cm) was dipped in the respective solution for five minutes. Pre-starved adult weevils were allowed to feed for 24 hours. Subsequently, the adult weevils were provided with untreated leaf sheaths. Mortality was observed at 24 hours interval up to 5 days and mycosis was observed at 3 and 6 day after treatment. The experiment was conducted in a completely randomized design with eleven treatments and three replications.

Statistical analysis

The concentration mortality response and time mortality responses were subjected to Probit analysis and the control mortality was corrected by Abbott's formula (Finney, 1971). After suitable transformation, the data on efficacy study was analyzed using Analysis of Variance (ANOVA) by completely randomized design using AGRES 3.01 and

Table 1. Concentration mortality response of *Odoiporus longicollis* to different entomopathogenic fungal isolates

Fungal isolates	Heterogeneity (2)*	Regression equation	LC_{50} ($x10^7$ spores ml^{-1})	Fiducial limit ($x10^8$ to 10^7 spores ml^{-1})
M. anisopliae (Ma-BW)	5.84	y = 0.599x - 1.529	1.07	6.43 - 1.79
M. anisopliae (Ma SBI)	4.11	y = 0.203x + 3.322	1.28	7.84 - 2.11
M. anisopliae (Ma-IIHR)	2.82	y = 0.627x - 1.41	1.58	9.71 - 2.57
B. bassiana (Bb-0367)	2.48	y = 0.547x - 0.854	3.69	2.06 - 6.55

*All lines are significantly a good fit at P≤0.05; No. of insects used in each isolate = 50

Table 2. Time mortality response of *Odoiporus longicollis* to different entomopathogenic fungal isolates @ 10^8 spores ml^{-1}

Fungal isolates	Heterogeneity (ϕ2)*	Regression equation	LT_{50} (h)	Fiducial limit (h)
M. anisopliae (Ma-BW)	4.25	y = 3.434x + 11.37	62.54	57.42 - 68.11
M. anisopliae (Ma SBI)	4.3	y = 3.212x + 10.47	67.41	61.66 - 73.69
M. anisopliae (Ma-IIHR)	3.49	y = 3.22x + 10.54	67.87	62.08 - 74.20
B. bassiana (Bb-0367)	4.74	y = 2.555x + 7.531	75.64	68.02 - 84.12

*All lines are significantly a good fit at P≤0.05

AGDATA software. Duncan's Multiple Range Test (DMRT) was applied for comparing treatment means at 5 per cent level of significance.

Virulence of *Metarhizium anisopliae* iaolates against adults of *Odoiporus longicollis*

All the four isolates of entomopathogenic fungi tested were proved to be pathogenic to the adult *O. longicollis* with significant differences in median lethal time and concentration (Table 1). The median lethal concentration (LC_{50}) for all pathogens was found to be in range of 1.07 x 10^7 to 3.69 x 10^7spores ml^{-1}. The native isolate of *M. anisopliae* (Ma-BW) showed higher virulence against adults with the lowest LC_{50} values 1.07 x 10^7spores ml^{-1}. This was followed by the other isolates of *M. anisopliae*, *viz*., Ma-SBI and Ma-IIHR which recorded LC_{50} values of 1.28 x 10^7 and 1.58 x 10^7 spores ml^{-1} respectively. The isolate of *B. bassiana* (Bb-0367) was found to be the least virulent among all the isolates tested with the highest LC_{50} value of 3.69 x 10^7spores ml^{-1}. Similarly, the isolate, Ma-BW had faster lethal effect on adult *O. longicollis* with the lowest LT_{50} value of 62.54 hours when tested at the concentration~1 x 10^8 spores ml^{-1} (Table 2). This was followed by the isolates Ma-SBI and Ma-IIHR which recorded LT_{50} of 67.41 and 67.87 h, respectively. The LT_{50} value for the isolate Bb-0367 was 75.64 h and recorded slower lethal effect on adult *O. longicollis.*

