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Review Article

ENTOMOPATHOGENIC POTENTIAL OF THE FUNGI *METARHIZIUM* (HYPOCREALES, CLAVICIPITACEAE) IN THE MANAGEMENT OF INSECT PESTS

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ABSTRACT
Metarhizium, a genus of entomopathogenic fungi belongs to Class Sordarimomycetes, is a soil-borne fungus and found in almost all habitats. Its action is epizootic in nature and does not hold any unwanted threat for humans or animals, as it a natural biocontrol agent and attacks only insects. Conservation biological control can be defined as manipulation of the environment to favor natural enemies, either by removing or mitigating adverse factors or by providing lacking requisites. Conservation of natural enemies can also be understood as one of the oldest form of biological control. Natural enemies, if established successfully, provide eco-friendly management of insect pests, which in turn reduces human and environmental burdens. Taking in to consideration the effective utilization of entompathogenic fungi in particular the *Metarhizium* sp. as an effective biocontrol agent in control of number of insect pests of agricultural and horticultural importance, the documentation of this fungal pathogen is important. In this review paper more than 150 insect species belonging to 11 different orders getting infected by *Metarhizium* sp. are detailed along with their classification.

KEY WORDS: *Metarhizium*, Hypocreales, Entomopathogenic Fungi, Biocontrol.

INTRODUCTION
Insects are the major culprits for considerable losses of wood in forest, crops, stored grains, textile industries, furnished wood etc. They affect the wood quality and thereby affect the revenue that also leads to accidents when it comes to building, bridges, rail tracks, where sleepers are made up of wood. Though synthetic insecticides/pesticides that are effective for small period of time are readily available commercially, their applications have serious consequences. Due to their broad spectrum nature, pesticides not only affect the targeted pest but also affect the beneficial organisms. Moreover, excessive and indiscriminate use of pesticides contaminates the soil, water and land. Pesticides have terrible adverse impact on humans, animals and the environment due to their toxic effects in food chain, development of resistance in target pest and destruction of natural enemies (Paray and Rajabalee, 1997; Joshi *et al*., 2000). Increased environmental awareness and concerns about the adverse effect of pesticides has made it imperative to identify safer and effective alternatives to the agro-chemicals (Logan *et al*., 1990). Due to the ill effects of pesticides, alternative eco-friendly strategies are being increasingly appreciated and practiced for pest management (Padmaja *et al*., 2005). During the last decade, there has been increased and continued interest in the employment of natural biological enemies for the management of forest pests (Sandhu *et al*., 2012). Ecologically compatible strategies were developed to manage insect pest populations by utilizing entomopathogenic virus, fungi, nematodes, protozoa and bacteria as biological control agents (Castillo *et al*., 2000; Lacey *et al* 2001). Once established, natural enemies has the capacity of self-replicating and surviving, which is one of the advantages of using biological control over synthetic pest control. Moreover, it reduces the excessive use of pesticides, hence reducing the risk to environment. Interestingly, biological control can even work with other pest management practices in an integrated pest management programme (IPM). Fungal entomopathogens with several life history specializations makes them a distinctive group from other types of pathogens. One of the important characteristics of entomopathogens is that they infect through the cuticle of their host (Hajek and Leger, 1994), usually not having to be ingested to cause infection. Deuteromycetes fungi that have been shown good potential as pest control agents for commercial production includes *B. bassiana*, *Metarhizium anisoplaie*, *Verticillium lecanii* and *Trichiderma viridae*. *Metarhizium* is known for its epizootic action and it also has been the subject of several molecular studies on fundamental topics such as virulence and host specificity. They can be grown on different artificial and natural media for field tests and application. High virulence, broad host range, quick mode of action, stability in culture, safety to workers, easy production and storage method are the essential characteristics required for a fungus to be used as an effective microbial insecticide (Mc Coy, 1990). *Metarhizium anisopliae* is an important entomopathogenic fungus, found in soils throughout the world. Fungi under the genus *Metarhizium* have been isolated from infected insects and soil of all continents, and there have been isolations

from near the Antarctic Circle also (Roddam and Rath, 1997). Although some isolates of these fungi have rather restricted host range, the group is better known for its ability to kill a wide spectrum of insects of at least seven orders (Veen, 1968) Four groups of insect pests (termites, locusts, spittlebugs and beetles) were previously targeted using *M. anisopliae* (Zimmermann, 1993). The common name for *Metarhizium*-induced disease is green muscardine, based on the encrustation of insect cadavers with green conidia. *Metarhizium anisopliae* was the first fungus worldwide to be mass produced and utilized for insect-pest control (Krassilstschik, 1888; Steinhaus, 1975). In the last few decades, many attempts with varying levels of success were made by utilizing *Metarhizium* sp. for pest control. In this paper. We attempt to review the effects of *Metarhzium* sp. on some major forest pests in Indian and international contexts. Further, considering the magnitude of risk factors associated with the prolonged use of commercially available insecticides, integrated pest management (IPM) methods have been suggested which have minimum side effects.

ISOLATION

Metarhizium is a soil-borne fungus found in almost all the habitats. The fungus can be isolated from soil samples, collected with the help of soil core borer or core sampler to the depth of 20 cm. The highly preferred method is insect bait method (Zimmermann, 1993). In this method the soil samples collected in clean polythene bags separately to be stored in refrigerated condition at 5°C**.** The samples should be subjected to insect bait method with in five to seven days by using the wax moth larvae *Galleria mellonella* as insect bait. The soil samples collected and kept in a polythene covers is to be moistened and filled in small 100 ml plastic containers and to release 10-15 grown up third instar larvae of *G. mellonella* in to the soil filled plastic containers for incubation for 14 days. Every day the soil has to be agitated to ensure that the larvae remain exposed to the soil. Diseased and mummified larvae should be collected from the container for isolation of the entomopathogenic fungi. If no external fungal sporulation is seen, the dead larvae will be surface sterilized by submersion in 1% sodium hypochlorite, followed by rinsing with sterilized water and allowed for sporulation in a moistened Petri plates. The fungi isolated from the cadavers are to be subcultured in Sabouraud dextrose agar medium (SDA). The fungi isolated from the artificial media will be again subcultured until the pure cultre is obtained (Raja Rishi *et al*. 2013).

