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EFFICACY OF ENTOMOPATHOGENIC FUNGI *Beauveria* (NFCCI -ACCESSION NUMBER: 4225). (HYPOCREALES: CORDYCIPITACEAE) AND *Metarhizium anisoplae* (METCHNIKOFF)) (HYPOCREALES: CLAVICIPITACEAE) AGAINST GREY WEEVIL *Myllocerus* Sp. (COLEOPTERA: CURCULIONIDAE) IN KASARAGOD DISTRICT OF KERALA, INDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author NRR designed the study, managed the literature searches performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SMG supervised the study. Both authors read and approved the final manuscript.

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ABSTRACT

Grey weevil, *Myllocerus* Schoenherr (Coleoptera: Curculionidae) has been a nuisance to the farmers over decades. The study revealed the efficacy of two potential entomopathogenic fungi, one the field collected *Beauveria* (Hypocreales: Cordycipitaceae) with NFCCI accession No. 4225 and the other *Metarhizium anisoplae* (Metchnikoff) (Hypocreales: Cordycipitaceae) against the adults and fourth instar larvae of *Myllocerus* sp. The result showed high adult mortality (100%) at $4.56x10^8$ Conidia/ml of field collected *Beauveria* after 240 hours of treatment. *M. anisoplae* treatment has resulted 90% adult mortality at $5.36x10^8$ after 240 hours of treatment. The larval mortality was very small when compared to adult mortality. The median lethal time for the most effective dose of *Beauveria* ($4.56x10^8$) was 70.48 hours and for the most effective dose of *M. anisopliae* ($5.36x10^8$ conidia/ml) was 99.14 hours. In the present study *Beauveria* sp. showed a more lethal effect against grey weevils.

Keywords: Entomopathogenic fungi; biopesticides.

1. INTRODUCTION

Weevils under the genus *Myllocerus* Schoenherr, 1823 (Curculionidae: Coleoptera) are characterized by a transverse head and stout rostrum [1]. Most of the species under the genus *Myllocerus* are polymorphic in their body colour and features like pattern of spots and blotches on the elytra [2]. Marshall [3] has described the geographical distribution of *Myllocerus* ranging from Africa to Australia covering Eastern Europe and Central & Southern Asia. Nearly all members in this heterogeneous and massive genus, *Myllocerus* are phytophilous and polyphagous. Globally, about 336 species are found to belong to

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this genus. The adults of these weevils have been reported to defoliate the cultivated plants ever since the beginning of 20th century and their larvae were found to feed on the roots of several host plants.

Biopesticides or biological pesticides are pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. They belong to three major categories that include biochemical pesticides; microbial pesticides and plant-incorporated protectants as defined by The Environmental Protection Agency (EPA) of U.S. [4,5]. Of about 700 different microbial products, 38 were fungal formulations isolated from or based on *Beauveria* sp., *Metarhizium* sp., *Trichoderma* sp. (Hypocreales: Hypocreaceae) [6].

Fungi generally have vast range of host organisms that may include almost all insect groups [7]. *Beauveria bassiana* (Bals.-Criv.) Vuill. (1912) (Hypocreales: Cordycipitaceae), fungus of the Sordariomycetes class, is commonly called 'white muscardine fungus'. It has a wide range of host including 175 members from all insect orders.

