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SCIENCE DIRECT
Bioresource Technology 97 (2006) 1801–1806
BIORESOURCE TECHNOLOGY

Prospects of using *Metarhizium anisopliae* to check the breeding of insect pest, *Oryctes rhinoceros* L. in coconut leaf vermicomposting sites

Murali Gopal *, Alka Gupta, George V. Thomas

Central Plantation Crops Research Institute, Kudlu P.O., Kasaragod 671 124, Kerala, India

Received 23 February 2005; received in revised form 3 September 2005; accepted 5 September 2005
Available online 17 October 2005

Abstract

During vermicomposting of coconut leaves by the earthworm *Eudrilus* sp., *Oryctes rhinoceros* L. (rhinoceros beetle), an insect pest of palms, was found to breed in the decomposing organic material. *Metarhizium anisopliae* var. *major* was tried as a biocontrol agent for management of this pest. The effect of pathogen at spore loads of 10^3, 10^4 and 10^5 per 10 g of substrate was tested in laboratory on *Eudrilus* sp. kept with *O. rhinoceros* grubs and on *Eudrilus* sp. alone for the pathogenic capability of the fungus on the pest and its possible toxicity towards the vermin. The efficacy of the entomopathogen was also tested in the field in vermicomposting tanks. In laboratory bioassay, 100% mycosis of *O. rhinoceros* grubs could be obtained while the entomopathogen had no toxic effect on the earthworms. There was a positive change in the number and weight of the earthworms on treatment with *M. anisopliae*. In the field, application of *M. anisopliae* reduced *O. rhinoceros* grubs in the vermicomposting tanks upto an extent of 72%. In conclusion, *M. anisopliae* could effectively control *O. rhinoceros* in vermicomposting sites and was non-hazardous to the vermicomposting process as well as the *Eudrilus* sp.

Keywords: Coconut palm; Earthworms; *Eudrilus* sp.; *Metarhizium anisopliae*; *Oryctes rhinoceros*; Vermicomposting

1. Introduction

Vermicompost is a very important low external input component in organic farming. It is prepared using earthworms, which feed on the agro-wastes and domestic refuse (Kale and Bano, 1988) and recycle them as manures, the process being termed vermicomposting. These manures are highly decomposed, organically rich and contribute immensely to the biomass of soil invertebrates, particularly in tropical regions. Coconut is important oil seed plantation crop grown in 1.91 million ha in India. Around 6–8 ton of leaf wastes are shed annually from a coconut garden of 1 ha area (175 coconut palms/ha). An indigenous strain of *Eudrilus* sp. had been identified at the Central Plantation Crops Research Institute (CPCRI), India, which was capable of decomposing the highly lignified coconut leaves effectively and efficiently into vermicompost in a period of 75–90 days (Prabhu et al., 1998). Production of vermicompost from coconut leaf waste with the help of this earthworm species is being extensively practiced as vermicompost is the most sought after low external input resource by farmers who cultivate high value, export oriented crops like pepper, cardamom, vanilla, tea, coffee, etc. However, the composting mixture of coconut leaves and cow-dung in vermicomposting sites was found to emanate an odour that attracted rhinoceros beetle, *Oryctes rhinoceros* L., a major insect pest of the coconut and oil palm (Nirula, 1955). Female beetles were attracted more in number than the males to the vermicomposting sites and they laid eggs in these sites so that the grubs on hatching got ready decomposed organic matter as feed for their growth. The adult beetles, which emerged out of these sites caused significant damage to the coconut and oil palms and already *Metarhizium anisopliae* had been reported to be efficient in managing this pest (Young, 1974; Abad et al., 1992).

* Corresponding author. Tel.: +91 4994 232894/5/6; fax: +91 4994 232322.
E-mail address: mgcpcri@yahoo.co.in (M. Gopal).

