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METARHIZIUM ANISOPLIAE: EFFECTIVE BIOLOGICAL PEST CONTROL FOR AGRICULTURAL CROP PESTS.

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ABSTRACT:
Sustainable agriculture in the 21st century will rely increasingly on microbial control and other alternative interventions for pest management that eco friendly. For biological control of insects pests, entomo-pathogenic microorganism and viruses play a major role. Entomopathogenic fungi are important naturally occurring antagonists of insect pests with a worldwide distribution. Research on these fungi has started about 100 years ago and after a decline, was reactivated since last 30 years. A number of entomopathogenic fungi have been identified and there potential as biological control agent. The present investigation were undertaken to study the isolation and screening of various entomopathogenic fungi viz., Beauveria and Metarhizium against third instars larvae of agricultural crop larvae the germination percentage of the isolates on different media were studied. Effective isolates *Metarhiziun anisopliae* 3 were used for percent germination studies on the different media and the media used were PDA, PYGA, . Out of four media tested the B. bassiana showed the highest germination on PYGA (92.30%). The soil sample from Agriculture College, shows presence of entomopathogenic fungi, which need the further more isolation and study for the efficiency against different insects pests. Among the five isolates *Metarhizium anisopliae* 3 show more effectiveness against third instars larvae of agricultural crop

Key words: Pest management, entomo-pathogenic fungi, *Metarhizium anisopliae.*

INTRODUCTION:

Several abiotic and biotic factors affect the production and productivity of the crops. Among the biotic factors, insect pests cause enormous damage to our crops and forest trees (Agarwal 1990). There are about 750 species of entomopathogenic fungi found through the five major taxa comprising division Eumycota (Basic,1835).The entomopathogenic fungi occupy the vital role in control of insects pests as entomopathogens. Some of the important entomopathogenic fungal genera are Beauveria, Metarhizium, Entomoprhora, Coelomomyces, Nomuraea, Aschersonian, Hirsutella, Verticuillum, Paecilomyces, etc. containing several species which are commonly used in microbial control (Agrwal and Rajak, 1985). In India, the entomopathogenic fungi have remained much less explored. In the present investigation, an attempt made to isolate and identify the entomopathogenic fungi for the different location of Pune district. Also the studies on germination percentage and virulence of isolated strains against agricultural larvae were carried out. The agricultural larva is widely distributed over the tropics and subtropics. It is represented by several species, which are among most dreaded agricultural pests, for they have define human efforts to check their spread, using chemical insecticide and consequent economic damage to several important crops. Any intervention in the environment towards the elimination of a species brings out parallel changes in the pest to overcome the hurdle. Thus, agricultural larvae overcome almost all the synthetic insecticide (Arms et al.1992, Basavana Goud, 1994). In India, Hardwick is the most widely prevalent and devastating one. It attacks more than 182 host plants belonging to 47 botanical families in the Indian subcontinent and it is now estimated to feed on more than 200 plant species (Pawar, 1998) typified by being highly mobile, plasticity in host suitability, fecundity, multi-voltine and voracious feeding.

Agricultural pest larvae cause severe damage to many food and fiber crops. ***Metarhizium anisopliea***, formerly known as ***Entomophthora anisopliea***, is a widely distributed soil-inhabiting fungus. The first use of *M. anisopliea* as a microbial agent against

insects was in 1879. *M. anisopliae* is categorized as a green muscardine fungus due to the green color of the sporulating colonies. It has been reported to infect approximately 200 species of insects and other arthropods. Although *M. anisopliae* is not infectious or toxic to mammals, inhalation of spores could cause allergic reactions in sensitive individuals. ***M. anisoplia****e* generally enters insects through spiracles and pores in the sense organs. Once inside the insect, the fungus produces a lateral extension of hyphae, which eventually proliferate and consume the internal contents of the insect. Hyphal growth continues until the insect is filled with mycelia. When the internal contents have been consumed, the fungus breaks through the cuticle and sporulates, which makes the insect appear "fuzzy." *M. anisopliae* can release spores (conidia) under low humidity conditions (<50%). In addition, *M. anisopliae* can obtain nutrition from the lipids on the cuticle. The fungus can also produce secondary metabolites, such as dextruxin, which have insecticidal properties on moth and fly larvae. Some insects have developed physiological mechanisms to reduce infection by fungi such as *M. anisopliae*. For example, the desert locust produces antifungal toxins, which can inhibit the germination of spores. In addition, insects can escape infection by molting rapidly or developing a new integument before the fungus can penetrate the cuticle. The successful mass culture of *M. anisopliae* and development of methods of mass-producing infective spores has led to the commercial development of this fungus as a microbial insecticide. *M. anisopliae* is grown on a large scale in semi-solid fermentation-- similar to that used in the production of *Bacillus thuringiensis*-- and the spores can then be formulated as a dust. The fungal spores can also be grown on sterilized rice in plastic bags for small-scale production. *M. anisopliae* is sensitive to temperature extremes; spore viability decreases as storage temperatures increase and virulence decreases at low temperatures. Bio blast is a commercially available formulation of *M. anisopliae* that is used to control termites such as *Reticulitermes* sp. The fungus is applied into wood known to contain active termite galleries. Termites in these galleries are exposed to direct contact with the fungus. In addition to direct contact with the fungus, infection of other termites in the colony occurs when grooming individuals exposed to the fungus spread the pathogen to healthy, non-infected individuals in the population. Laboratory studies have shown that death occurs within 4 to 10 days, depending on temperature.

