Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2022; 10(4): 136-139

Received: 16-04-2022
Accepted: 23-05-2022

Sharanabasappa MG
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

Patil RK
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

Corresponding Author:
Sharanabasappa MG
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad, Karnataka, India

Bioefficacy of different formulations of *Metarhizium anisopliae* (Metchnikoff) Sorokin IOF strain (KF408075) against *Oryctus rhinoceros* (Linnaeus) *in vitro* condition

Sharanabasappa MG and Patil RK

DOI: https://doi.org/10.22271/j.ento.2022.v10.i4b.9025

Abstract
The laboratory experiments were carriedout at the Institute of Organic Farming (IOF), UAS, Dharwad, during 2014-2015 to study the bioefficacy of different formulations of *Metarhizium anisopliae* against coconut rhinoceros grubs, *Oryctus rhinoceros* (Linnaeus). Among different oil based formulation, rice bran oil (60%) + corn oil (40%) formulation found least LC_{50} value (1.99 x 10^6 cfu/ml) at 432 h. In granular and wettable powder formulation, the granular formulation recorded least LC_{50} value 0.44 x 10^8 cfu/g at 240 h. The medean lethal time (LT_{50}) of different oil based formulations, the rice bran oil (60%) + corn oil (40%) formulation @ 3 x 10^6 cfu / ml recorded lower LT_{50} value of 319.89 h (13.32 days). Among granular and wettable powder formulation, the granular formulation @ 5 x 10^8 cfu / g, proved most effective exhibiting 50 per cent of mortality with least LT_{50} value of 162.88 h (6.78 days).

Keywords: Rhinoceros grubs, *Metarhizium anisopliae*, formulations

Introduction
The entomopathogenic fungi are normal components of the natural agents that affect insect populations and when host densities are high, they are been known to produce rapid and spectacular epizootics. Among the existing several insect pathogens, entomopathogenic fungus, *M. anisopliae* found most promising one because of its wide spread occurrence and relative abundance due to its wide host range which includes all of the major insect pests. For instance, *M. anisopliae* can parasitise as many as 200 different species of insects (Hajek and Leger 1994) [3]. Entomopathogenic fungus *M. anisopliae* has been intensively studied to control a wide species of insect pests, including the coconut *O. rhinoceros* (Sivapragasam and Tey, 1995; Ramle *et al.*, 1999) [11, 8].
In the early establishment stage of coconut palm, the grubs develop in the rotting residues that are left to rot naturally in the fields. Application of *M. anisopliae* by spraying of spore solutions and broadcasting of sporulation media into the habitat has significantly reduced the grub's population (Ramle *et al.*, 1999) [13].
Application of oil based and wettable powder formulations are restricted in hilly areas where carrying of water is difficult. As an alternative, the use of granule formulation for controlling the pest was studied. The granule formulation of *M. anisopliae* has been used to control the black vine weevil, *Otiorhynchus sulcatus*, and an insect that has a similar life cycle to *O. rhinoceros*, which spends a part of its life cycle in the soil.
In this study, the efficacy of different formulations of *M. anisopliae* IOF strain (KF408075) pathogenicity of the oil based, granular and wettable powder formulations against the grubs of the *O. rhinoceros* was taken up.

Material and Methods
A laboratory experiment was carried out to prepare the different formulations of *M. anisopliae* at the Institute of Organic Farming (IOF), UAS, Dharwad during 2015. The detailed methodology followed for preparation of formulations as detailed below:

Preparation of granular formulation

The granular formulation was prepared by using 70 g of freshly harvested *M. anisopliae* spores (10^9 spores/ g) developed on broken rice, mixed with one kg of talc powder + 200 g Bentonite clay + 200 ml Rice bran oil + 200 ml gum and required quantity of distilled water was added. All the ingredients were mixed thoroughly to get a fine paste, which was passed through manually operated device (chakali making device) for making uniform sized granules of proper size (< 1mm) and shape and further, it was shade dried for 24 h in a tray. After shade drying, the dried granules were stored in a polythene bag for further study.

