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UTILIZATION OF ENTOMOPATHOGENIC FUNGI AS BIOLOGICALCONTROL AGENT AGAINST MAJOR INSECT PESTS OF MANGO

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ABSTRACT

Survey and collection of entomopathogenic fungi infecting important pests of mango were done and incidence of infection was recorded. Isolates were also collected from different sources such as *Sporothrix insectorum* and *Aphanocladium album* from Insect Pathology Resource Center, Boyce Thompson Institute, New York (ARSEF). *Metarhizium anisopliae*(ARSEF, Leyte State University and RCPC, Cebu). *Beauveria bassiana* and *Verticillium lecanii* (ARSEF and Leyte).

The potential of the fungal isolates for maximum growth and conidial production was studied using locally available substrates in combination with water and coconut milk. Conidia production of *Metarhizium anisopliae* (Cebu and Leyte) did not vary significantly for most types of substrates used except on palay plus coconut milk. However, *M. anisopliae* (ARSEF) produced the significantly highest spores on Rice broken + water. For *B. bassiana* (Leyte), Rice broken + water supported the highest conidial production of 7.78/gram while, for *Bb*(ARSEF) comparable results were obtained on Corn grits + water, Rice broken + water, Rice whole + water and Palay + water. Spore production for *Verticillium lecanii* grown on both types of rice and corn grits with addition of water did not vary significantly. On the other hand, no sporulation was observed on either types of corn substrate combined with coconut milk. For *Aphanocladium album* (ARSEF), Rice whole + water supported the highest conidial production. Satisfactory yield of conidia/ gram was achieved after 14 days of incubation. Rice with water gave the best results for conidial production and corn can be a good substitute for some isolates.

The efficacy of different entomopathogenic fungi like *Metarhizium anisopliae, Beauveria bassiana, Verticillium lecanii* and *Aphanocladium album* as biological control agent against mirid bugs *(Helopeltis collaris.)*; mango leafhoppers *(Idioscopus clypealis)* and fruit flies *(Bactrocera philippinensis* and *Bactrocera occipitalis*) were evaluated under laboratory and field condition.

B. bassiana at 1x108 and 1x109 conidia/ml caused 100% mortality on mirid bug at 2-4 days after treatment.

Mortality and mycosis of fruit flies differed significantly based on conidial concentrations of the four fungi. *B. bassiana* and *M. anisopliae* at 1x108 and 1x109 conidia/ml caused the highest mycosis of 82 – 93% on *B. philippinensis* and 88 – 92% on *B. occipitalis*. *V. lecanii* at 1x109 conidia/ml caused 57 and 68% mycosis on *B. philippinensis* and *B. occipitalis*, respectively. The lowest mycosis of 35% on *B. philippinensis* and 40% on *B. occipitalis* was caused by *A. album* at 1x109 conidia/ml.

The LT50 occurred at 3.29 days at 1x109 conidia/ml on *Beauveria bassiana* for *Bactrocera philippinensis* and 3.48 for *Bactrocera occipitalis.*

Field trial on mango leafhoppers treated with *Beauveria bassiana* and *Metarhizium anisopliae* 16 days after treatment showed a cumulative mortality of 56.50 and 42.83 percent, respectively. Mycosis on insects treated with *Beauveria bassiana* ranged from 0.83 to 7.67% while no mycosis was observed on *Metarhizium anisopliae* treatments. It was observed that at high insect population, the application of entomopathogenic fungi alone did not give good control, such that combination of other control strategies should be applied for effective control.

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INTRODUCTION

'Carabao' mango ranks third next to banana and pineapple as the most important fruit crop in the Philippines. The total land area devoted to this crop has reached 184,174 hectares in 2007, producing 1,023,906.89 mt fruits. The mango industry supports the country's economy in terms of local fruit production. (P 41.6 B), export (31 M U$ fresh and 29.7 M U$ processed) and contribution to GVA in agriculture (P 15 B). In addition, about 2.5 million farmers are directly or indirectly dependent on the industry.