Metarhizium anisopliae (Metchnikoff) Sorokin (1883) (Hypocreales: Cordycipitaceae) commonly called 'green muscardine fungus' is also a Sordariomycetes fungus and is found inhabiting in soil and attacking a wide range of insects [8]. It was Elie Metchnikoff who first introduced *M. anisopliae* for pest management in the field [9] while *B. bassiana* derived product called Boverin was used against Colorado potato beetle in former USSR in 1965 [10]. Maina et al. [8] reported that there were one hundred and seventy-one products developed from entomopathogenic fungi, of which 33.9% were derived from *B. bassiana* and *M. anisopliae*. Fancelli et al. [11] reported three isolates of *B. bassiana* viz CNPMF407, CNPMF218 and CNPMF 416 as effective against *Cosmopolites sordidus* (Germ.) (Coleoptera: Curculionidae). The study revealed that strain CNPMF218 with 20% as most efficient that has led to the reduction in population size of the pest by 40% after 12 months. Erler and Ates [12] evaluated the potential of different strains of both *B. bassiana* and *M. anisopliae* against the larvae of june beetle, *Polyphylla fullo* (Linnaeus) (Coleoptera: Scarabaeidae) and found *B. bassiana* strain PPRI 5339 as the most effective with 79.8% mortality in young larvae and 71.6% mortality in old larvae at $4x10^9$ conidia/ml. *Metarhizium* strain F52 caused 74% and 67.6% mortality at $9x10^8$cfu/g in young and older larvae respectively.

Malik et al. [13] evaluated the effect of entomopathogenic fungus *B. bassiana* against *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) and reported 100% mortality for second instar at $1x10^6$ conidia /ml and so as the chemical Imidacloprid (1 ppm) after 20 days. Baysel et al. [14] characterized ten isolates of *B. bassiana* from *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) and *Gonioctena fornicata* (Brugg.) (Coleoptera: Chrysomelidae) against larvae of *H. postica* and revealed that all strains of *B. bassiana* caused 100% mortality at$1x10^7$ conidia/ml, exactly one week after treatment. Sankaran et al. [15] evaluated the efficacy of the entomopathogenic fungi *B.bassiana* against *M.viridanus* Fabricius (Coleoptera: Curculionidae) and found 53% mortality when applied directly and 43% mortality when given along with food, both at a concentration of $1x10^5$ spores /ml.

2. MATERIALS AND METHODS

2.1 Collection of *Beauveria* Species

Extensive survey was carried out throughout Kasaragod district, Kerala, India, for the fungal infested weevil. The study was done during August to Septermber, 2019. From the plantations of Padannakkad, Kasaragod, the weevil pest *Myllocerus undatus* Marshall (Coleoptera: Curculionidae) was found infested by a fungus. The infested weevil was collected and brought to the lab, and then the fungal infested weevil was kept in 0.01% mercuric chloride ($HgCl_2$) for one minute and washed several times with sterile water and cut into small pieces using a sterilized blade. The laboratory conditions maintained at 25±2°C, 71±5% RH.

2.2 Preparation of Potato Dextrose Agar (PDA) Media for the Subculture

200 g of potato starch was dissolved in 1 litre of water and boiled. It is filtered and to the filterate 20 g of glucose solution was added along with 15 g of agar and was boiled again. Thereafter, the medium was transferred to test tubes (slant) or petridishes without any bubble and manipulated in a laminar airflow chamber. The test tubes were then sealed tightly with cotton plug and the petridishes were covered with polythene cover.

2.3 Fungal Culturing and Sub-culturing

Transfer of the collected fungal specimen was done in highly sterile and clean surroundings in a laminar airflow chamber. First, the inoculating forceps and half spear point needle were sterilized by heating followed by air cooling. The pieces of insect body were then transferred to a petridish by gently raising the cover keeping near the flame to avoid contamination. After transferring all the leftover pieces to culture media, the Petri dishes were sealed

with plastic wrappers, kept at room temperature and observed for fungal growth. The culture thus obtained from the infested weevil were sub cultured in test tubes and sent to National Fungal Culture Collection of India (NFCCI) for fungal culture deposition and accession number.

2.4 Isolation and Subculturing of *Metarhizium anisopliae*

Culture of *Metarhizium anisopliae* was obtained from NFCCI (NFCCI -Accession Number: 1872). The fungal culture obtained from NFCCI was cultured and sub cultured in PDA medium. From the subculture a stock solution was prepared by mixing 1gram conidia in 100 ml. From this stock culture different dilutions were prepared.