Preparation of oil-based formulation

The oil-based formulation of *M. anisopliae* was prepared by using freshly harvested four grams of *M. anisopliae* dry conidia (10^9 spores/ g) obtained from broken rice for which 20 ml of oil (rice bran oil (60%) + corn oil (40%), rice bran oil and olive oil) + 20 ml glycerol, were mixed and homogenized by using vertical mixture for five minutes for proper encapsulation of spores for which 956 ml of distilled water was added + 0.1% of tween-80 as spreading agent of the spores.

Preparation of wettable powder formulation

Ten grams of dried conidia of *M. anisopliae* cultured on broken rice grains (10^9 cfu / g) was mixed with 90 g of carrier material (talc) to get $1.9x10^8$ cfu / g of product. Before mixing the carrier material sieved through 355 mesh size sieves to maintain uniformity in particle size of conidial powder. The carrier material sterilized in an autoclave at 121^0C and 15 Psi for 30 min and mixed with conidial after two days for which CMC (…….) was added.

Invitro studies bio-efficacy of different formulations of *M. anisopliae* against rhinoceros grubs was carried out in the Entomology laboratory, Institute of Organic Farming (IOF), UAS Dharwad. Bio-efficacy of granular, oil based and wettable powder (standard check) formulations of *M. anisopliae* were carried out against third instar rhinoceros grubs.

The third instar grubs of uniform size and weight were collected from FYM pit, maintained at MARS, Dharwad brought to the laboratory and released in to plastic cups (diameter 25 cm and height 30 cm) containing 1 kg of FYM mixed with different concentration of *M. anisopliae* granular formulations (0.60, 1.25, 2.50, 5.00 and 10.00 g of granules / kg of FYM), oil based formulations (1.00, 1.50, 2.00, 2.50 and 3.00 ml of stock solution containing tween 80 added to 1 kg of FYM) and wettable powder formulation 0.60 g, 1.25 g, 2.50 g, 5.00 g and 10.00 g of WP formulation of *M. anisopliae* per kg FYM was added and maize seeds were sown in pot, to provide food for grubs and sprinkled with water daily to maintain humidity, (> 80% RH), for sporulation of grubs. Each treatment was replicated three times, for each replication 10 grubs were released. The number of dead grubs recorded from 5th day onwards till the death of all the grubs. Finally, per cent corrected mortality of grubs was worked out by using Abbott's formula:

$$\text{Per cent corrected mortality} = \frac{\text{Y Number of grubs dead in control} - \text{X Number of grubs dead in treatment}}{\text{X Total number of grubs used in control} - \text{Number of grubs dead in control}} \text{ X } 100$$

A total of five concentration of each formulation of *M. anisopliae* with three replications was maintained to get mortality range between 20 to 80 per cent to find out LC_{50} and LT_{50} values by using Proc Life Reg procedure and data were fitted to a Weibull distribution (SAS OnlineDoc 9.1.3) for different formulation of *M. anisopliae* developed against third instar grubs of rhinoceros beetle.

Results and Discussion

The results pertaining to LC_{50} and LT_{50} of different formulations *viz.*, oil based, granular and wettable powder formulations against *O. rhinoceros* grubs are presented.

The mortality response of coconut rhinoceros grubs to rice bran oil (60%) + corn oil (40%) formulation of *M. anisopliae* which was found to be superior over the rice bran oil and olive oil formulations. The results from the laboratory studies, revealed that, among the oil based formulations evaluated, the combination of rice bran oil (60%) + corn oil (40%) formulation found superior in getting higher mortality of rhinoceros grubs with lower LC_{50} value of 1.99×10^6 cfu / ml, followed by rice bran oil formulation (2.19×10^6 cfu / ml) and olive oil formulation (2.39×10^6 cfu / ml) at 432 h (Table 1).