Metarhizium anisopliae

Metarhizium as conidiophores on low mounds, covered by conidia branched closely or loosely grouped, forming a sporulating layer sporogenus cells, borne singly, in pair or in wholes; conidia apical, produced in basipetal chains, compacted into the columns, long avoid to cylindrical with rounded ends, one celled, olive green in mass. *Metarhizium anisopliae* Sorokin, the green muscardine fungi, also belong to subdivision-Dueteromycotina, Class-Hypomycetes, Order- Moniliales and Family-Moniliaceae. This fungus are known to be attacking over 200 species of insects covering seven orders (Roberts,1973b.)

MATERIALS AND METHODS :

Field survey was conducted around Pune region to collect the different vegetables and agricultural crop larvae from different location. Laboratory studies were carried out to isolates the promising entomopathogenic fungi from agricultural crop pest larvae. Also the studies were carried out to find to germination percentage of promising isolates on different media. The relatively efficacy of the same isolates was carried out against agricultural crop larvae. Experiment was conducted of Entomology section, Agriculture, Pune-5.

For the isolation of entomopathogenic fungi directly from the agricultural crop.To collect the infected agricultural crop from the PimpriMandai, AkurdiMandai, MancharMandai and some farm region.

1. To make the PDA media.
2. Then isolates the ***Metarhizium anisopliae anisopliae*** fungus on this media Petri plate The plate and conical flask were incubated at room temperature for 4-5 days.
3. The fungus was purified by 5-6 sub culturing.
4. Then isolated fungi were identified using the key as started earlier
5. To take the agricultural crop larvae on this sub culturing.

All the isolates maintained on PAD. All isolates were purified by 3-4 sub culturing on PDA and PYGA slants at room temperature.

Project set-up and Precaution

The rearing cages and culture room were disinfected with formalin to avoid microbial contamination. Rearing cages were kept away from ants by keeping ant wells below the base.

Observation

The mortality was recorded at an interval of 24 hrs. up to 14 days. The exact time required to kill the test larvae was strictly recorded. The dead larvae were removed and maintained in separate vials and place in humid chamber to allow outgrowth of relevant fungus.

The larvae that had died in 24 hrs. Therefore, such larvae were excluded from mortality analysis. Then the mortalities due to fungus treatment were corrected with control.

Studies on germination percentage of effective isolates. The germination percentage was detected for all the isolates for two different media as given in table

RESULT & DISCUSSION :

Screening of various fungal isolates against Agricultural Pests

The conidial viability of all fungal isolates was consistently high (75-90%) in all bioassay. The mean germination rate of isolates was 90 per cent in all cases. All isolates were able to infect the Agricultural plant. The data on mean mortality induced by each isolate at a concentration of 1 * 10^7conidial after 14 days are presented in Table.

The cumulative mortality data shows a non-significant variation between isolation for the agricultural crop larvae. The highest mortality was caused by M. a (83.33%) followed by M.a. 4 (73.33%)..Regardless of the isolate mortality rate ranged from the 56.66 to 83.33 percent.

the lowest germination on PYGA (89.40%). Regardless of medium germination percentage of M. anisopliae ranges from 89.40 to 90.00.

This shows that the effective media for M. anisopliae was PDA.

larvae under laboratory condition.

The efficacy of the effective isolate M. anisopliae was tested under laboratory condition by using conc. 1 *10^4 to 1 *10^8 spores. The dead cadavers

when kept on moist filter paper, white mycelia growth appeared within 24 hrs. after the death on inter segmental region followed by segmented region. In next 24 hrs. cadavers were fully covered with dense white map and fungi started sporulating. Sporulation of M. anisopliae took 3-4 days. Sporulation of M. anisopliae was characterized by green powdery mass.All the mortality percentage were recorded 14 days after inoculation. The M. anisopliae showed mortality range between 33.30 to 96.66 at 1 *10^4 to 1 *10^8 spores.

SUMMERY AND CONCLUSION

The present investigation were undertaken to study the isolation and screening of various entomopathogenic fungi viz., Beauveria and Metarhizium against third instar larvae of agricultural crop larvae the germination percentage of the isolates on different media were studies. This laboratory studies were conducted at the Entomology section, College of Agriculture, Pune-5 2012-2013.