2.5 Bioassay Using Fungal Strains

Pest control studies using the fungal strain were carried out by picking up the spores / conidia from 7-10 days old well sporulating culture using a sterilized scalpel. About 1 g of conidia was weighed and diluted in 100 ml of water. The solution was homogenized by stirring vigorously with utmost care.

For *Beauveria* sp three major doses of the conidial suspension, ranging from $4.56x10^8$ (dose I), $3.2x10^7$ (dose II), and $1.28x10^7$ (dose III) conidia per milliliter of water were evaluated in the bioassay. Dose I is obtained from the culture on PDB medium prepared in shaker system, dose II and dose III were obtained from subculture in Petri dishes after 10 and 7 days respectively. Six conidial concentrations were prepared by diluting in different proportions.

Similarly for *Metarhizium,* two major doses viz Dose I with $5.36x10^8$ conidia/ml was obtained from culture on PDB medium prepared in shaker system and Dose II with $7.5x10^6$ conidia/ml was obtained from plate culture on PDA medium. Four other conidial concentrations were prepared by diluting in different rates. Each of the four resulting doses was again diluted. All of the doses were applied on larva and adult of *Myllocerus* sp. collected in petridishes of 10 cm diameter. A batch of ten adults and larvae each were dipped directly in respective doses of fungal suspension of for about one minute. The treated larvae and adult were allowed to feed on ragi rootlets and mango leaves (or any plant leaf as the pest is polyphagous). Maintained five set replicas (each set containing 10 numbers) of both larvae and adult for each doses. The percentage of mortality of both larvae and adult was calculated for each dose. Various lethal concentrations (LC10 –LC90) were calculated by probit analysis using SPSS22.

3. RESULTS AND DISCUSSION

The entomopathogenic fungus collected from the field that showed potent lethal effect on *Myllocerus* was sent to NFCCI and were identified as *Beauveria* sp. It was provided with NFCCI accession number 4225. From the subculture of the *Beauveria* sp. maintained in the lab, different dilutions are taken for treating against *Myllocerus* sp. The data on the efficiency of different doses of *Beauveria* sp. are tabulated and presented in the Table 1.

Dose I of *Beauveria* sp. had a concentration of $4.56 x10^8$ conidia /ml and showed the highest efficacy against adult *Myllocerus* compared to other two dosages. At 72 hours after treatment, 40% adult mortality was recorded which reached to80% by 120 hours and by 240 hours of treatment 100% adult mortality was recorded. Even though high adult mortality was observed it was less effective against the larval stages.

Dose II of *Beauveria* sp. contained $3.2 x10^7$ conidia /ml (Treatment 3). When treated against the adult weevil caused 30% mortality after 72 hours of

Table 1. Effect of *Beauveria* sp.on *Myllocerus* sp.(Coleoptera: Curculionidae)

Treatments	conidia /ml	Percentage of adult mortality						Mean larval mortality		
		24 HAT[xx]	48 HAT	72 HAT	120 HAT	192 HAT	240 HAT	72 HAT	120 HAT	240 HAT
	Control	0	0	0	0	0	0	0	0	0
1	$4.56x10^8$	10	30	40	80	90	100	0	20	40
2	$4.56x10^7$	0	10	20	60	70	90	0	0	20
3	$3.2x10^7$	0	10	30	60	70	80	0	10	20
4	$1.28x10^7$	0	10	16	30	50	70	0	0	10
5	$4.56x10^6$	0	10	10	40	60	70	0	0	00
6	$3.2x10^6$	0	0	0	60	60	60	0	0	10
7	$1.28x10^6$	0	0	0	10	20	40	0	0	0
8	$3.2x10^5$	0	0	0	0	20	30	0	0	0
9	$1.28x10^5$	0	0	0	0	0	1	0	0	0