In the present findings of the granular formulation recorded least LC_{50} value 0.441×10^8 cfu / g, compared to wettable powder formulation 0.684×10^8 cfu / g at 240 h (Table 2). However, the granular formulation found least LC_{50} value compared to wettable powder formulation. As the concentration of spore load increases with increase in mortality percentage and decrease in time. Similarly, (Ramle *et al.*, 2006) [9] reported that, the dipping the rhinoceros grubs in a spore solution of *M. anisopliae* of 10^8 cfu / ml, caused 100% mortality after 12 and 14 days. Varma and Yamini (2013) [12] reported that, the application of *Metarhizium anisopliae* @ 300 ml/lit of water / m^2 area (10^8 cfu / lit of water) in manure pits coconut basins and other breeding sites during wet conditions. Observations after three weeks of treatment showed 100% mortality of *O. rhinoceros* grubs by *M. anisopliae* and after 6 months, reduction of pest attack from 85% to 10%. Similarly, In glasshouse pot experiment with perennial ryegrass (*Lolium perenne* L.), the *M. anisopliae* isolate CLO 53 caused mortalities of 50 and 88% of *Hoplia philanthus* larvae 10 weeks after application of 10^4 and 10^6 cfu / cm^2 soil surface (Ansari, 2004) [1]. The variation in virulence of different isolates, origin from different geographical locations were reported by various workers (Fernando *et al.*, 1995) [2] reported that the *M. anisopliae* caused 100% mortality of *Oryctes rhinoceros* larvae and adults when treated with suspensions of 10^7 cfu / ml, in laboratory condition. (Khudhair *et al.*, 2015) [5] reported that, the virulence of entomopathogenic fungi *Metarhizium anisopliae* was tested against Arabian Rhinoceros Beetle, *Oryctes agamemnon arabicus* (Illiger) larvae at (1×10^9 cfu /ml) locally isolated entomopathogenic fungi spore suspensions directly sprayed on grubs to cause high mortality rate reaching 100% after 29 days. Makaka and Caston (2008) [7] reported that, oil based formulation of *M. anisopliae* isolate (IMI098376) found least LC_{50} value ($4.47x10^4$ cfu / ml) against black maize beetle, *Heteronychus licas* (Seidlitz) at 13.30 weeks.

Bioefficacy of entomopathogenic fungus, *M. anisopliae* formulations *viz.*, oil, granular and wettable powder formulations to the third instar rhinoceros grubs *in vitro* condition. The present findings indicated that in general, the concentration of inoculum and time required to kill 50 per

cent mortality of rhinoceros grubs was inversely correlated. Among different formulations evaluated under laboratory condition, rice bran oil (60%) + corn oil (40%) formulation @ 3 x 10^6 cfu / ml, recorded lower LT_{50} value of 319.89 h (13.32 days) followed by rice bran oil formulation 343.46 h (14.31 days), and olive oil formulation 357.12 h (14.88 days) (Table 3).

The granular formulation @ 5 x 10^8 cfu / g, proved to be most virulent exhibiting 50 per cent of mortality with least time of LT_{50} value of 162.88 h (6.78 days), followed by wettable powder formulation @ 5 x 10^8 cfu / g recorded more LT_{50} value of 177.80 h (7.40 days) (Table 4). However, the present study revealed that the, concentration of the doses increases with increasing in the mortality with decrease in time. The present findings are in line with, (Latifina and Rad, 2012) [6] reported that, conidial suspension of *M. anisopliae* isolate (DEMID 01) were found lowest LC_{50} value of (5.69 × 10^8 cfu /ml), against *Oryctes elegans* and LT_{50} values of *M. anisopliae* ranges, from 6.45 to 10.12 days under laboratory condition. Sharma *et al.* (1999) [10] who reported that, first instar grub *H. consanguinea* which has taken least time to kill 50 per cent of mortality compared to second and third instars at 4 x 10^7 cfu / g. Similar finding was also made by Inglis *et al.* (1993) [4] who reported that young larvae of European corn borer was more susceptible to *M. anisopliae* oil based formulation than the older larvae. Makaka and Caston (2008) [7] reported that, median lethal time LT_{50} of oil based formulation *M. anisopliae* isolate (IMI098376) against black maize beetle, *Heteronychus licas* at the lowest concentration (3.10x10^4 cfu / ml) causes 50 per cent of mortality in 13.3 week.