0960-8524/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2005.09.005

Therefore, the present work was carried out to evaluate the efficacy of *M. anisopliae* in controlling the grubs of *O. rhinoceros* in vermicomposting sites and to study the safety of this entomofungal pathogen towards the earthworm, *Eudrilus* sp.

2. Methods

M. anisopliae var. *major* (Metsch.) Sorokin was isolated from field-collected infected *O. rhinoceros* grubs using a modified isolation medium (Mohan et al., 1982). The fungus was mass-multiplied in sterilized coconut water (Dangar et al., 1991); the fungal spores were harvested and used for the studies. The III instar grubs of *O. rhinoceros* were collected from fallen coconut logs, farmyard pits, coir waste dumps, etc. for the laboratory bioassays and were screened in the laboratory for 15 days for any natural pathogenicity. Only the healthy ones were taken for the experiment. The laboratory experiments were performed in plastic containers of 0.5 m height and 0.3 m diameter filled with 200 g of decomposing coconut leaves and cow-dung mixture in 1:1 ratio. In one set of containers, 50 fully grown *Eudrilus* sp. (each 6–8 inch long weighing 1.5–1.8 g) were introduced while in another set, 20 *Eudrilus* worms and 3 III instar grubs of *O. rhinoceros* (each weighing 15–20 g) were introduced. Spores of *M. anisopliae* var. *major* were added to give a spore load of 10^3, 10^4 and 10^5 per 10 g of the substrate. The boxes were closed with a lid in which holes were made. A control treatment without *M. anisopliae* was maintained. Each treatment consisted of 10 replicates. Observations on the response of the earthworms to the external stimuli, total number of worms, weight of worms and mycosis of *O. rhinoceros* grubs were recorded at the end of 60 days period. Sample earthworms were drawn at random and washed with sterile water (10 ml/worm), which was serially diluted and plated on *M. anisopliae* specific media (Mohan et al., 1982) to count the number of colony forming units. Twenty-five earthworms were removed and placed in clean basins. The fresh vermicast excreted by these worms (1 g from each replication of all treatments) was analyzed for the presence of *M. anisopliae* spores. This was done to see if earthworms consumed the spores of the pathogen and then voided it in their excreta.

Field trials to study the effect of *M. anisopliae* on the number and vermicomposting capacity of *Eudrilus* sp., and its pathogenic effect on *O. rhinoceros* grubs proliferating in the vermicomposting substrates were conducted in cement tanks of $7.5 \times 2.5 \times 1$ m dimensions ($l \times b \times h$) with proper drainage facilities. The tanks were located at 12° 30′ north latitude, 75° longitude and 10 m above MSL; ≈250 m away from the Arabian Sea (western coast of India) and had slanting roofs made of thatched coconut leaves to prevent sunlight and rainwater falling directly into them. Shed coconut leaves from coconut garden (weighing ≈2.5–3.0 kg each) were placed inside the tank with abaxial side up to about one foot height. A layer of fresh cow-dung slurry was spread uniformly over the stacked coconut leaves. Three such layers (coconut leaves + cow-dung slurry) were accommodated in a tank. This way ≈1000 kg of coconut leaves were heaped inside the tank and 100 kg of fresh cow-dung slurry was spread (25–30 kg at each one foot layer of coconut leaves). Water was sprinkled regularly to keep the substrate at 50–60% moisture. The surface was mulched with grass or coconut leaves to prevent moisture loss. The whole material was allowed to undergo preliminary microbial decomposition for 15–20 days, after which, 1000 *Eudrilus* worms were introduced into the substrate for vermicomposting. After 75 days of vermicomposting, watering was stopped to allow the earthworms to move to deeper layers. Fifteen days after stopping watering, the ready vermicompost was separated from the un-decomposed/un-digested debris and the earthworms were collected by hand sorting. In the first field trial of the series conducted during October–December 2002, *M. anisopliae* was applied @ 1.5×10^{11} spores to 1.1 ton of substrate (1000 kg coconut leaves + 100 kg cow-dung slurry), which was approximately 9.0×10^5 spores/10 g of substrate, equivalent to the highest dose tested in the laboratory bioassays. The above dosage was obtained by mixing 1.5 l of coconut water culture of *M. anisopliae* (containing 10^8 spores/ml of coconut water at harvest) with 30 kg of mature vermicompost and was spread along with the cow-dung slurry on the coconut leaf stack, 10 kg in each layer. In the control tank, 1.5 l of coconut water mixed with 30 kg of vermicompost was spread along with the cow-dung slurry, 10 kg in each layer. In the second field trial *M. anisopliae* was multiplied in 1.5 l of coconut water and this culture (containing 10^8 spores/ml of coconut water at harvest) was mixed with 30 kg of vermicompost and spread along with cow-dung slurry @ 7.5 kg in the first layer from the bottom, 15 kg in the middle layer and 7.5 kg in the top layer. This trial was again repeated. Each field trial had two replicates.