[xx]*Hours After Treatment*

treatment, 60% mortality was recorded at120 hours after treatment and 80% mortality was recorded at 240 hours after treatment. The one by tenth dilution of dose II (3.2 x10^6 conidia / ml, treatment 6) caused 60% mortality after 240 hours of treatment and another dilution of dose II with a concentration of 3.2x10^5 (Treatment 8) caused 30% at the same time interval (after 240 hours of treatment). Dose III of *Beauveria* sp. had1.28 x 10^7 conidia/ml (Treatment 4) and when treated against *Myllocerus* 16% mortality was recorded after 72 hours of treatment. Mortality rate was 30% after 120 hours of treatment and 70% after 240 hours of treatment. Thus, *Beauveria* sp. at a concentration of 4.56 x10^8 conidia /ml had the maximum lethality causing 100% death in 240 hours after treatment (Figs. 1 and 2).

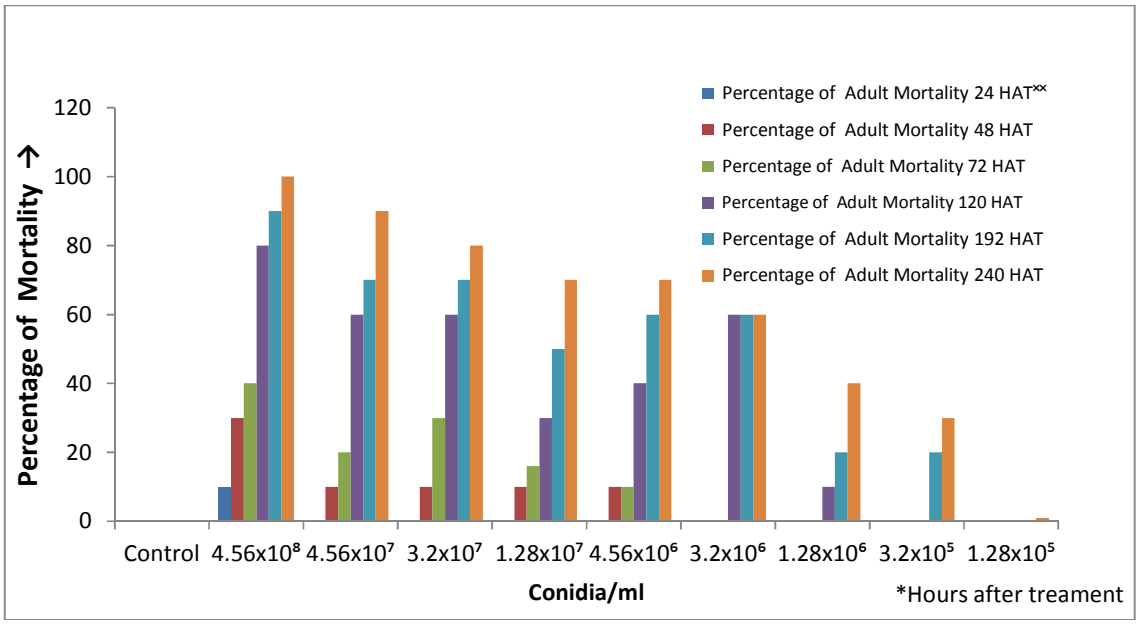

Fig. 1. Effect of *Beauveria* sp. against adults of *Myllocerus sp.*

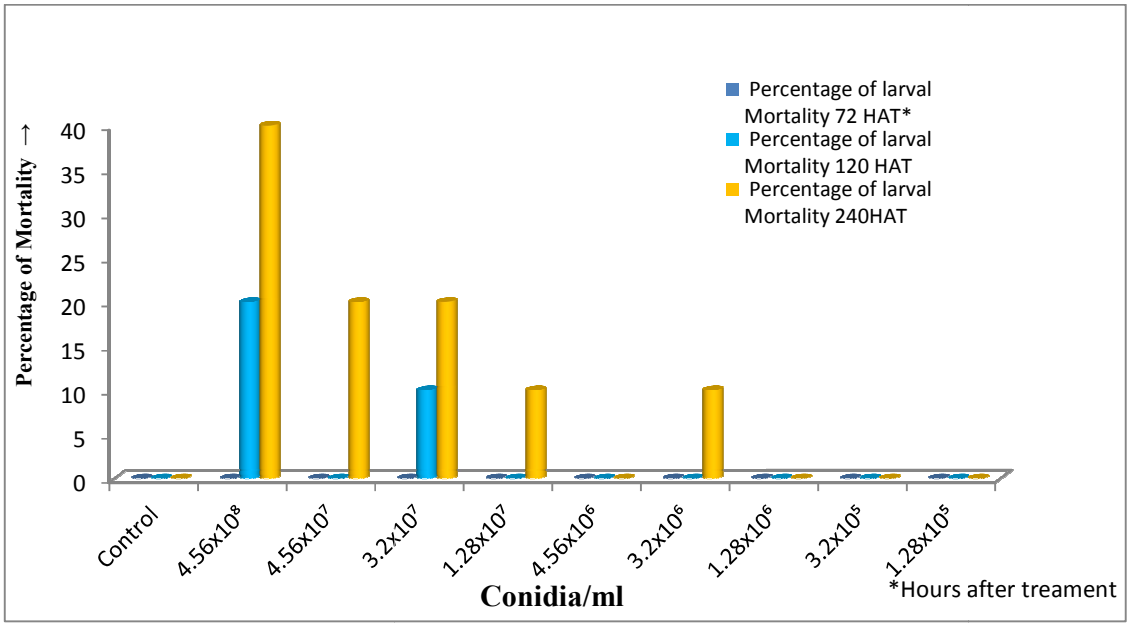

Fig. 2. Effect of *Beauvaria* sp against IV instar of *Myllocerus* sp.

Metarhizium anisopliae was also found effective against *Myllocerus* sp. Table 2 represents the data on the efficacy of *M.anisopliae* against adults and fourth instar larvae of *Myllocerus* sp.