Fully aware of the significant impact to our economic, research and extension activities are geared toward increase production of better quality fruits. Year round production of mango through flower induction technology and improved cultural management are some important research breakthroughs.

However, despite advancement in these technologies, the average yield of mango per hectare is relatively low (7.2 mt) compared to other mango growing countries. In addition, the percent rejects for exportable fruits is high. The main cause of low yield and poor quality fruits is attributed to high incidence of insect pests and diseases. If unchecked, the presence of insects alone can result to complete crop failure.

The control of important insect pests of mango still relies on the use of insecticides. With high incidence and severity of damage, growers are compelled to increase frequency and dosage of applications, making production less profitable. An average of 6 to 8 sprayings is common, increasing the cost of production from 60 to 80 percent. Moreover, because of the indiscriminate use of insecticides, insect resistance developed, secondary pest emerged and environmental contamination becomes a big problem.

Fruit flies, *Bactrocera philippinensis* and *Bactrocera occipitalis* are the two species known to attack mango in the Philippines. The adult fly is about the size of a common house fly. It has a light brown body with yellow stripes on the thorax. The wings are transparent with narrow black bands along the margin. The destructive pest deposits its eggs on the mango fruit. During egg laying, the female fruit fly damages the fruit surface by producing ovipositional punctures, the larvae feed on the tissues, causing fruit drop. Mango leafhopper *(Idioscopus clypealis)* is the dominant species that attacks mango flowers. Adult leafhoppers are wedge-shape, light green to brownish green and measure 3.5 to 4.0 mm. They spend most of their time under the leaves and later go to flowers as young emerge. The female mango leafhopper lays 100-200 eggs, which are deposited on the buds or the main stalk of the panicle. They damage the flowers by piercing their proboscis or (mouthparts) in the tissues and sucking the plant sap. The insect excretes sticky and sweet fluid, which is an excellent medium for the development of the fungus, sooty mold. Under high insect population, the whole canopy is covered with sooty mold wherein leaves and flowers blacken.

One management tactics, which can help address these problems, is Integrated Pest Management. IPM involves the combination of proper cultural practices to improve tree vigor, use of resistant varieties and biological control to regulate pest populations. Pesticide is an important component of IPM and should be used only when necessary.

In the actual implementation of mango IPM, only cultural management and pesticide usage are given emphasis. Use of resistant varieties in mango is still limited, while the role of natural enemies is not fully understood. Hence, attempts to conserve and augment their populations as effective biological control agents are not fully realized.

Entomophagous fungi regulate insect pest population in the field. They are abundant in the mango-agro ecosystem due to favorable microclimate, which sustain development and spread. They produce natural metabolites that are toxic to insects and prove to be useful as insect control agents. Basic information on the nature and incidence of infection, identity of the species and factors related to their growth/spread are vital information needed in the mass production of species/strains. Hence, the study on the bioefficacy tests to determine virulent species/strains against important insect pests was conducted.

OBJECTIVES

General :

To utilize promising Entomopathogenic fungi as biological control agent against major insect pests of mango.

Specific :

1. To survey and collect, isolate (pure culture) and identify entomopathogenic fungi existing in mango agro-ecosystem (Guimaras, Iloilo, Negros Occidental).

2. To rear and mass produce important pests of mango as test insects for the fungus. This will include mango leafhoppers, helopeltis and fruit flies.

3. To mass produce the fungus using locally available materials.

4. To conduct bioefficacy tests involving promising entomopathogenic fungi against major insect pests of mango.

5. To test promising isolates in the field.

REVIEW OF LITERATURE

The term entomogenous fungus was originally intended to designate pathogens of insects (Steinhaus, 1949 and Ferron, 1978). Today, this term is expanded to include fungal association with other terrestrial anthropods namely : mites, spiders, centipedes and millipedes. Humber (1996) equated this term to fungal entomopathogens or entomopathogenic fungi. The later, is commonly used in many publications.