This showed that the native isolate was more virulent against the insect. It is generally admitted that the most virulent fungal isolates are those isolated from the original host.The local isolates of *Metarhizium* spp. depicted promising preliminary results that could pave the way towards production of new and highly effective entomopathogenic fungi in curbing different agricultural insect pests (Pajar *et al.*, 2013). The above results are in tune with Padmanaban *et al.,* (2009) who reported that native isolate *B. bassiana* 176 was virulent against banana pseudostem weevil, *O. longicollis* and rhizome weevil *Cosmopolites sordidus* (Germar) causing cent per cent mortality of both test insects at six days after treatment. Similarly, liquid formulation of *B. bassiana* (beauvericide) was found highly effective in managing pseudostem borer, *O. longicollis*. Spraying with beauvericide resulted in the highest per cent reduction (64.43) of pseudostem borer infestation, while it was 36.38 in case of swabbing of beauvericide in pseudostem (Sharmila Bharathi and Mohan, 2015). Effectiveness of *M. anisopliae* against pseudostem borer is also reported by Anitha (2000).

Evaluation oil in water based formulations against *Odoiporus longicollis*

The results on efficacy of different formulations of entomopathogenic fungi against adult *O. longicollis* revealed that, the highest adult mortality of 100 per cent was recorded in standard check monocrotophos 36 SL (Std. check @ 0.054%), 24 Hours After Treatment (HAT) (Table 3). Among the treatment with entomopathogens, crude Ma-BW and 2% formulation of Ma-BW in ground nut oil recorded highest adult mortality of 83.33 per cent and was found on par with crude Bb-0367 and 2% formulation of Ma-BW in castor oil with 80 per cent mortality at 120 HAT. Next effective treatment was 2% oil formulations of Ma-BW in sunflower oil, gingelly oil and neem oil with per cent adult mortality of 76.67. Talc formulation of Ma-BW and 2% formulation of Ma-BW in mineral oil recorded 66.67 and 60 per cent mortality of adult pseudostem borer at 120 HAT. Highest mean per cent mycosis of adult pseudostem borer was observed in treatment with crude Ma-BW *i.e*., 51.29, and was followed by 2% castor oil + Ma-BW and 2% ground nut oil + Ma-BW with mean per cent mycosis of 50.05 and 47.95 respectively. 2 % mineral oil + Ma-BW recorded least mycosis in adults which was 38.72 per cent.

Table 3. Efficacy of oil in water formulation of *Metarhizium anisopliae* (Ma-BW) against *Odoiporus longicollis*

S. No	Treatments	Mortality of adults* (%) Hours after treatment (HAT)					Mean Mortality (%)	Mycosis of adults* (%) Hours after treatment (HAT)		Mean Mycosis (%)
		24	48	72	96	120		72	144	
1	2 % Sunflower oil + Ma-BW	06.67^{bcd} (14.96)	10.00^{cd} (18.43)	36.67^{b} (37.27)	70.00^{bc} (56.79)	76.67^{bc} (61.12)	26.00	33.33^{ab} (35.26)	73.33^{bc} (58.91)	46.12
2	2 % Gingelly oil + Ma-BW	03.33^{cd} (10.52)	10.00^{cd} (18.43)	33.33^{bc} (35.26)	70.00^{bc} (56.79)	76.67^{bc} (61.12)	24.67	30.00^{ab} (33.21)	76.67^{abc} (61.12)	45.56
3	2 % Ground nut oil + Ma-BW	10.00^{bc} (18.43)	10.00^{cd} (18.43)	30.00^{bc} (33.21)	73.33^{bc} (58.91)	83.33^{b} (65.91)	26.67	30.00^{ab} (33.21)	83.33^{a} (65.91)	47.95
4	2 % Castor oil + Ma-BW	06.67^{bcd} (14.96)	16.67^{bc} (24.09)	36.67^{b} (37.27)	56.67^{d} (48.83)	80.00^{b} (63.43)	28.00	36.67^{a} (37.27)	80.00^{ab} (63.43)	50.05
5	2 % Neem oil + Ma-BW	16.67^{b} (24.09)	20.00^{b} (26.57)	36.67^{b} (37.27)	76.67^{b} (61.12)	76.67^{bc} (61.12)	30.00	36.67^{a} (37.27)	76.67^{abc} (61.12)	48.89
6	2 % Mineral oil + Ma-BW	03.33^{cd} (10.52)	06.67^{d} (14.96)	26.67^{c} (31.09)	56.67^{d} (48.83)	60.00^{d} (50.77)	19.33	26.67^{b} (31.09)	60.00^{d} (50.77)	38.72
7	Talc Ma-BW	06.67^{bcd} (14.96)	16.67^{bc} (24.09)	30.00^{bc} (33.21)	53.33^{d} (46.91)	66.67^{cd} (54.74)	24.00	30.00^{ab} (33.21)	66.67^{cd} (54.74)	42.37
8	Crude Ma-BW	16.67^{c} (24.09)	20.00^{b} (26.57)	36.67^{bc} (37.27)	76.67^{b} (61.12)	83.33 (65.91)^{b}	31.33	36.67^{a} (37.27)	83.33^{ab} (65.91)	51.29
9	Crude Bb-0367	13.33^{b} (21.42)	16.67^{bc} (24.09)	33.33^{bc} (35.26)	66.67^{c} (54.74)	80.00^{b} (63.43)	28.67	33.33^{ab} (35.26)	76.67^{abc} (61.12)	47.22
10	Monocrotophos 36 SL (Std. check @ 0.054 %)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)	80.00	0.00^{c} (0.00)	0.00e (0.00)	0.00
11	Untreated Control	00.00^{d} (00.00)	00.00^{e} (00.00)	00.00^{d} (00.00)	00.00^{e} (00.00)	00.00^{e} (00.00)	0.00	0.00^{c} (0.00)	0.00e (0.00)	0.00
	SE d	6.042	3.2413	2.2992	2.329	3.9742		2.1340	3.6561	
	CD (P=0.05)	12.53	6.7220	4.7682	4.831	8.2420		4.4257	7.5824	