At the end of each trial, the number of earthworms present in the tanks, *O. rhinoceros* grubs recovered (dead or alive), and percentage of matured vermicompost produced was recorded. The grubs that were found alive (from both entomopathogen treated and control tanks) were maintained separately in a box containing fresh vermicompost and their further growth was observed.

3. Results and discussion

The response of earthworms to light and touch stimuli was tested at weekly intervals till 60 days period in the laboratory bioassay. It was observed that the *Eudrilus* responded in similar manner in control as well as *M. anisopliae* applied containers, i.e. they tried to burrow inside the substrate the moment they were exposed to natural light and wriggled vigorously to the touch stimulus.

The effect of *M. anisopliae* on the population of *Eudrilus* sp. is presented in Table 1. The number of earthworms increased to a maximum of 146% in the lowest dilution (10^3 spores of *M. anisopliae*/10 g substrate) during first trial and

Table 1
Effect of *M. anisopliae* on earthworm (*Eudrilus* sp.) numbers during different periods[a]

M. anisopliae spore concentration	August–September 2002				October–November 2002				April–May 2003			
	Worm numbers		% Change	Factor change	Worm numbers		% Change	Factor change	Worm numbers		% Change	Factor change
	Initial	Final			Initial	Final			Initial	Final		
When maintained alone in the substrate												
10^3	50	123	+146	2.45	50	113	+126	2.27	50	106	+112	2.12
10^4	50	114	+128	2.28	50	113	+126	2.62	50	94	+88	1.87
10^5	50	113	+126	2.22	50	106	+112	2.10	50	96	+92	1.92
Control	50	112	+124	2.24	50	90	+80	1.81	50	78	+56	1.50
CV				19.5				17.95				19.65
CD ($p = 0.05$)				0.33				NS				0.34
When maintained in presence of O. rhinoceros grubs in the substrate												
10^3	20	43	+115	2.12	20	47	+135	2.3	20	45	+125	2.25
10^4	20	45	+125	2.00	20	44	+120	2.2	20	40	+100	1.74
10^5	20	37	+85	1.84	20	41	+105	2.0	20	34	+70	1.69
Control	20	40	+100	1.96	20	46	+130	2.2	20	32	+60	1.58
CV				16.28				23.3				15.91
CD ($p = 0.05$)				NS				NS				0.26

[a] Average of 10 replications.

the minimum was 88% increase in the middle dilution (10^4 spores/10 g substrate) during next trial. The increase in *Eudrilus* numbers in control treatment ranged from 56 to 124%. At the end of 60 days period, the weight of worms in *M. anisopliae* treated containers varied slightly among the different doses. The lowest dose of 10^3 spores/10 g substrate resulted in least weight loss when compared to initial weight of the worms in the same treatment. It was seen that the worms in pathogen-treated containers had slightly more weight or were on par with control (Table 2).