Survey & identification of entomopathogenic fungi

During the course of the present investigation soil samples and insect cadavers were collected from the College of Agriculture, Pune, Manchar Pune district. Out of this location the College of Agriculture, shows the presence of the entomopathogenic fungi in the soil the fungi isolated in case of two ***Metarhizium anisopliae*** (M. a. 3 and M. a. 4) respectively.

Percent germination of entomopathogenic fungi

Only effective isolates ***Metarhizium anisopliae*** 3 were used for percent germination studies on the different media and the media used were PDA, PYGA, Out of four media tested the B. bassiana showed the highest germination on PYGA (92.30%).

Efficacy of effective fungal isolates against agricultural crop larvae.

The efficacy of effective isolates M.a.3 were tested under laboratory condition at 1 * 104 to 1 * 108 spores ml-1. . The M. anisopliaeshdpowed mortality range between 33.30 to 96.66% with LC50 value 0.90 * 105 spores ml-1. Thus according to present investigation M.a. 3 is the most effective iosolates of entomopathogenic fungi tested against third instar larvae ofagricultural crop .

CONCLUSION :

Isolation and screening of various entomopathogenic fungi against agricultural crop larvae show results as follows:

1. The soils of College of Agriculture shows presence of entomopathogenic fungi which need the further more isolation and study for the efficacy against different insects pests. Among the five isolates . M. a3 showed more effectiveness against third instars larvae of agricultural crop.

2. Among the four media tested for percent germination PDA is effective for MEPA for M. anisopliae, which need further investigation on cost of media to find out more cost effective medium.

3. The efficacy study showed that two isolate selected for bioassay were effective as a bio control agent. It needs further studies on mass production of isolate on different media, suitable formulation and persistence field experiment on efficacy of these isolates.

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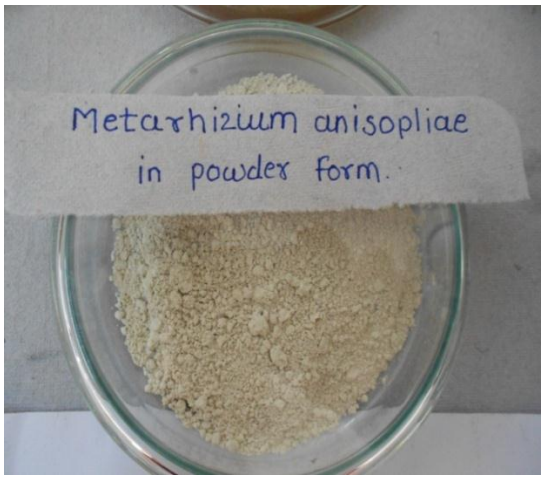
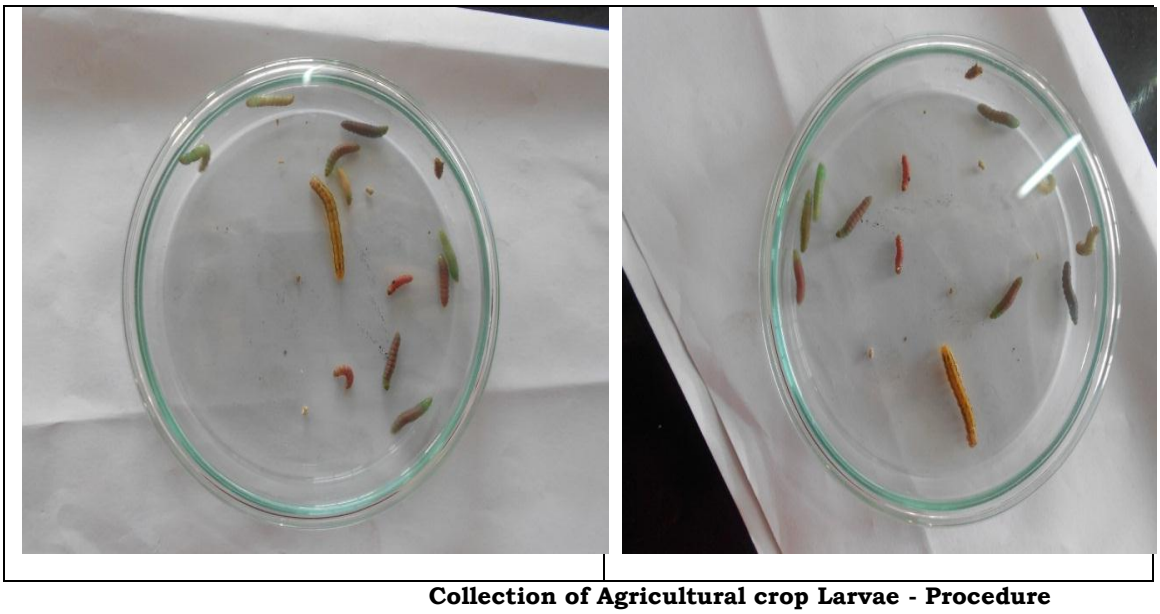