In another method *Metarhizium* can also be isolated from roots. In this the roots were washed with sterile distilled water to remove excess soil. Soil that adhered closely to the root was kept as representative of the rhizosphere. The roots were cut into 0.5 cm pieces, placed in 5 ml distilled water and homogenized using a rotary homogenizer (Greiner Scientific). Samples (100 ml) of homogenate were spread, in duplicate, onto selective media, containing 39 g potato-dextrose agar (PDA) l21 (Difco), 0.5 g cycloheximide l21, 0.2 g chloramphenicol l21, 0.5 g 65% dodine l21 and 0.01 g crystal violet l21. The plates were incubated at 27 °C for 20 days. Metarhizium isolates were identified by colony morphology, namely white mycelia with green conidia, as well as microscopic identification of conidial morphology. Morphologically differing colonies were individually isolated from the selective plate of each plant root sample and grown on PDA plates at 27 °C for 10 days. Morphologically similar colonies were also isolated multiple times from the same plant root sample (Michael Wyrebek *et al*. 2011). Metarhizium fungal pathogens were also isolated directely from the naturally infected cadavers by incubating or streaking a sub-sample on surface of selective media (potato dextrose agar, PDA, Criterion, USA with 0.1% chloramphenicol and 0.05% cyclohexamind) (Tangthira sunun *et al*. 2010).

In another process of isolation, the soil samples were processed by diluting 10 g in 100 ml of sterile water that was added 0.01% Tween-80. One milliliter of the soil dilution was spread over those selective media. Then, single colony of *Metarhizium* spp. was transferred to PDA for purification. All were propagated and maintained with a 5 mm diameter mycelial plug taken from the growing edge of a 7 days old culture grown on PDA plates for 28 days at room temperature. Pure culture can be obtained and the identification has to be done by observation of conidia, colony and mycelia (Tangthirasunun *et al*. 2010).

SUCCESS STORIES

Metarhizium was tested against almost 150 species of insect pests in about more than 200 experiments on insects of different orders namely Coleoptera, Lepidoptera, Blattoidea, Isoptera, Hemiptera, Orthoptera, Diptera, Hymenoptera, Thysanoptera, Neoptera and Trombidiformes. Review of the literature reveals that *Metarhizium* has been most effectively and extensively used for the order Coleoptera. More than 50 species under the order Coleopters were studied and most of them were found to be susceptible to attack by *Metarhizium*. The second most studied order was Lepidoptera. More than 25 different species belonging to order Lepidoptera were recorded to be attacked by *Metarhizium,* in more than 30 separate studies. Hemiptera was the third extensively studied order where more than 20 experiments have been conducted. Isoptera was the fourth extensively studied order which included 16 species with more than 30 cases. Ten species of Blattoidea were recorded in more than 40 studies, both in lab as well as in field conditions. In addition to these orders, Orthoptera, Diptera, Hymenoptera, Thysanoptera, Neoptera and Trombidiformes were also observed to be attacked by *Metarhzium*. Under the order Coleoptera *Metarizium* was found to be most effective against members of the family Curculionidae. Sixteen species of this family were infected by *Metarizium*, followed by Cerambycidae, Scarabaeidae and Chrysomelidae. Interestingly, among different stages, larval stages were most prone to get attacked. Results varied from species to species where mortality percentage varied from a minimum of 15% to a maximum of 100%. The underlying reasons for this level of variation could be attributed to the climatic factors that influence the virulence of the fungus, and the differences in the susceptibility of the insects and there stages (Table-1).

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TABLE 1. Pathogenicity of *Metarhizium* against forest and crop pests

S.No	Target organism	Order	Family	Stage	Effect	Reference
1.	*Adoryphorus couloni*	Coleoptera	Scarabaeidae	Grub	90-100% mortality	Brownbridge *et al*., 2009
2.	*Aeolesthes sarta*	Coleoptera	Cerambycidae	Grub	50.99% mortality	Farashiani *et al*., 2008
					47.50% mortality	Mohi-Uddin *et al*., 2009
3.	*Agrilus auriventris*	Coleoptera	Buprestidae	Grub	72.7-94.8% mortality	Fan *et al*., 1990
					59.7% mortality	Fan *et al*., 1988
4.	*Amrasca devastans*	Hemiptera	Cicadellidae	Larva	$9x10^9$ cfu/g @ 2500 g/ha produced 12.3 % Mortality in field	Neelima *et al*., 2011
5.	*Anomala cuprea*	Coleoptera	Scarabaeidae	Grub	Pathogenic	Shimazu *et al*., 1993
6.	*Anopheles gambiae*	Diptera	Culicidae	Adult	Susceptiblity to the funguswas more with mineral oil formulation applied to polyester net than pyrethroid	Howard, 2010
				Larva	Pathogenic	Wang *et al*., 2010
7.	*Anoplophora glabripennis*	Coleoptera	*Cerambycidae*	Adult	Shortened longevity and decreased oviposition. Percentages of eggs that did not hatch were greater in fungal-treated females and 60% of unhatched eggs contained signs of fungal infection	Hajek *et al*., 2008
				Adult	Beetles in two density treatments died in fewer days than beetles exposed to environments without conidia.	Shanley and Hajek, 2008
				Adult	Numbers of adult beetles from plots of each fungal species died in <10 days were greater than controls	Hajek *et al*., 2006
				Adult	Survival times for 50% of the beetles tested (ST_{50}) was 5 days.	Dubois *et al*., 2008
				Grub and adult	Decreased ALB longevity and fitness in two application methods. Longer activity of fungus in cages treated with fungal bands compared with sprays.	Hajek *et al*., 2007
8.	*Aphis craccivora*	Hemiptera	Aphididae	Adult	Pathogenic with a varying degree of infectivity.	Pegu *et al*., 2013
9.	*Aphis gossypii*	Hemiptera	Aphididae	Adult	Pathogenic with a varying degree of infectivity.	Pegu *et al*., 2013
10.	*Apriona germari*	Colelptera	Cerambycidae	Grub	Cumulative corrected mortality reached to 89.47% and 88.92% after 14 d ay treatment.	Chen DeLan, 2013
11.	*Apriona rugicollis*	Coleoptera	Cerambycidae	Grub	Produced 81.2% mortallity	Fan *et al* .,1988
12.	*Atta bisphaerica*	Hymenoptera	Formicidae	Adult	More than 80% mortality recorded	Castilho *et al*., 2010
13.	*Atta sexdens piriventris*	Hymenoptera	Formicidae	Adult	Among the strains E_6, Al Rj and E_{sa} ,E_{sa} were most pathogenic.	Silvaand Diehl,1988
14.	*Atta sexdens rubropilosa*	Hymenoptera	Formicidae	Adult	More than 80% of mortality recorded	Castilho *et al*., 2010
15.	*Atta sexdens sexdens*	Hymenoptera	Formicidae	Adult	Above 80% mortality obtained	Loureiro*et al*., 2005
16.	*Batocera horsfieldi*	Coleoptera	*Cerambycidae*	Grub	78.3 % mortality recorded	Fan *et al*., 1988
17.	*Bemisia tabaci*	Hemiptera	Aleyrodidae	Maggot	Effective against nymphs	Azevedo *et al*., 2005
				Maggot	Pathogenic with varying degree of infectivity	Pegu *et al*., 2013