In the experiments, where *Eudrilus* and *O. rhinoceros* grubs were put together in the substrate, the final population of earthworms in all the *M. anisopliae* applied treatments was slightly more or on par with that of the control treatment (Table 1). The maximum number of earthworms was found in the lowest dosage. Among the three doses applied, the highest dose of the entomofungal pathogen (10^5 spores/10 g substrate) marginally reduced the earthworm numbers, but it was on par with the numbers in control treatment. However, the weight of the earthworms was slightly high or on par in all the *M. anisopliae* treatments as compared to control (Table 2). The possible reason is discussed in later part of this paper. These experiments proved the non-lethal nature of *M. anisopliae* towards *Eudrilus* sp.

The fungal pathogen, *M. anisopliae*, inflicted 100% mortality on *O. rhinoceros* grubs at all the three dosages tried; while in the control treatment, the grubs remained alive

Table 2
Effect of *M. anisopliae* on earthworm (*Eudrilus* sp.) weight[a]

M. anisopliae spore concentration	August–September 2002				October–November 2002				April–May 2003			
	Earthworm weight (g)		% Change	Factor change	Earthworm weight (g)		% Change	Factor change	Earthworm weight (g)		% Change	Factor change
	Initial	Final			Initial	Final			Initial	Final		
When maintained alone in the substrate												
10^3	17.8	17.0	−4.2	0.95	18.6	18.9	+2.0	0.9	17.6	16.0	−9.0	0.90
10^4	17.9	16.3	−8.5	0.91	19.2	17.9	−6.7	0.93	17.3	16.3	−5.7	0.95
10^5	18.5	16.9	−8.6	0.91	17.9	17.6	−1.6	0.98	17.8	14.8	−16.8	0.83
Control	18.2	17.3	−5.0	0.95	18.5	17.4	−6.0	0.93	18.2	15.0	−17.5	0.77
CV				7.33				5.21				10.1
CD ($p = 0.05$)				NS				NS				0.07
When maintained in presence of O. rhinoceros grubs in the substrate												
10^3	18.6	17.5	−6.0	0.94	18.3	18.5	+1.1	1.01	17.5	14.4	−17.7	0.82
10^4	17.9	17.5	−2.2	0.97	18.5	18.4	−0.5	0.99	17.5	14.6	−16.5	0.83
10^5	17.5	17.1	−2.2	0.97	19.2	18.2	−5.2	0.94	17.9	14.1	−21.2	0.78
Control	18.2	17.3	−4.9	0.95	18.8	17.8	−5.3	0.94	17.8	13.8	−22. 5	0.77
CV				13.65				19.17				8.04
CD ($p = 0.05$)				NS				NS				NS

[a] Average of 10 replications, 10 worms' weight per replication.

and continued their life cycle. However, the time taken by the pathogen for the mycosis of the insect grubs depended primarily upon the concentration of the spores of *M. anisopliae* applied. The highest dose (10^5 spores/10 g substrate) gave the quickest killing time of 8 days. The spore inoculum of 10^4/10 g substrate took 10–18 days while the lowest dose of 10^3 spores/10 g substrate took the longest of 22 days to kill all the grubs. Though the experiments were carried out in laboratory conditions (non-air-conditioned), the weather played an important role in regulating the period of mortality. High humidity (>90%) coupled with moderate temperature (30 °C) favoured the entomofungal activity resulting in quicker mycosis at all the three concentrations. Mortality of the grubs took more than 15 days even at the highest *M. anisopliae* spore concentration when the humidity was around 84% and temperature at 34 °C, indicating that dry weather conditions retarded the pathogenic efficiency of *M. anisopliae*. Our earlier studies on the pathogenicity of *M. anisopliae* also indicate similar activity in different weather conditions (Gopal et al., 2002).