Table 1: Median lethal concentration (LC_{50}) of oil based formulations of *M. anisopliae* IOF_1 strain (KF408075) against 3rd instar coconut rhinoceros grubs, *Oryctus rhinoceros* (Linnaeus)

Formulation	LC_{50} (cfu/ml)	Fiducial limits of LC_{50} (cfu/ml)		Regression equation (Y=a+bx)	LC_{95} (cfu/ml)	χ^2
	Lower limit		Upper limit			
Rice bran oil (60%) + corn oil (40%) formulation	1.992 x 10^6 (cfu/ml)	1.754 x 10^6 (cfu/ml)	2.253 x 10^6 (cfu/ml)	Y= 2.076+0.448x	3.438 x 10^6 (cfu/ml)	**3.325**
Rice bran oil formulation	2.194 x 10^6 (cfu/ml)	1.954 x 10^6 (cfu/ml)	2.471 x 10^6 (cfu/ml)	Y= 2.633+0.590x	3.584 x 10^6 (cfu/ml)	**0.386**
Olive oil formulation	2.388 x 10^6 (cfu/ml)	2.065 x 10^6 (cfu/ml)	2.943 x 10^6 (cfu/ml)	Y= 1.991 + 0.438x	4.900 x 10^6 (cfu/ml)	**5.200**

Table 2: Median lethal concentration (LC_{50}) of granular and wettable powder formulations of *M. anisopliae* IOF_1 strain (KF408075) against 3rd instar coconut rhinoceros grubs, *Oryctus rhinoceros* (Linnaeus)

Formulation	LC50 (cfu/g)	Fiducial limits of LC50 (cfu/g)		Regression equation(Y=a+bx)	LC95 (cfu/g)	χ2
		Lower limit	Upper limit			
Granular formulation	0.441 x 10^8 (cfu/g)	0.177 x 10^8 (cfu/g)	0.631 x 10^8 (cfu/g)	Y= 0.946 + 0.172x	1.829 x 10^8 (cfu/g)	0.307
Wettable powder formulation	0.684 x 10^8 (cfu/g)	0.443 x 10^8 (cfu/g)	0.893 x 10^8 (cfu/g)	Y= 0.446 + 0.153x	2.283 x 10^8 (cfu/g)	4.226

Table 3: Median lethal time (LT_{50}) of oil based formulations of *M. anisopliae* IOF_1 strain (KF408075) against 3rd instar coconut rhinoceros grubs, *Oryctus rhinoceros* (Linnaeus)

Formulation	Dose (cfu/ml)	LT_{50} (hrs)	Fiducial limits of LT_{50} (hrs)		Regression Equation(Y=a+bx)	LT_{95} (hrs)	χ^2
			Lower limit	Upper limit			
Rice bran oil (60%) + corn oil (40%) formulation	2.5 x 10^6 (cfu/ml)	338.36	302.23	384.09	Y= 20.200 + 4.825x	543.68	1.012
	3.0 x 10^6 (cfu/ml)	319.89	285.68	358.79	Y= 20.728 + 4.711x	505.57	2.046
Rice bran oil formulation	2.5 x 10^6 (cfu/ml)	384.10	343.16	470.25	Y= 19.706 + 5.468x	631.20	1.854
	3.0 x 10^6 (cfu/ml)	343.46	311.24	381.75	Y= 24.779 + 5.824x	506.07	2.223
Olive oil formulation	2.5 x 10^6 (cfu/ml)	394.99	348.54	511.21	Y= 17.721 + 4.992x	688.02	1.010
	3.0 x 10^6 (cfu/ml)	357.12	318.36	417.73	Y= 19.064 + 4.830x	593.04	0.251

Table 4: Median lethal time (LT_{50}) of granular and wettable powder formulations of *M. anisopliae* IOF_1 strain (KF408075) against 3rd instar coconut rhinoceros grubs, *Oryctus rhinoceros* (Linnaeus)