18.	*Blitopertha pallidipennis*	Coleoptera	*Scarabaeidae*	Grub	53.8% mortality observed	Li *et al*., 1992
19.	*Brontispa longissima*	Coleoptera	Chrysomelidae	Grub	Spraying of improved strains of the entomofungal pathogen has showed greater efficacy	Retinam and Singh, 2007
20.	*Bruchus rufimanus*	Coleoptera	Chrysomelidae	Adult	Gave good protection to broad beans.	Sabbour *et al*., 2007
21	*Bupalus piniaria*	Lepidoptera	Geometridae	Larva	100% mortality was seen in 18 days	Nedveckyte *et al*., 2011
22.	*Carposina nipponensis*	Lepidoptera	*Carposinidae*	Larva	84.5-95.3% mortality recorded	Fan *et al*., 1990
23	*Catopsilia crocale*	Lepidoptera	Pieridae	Larva	Mortalitiy of 6.66, 13.33 and 73.33% at 1, 3 and 6 DAT.	Barman and Nath , 2002
24	*Choristoneura fumiferana*	Lepidoptera	Tortricidae	Larva	The LD_{50} after 72 h was 1.4µ g/larva for destruxins.	Brousseau *et al*., 1998
				Larva	Destruxins were toxic to 3rd, 4th and 5th instar larvae with LD_{50} of 0.116, 0.35 and 1.52 µ g/larva.	Brousseau *et al*., 1996
25.	*Chrysoperla carnea*	Neuroptera	Chrysopidae	Adult	The treatment was least toxic compared to commercially available insecticides.	Nitharwal *et al*., 2013
26.	*Clostera anachoreta*	Lepidoptera	Notodontidae	Adult	*Ma*789 was the most virulent strain	Pan *et al*., 2012
27.	*Coccidohystrix insolita*	Hemiptera	*Pseudococcidae*	Adult	37.35-43.13% mortality recorded	Haque and Ghosh, 2007
28.	*Coelomera lanio*	Coleoptera	Chrysomelidae	Pupa	Affected pupae	Silveira*et al*., 2002
29.	*Conotrachelus humeropictus*	Coleoptera	Curculionidae	Grub	42.7% mortality observed	Mendes *et al*., 2001
30.	*Conotrachelus psidii*	Coleoptera	Curculionidae	Adult	57.3 and 88.6% mortality recorded	Brito *et al*., 2008
31.	*Coptotermes frenchi*	Blattodea	Rhinotermitidae	Adult	Of the 93 isolates screened in the grooming assay, 26 were identified as promising	Milner *et al*., 1998
32.	*Coptotermes acinaciformis*	Blattodea	Rhinotermitidae	Adult	Of the 93 isolates screened in the grooming assay, 26 were identified as promising	Milner *et al*., 1998
					No consistent effect on pathogenicity	Milner *et al*., 1997
33.	*Coptotermes curvignathus*	Blattodea	Rhinotermitidae	Adult	100% mortality recorded	Hoe *et al*., 2009
					100% mortality recorded	Sajap and Kaur, 1990
34.	*Coptotermes formosanus*	Blattodea	Rhinotermitidae	Adult	100% mortality observed	Wright *et al*., 2005
					Infection eached Epizootic level	Sun *et al*., 2003
					Termite workers dusted with dry conidia were capable of transmitting the pathogen to other colony members.	Jones *et al*., 1996
					99% mortality recorded	Sun *et al*., 2008
					Higher percentage of exposed individuals could transfer inoculum resulting in high mortality	Hussain and Yi, 2013
					100% mortality recorded	Wright and Cornelius, 2012
					M. anisopliae was found to be highly virulent (LT_{50}- 3.10 days)	Hussain *et al*., 2010
					100% mortility recorded	Chai, 1995
				Adult	Rapid termite mortality	Delate *et al*., 1995
				Adult	100% mortality within 2 days	Wells *et al*., 1995
35.	*Coptotermes gestroi*	Blattodea	Rhinotermitidae	Adult	100% termite mortality after 1 month	Maketon *et al*., 2007
36.	*Coptotermes heimi*	Isoptera	Rhinotermitidae	Adult	LT_{50} were comparatively more (65-106 h) in soil than on filter paper (50-83 h).	Ashraf *et al*., 2009
				Adult	LT_{50} was comparatively more (93-175 hrs) in soil than on filter paper (91-135).	Ashraf *et al*., 2008