Dose I of *M. anisopliae* has $5.36x10^8$ conidia/ml (Treatment 1). When treated against adults of *Myllocerus* resulted in 30% mortality at 72 hours after treatment. The mortality rate increased to 50% at 120 hours after treatment and to 90% at 240 hours after treatment.100% adult mortality was observed at 360 hours after treatment. Dose II of *M. anisopliae* had $7.5x10^6$ conidia /ml (Treatment 3). When treated against adults of *Myllocerus* 30% mortality was recorded after 120 hours of treatment. Mortality rate has increased to 60% after 192 hours of treatment and 240 hours after treatment, 70% mortality was recorded (Figs. 3 and 4).

The LC50 value for the entomopathogenic fungus *Beauveria* was found to be $2.01x10^6$conidia/ml and that of *M.anisopliae* was found to be $9.04x10^5$ conidia/ml by probit analysis. The median lethal time for the most effective dose of *Beauveria* ($4.56x10^8$) was 70.48 hours and that for the most effective dose of *M. anisopliae* ($5.36x10^8$ conidia/ml) was 99.14 hours. The tabulated values are represented in Table 3.

Shantipriya and Misra [16] showed the efficacy of some biopesticides against *Myllocerus maculosus*. The study effectively utilised two entomopathogenic fungi *Metarhizium anisopliae* (1%WP) in as Kalachakara, Biodart (1%) and *Beauveria bassiana* (1000 g, in as Daman (1%WP)). Along with them 1000 g of Padan (50SP/ha), spinosad 45 SC/ha (125 ml), Servo Agrospray/ha (5000 ml) and Ozoneem/ha

Table 2. Effect of *Metarhizium anisopliae* on *Myllocerus* sp.