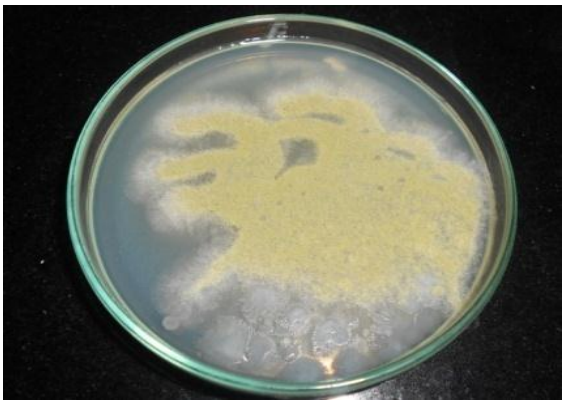
M. anisopliae in powder form

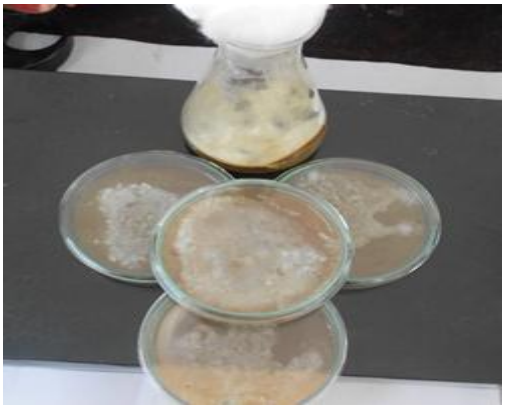
Collection of Agricultural crop Larvae



Collection of Agricultural crop Larvae - Procedure

1.





Culture of M. anisopliae Maintenance of entomopathogenic fungi

Sr.	Name of Media	Contents	
1	Potato Dextrose Agar (PDA)	Peeled potato	250g
		Dextrose	20g
		Agar-agar	20g
2	Peptone Yeast Glucose Agar (PYGA)	Glucose	10g
		Yeast Extract	5g
		Peptone	5g
		Agar-agar	15g

Note: All above contents are from 1 liter of media , all the media antibiotic Streptomycin

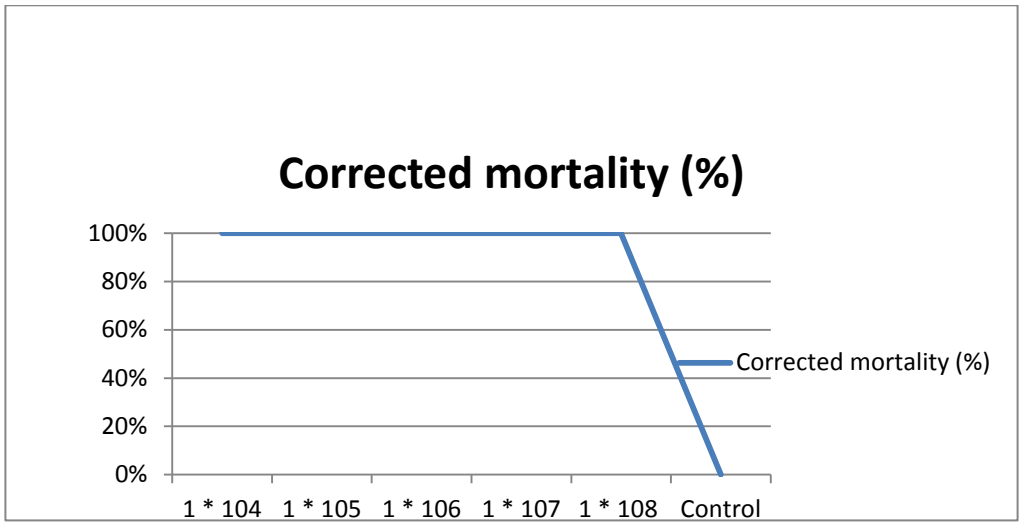
Table1. Isolation of entomopathogenic fungi obtained from soil Pune, **India**

Isolate	Method of isolation	Location
M. a. 3	Galleria bait method	Ramkrishn More college Akurdi., Pune
M. a. 4	Soil plating method	Agricultural College. Pune

Screening of various fungal isolates against Agricultural Pests

Table no.2. Germination percentage of *Metarhizium anisopliae* on different media

Media	Spore germination	Time required for germination (hrs.)
PDA	**90.00 (71.56)**	**18**
PYGA	**89.40 (71.00)**	**18**



Graph:1. Efficacy of *Metarhizium anisopliae* against the agricultural crop pest