37.	*Coptotermes lacteus*	Blattodea	Rhinotermitidae	Adult	Repellency of *Metarhiziumanisopliae* conidia to termites	Milner, 2000
				Adult	Isolates such as FI-1037, FI-1099 and FI-1186 were markedly less repellent	Staples and Milner, 2000
				Larva	20 weevil larvae were applied to the soil surface and the tiles were dug up 20 days later and examined for living and dead larvae	Tedders *et al*., 1973
				Adult	*M. anisopliae* may have potential to cause significant infection.	Ilan *et al*., 2009
				Larva	*M. anisopliae* colonies were restricted to larval cadavers and to the inside of the larval soil cells.	Gottwald and Tedders, 1984
				Larva	75% mortality achieved	Ilan *et al*., 2009
38.	*Corythucha ciliata*	Hemiptera	Tingidae	Adult and nymph	Pathogenic	Sevim *et al*., 2013
39.	*Curculio nucum* L	Coleoptera	*Curculionidae*	Grub	80% mortality recorded	Carrera *et al*., 2013
					The median lethal concentration of *M. anisopliae* was $6.38x10^8$spores $litre^{-1}$. Median lethal time was 8.60-15.19 days for $1.0x10^{11}$-$1.0x10^7$ spores $litre^{-1}$.	Cheng, 2000
					The total number of species and individuals were slightly lower in the sprayed areas than in the unsprayed areas.	Jiang *et al*., 2005
40	Curculio sayi	Coleoptera	*Curculionidae*	Grub	56% mortality recorded	Ihara *et al*., 2003
41.	*Curculio sikkimensis*	Coleoptera	*Curculionidae*	Grub	At 25°C pathogenecity of *Metarhizium* was Maximum	Ihara *et al*., 2009
42.	*Cyclocephala comata*	Coleoptera	*Dynastidae*	Grub	Susceptible to *M. anisopliae*, with LD_{50} of $2.1x10^{11}$ conidia per larva.	Ponce *et al*., 2004
43.	*Cydia funebrana*	Lepidoptera	*Tortricidae*	Adult	Caused the highest mortality to moth species.	Stephan and Herker , 2011
44.	*Dendroctonus frontalis*	Coleoptera	*Curculionidae*	Grub	LD_{50} and LD_{90} following topical application of conidia were7.2 x 10^3 and 1.1 x $10^5$for *M. anisopliae*.	Pabst and Sikorowski, 1980
45.	*Dociostaurus maroccanus*	Orthoptera	Acrididae	Larva	Strong mortality in the insects treated at the larval and adult stages.	Outtar *et al*., 2011
46.	*Dysdercus cingulatus*	Hemiptera	*Pyrrhocoridae*	Nymph and Adult	Shown highly effective against both 6±1 day old nymphs and adults of red cotton bugs.	Rai *et al*., 2013
				Adult	The LC_{50} values ranged from $2.25x10^5$ to $3.66x10^8$ spores/ml.	Sahayaraj and Borgio, 2009
47.	*Epilachna* beetle	Coleoptera	Coccinellidae	Grub	90.42% mortality on 2nd and 7^{th}day.	Haque *et al*., 2007
48.	*Esfandiaria obesa*	Orthoptera	*Acrididae*	Larva	98 - 100% mortality in larval stages.	Bagheri *et al*., 2007
49.	*Euproctis pseudoconspersa*	Lepidoptera	Lymantriidae	Larva	77.4% mortality recorded.	Fan *et al*., 1988
50.	*Eutectona machaeralis*	Lepidoptera	Pyralidae	Larva	*M. anisopliae* with 3 g/L found to be the best treatment causing 50.77 to 68.07% mortality.	Chavan *et al*., 2012
51.	*Frankliniella occidentalis*	Thysanoptera	Thripidae	Adult	Control of thrips	Ansari *et al*., 2007
52.	*Galleria mellonella*	Lepidoptera	Pyralidae	Larva	Inhibition of proteinase by phenyl-methylsulfonyl fluoride, decreased toxicity.	Kucera,1980
				Adult	The strong effect of destruxins (DE) was characterized by changes in the morphology of the cells.	Vey and Quiot, 1989

				Larva	97% mortality recorded	Valdes ,1976
53.	*Glycaspis brimblecombei*	Hemiptera	*Psyllidae*	Nymph	Tested products were pathogenic to the red gum lerp psyllid	Pogetto *et al*., 2011
54.	*Gonipterusscutellatus gyllenhal*	Coleoptera	Curculionidae	Grub	Weak toxicity noticed	Molina and Carbone, 2010
55.	*Gynaikothrips uzeli*	Thysanoptera	*Phlaeothripidae*	Adult	75% mortality occurred	Pena *et al*., 2011
56.	*Hedypathes betulinus*	Coleoptera	Cerambycidae	Grub	10^7 conidia mL^{-1}caused mortality in insect.	Leite *et al*., 2011
57.	*Helicoverpa armigera*	Lepidoptera	*Noctuidae*	Larva	29.12-58.25% mortality recorded	Haque *et al*., 2007
				Larva	73% mortaliy and77% weight reduction over control	Rao *et al*., 2011
58.	*Helopeltis antonii*	Hemiptera	Miridae	Adult	32.5% mortility.	Patil and Naik, 2004
59.	*Henosepilachna vigintioctopunctata*	Coleoptera	Coccinellidae	Grub	Decrease in feeding	Swaminathn and Hussain, 2010
				Adult	74.13 % mortility	Vishwakarma *et al*., 2011
60.	*Heterotermes tenuis*	Isoptera	*Rhinotermitidae*	Adult	100% of insects eliminated in six days.	Sterling *et al*., 2011
					Nimkol-L was compatible with *M. anisopliae* strain up to 1% a.i.	Castiglioni *et al*., 2003
					Pathogenic.	Castiglioni *et al*., 2005
					The penetration, colonization and conidiogenesis phase is faster for *M. anisopliae* which results in a faster rate of insect mortality.	Moino Junior *et al*., 2002
61.	*Hieroglyphus banian*	Orthoptera	*Acrididae*	Adult	Four species of entomogenous fungi were isolated from 8 insect species .Confirmed their pathogenic relationship with varying degree of infectivity.	Pegu *et al*., 2013
62.	*Hodotermes mossambicus*	Isoptera	Hodotermitidae	Adult	Large diversity in susceptibility to disease.	Chouvenc *et al*., 2009
63.	*Hodotermopsis sjoestedti*	Isoptera	Termopsidae	Adult	Large diversity in susceptibility to disease.	Chouvenc *et al*., 2009
64.	*Holotrichia consanguinea*.	Coleoptera	Scarabaeidae	Grub	Penicillium was the most antagonistic towards the pathogenic fungi.	Sharma *et al*., 1999
65.	*Hyadaphis coriandri*	Hemiptera	*Aphididae*	Adult	Reduced maximum population after first spray (32.3 aphids/floret).	Ramanujam *et al*., 2013
66.	*Hyblaea puera*	Lepidoptera	Hyblaeidae	Larva	37.69 % mortality.	Sakchoowong, 2002
				Larva	Pathogenic to larvae.	Kalia and Harsh, 2003
				Adult	The LC_{50} of the isolates ranged from $0.01x10^5$ to $759.21x10^5$ for the different pests.	Remadevi *et al*., 2010
67.	*Hylobius pales*	Coleoptera	Curculionidae	Adult	High mortality. When larger doses were employed, the insects succumbed faster.	Schabel, 1976
68.	*Hyphantria cunea*	Lepidoptera	Arctiidae	Larva	Mortality up to 86.8%	Tserodze and Murvandze, 2009
					Mortality ranged from 52% to 68%	Burjanadze *et al*., 2013
					83%-100 mortality recorded	Montermini *et al*., 1985
69.	*Hypsipyla grandella*	Lepidoptera	Pyralidae	Larva	Larvae are most susceptible to *Metarhiziumanisopliae* during the fifth instar.	Salvatierra and Berrios, 1972
70.	*Hypsipyla robusta*	Lepidoptera,	Pyralidae	Larva	The LC_{50} of the isolates ranged from $0.01x10^5$ to $759.21x10^5$	Remadevi *et al*., 2010
					Highly lethal with significantly lower LD_{50} value	Balachander *et al* ., 2012