Treatments	conidia /ml	Percentage of adult mortality						Percentage of larval mortality		
		24 HAT^{xx}	48 HAT	72 HAT	120 HAT	192 HAT	240 HAT	72 HAT	120 HAT	240 HAT
	Control	0	0	0	0	0	0	0	0	0
1	$5.36x10^8$	20	20	30	50	70	90	0	10	30
2	$5.36x10^7$	0	10	20	40	60	80	0	0	10
3	$7.5x10^6$	10	10	20	30	60	70	0	0	20
4	$5.36x10^6$	0	0	10	20	40	60	0	0	10
5	$7.5x10^5$	0	0	10	40	40	50	0	0	10
6	$7.5x10^4$	0	0	0	20	30	30	0	0	0

^{xx}*Hours After Treatment*

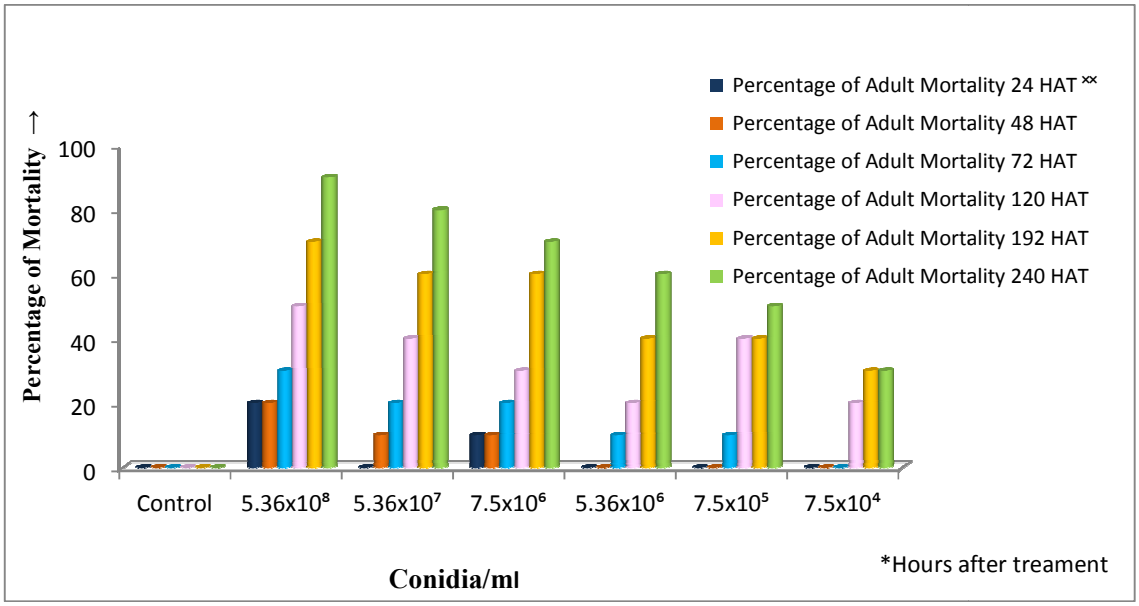

Fig. 3. Effect of *Metarhizium anisopliae* against adults of *Myllocerus* sp.