Formulation	Dose (cfu/g)	LT_{50} (hrs)	Fiducial limits of LT_{50} (hrs)		Regression Equation(Y=a+bx)	LT_{95} (hrs)	χ^2
			Lower limit	Upper limit			
Granular formulation	2.50 x 10^8 (cfu/g)	164.42	136.69	172.71	Y= 25.029 + 5.844x	223.97	0.380
	5.00 x 10^8 (cfu/g)	162.88	144.14	172.44	Y= 33.549 + 8.034x	209.09	0.410
Wettable powder formulation	5.00 x 10^8 (cfu/g)	177.80	152.81	188.94	Y= 31.306 + 8.317x	205.88	0.390

References

1. Ansari MA, Vestergaard S, Tirry L, Moensa M. Selection of a highly virulent fungal isolate, *Metarhizium anisopliae* CLO 53, for controlling Hoplia philanthus. J. Invert. Path. 2004;85:89-96.
2. Fernando LCP, Kanagaratnam P, Narangoda NK. some studies on the use of *Metarhizium anisopliae* (Metsch.) Sor. for the control of *Oryctes rhinoceros* in Sri Lanka. COCOS Printed in Sri Lanka. 1995;10:46-52.
3. Hajek AE, Leger RJ St. Interactions between fungal pathogens and insect hosts, Ann. Rev. Entom. 1994;39:293-322.
4. Inglis GD, Goettel MS, Jhonson DL. Persistence of the entomopathogenic fungus, *Beauveria bassiana* on phylloplanes of crested wheat grass and alfalfa. J. Biol. Control. 1993;3:258-270.
5. Khudhair MW, Khalaf MZ, Alrubeai HF, Shbar AK, Hamad BS, Khalaf HS. Evaluating the virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) and *Beauveria bassiana* (Ascomycota: Hypocreales) isolates to Arabian rhinoceros beetle, *Oryctes agamemnon arabicus*. J Entom. Acarological Res. 2015;47:518.
6. Latifina M, Rad B. Pathogenicity of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillmin, *Beauveria brongniartii* Saccardo and

Metarhizium anisopliae Metsch to adult *Oryctes elegans* Prell and effects on feeding and fecundity. Int. J Agri. Crop Sci. 2012;4(14):1026-1032.
7. Makaka D, Caston T. Efficacy of two isolates of *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) against the adults of the black maize beetle *Heteronychus licas* Klug (Coleoptera: Scarabidae) under laboratory conditions. Afr. J Agri. Res. 2008;3(4):259-265.
8. Kartik Chandra Sahu, Dr. Mahendra Kumar Satapathy. Rooftop garden an organic farming to grow uncontaminated vegetable improved food quality and food security in Bhubaneswar City, India. Int. J Agric. Extension Social Dev. 2021;4(2):110-116. DOI: 10.33545/26180723.2021.v4.i2b.110
9. Ramle M, Wahid MB, Kamarudin N, Ali SRA, Hamid NH. Research into the commercialization of *Metarhizium anisopliae* (Hyphomycetes) for biocontrol of the rhinoceros beetle, *Oryctes rhinoceros* (Scarabaeidae), in oil palm. J. Oil Palm Res. 2006;4:37-49.
10. Sharma S, Gupta RBL, Yadav CPS. Mass multiplication and formulation of entomopathogenic fungi and their efficacy against white grubs. J. Mycol. Plant Path. 1999;29(3):299-305.
11. Sivapragasam A, Tey CC. Susceptibility of *Oryctes rhinoceros* (L.) larvae to three isolates of *Metarhizium anisopliae* (Metsch.) Sorokin. MAPPS Newsletter. 1995;18(2):13-14.
12. Varma CK, Yamini. Efficacy of ecofriendly management against rhinoceros grubs in coconut. J BioPestic. 2013;6(2):101-103.
13. Ramle M, Basri MW, Norman K, Mukesh S, Ramlah AA. Impact of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) applied by wet and dry inoculums on oil palm rhinoceros beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). J Oil Palm Research. 1999;11(2):25-40.