					of 2.6%	
71.	*Icerya purchasi*	Hemiptera	Monophlebidae	Adult	20.3% Mortality recorded	Fan *et al*., 1988
72.	*Ips typographus*	Coleoptera	Curculionidae	Adult	Infection was highest when beetles had contact with freshly suspension treated bark for five minutes	Herrmann and Wegensteine, 2011
					97% mortality recorded	Mudroncekova *et al*., 2013
					Metarhizium anisopliae was found for the first time to attack *I. typographus*	Keller *et al*., 2004
73.	*Kalotermes flavicollis*	Dictyoptera	*Kalotermitidae*	Adult	Large diversity in disease susceptibility	Chouvenc *et al*., 2009
74.	*Lepidiota stigma*	Coleoptera	*Scarabaeidae*	Grub	Lower number of grubs observed in fields applied with $1x10^{13}$/ha (0.33/m row)	Rachappa *et al*., 2004
75.	*Leptispa pygmaea*	Coleoptera	*Chrysomelidae*	Adult	42 % effective mortality	Japur *et al*., 2012
76.	*Leptocorisa oratorius*	Hemiptera	*Alydidae*	Adult	Effective against gundhi bug, damaged grain	Kalita*et al*., 2009
77.	*Leptoglossus occidentalis*	Hemiptera	Coreidae	Adult	Sensitive to infection, killed the treated individuals within 10 to 30 days.	Rumine and Barzanti, 2009
78.	*Leptoglossus zonatus*	Hemiptera	Coreidae	Adult	94%Mortality recorded.	Grimm and Guharay, 1998
79.	*Leptopharsa heveae*	Hemiptera	Tingidae	Larva	Large variation of virulence observed	Silva*et al*., 2012
80.	*Leucopholis irrorata*	Coleoptera	Melolonthidae	Grub	30% larval mortality recorded	Braza, 1990
81.	*Leucopholis lepidophora*	Coleoptera	*Scarabaeidae*	Grub	With $4x10^8$ conidia 33.33% mortality was achieved	Rakesha *et al*., 2012
					60.94 % mortility was recorded	Channakeshavamurthy*et al*., 2010
					The applications of *Metarhiziumanisopliae* 2 x 10^8 at 20 g/palm caused 60.94 and 50.97% mortality	Murthy *et al*., 2010
82.	*Lipaphis erysimi*	Hemiptera	Aphididae	Adult	Recorded 64% mortality in 3.8 days	Araujo Junior *et al* ., 2009
83.	*Liriomyza trifolii*	Diptera	Agromyzidae	Larva	Efficient compound	El-Salam *et al*., 2013
84.	*Locusta migratoria*	Orthoptera	Acrididae	Adult	*Metarhizium* can suppress small local populations of *L. migratoria*	Hunter *et al*., 1999
				Nymph	Walking activity was reduced to 24%	Ranaivo *et al*., 1996
85.	*Locusta migratoria cinerascens*	Orthoptera	Acrididae	Larva	High mortality in the insects treated at both the larval and adult stages	Outtar *et al*., 2011
86.	Lymantria dispar	Lepidoptera	Erebidae	Larva	100% mortality within 120h of treatment	Wasti and Hartmann, 1982
				Larva	The insecticide activity was studied in relation to the fungus spore concentration, the incubation time and the larval instar, in order to establish the different lethal doses.	Ouakid *et al*., 2005
87.	*Maruca vitrata*	Lepidoptera	*Crambidae*	Larva	*Metarhizium anisopliae* was moderately effective	Sunitha*et al*., 2008
88.	*Mastotermes darwiniensis*	Blattodea	Mastotermitidae	Adult	Large diversity in disease susceptibility	Chouvenc *et al*., 2009
89.	*Melanotus cribricollis*	Coleoptera	Elateridae	Grub	Mortality increased with increase of temperature and reached 96.7% at 30°C	Wang *et al*., 2010
90.	*Micadina yingdeensis*	Phasmida	Heteronemiidae	Adult	Dosage of $1.5x10^{13}$ spores/ha resulted in 70% mortality.	ShiLan *et al*., 1994
91.	*Microcerotermes diversus* Silvestri	Isoptera	Termitidae		Treated-sawdust bait was applied by two methods, LC_{50} and LC_{90} were $8.4x10^6$ and $3.9x10^7$spore/ml, respectively	Habibpour *et al*., 2011
92.	*Monochamus alternatus*	Coleoptera	Cerembycidae	Adult	90% mortality recorded	He *et al*., 2009