Roberts and Humber (1981) classified entomopathogenic fungi into the following categories: a) pathogens, if they cause early death of the host by depriving nutrients or releasing toxins (*Beauveria, Metarhizium and Entomophtora*); b) parasites, if they impair host activities and cause severe debilitation but do not cause early death *(Laboulbeniales)*; c) facultative pathogens, if they attack old weakened or wounded hosts *(Conidiobolus)*; and d) wound pathogens, if incapable of penetrating the cuticle but enter the insects body through wounds/abrasion.

According to Rombach and Roberts (1989) entomopathogenic fungi comprise a heterogenous group with over 100 genera. They are present in all major groups of fungi namely: Ascomycotina, Basidiomycotina, Zygomycotina and Deuteromycotina. However, the more important genera are: *Entomopthora, Aphanocladium, Beauveria, Hirsutella, Metarhizium, Paecilomyces, Sporothrix* and *Verticillium*. These fungi are found to regulate pest population and have been mass produced as biological control agents.

Metarhizium anisopliae is frequently reported for the control of stored-grain insects although it has been used to actively control other insect species infestations (Batta **et al.,** 2003). Mixtures of *Metarhizium anisopliae* conidial suspensions with that of Beauveria bassiana has been used against *S. oryzae* on wheat grains (Dal-Bello **et al.,** 2001). Beauveria bassiana as well as *Metarhizium anisopliae* generally have a wide host range but other isolates of these species may be host specific (Leland, 2001). These isolates are known for its ability to kill a wide spectrum of insects, including insects in at least seven orders (Roberts and Leger, 2004). The effectiveness of Verticillium lecanii on scale insect *(Coccus viridis)* was demonstrated in India by Easwaramoorthy & Jayaraj (1978) and used to control whitefly in cucumbers (Hall, 1982). The fungus is a well known cosmopolitan species and restricted to insect hosts.

Lacey and Brooks (1994) described the infection process to start with the entry of the propagules into the host's cuticle. Once inside, the fungus multiply and invade the hemocoel. In many cases toxin is released. Hence, the fungus kills the host soon after infection. Following death, infectious spores are produced outside the body. Insect cadavers are mummified (mycosis), allowing gross pathology and fungal identity.

Reports of entomopathogenic fungi attacking mango pests are limited. However, their occurrence has regulated pest populations below economic threshold level. In India

(Kuman **et al.,** 1983) reported large number of mango hoppers (*Amritodus atkinsoni* and *Idioscopus clypealis*) infected with *Isaria tax* and *Verticillium lecanii*. Similarly, two entomopathogenic fungi, *V. lecanii* and *B. bassiana* were also observed infecting mango hoppers in Uttar Pradesh, India (Srivastara and Tandon, 1986). In the Philippines, *Hirsutella versicolor* was found to infect the mango leafhopper, *Idioscopus clypealis*. Infected insects were found underneath the leaves, mummified by the fungus. Golez (1998) reported the difficulty of growing Hirsutella on *B. bassiana* was found infecting *I. clypealis* and *I. nitidulus* (Tripathi **et. al.,** 1990). This fungus was the first species isolated from hoppers in India.

For other mango pests, *Aspergillus* sp. was found to infect the immature stages of the mango seed weevil, *Sternochetus gravis* (De and Pande, 1988). While Srivastava and Fasih (1988) observed infestation on mango mealybug by the fungus *B. bassiana*. On the other hand, (Sheng **et al.,** 1998) reported that larvae of the long horned beetle (*Rhytidodera sp.*) succumbed to attack a new strain of *B. bassiana*.

Advancement in handling diseased insects, identification, preservation and culture has led to discovery of new species and strains of entomopathogenic fungi. However, their potential roles as biological control agents have yet to be assessed through laboratory/field bioassays. Paparok and Hayek (1994) reported two methods of exposing healthy insects to pathogenic fungi. The first approach is to use conidial inoculum, whereby insects are showered with conidia or insects are brought on a surface covered with conidia. In such case, insects are placed in suspension with known concentration of conidia. The second method is injecting the fungal materials into the insect's body.