* Mean of three replications, figures in parentheses are arc sin transformed values, in a column, means followed by the common letter(s) are not significant in DMRT @ 5% level of significance.

Literature on the use of oil formulation of entomopathogenic fungi against coleopteran insects is scanty, though few researchers have attempted to test the efficacy of oil formulations against few coleopteran insects. Ibrahim *et al.,* (1999) reported that conidia of oil formulated *M. anisopliae* increasingly germinated over insect surface which lend support to the present finding. Coconut oil based formulation of *B. bassiana* was found effective against cocoa weevil adults, *Pantorhytes plutus* Faust as compared to water + 0.01 per cent Tween 80 (Prior *et al.,* 1988). A suspoemulsion, containing emulsifiable adjuvant oil plus conidia of *M. anisopliae* and water was as infective as oil-based fungal formulations and more infective than conventional water-based fungal formulations against the yellow mealworm, *Tenebrio molitor* (Linn.) larvae (Alves *et al*., 1998). The main issues in the successful use of fungi for pest control include infectivity and persistence of their inoculums in the environment (Moore and prior, 1993). Suspending entomopathogenic fungal conidia in oil frequently improves their persistence and virulence against insects, compared to water suspensions (Prior *et al.,* 1988; Bateman *et al.,* 1993). The above finding supports the present finding. The results of the present study indicated the potential of the oil in water formulations of the native isolate *M. anisopliae* can be successfully implemented for the management of the banana pseudostem borer. Further studies are under progress to evaluate the shelf-life of the oil in water formulations and field efficacy.

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REFERENCES

Alves RT, Bateman RP, Prior C and Leather SR. 1998. Effects of simulated solar radiation on conidial germination of *Metarhizium anisopliae* in different formulations. *Crop Prot.* **17**: 675-679. Crossref.

Ambethgar V. 2009. Potential of entomopathogenic fungi in insecticide resistance management (IRM): A review. *J Biopestic.* **2**(2): 177 - 193

Anitha N. 2000. *Bioecology and Integrated Management of Banana Pseudostem Weevil Odoiporus longicollis Oliv.* Ph.D. Thesis, Kerala Agriculture University, Vellayani, Thiruvananthapuram.

Bateman RP, Carey M, Moore D and Prior C. 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. *Ann Appl Biol.* **122**: 145-152. Crossref.

Baverstock J, Roy H and Pell J. 2009. Entomopathogenic fungi and insect behaviour: From unsuspecting hosts to targeted vectors. *BioControl*. **55**(1): 89–102. DOI: 10.1007/s10526-009-9238-5. Crossref.