					Results showed that *Metarhiziumanisopliae*789 (Ma789) was the most virulent one.	YongSheng *et al*., 2010
				Adult	85% mortality recorded	He *et al*., 2008
				Adult and grub	The mortality was 80.0% and 76.0% for larvae and 83.4% and 88.9% for adults	He *et al*.,2007
				Grub	With the cadaver rates being as high as 76.9-93.1% (1.0x10^7 conidia/ml) and 57.9-75.0% (6.5x10^5-3.4x10^6 conidia per individual) for the larvae at 15 days after inoculation and adults at 20 days after inoculation, respectively	He *et al*., 2005
93.	*Myzus persicae*	Hemiptera	Aphididae	Adult	Pathogenic relationship with varying degree of infectivity	Pegu *et al*., 2013
94.	*Nasutitermes exitiosus*	Isoptera	Termitidae	Adult	Isolate FI-610 was found to be one of the most effective	Milner *et al*., 1998
					Almost half the treated colonies became moribund.	Hanel, 1983
					Samples from treated colonies showed high levels of contamination	Hanel and Watson, 1983
					Decreasing RH reduced germination but no consistent effect on pathogenicity	Milner *et al*., 1997
95.	*Nasutitermes sp*	Isoptera	Termitidae	Adult	Infected termites died 1 to 3 days post-inoculation	Gutierrez *et al*., 2004
96.	*Nasutitermes voeltzkowi*	Isoptera	Termitidae	Adult	Large diversity in susceptibility to disease	Chouvenc *et al*., 2009
97.	*Neocerambyx mandarinus*	Coleoptera	Cerembycidae	Grub	Recorded 92.7% mortalitity	Fan *et al*., 1988
98.	*Ocinara varians*	Lepidoptera	Bombycidae	Larva	Fungal spores retarded larval growth by 35% -76%	Hussain *et al*., 2009
99.	*Odontotermes sp.*	Isoptera	Termitidae	Adult	LC_{50} of the isolates ranged from 0.01x10^5 to 759.21x10^5. Effective	Remadevi *et al*., 2010
100.	*Odontotermes formosanus*	Isoptera	Termitidae	Adult	100% mortality 3 days post-inoculation	ChangJin *et al*., 2009
101.	*Odontotermes obesus*	Isoptera	Termitidae	Adult	Pathogenic relationship with varying degree of infectivity.	Pegu *et al*., 2013
				Adult	Transmission of *Metarhizium anisopliae* was 50 to 98% and 16 to 78% for the isolates tested	Balachander *et al*., 2013
				Adult	At 5 ml/litre showed lower efficacy	Premalatha *et al*., 2008
102.	*Odontotermeswallonensis*	Isoptera	Termitidae	Adult	100% of mortality of termites recorded	Nagaraju *et al*., 2013
103.	*Oryctes rhinoceros*	Coleoptera	*Scarabaeidae*	Adult and grub	Killing 91.7% of the larvae and between 63% and 69% adults	Moslim *et al*., 2011
				Grub	Recorded 90% mortality after treatment	Moslim *et al*., 2009
				Grub	The fungus was highly virulent against all stages of the pest except egg.	Kalidas and Prasad, 2001
				Adult	Wet inoculum was effective for the control of *Oryctes rhinoceros* on oil palm	Moslim *et al*., 1999
				Adult	83% infection in 4 months after treatment	Tey and Ho,1995
				Grub	Dipping 3rd-instar larvae briefly in conidial suspensions at 10 million spores/ml led to the development of brown lesions on the body of the larvae	Sivapragasam and Tey, 1994
				Adult	The pathogen can reduce *O. rhinoceros* populations	Bedford, 2013
104.	*Otiorhynchus ovatus*	Coleoptera	Curculionidae	Grub	The fungus was highly virulent even at the low	Fisher and Bruck, 2008

					dose of $1x10^4$	
				Adult	Over 98% control achieved	Shah *et al*., 2007
				Grub	Temperatures below 20°C (68F) significantly slowed fungal growth and the speed at which *M. anisopliae* infected BVW larvae.	Bruck , 2007
					Reduction in larval numbers ranging from 0 to 96% and 0 to 90% at East Mailing and Little hampton respectively	Moorhouse *et al*., 1993
					Three of the 5 isolates of *Metarhiziumanisopliae* controlled larvae	Tol and Van, 1993
					Pathogenic at 1 x 10^6 spores/cm^3.Ultimately gave complete kill even at 1 x 10^5conidia ml^{-1}	Prado, 1980
				Grub	74 and 81% control of the pest achieved	Stenzel *et al*., 1992
					Significant reduction on root herbivory	Oddsdottir *et al*., 2010
					Pathogenic at 1 x 10^6 spores/cm^3. Ultimately gave complete kill even at 1 x 10^5 conidia ml^{-1}	Prado, 1980
				Grub	Higher efficacy when conidia mixed with neem seed cake (5 g/l of peat)	Shah *et al*., 2008
					The fungus was highly virulent even at the low dose of $1x10^4$ spores per g dry soil.	Fisher and Bruck, 2008
105.	*Pachycoris klugii*	Hemiptera	Scutelleridae	Adult	Recorded 65% mortality	Grimm and Guharay, 1998
106.	*Paliga machoeralis*	Lepidoptera	Crambidae	Larva	The LC_{50} of the isolates ranged from $0.01x10^5$ to $759.21x10^5$	Remadevi *et al*., 2010
					Among the 25 isolates tested, MIS2, MIS7, MIS1 and MIS3 were found to be more effective	Sapna Bai *et al* ., 2013
107.	*Pantana phyllostachysae*	Lepidoptera	*Lymantriidae*	Larva	All strains showed pathogenicity to 2nd- and 3rd-instar larvae.	Zhang *et al*., 2002
108.	*Phyllophaga* sp.	Coleoptera	Scarabaeidae	Grub	47.16% decrease in plant mortality over control in treated plots.	Bhagat, 2005
109.	*Pityogenes chalcographus*	Coleoptera	Curculionidae	Adult	Capable of causing high mortality.	Pehl and Kehr, 1994
110.	*Plagiodera versicolora*	Coleoptera	*Chrysomelidae*	Grub	Pathogenic.	Demir *et al*., 2013
111.	*Platypus cylindrus*	Coleoptera	Curculionidae	Adult	Infected adults were able to contaminate larvae by transfer of spores.	Glare *et al*., 2002
112.	*Pleonomus canaliculatus*	Coleoptera	Elateridae	Grub	Pathogenic.	HuaChao *et al*., 2002
113.	*Plocaederus ferrugineus*	Coleoptera	Cerambycidae	Grub	All the treatments were effective and superior over control.	Sahu and Sharma, 2008
				Grub	Pouring conidial suspension achieved 33.3-36.4% recovery of infested trees followed by swabbing conidial slurry with 23-25% and soil application with 15.4-16.6%.	Ambethgar , 2010
					91.66% mortality recorded	Ambethgar , 2001
				Grub	Fungus caused a green muscardine disease in larvae	Choudhuri , 1973
				Grub	100% mortality recorded	Ambethgar *et al*., 1999
114.	*Polyphagotarsonemus latus*	Trombidiformes	Tarsonemidae	Larva and Adult	Successful in controlling both larvae and adult	Maketon *et al*., 2008
115.	*Popillia japonica*	Coleoptera	Scarabaeidae		Infected 1.2% of the population	Hanula anf Andreadis,