Barson **et al.,**(1994) evaluated six species of entomopathogenic fungi for the control of housefly by immersing the third instar larvae in conidial suspension of different concentrations. In the case of adult flies, these were immobilized first and 1.0 ml of the spore suspension was applied on the ventral surface of the abdomen using a micropipette. The effectiveness of *B. bassiana* against sand flies was also determined by spraying coffee plants with the fungus and releasing the fly thereafter (Reithinger **et al.,**1997). In addition, flies maybe allowed to hover on filter paper containing the fungus. For stink bug, laboratory bioassay involved dusting the surface of plastic boxes with *Metarhizium* and *Beauveria*, allowing the insects to walk on them for 10 seconds (Gomez and Moscardi, 1998). The potential of *Verticillium* against thrips was evaluated but immersing tobacco leaf (10mm diameter) inside petridish containing various spores suspension for 10 seconds.

Goettel and Inglis (1994) reported that although bioassay can provide valuable information on pathogen-insect-environment interaction, the value of results depend on the design, execution, analysis and interpretation.

The interest in the use of entomopathogenic fungi as compliment for chemical control has greatly increase in recent years. Several species, particularly members of Deuteromycetes have shown considerable promise and yielded to laboratory manipulation and are suitable for mass production.

Barnes **et al.,**(1975) enhanced the development of *M. anisopliae* and *B. bassiana* by growing them in liquid media containing peptone of various sources. Try tone, capstone and yeast extract were effective for mycelial growth while yeast extract was most effective in production of spores. A simple liquid medium containing 2.5% glucose, 2.5% starch, 2% corn strip, 0.5 % Nail and 0.2% CaCO3 in 500 ml water improved spore production of *B. bassiana* (Samsinakova and Kalalova, 1981). The fungus *B. bassiana* and *Paecillomyces fumosoroseus* were successfully mass produced using rice (loose solid media) and coconut water liquid media (Ibrahim. Cow, 1993). Spore production was much higher in rice compared to other materials and similar result was obtained with coconut water. Sharma **et al.,** (1999) mass produced *M. anisopliae, B. bassiana* and *B. brongniastii* using molasses yeast broth. These fungi produced 8 x 108. 1 x 109 and 2 x 109 conidia per ml. Among the grain media used. crushed maize was effective in the production of *M. anisopliae* while cowpea for Beauveria sp.

Rice substrates are also used for production of *M. anisopliae* and *P. fumosoroseus* in the West Indies for management of froghoppers. *Bemisia tabaci* and, potentially, Thrips palmi. on sugarcane and vegetable crops (Hall **et al.,** 1994). Rice is also the favored medium for production of *M. anisopliae* and *B. bassiana* in Brazil (Moscardi, 1989).

METHODOLOGY

1. Survey and collection of entomopathogenic fungi

Random samples of mango trees grown in the island of Guimaras (5 municipalities). Iloilo (5 municipalities) and Negros Occ. (3 municipalities) representing Western Visayas were surveyed for the presence of fungal pathogens attacking major insect pests of mango. These included mango leafhoppers. fruit fly and helopeltis bug.

Monthly survey involved examination of 50 trees from each location. to include 25 backyard and 25 orchard trees. In cases. where orchard trees are abundant. the 25-tree samples were increased to 50. Each tree was divided into 4 quadrants and from each quadrant. 25 leaves/flowers/fruits were randomly examined for the presence of insect cadaver. Hence. in one tree. 100 leaves/flowers at random were examined. For pests that spent part of their life cycle in the soil, sample consisting of 10 cm3 soil was collected underneath the tree canopy. Ten samples were taken at random from an orchard and another 10 from backyard. The latter. were sieved and infected insects were collected and preserved.

Handling and sterilization

Infected insects were removed from the substrate and placed individually in small, clean box made of bond paper and lined with tissue paper. Specimens were kept dry and refrigerated to slowdown the growth of saprophytic microorganisms.