The presence of *M. anisopliae* spores on the body surface of *Eudrilus* sp. was detected in body wash experiments (Fig. 1). The number of spores (cfu/ml of washing) attached to the worm surface normally increased with increasing concentration of *M. anisopliae.* The number of spores was found to be high in body washes in all the *M. anisopliae* applied treatments. The number of spores was significantly high in the body washes of *Eudrilus* in presence of *Oryctes* grubs. This was because the mycosed grub produces heavy load of *M. anisopliae* spores on its body surface, which adds to the initial inoculum concentration, resulting in more number of spores sticking to the earthworm body. Despite this, *M. anisopliae* was unable to cause any pathogenicity to the earthworms.

The pathogen *M. anisopliae* gains entry into an insect body, including that of *O. rhinoceros* grubs, by direct penetration of the fungal hyphae into the body integuments, through breathing apertures on the body, ingestion into the digestive tract or through wounds. However, in case of *Eudrilus* sp., presence of a mucus layer containing exudates of epidermal mucus cells as well as coelomic fluid ejected through the dorsal pores on the body surface might have prevented the entry of *M. anisopliae* through integuments. Moreover, coelomic fluid is well known to have antimicrobial activity (Cotuk and Dales, 1984; Lassegues et al., 1989). Recent studies have identified lysozyme-like molecule and a pattern recognition protein called coelomic cytolytic factor (CCF) in the coelomic fluid of *Eisenia foetida* earthworms (Kohlerova et al., 2004), which help them to protect against invading pathogens. It is likely that the mucus layer and coelomic fluid, together with innate defense mechanisms (Lee, 1985) might have protected *Eudrilus* sp. earthworms from *M. anisopliae* infection. Similar report of non-toxic effect of the entomofungal pathogen *Beauveria bassiana* on five different types of soil dwelling earthworms (Pizl, 1993) is available.

We could also detect the presence of *M. anisopliae* spores in the vermicasts of the earthworms. The colony forming units of *M. anisopliae* were many fold higher in the vermicasts when earthworms were kept together with *Oryctes* grubs, than when they were alone in the substrate (Fig. 2). The vermicasts from control treatment were totally free of any *M. anisopliae* propagules. This observation is parallel to the counts of *M. anisopliae* spores present in body wash of the worms as mentioned earlier. We also recorded an increase in the final body weight of the earthworms when *M. anisopliae* was applied. These two observations, i.e., presence of spores in vermicasts and increase in weight of earthworms mean that the pathogen

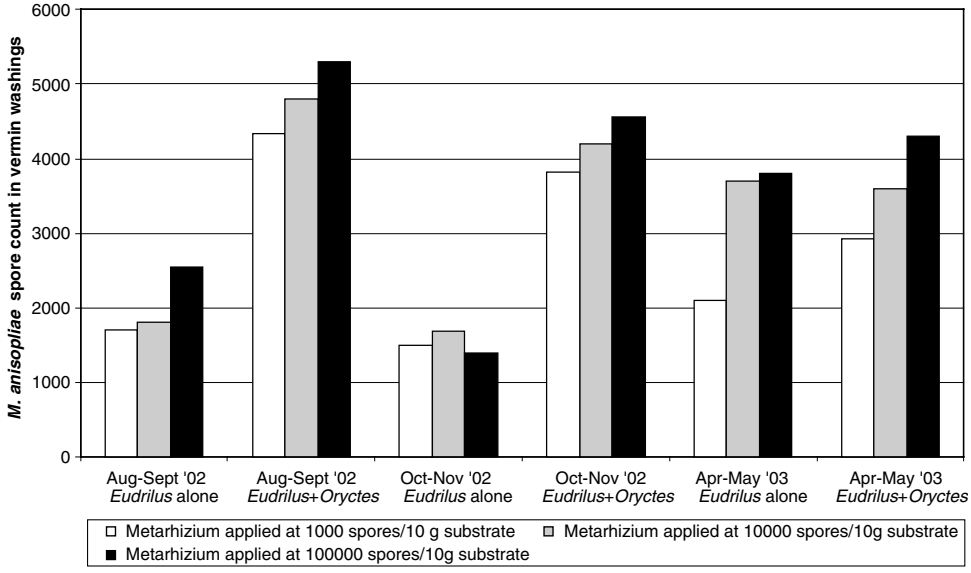

Fig. 1. *M. anisopliae* spore count in body washings of *Eudrilus* sp. when maintained alone and along with *O. rhinoceros* grubs (average of 3 replicates, 10 ml body washings per replication).