						1988
116.	*Prorhinotermes canalifrons*	Isoptera	Rhinotermitidae		Large diversity in susceptibility to infection	Chouvenc *et al*., 2009
117.	*Pseudacysta perseae*	Hemiptera	Tingidae	Adult and Nymphs	At 30 day, treatments showed a biological efficacy exceeding 90%.	Romero *et al*., 2012
118.	*Pseudophacopteron canarium*	Hemiptera	Phacopteronidae		Recorded8.7-53% mortality	RongYing *et al*., 1995
119.	*Pyrausta coclesalis*	Lepidoptera	*Crambidae*	Larva	Recorded 36 % mortality	Rishi *et al*., 2012
120.	*Raphidopalpa foveicollis*	Coleoptera	*Chrysomelidae*		Reduction in damage 64.7% at 3.0 g/lit.	Vishwakarma *et al*., 2011
121.	*Reticutermes speratus*	Blattodea	Rhinotermitidae	Adult	The conidia affiliated with the termites reared individually did not show a marked reduction. Within 3 h, almost all of the termites held in groups contained the conidia in their foreguts, but no conidia were detected in the foreguts of the termites reared individually.	Shimizu and Yamaji , 2003
122.	*Reticulitermes flavipes*	Isoptera	Rhinotermitidae		Large diversity observed in susceptibility to disease	Chouvenc *et al*., 2009
				Adult	Physiological cost to successfully encapsulate *M. anisopliae* varied greatly	Chouvenc *et al*., 2011
				Adult	None of the conidia found in the alimentary tracts germinated	Chouvenc *et al*., 2010
				Adult	90% mortality recorded	Chouvenc *et al*., 2008
				Adult	The relative number of hemocytes per termite increased 24 h after fungal exposure	Chouvenc *et al*., 2009
				Adult	Hyphal growth was generalized in the body cavity of the cadaver	Chouvenc *et al*., 2009
				Adult	Recorded 100% mortality	Wang and Powell, 2004
				Adult	Recorded 41.6% mortality	Ramakrishnan *et al*., 1999
				Adult	Recorded 100% mortality	Zoberi,1995
				Adult	Recorded 92% mortality	Quarles,1999
					Good controlling agent	Milner *et al*., 1996
123.	*Reticulitermes grassei*	Blattodea	Rhinotermitidae		Recorded 93.44% mortality	Alvarez *et al*., 2005
124.	*Reticulitermes sp.*	Blattodea	Rhinotermitidae	Adult	Healthy termites concentrate in grooming activity on diseased individuals and thereby became infected	Kramm *et al*., 1982
				Adult	Fungi reisolated from the hind gut of diseased termites were pathogenic to healthy termites	Kramm and West , 1982
125.	*Rhagoletis cerasi*	Diptera	Tephritidae	Larva and Adult	Fungal strains were able to cause mycosis	Daniel *et al*., 2008
126.	*Rhyacionia frustrana*	Lepidoptera	Tortricidae		Population of *R. frustrana* decreased to very low levels on the treated plots	Duarte *et al*., 1992
127.	*Leucopholis lepidophora*	Coleoptera	*Scarabaeidae*	Grub	2 x 10^8 at 20 g/palm caused 60.94 and 50.97% mortality	Murthy *et al*., 2010
128.	*Saccharosydne saccharivora*	Hemiptera	*Delphacidae*		Reduced population of the pest from 61.25 to 20.14%	Vanegas *et al*., 2006
129.	*Saperda populnea*	Coleoptera	Cerambycidae		Recorded 94.1% mortality	Fan *et al*., 1988
130.	*Schistocerca gregaria*	Orthoptera	Acrididae	Larva and adult	Recorded 100% mortality	Miscellaneous. 1992