For surface sterilization, infected insects were placed in 70% alcohol for 2 seconds, rinsed in distilled water and placed in petri dish containing 1% sodium hypochlorite solution. Sterilized insects were rinsed with distilled water (2x) and blot dried.

2. Isolation of fungal pathogen

Two methods were employed. The first was the descending conidia procedure. Insects showing mycelia and spores on their bodies were placed on a wet sterile filter paper and fastened to the lid of a petri dish. This represented the cover and spores from the insect's body were collected at the bottom of the dish. With a sterile wire loop, spores were transferred and streaked on a suitable solid media (PDA + YE). The second procedure involved isolation of the fungus from the insect. This was intended for dead insects without signs of the fungal propagules. After sterilization, the insect's body was dissected and aseptically transferred to a solid medium until the fungus grew and sporulated.

Reisolation of the fungus from the original medium was done to obtain pure culture. This was done by picking uncontaminated colonies and streaking them over a freshly prepared medium. Pure cultures were served as fungal stocks for succeeding experiments.

Fungal identification

Infected insects were dissected under a microscope and mounted on a glass slide. Staining was done to determine the important parts of the fungus. Using taxonomic

key with pictorial illustrations, the fungus was identified to generic level. Specimens were also sent to Dr. Lina Villacarlos at Leyte State University for assistance in species identification. In addition, pure culture was also sent to USDA-ARS (Collection of Entomopathogenic Fungal Cultures) at Cornel University, Ithaca, New York, USA for identification and confirmation.

3. Mass production of the fungus.

Mass production of promising fungal isolates (Ma, Bb, Vl, Aa) were done utilizing locally available materials such as palay (unpolished rice) , white rice (whole and broken), yellow corn (cracked and grits) and sorghum. Fifty grams of each material was placed inside transparent plastic polyethylene bag (5 x 9 mm). To obtain a solid-loose medium for fungal development, the following materials were added: a) distilled water (50 ml) and b) coconut milk (50 ml). After the liquid have been incorporated in the medium, these were mixed thoroughly and sterilized at 15 psi for 20 to 30 minutes and allowed to cool.

One ml spore suspension of the isolate (1 x 108 spores/ml) was injected inside each bag using a hypodermic syringe. The spore suspension was mixed with the loose medium and incubated for 14 days in a dark room at 28oC. After which, these were harvested and examined.

The effectiveness of the medium to support growth and sporulation of the fungus was evaluated in terms of the number of spores produced per gram of the medium and percent germination. Appropriate medium/media was used for the mass production of the fungal pathogens.

Six trials were conducted to produce reliable results.

4. Bioefficacy test of different entomopathogenic fungi

Laboratory test

The efficacy of entomopathogenic fungi as biological control agent was evaluated using the following procedures:

a. General test. Fungal isolates from different sources were grown as stock cultures and initiated on potato dextrose agar supplemented with 1.0% yeast extract. To enhance sporulation, these were kept in dark room at 28oC for 14 days. Conidia formed was collected on the surface of the medium and placed in test tubes containing 5 ml sterile water with 0.1 % Tween 80. The suspension was shaken vigorously and the number of conidia was estimated using a hemacytometer. Using the dip-suspension method, the nymphs and adults of the test insects (hopper, fruit fly and helopeltis) were placed inside steriled gauze and dipped in the spore concentration of 1 x 108 spores/ml for 10 seconds. After the treatment, the insects were placed in plastic cups lined with moist filter paper and provided with food and water. An alternate procedure was also used where insect was sprayed with known concentration of the fungus using an automized spray or allowed to walk on a treated surface containing 1 ml of the spore suspension.