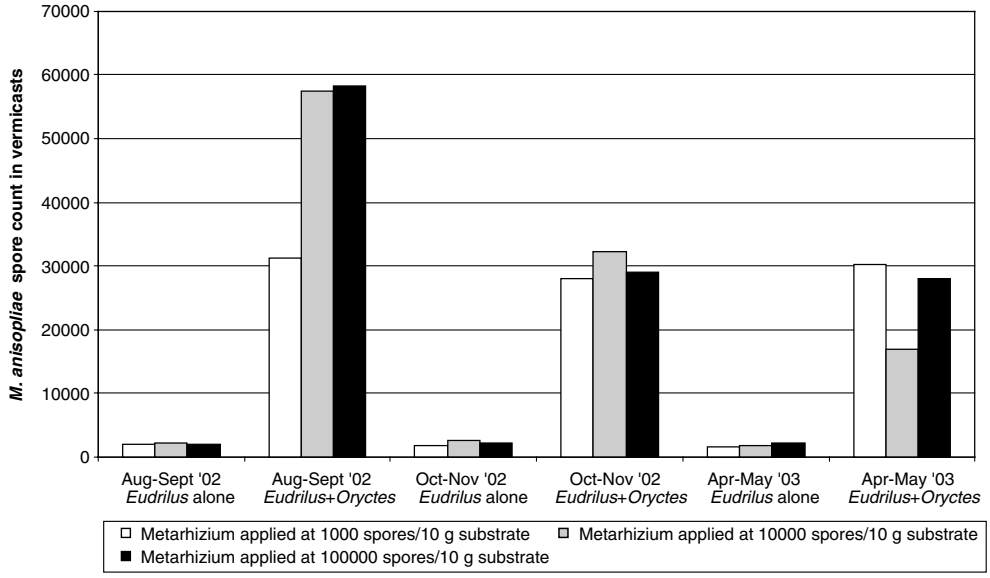

Fig. 2. *M. anisopliae* spore count in vermicasts of *Eudrilus* sp. when maintained alone and along with *O. rhinoceros* grubs (average of 3 replicates, 1 g vermicast per population).

is partially used as diet and some of it is voided in the vermicast. Microorganisms have been reported to be a component of the diet of some earthworms (Lee, 1985). Amendment of vermicomposting substrate with fungi such as *Aspergillus* and *Penicillium* spp. was found to increase the growth rate of *E. foetida* (Pizl and Novakova, 2004). However, some microorganisms are not digested and survive the passage through the earthworm gut (Satchell, 1983). Evidently, *M. anisopliae* was not affected during passage through *Eudrilus* gut. This may be advantageous as the application of such vermicompost in fields, particularly near the rhizosphere of plants, can enable suppression of many soil dwelling insect pests particularly coleopterans, lepidopterans and homopterans by *M. anisopliae* (Leatherdale, 1970). Similar pesticidal application of vermicompost prepared from neem leaves had been reported by Gajalakshmi and Abbasi (2004).