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				Larva and adult	Strong mortality in the insects treated at the larval and adult stage.	Outtar *et al*., 2011
131.	*Scolytus multistriatus*	Coleoptera	Curculionidae	Grub	Recorded 100% mortality	Houle *et al*., 1987
132.	*Scolytus scolytus*	Coleoptera	Curculionidae	Adult	Limited mortility observed	Doberski, 1981
133.	*Sitophilus oryzae*	Coleoptera	Curculionidae	Adult	Percentages of infestation were significantly decreased after treatment	Sabbour, 2012
134.	*Spilarctia obliqua*	Lepidoptera,	Acrididae	Adult	Tested isolates were virulent	Remadevi *et al*., 2010
135.	*Spodoptera litura*	Lepidoptera	Noctuidae	Larva	73% mortality and 77% weight reduction	Rao *et al*., 2011
					Pathogenic relationship with varying degree of infectivity.	Pegu *et al*., 2013
136.	*Spondylis buprestoides*	Coleoptera	*Cerambycidae*	Grub	Effectively controlled.	ChengRun , 2005
137.	*Stigmella populnea*	Coleoptera	Nepticulidae	Grub	Recorded 70.8% control	Fan *et al*., 1990
138.	*Strophosoma capitatum*	Coleoptera	Curculionidae	Adult	Average survival time at 20° C ranged between 23 and 28 days.	Nielsen *et al*., 2006
				Adult	80% mortality in lab and 60% mortality in the field.	Nielsen *et al*., 2007
139.	*Strophosoma melanogrammum*	Coleoptera	Curculionidae	Adult	Direct inoculation caused over 80% mortality. Indirect inoculation resulted in 72% mortality.	Kram, 2010
				Adult	80% mortality in lab and 60% mortality in the field.	Nielsen *et al*., 2007
				Adult	Recorded 90% mortality	Nielsen *et al*., 2006
140.	*Taeniothrips inconsequens*	Thysanoptera	*Thripidae*	Larva	The highest rates of infection were seen in larvae recovered from soil samples (11.9%)	Brownbridge *et al*., 1999
141.	Termites	Isoptera	Rhinotermitidae	Adult	Efficient for controlling termites	Nyeko *et al*., 2010
142.	Termites	Isoptera	Rhinotermitidae	Adult	Behavioural defence mechanisms by termites can limit the effectiveness of conidia applications	Lenz , 2005
143.	*Thaumetopoea pityocampa*	Lepidoptera	Thaumetopoeidae	Larva	Mortality was significantly high	Er *et al*., 2007
144.	*Toxoptera aurantii*	Hemiptera	Aphididae	Adult	Observed pathogenic	Pegu *et al*., 2013
145.	*Trialeurodes vaporariorum*	Hemiptera	*Aleyrodidae*	Maggot	Recorded 75%mortality	Pena *et al*., 2011
					Recorded 98% efficacy	Prishchepa *et al*., 2005
146.	*Tuta absoluta*	Lepidoptera	Gelechiidae	Egg and Larva	Pathogenic to eggs and larvae	Pires *et al*., 2010
147.	*Vatiga illudens*	Hemiptera	Tingidae	Adult	Recorded 74% Mortality	Oliveira *et al*., 2001
148.	*Zaprionus indianus*	Diptera	Drosophilidae	Adult	Recorded 100% mortality	Svedese *et al*., 2012
149.	*Zeuzera pyrina*	Lepidoptera	Cossidae	Larva	Recorded 95-99% mortality	Deseo and Docci, 1985

CONSERVATION OF ENTOMOPATHOGENIC FUNGUS *METARHIZIUM*

Biological control is defined as the reduction of pest populations by natural enemies and typically involves an active human role. Conservation biological control (CBC) is a biological control strategy in which farming practices and environmental manipulations are adopted to enhance the living conditions for specific natural enemies of pests (Meyling and Eilenberg, 2007). Avoiding measures that are harmful to natural enemies and adapting measures that can benefit them may provide successful biological control. Probably, the first comprehensive review of the relationships of crop pests and beneficial insects with uncultivated land (van Emden, 1965) was able to cite examples of all the components of Conservation Biological Control by habitat modification used in practice today. There are three basic types of biological pest control strategies, importation (sometimes called classical biological control), augmentation and conservation. Importation is the introduction of a species in some area with the intention of biological control. Augmentation defines the actions or processes by which population of biocontrol agent can be increased. Conservation means act of preserving or guarding a biocontrol agent. Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, and pathogens. A predator is an organism which naturally preys on another organism, a parasitoid is an insect whose larvae lives as parasite and eventually kills the host, and a pathogen is an agent that causes illness in the host or kills it. *Metarhizium* as an entomopathogen is a natural enemy of pests found in forest and agricultural land and is a candidate for future conservation biological control in temperate regions. However, compared with classical and augmentation biological control, it has received relatively little attention as a method of arthropod pest suppression (Ehler, 1998; Landis *et al*., 2000). It is generally acknowledged that biological control alone is unlikely to provide adequate pest control, but through careful integration with other pest suppression tactics, it could represent a significant source of sustainable control (Dowell, 1990; Gerling, 1992; Cock, 1994; Heinz, 1996). Here question arises what can be done for conservation of this natural enemy of pests, and the answer is hidden somewhere in the biology and ecology of this entomopathogen. Like other organisms, these natural enemies too require food, water, and shelter, and protection from adverse conditions. One the best way could be avoiding use of insecticides and pesticides which has both direct and indirect effect of eliminating natural enemies. Insecticides not only kill harmful insects but also significantly affects biological control agent. In soil, this species can withstand drought or any adverse climatic condition by forming sclerotium and can start multiplying under favourable conditions. Since it is a soil-borne fungus, it is perfectly protected from UV radiation. It is important to consider biological and ecological need of a natural enemy for the success of any biological control method. Environmental modifications may be made to increase natural enemy effectiveness. Some of these modifications include, A. Construction of artificial structures, B. Provision of supplementary food, C. Provision of alternative hosts, D. Improvement of pest-natural enemy synchronization, E. Modification of adverse agricultural practices.

CONCLUSION

The forest is a dynamic ecosystem constituting principal natural renewable resources of multifarious uses which fulfill the requirement of the society and sustainability of the earth. Insect pests are major biological determinants of forest productivity, and integrated pest management is not new to Indian forestry (Sundararaj, 2014). Biological control is a self sustaining measure that could be adopted for long term results in forest habitat as well as in field conditions. It can also be used in formulation form on timber along with timber polish, or can be sprayed and left as such. Though biological control with entomopathogens like *Metarhizium* is most frequently encouraged to be practiced, there are some limitations with this method. Not all the target pests are affected up to the expectation. Field and lab trials results some times vary greatly. Some insects get infected when they are in their larval stage and some in their adult stage. In case of some insects, results were almost 100% whereas for others mortality was as low as 15% only. Death time also varies with different pests, which vary from 3 to several days. Infection rates were high in freshly cultured isolates as compared to old and stored cultures. Temperature and humidity plays vital role in attachment and growth of conidia. If temperature is lower than 20°C it slows the infection rate of the fungus. When *Metarhizium* conidia were applied with sunflower oil or IMI, comparatively higher mortality rate was observed. Pests exposed to *Metarhizium* also showed shortened longevity and decreased oviposition. It also reduces hatching percentage of laid eggs. Wet inoculum is more effective as compared to dry inoculum. Biopesticide has been mostly used in agricultural field compared to forests. Introduction of an effective bioconrol agent in the forest plantaion may provide a solution for the damage caused by notorious insect pests.

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