The different fungal isolates were initially screened for their efficacy using the standard concentration of 108 conidia per ml. Daily observation was taken to determine the LT 50 or lethal time.

b. Specific test. *Metarhizium anisopliae* (Cebu), *Beauveria bassiana* (Leyte), *Verticillium lecanii* (Leyte) and *Aphanocladium album* (ARSEF) at concentrations of 1x106, 1x107, 1x108 and 1x109 conidia ml-1 were tested for 2 fruitfly species, *Bactrocera philippinensis* & *B. occipitalis*. Tween 80 (0.1%) was used for the control the test insects were inoculated either by dipping or exposing in filter papers dipped in fungal suspensions. After treatment, the insects were held in plastic cups and provided with food and water. Inoculated insects were held under room temperature and checked daily for mortality. Dead insects were observed for development of the fungus to confirm mycosis. The efficacy was calculated based on percentage cumulative mortality (Abbott's) caused by mycosis.

The quality of the fungal isolate was evaluated in terms of percent germination determined from 300 randomly selected spores. This experiment was replicated 3 times using an appropriate design recommended by statistician

Field efficacy

Promising fungal isolates was prepared at concentration of 1 x 1012 and tested against mango leafhoppers under field condition. Nine (9) mango experimental trees including control with three (3) replications were induced to flower using KNO3. After ten (10) days, cloth bags measuring 16" x 24" dimension were set up on trees, five (5) bags per tree. Freshly collected insects were placed inside cloth bags (40 test insects per bag). Spraying of fungal suspension on insects, was done at three days interval using hand sprayer at 1 Liter per tree.

Preparation of fungal suspension was done by extracting the spores produced in 50g rice medium suspended to 100 ml water. The liquid was filtered using a muslin cloth to sieve large particles that cause blockages on sprayer. Counting of spores on the extract liquid was done and the volume was then adjusted to a concentration of 1 x 1012 spores/ml. Germination test on spores was done before the field application in order to check the viability of the isolates.

The efficacy was evaluated based on the number of dead insects. Mortality was collected two (2) days after spraying of fungal solution. Dead insects were surface sterilized with 5% sodium hypochlorite (NaOCl) solution, rinsed twice with sterile distilled water and blot dried on moistened sterile filter paper inside petri plates to enhance the development of mycosis. Observation of mycosis was done daily.

5. Statistical analysis

Data obtained from experiments was subjected to analysis of variance for a randomized complete block design using Statistical Analysis System (SAS). Differences between treatment means were determined using Duncan's Multiple Range Test (DMRT) at 5% level of significance when a significant F value ($P<0.05$) was obtained. Mortality data was computed based on Abbott formula (Abbott, 1995). LT50 (lethal time required to kill 50% of the treated insect population) was determined using Reed-Muench computation.

RESULTS AND DISCUSSION

1. Survey and collection of entomopathogenic fungi

Survey and collection of entomopathogenic fungi were conducted in mango growing areas of Guimaras, Iloilo and Negros Occidental. From each site, 25 trees were surveyed and 100 leaves per tree were examined for the presence of the entomopathogenic fungi. At present 2 species were recovered, Hirsutella versicolor from mango leafhoppers and Verticillium sp. from June beetle. In Guimaras, Hirsutella infection on mango leafhoppers range from 0.12 to 1.80%. In Iloilo, the incidence range from 0.08 to 24% while in Negros Occidental, the ranged was 0.16 to 0.48%. The presence of infected insects in the field was higher during rainy months (October & November) and none during the dry season.

Isolates of other entomopathogenic fungi were obtained from different sources. These included Sporothrix insectorum and Aphanocladium album from Insect Pathology Resource Center, Boyce Thompson Institute, New York (ARSEF). *Metarhizium anisopliae*(ARSEF, Leyte State University and RCPC, Cebu), *Beauveria bassiana* and *Verticillium lecanii* (ARSEF and Leyte).

2. Isolation of the fungus

Isolation of the fungus following the proper protocol in sterile conditions was employed. Culture media such as potato dextrose agar + yeast extract (PDA + YE), sabourauds agar (SA) and nutrient agar (NA) were used for isolation of the fungi. Verticillium grew abundantly on PDA + YE compared to SA and NA media. On the other hand, *Hirsutella versicolor* was slow grower and no synemata was produced. Further isolations failed to produce a pure culture of the fungus because of the presence of mycoparasite, Calcarisporium sp.