In the first field trial of the series, an average of 20 *O. rhinoceros* grubs could be collected from the tanks to which the entomofungal pathogen was applied, whereas, the control tanks yielded 64 grubs. The total number of earthworm harvested and mature compost produced were on par in pathogen-treated (2450 ± 250 earthworms, 65–70% compost) and control (2375 ± 250 worms, 65–70% compost) tanks. In the second field trial *M. anisopliae* spores were spread in such a way that the middle layer of the cow-dung slurry received more of the inoculum (15 kg). At the end of the trial, an average of 38 *O. rhinoceros* grubs were collected from the entomopathogen-applied tanks and 134 grubs were recovered from the control tank. The yield of earthworm and mature compost were on par in *M. anisopliae* treated (850 ± 100 worms, 55–60% compost) and control (935 ± 100 worms, 55–57% compost) tanks. The third trial yielded 23 *O. rhinoceros* grubs, 2370 ± 250 worms and 60–65% compost in *M. anisopliae* treatment and 64 grubs, 2500 ± 250 worms and 60–65% compost in control tanks. *O. rhinoceros* grubs retrieved from *M. anisopliae*-treated tanks and maintained in fresh vermicompost succumbed to the disease within 10–20 days period; while those collected from control tanks went on to complete their life-cycle without getting mycosed.

During the field trials, presence of large number of eggs and I instar grubs of *O. rhinoceros* was observed mostly around the mid point of the total vermicomposting period, around 45 days onwards. This meant that after almost 30–40 days of earthworm activity in the tanks, the composting substrate produced the odour that attracted *O. rhinoceros* beetles to the sites, particularly the females, for laying the eggs. Since it was not possible to apply *M. anisopliae* at mid-stage of the vermicomposting process, the first field trial was conducted in which the fungal inoculum (1.5×10^{11} spores/1.1 ton of substrate) mixed with 30 kg of vermicompost was spread equally (10 kg) in the three layers of cow-dung slurry while filling the tank with coconut leaves. The result indicate that the entomofungal pathogen was effective as 69% less *O. rhinoceros* grubs were recovered from the pathogen-treated tank as compared to control. During the progress of this field trial, random observations revealed the presence of cadavers of *O. rhinoceros* grubs covered with green coloured spores, a typical symptom of *M. anisopliae* infection. It was also noted that the earthworm numbers as well as the population of the insect grubs were generally high in the middle zone of the heaped substrate, which coincided with the central layer of cow-dung slurry. This prompted us to take up more field trials with differential distribution of *M. anisopliae* inoculum. In the next field trial, instead of adding the spores equally in all the three cow-dung layers, 15 kg of inoculum + vermicompost was added to the central layer with top and bottom cow-dung layers receiving 7.5 kg each.

However, the increased input of the pathogen inoculum in the biologically active zone did not bring about any significant reduction in the population of the insect pest, as 65–70% less *O. rhinoceros* grubs were recovered from this second trial, which was not much different from the first trial. The observation led us to believe that 10 kg of the *M. anisopliae* containing vermicompost in the middle layer is sufficient enough to curb *O. rhinoceros* breeding. This could be attributed to the presence of higher number of earthworms in the middle zone of the substrate and their active movement laterally and vertically might have acted as an effective vector to transport the fungal spores and infect the grubs. Further, the fact that all the live *O. rhinoceros* grubs retrieved from the pathogen applied tanks later on contracted the fungal disease lends support to our assumption. Thus, it is concluded that cent percent of the grubs eventually die in the *M. anisopliae* treated tanks thereby suppressing the build up of the insect pest population.

The field experiments also confirmed the influence of weather factors, particularly relative humidity and temperature, on the vermicomposting process (Gopal et al., 2004). High temperature (34 °C) and low humidity (80% RH) resulted in production of less number of earthworms in *M. anisopliae*-treated and control tanks as compared to trials when maximum temperature was 32 °C and RH ranged between 86–90%. The mature vermicompost turnover was also less by 10–15% during this period. However, the infectivity of *M. anisopliae* remained maximum, implying that the moisture available in the vermicomposting substrate was sufficient for its pathogenicity.

4. Conclusion

Thus, from the laboratory and field experiments, it was amply clear that *M. anisopliae* var. *major* could be safely used as a bioagent for management of *Oryctes rhinoceros* breeding in the coconut leaf vermicomposting sites, and was non-lethal to the *Eudrilus* sp. The application of *M. anisopliae* in the vermicomposting substrates did not affect the capacity of the *Eudrilus* sp. to convert the substrates to mature compost.

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