Finney DJ. 1971. *Probit analysis*, 3rd edn. Cambridge University Press, Cambridge, UK. pp. 333.

Fuxa JR and Tanada Y. 1987. *Epizootiology of insect diseases.* Wiley-Interscience, New York. pp. 450–478.

Goettel MS and Inglis GD. 1997. Fungi Hyphomycetes. In: Lacey LA (Ed.) *Manual of techniques in Insect Pathology.* Academic press, New York. pp. 213-250. Crossref.

Ibrahim L, Butt TM, Beckett A and Clark SJ. 1999. The germination of oil formulated conidia of the insect pathogen *Metarhizium anisopliae. Mycol Res.* **103**: 901–907. Crossref.

Jeyarajan R, Ramakrishnan G, Dinakaran D and Sridhar R. 1994. Development of product of *Trichoderma viride* and *Bacillus subtilis* control of root rot disease, In: Dwivedi BK (Ed.) *Biotechnology in India*. Bio Research Society, Allahabad, India. pp. 25–36.

Lingappa S, Saxena H and Vimala Devi PS. 2005. Role of biocontrol agents in management of *Helicoverpa armigera* (Hubner). In: Hem Saxena, Rai AB, Ahmad R and Sanjeev Gupta (Eds). *Recent advances in Helicoverpa armigera Management*, Indian Society of Pulses Research and development, IIPR, Kanpur. pp. 159-184.

Lopes RB, Michereff-Filho M. Tigano MS, Neves PMOJ, LemaLópez E, Fancelli M, da Silva JP. 2011. Virulence and horizontal transmission of selected brazilian strains of *Beauveria bassiana* against *Cosmopolites sordidus* under laboratory conditions. *Bull Insectol.* **64**(2): 201–208.

Moore D and Prior C. 1993. The potential of mycoinsecticides. *Biocontrol News Info.* **14**: 31–40.

Padmanaban B, Thangavelu R, Gopi M and Mustaffa MM. 2009. Effect of mass multiplication media on sporulation, field efficacy and shelf life of Beauveria bassiana against rhizome and pseudostem weevils of banana. *Biol. Control* **23**(3): 277-283.

Pajar JAL, Cabahug DV, Sumaya NHN, Joey Genevieve T. Madamba MRSR, Rivero HI. 2013. Virulence of local *Metarhizium* spp. Isolates against *Tenebrio Molitor* (Linn): An initial comparison with non-native and commercially available strains. *Int J Comput Internet Manage.* **21**(1): 48–52.

Prior C, Jollands P and Patourel G. 1988. Infectivity of oil and water formulations of *Beauveria bassiana* to the cocoa weevil pest, Pantorhytes plutus. *J Invertebr Pathol.* **52**: 66–72. Crossref.

Rajendran L, Samiyappan R, Raguchander T and Saravanakumar D. 2007. Endophytic bacteria mediate plant resistance against cotton bollworm. *J Plant Interaction.* **2**: 1–10. Crossref.

Sharmila Bharathi C and Mohan B. 2015. Bioefficacy of liquid *Beauveria bassiana* for the management of pseudostem borer Odoiporus longicollis (Olivier) in hill banana of Kolli hills. *Asian J Plant Sci Res. Res*. **5**: 55-60.

Soper RS and Ward MG. 1981. Production, formulation and application of fungi for insect control, pp 161-180. In: Papavizas. G.C.(Eds) *Biological Control in Crop Production*, BARC Symposium No. 5".Allanheld, Osmun, Montclair, New Jersey.

Ummidi VRS and Vadlamani P. 2014. Preparation and use of oil formulations of *Beauveria bassiana* and *Metarhizium anisopliae* against Spodoptera litura larvae. *Afr J Microbiol Res.* **8**(15): 1638–1644. Crossref.

Vega FE, Dowd PF, Lacey LA, Pell JK, Jackson DM and Klein M. 2007. Dissemination of beneficial microbial agents by insects. In: Lacey, L.A., Kaya, H. (Eds.) Field manual of techniques in invertebrate pathology. Second edition. *Application and evaluation of pathogens for control of insects and other invertebrate pests.* Springer, The Netherlands. pp. 127–146.