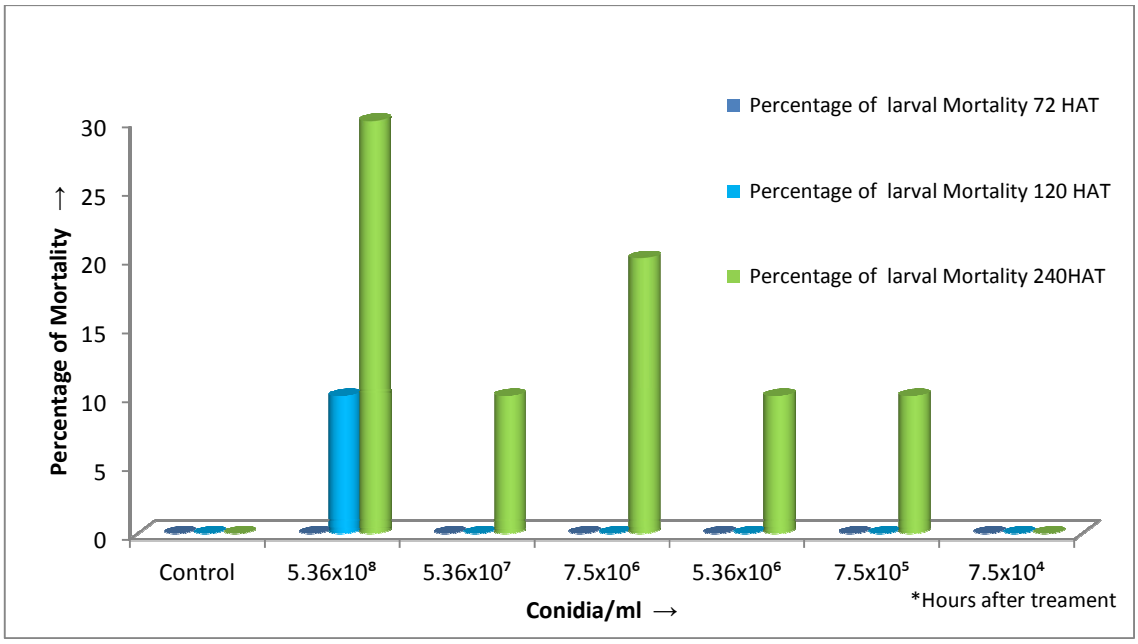

Fig. 4. Effect of *Metarhizium anisopliae* against IV instars of *Myllocerus* sp.

Table 3. Details lethal concentrations of both *Beauveria* sp. and *Metarhizium* sp. on adult *Myllocerus* sp.

	Lethal concentration	Conidia /ml	Lower limit	Upper limit
Beauveria sp.	LC10	$7.26x10^4$	$2.06x10^4$	$1.66x10^5$
	LC50	$2.01x\ 10^6$	$1.207x10^6$	$3.16\ x10^6$
	LC90	$5.56x10^7$	$2.87\ x10^7$	$1.47\ x10^8$
Metarhizium anisopliae	LC10	$1.66x10^3$	14.27	$1.62x10^4$
	LC50	$9.04x10^5$	$2.06\ x10^5$	$2.45\ x10^6$
	LC90	$4.93\ x10^8$	$1.05\ x10^5$	$1.05\ x10^{10}$

in two different concentration - (1500 ppm) in 2500 ml and (10,000 ppm) in 1500 ml were also used. Anitha et al. [17] reported *Bacillus thuringiensis* var kurstaki (Bacillales: Bacillaceae) and *M. anisopliae* as effective biopesticides against grey weevil *Myllocerus* sp., a pest of sunflower. The study utilised *B. thuringiensis* at 5.2 x 10^7 conidia per ml and *M. anisopliae* at 2.2 $x10^4$ conidia per ml and proved that these micropathogens could potentially control the leaf damage by the weevil pest. Prabhuraj et al. [18] he evaluated the efficacy of *Heterorhabditis* sp. (Rhabditida: Heterorhabditidae) and *Steinernema glaseri* (Steiner) (Rhabditida: Steinernematidae), two entomopathogenic fungi against *Myllocerus discolor* Boheman (Coleoptera: Curulionidae). 10 days after application, mortality level recorded was 93.8% for *Hetero rhabditis* sp. and 92.8% for *S. glaseri.*

4. CONCLUSION

The present study proved the efficacy of entomopathogenic fungi against the polyphagous pest, *Myllocerus* sp. Several fungi, as mycoinsecticides are yet to be discovered. If properly improved and developed, use of fungi as a biocontrol agent could be one among the best pest management strategies. Furthermore mycoinsecticides application is getting popularized as the method is100% ecofriendly that reduces the use of synthetic pesticides. In future by improving the pathogenicity, persistence of the entomopathogenic fungi and by making its application more farmer friendly our country can go far forward in the field of agriculture.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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