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Isolation and Identification of Entomopathogenic Fungi, *Metarhiziumanisopliae*

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Abstract: Armyworm is a common spring pest that occasionally causes catastrophic losses in small grains. *Metarhiziumanisopliae*is one of several natural agents for controlling a broad range of insects by direct penetration of the host cuticle. In this study, the indigenous strain of *M. anisopliae*was isolated and identified.
Keywords: Army worm, dead insect, entomopathogenic fungi, *Metarhiziumanisopliae*

1. Introduction

Agriculture is one of the building stones of Myanmar. Fertilizers such as chemical, natural and biofertilizer, etc have been used for plant growing but crops are host to several insect pests. Insects have proven to be among the most formidable adversaries of mankind. One such estimate claims a total of 35% of reproduction less with a break up of 14% by insects and pests, 11% by diseases and 10% by weeds. While pests and diseases directly invade plants, weeds compete with them for space, water and nutrition. Generally, these pests have been fought by synthetic agro-chemicals [1].

Concerns over environmental issues related to chemical pesticide use such as; impact on humans, livestock and wildlife including natural enemies, water contamination and pesticide disposal issues have led to changes in chemical strategies and development of Integrated Pest Management (IPM) programs [2]. Biological control with pathogens may play an important role in this integrated pest management strategy. Biological pest control agents include viruses, bacteria, fungi, and nematodes. The use of microorganisms as selective pesticides has had some notable successes.

Entomopathogenic fungi were among the first organisms to be used for the biological control of pests. More than 700 species of fungi from around 90 genera are pathogenic to insects. Most are found within the deuteromycetes and entomophthorales. Some insect-pathogenic fungi have restricted host ranges, for example, *Aschersoniaaleyrodis*infects only scale insects and whiteflies, while other fungal species have a wide host range, with individual isolates being more specific, for example, *Metarhiziumanisopliae*and *Beauveriabassiana*.

Fungal species such as *M. anisopliae*and *B. bassiana*are well characterized in respect to pathogenicity to several insects and they have been used as agents for the biological control of agriculture pests worldwide. In Colombia, about 11 companies offer at least 16 products based on the entomopathogenic fungi *B. bassiana*. These products are used not only in the coffee crop but also in other crops such as cabbage, corn, bean, tomato, potato. They are also used to treat public disease vectors (e.g, flies and mosquitoes) [3]. The aim of this research is isolate and identifyentomopathogenic fungi, *Metarhiziumanisopliae.*

2. Materials and Methods

2.1 Sample Collection for *Metarhiziumanisopliae*

The indigenous strain of *M. anisopliae* was isolated from the dead army worm.

2.2 Sample Collection for Insects

Armyworm (*Spodopteralitura*) larvae were collected from cabbage plantation at Kyaukse Township, Mandalay division, Myanmar.

2.3 Isolation of *Metarhiziumanisopliae*

The dead cabbage looper was soaked with 1% normal saline in a test tube for 10 minutes and then crushed by a glass rod. After complete crushing, the sample was centrifuged down at 7000 r.p.m for 10 minutes, supernatant decant, pellet was immediately streaked onto the surface of Potato Dextrose (PDA) agar in petridish and incubated at 30±2 ^{o}Cfor one week.The individual pure colony on the media was transferred to medium slants in test tube by streaking. Isolated cultures were frequently transferred from the agar slants to the same kind of another set of agar slants in order to obtain pure culture.

2.4 D irect Microscopic Examination of *M. anisopliae*

*M.anisopliae*was grown on PDA agar by slide-culture method. The morphology of spore and mycelium were examined under binocular microscope with 400 magnification.

2.5 Investigation of Growth Condition

*M. anisopliae*were grown on nutrient agar (NA), sabouraud dextrose agar (SDA), czapek-dox agar (CDA), yeast peptone dextrose agar (YPDA) and potato dextrose agar (PDA). The cultures were grown for fivedays at 30±2 ^{o}C. The colony diameters were measured following the one-week incubation period in order to quantify growth.

2.6 Mass Production of Fungal Spores

The spores of *M. anisopliae*have been successfully produced using solid state fermentation on rice. The production process involves two fermentations- propagation of the fungal mycelia in liquid medium and sporulation on solid substrate. The rice (300 g) was placed in each autocalvable plastic bag, sterilized, inoculated with the fungal mycelia then incubated at 30±2 ^{o}C until maturity. Separation of spores from the rice was by washing in water with 0.0002% Tween 80 to first form a spore solution. The solution was then filtrated and dried at low temperature (10^{o}C - 15^{o}C) for several hours.

3. Results and Discussion

3.1 Growth Condition of*M. anisopliae*

M. anisopliae was isolated from the dead insect, army worm andgrown on various culture media and presented in Table 1. In the case of growth study, the growth condition of *M. anisopliae*was determined by growing on various media such as NA, SDA, CDA,YPDA and PDAwere used. As a result this study,sabouraud dextrose agar (SDA) was the best one for growth.

Table 1. Culture Diameters of*M. anisopliae*

Media	Diameter (cm)
NA (Nutrient Agar)	4.7
SDA (Sabouraud dextrose Agar) agar	6.3
CDA (Czapek-dox agar)	4.1
YPDA (Yeast peptone dextrose agar)	5.3
PDA (Potato dextrose agar)	5.1

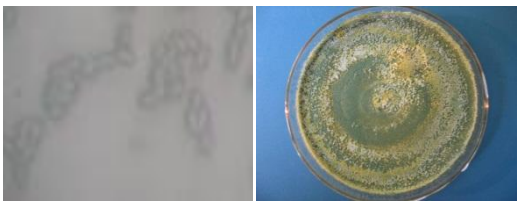

Figure 1. Colonial and microscopic morphology of *M. anisopliae*

3.2 Mass Production of Spore on Rice

The spores of *M. anisopliae*strains have been produced using solid state fermentation on rice. The yields of spores after 2 weeks and 4 weeks were shown in Table 2.

Table. 2 The Yield of Spore of *Metarhiziumanisopliae*Strain Aftar One Month and Two Month

Metarhiziumanisopliae	Yield
Two weeks	8.4 g
Four weeks	10.8 g

Nowadays, biopesticides are more interested than natural and synthetic agrochemical pesticides in plant protection from insect damage, disease and weed because they attack and fight only the unbeneficial target insects. Two advantages, cost effective and harmless to animals made the biopesticides more attractive.In the present work, indigenous strain of *Metarhiziumanisopliae*was isolated from dead insect, army worm using PDA

media. Only one strain was obtained and then characterized by standard microbial methods especially morphological (cultural, colonial and microscopic) investigations.

Conclusion

Myanmar is the agricultural country and biopesticides are necessary in agriculture. There are two different pathways in considering biopesticide approach: antagonistic pathway and entamopathogenic pathway. In this research work, the main concern is to isolate and identify theentamopathogenic fungus *Metarhiziumanisopliae.*. This strainwas grown on various culture media. Among them, it was found that SDA was the best one.

The information from this research work will fulfill one of the needs for biopesticide research implementing at the Department of Biotechnology underTechnological University (Kyaukse).Kyaukse Township, Mandalay Division, Myanmar.

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