Re-isolation of fungal isolates from other sources was done on several culture media. *Metarhizium* and *Verticillium* isolated were grown using SA slants. The rest of the isolates were grown in test tube slants using PDA + YE and NA. Isolates grown in PDA + YE showed better growth and development compared to SA and NA medium.

To enhance the virulence of the fungus under pure culture, fungal transmission test using adult fruit fly and mango leafhoppers were done for the isolates *Metarhizium anisopliae*, *Verticillium lecanii*, *Beauveria bassiana* and *Aphanocladium album*.

Fig. 1. Entomopathogenic fungal isolates grown on culture medium.

3. Mass production of fungus using different indigenous materials

The fungal isolates used were: *Metarhizium anisoplae* (ARSEF, Leyte and Cebu isolates), *Beauveria bassiana* (ARSEF and Leyte isolates), *Verticillium lecanii* (Leyte isolate) and *Aphanocladium album* (ARSEF).

The conidial yield of the fungal isolates tested varied significantly with the growth of substrates used. Table 1 showed that fungal isolates grown on selected grain substrates in plastic bags obtained high yield of x109 spore/gram substrate. Conidia production of *Metarhizium anisopliae* (Cebu and Leyte) did not vary significantly for all types of substrates used except on palay plus coconut milk. However, for Ma (ARSEF), RbW significantly gave the highest yield. For *Beauveria bassiana* (Leyte),RbW supported the highest conidial production of 7.78/gram substrate while, for Bb(ARSEF) comparable results was obtained on CgW, RbW, RwW and PW. Spore production of *Verticillium lecanii* grown on both types of rice and corn grits with addition of water did not vary significantly, on the other hand, no growth was observed on either types of corn combined with coconut milk. For *Aphanocladium album* (ARSEF), RwW supported the highest conidial production. Satisfactory yield of conidia/gram was achieved after 14 days of incubation. Generally, rice with water gave the best results for conidial production, although, corn can be a good substitute for some isolates. Sorghum as one of the substrates tested was eliminated due to limited supply and cost.

Table 1. Conidial production of fungal isolates grown on different substrates in plastic (polypropylene) bags.

Substrate	Number of conidia ($x10^9$)/gm substrate Fungal Isolates Tested						
	Ma[c]	*Ma*[d]	*Ma*[b]	*Bb*[d]	*Bb*[b]	*Vl*[d]	*Aa*[b]
Rice whole + water (RwW)	1.904^{ab}	1.516^{abc}	1.214^{bc}	6.044^{b}	1.690^{abc}	4.913^{a}	8.533^{a}
Rice broken + water (RbW)	2.239^{a}	1.877^{ab}	1.879^{a}	7.768^{a}	2.140^{ab}	5.908^{a}	4.971^{b}
Corn cracked + water (CcW)	1.70^{ab}	1.238^{abc}	0.575^{cd}	4.377^{c}	1.108^{bc}	3.302^{b}	4.050^{b}
Corn grits + water (CgW)	1.956^{ab}	2.245^{a}	0.61^{cd}	4.094^{cd}	2.810^{a}	5.319^{a}	4.085^{b}
Palay + water (PW)	1.371^{ab}	0.802^{abc}	0.638^{cd}	2.592^{def}	1.712^{abc}	1.062^{cd}	4.644^{b}
Rice whole + coconut milk (RwCm)	2.215^{a}	0.915^{abc}	1.175^{bc}	2.869^{cdef}	1.325^{bc}	2.046^{bc}	3.402^{bc}
Rice broken + coconut milk (RbCm)	1.048^{ab}	1.281^{abc}	1.269^{b}	3.452^{cde}	1.052^{bc}	1.142^{cd}	3.625^{b}
Corn cracked + coconut milk (CcCm)	1.421^{ab}	0.571^{bc}	0.329^{cd}	2.096^{ef}	1.504^{bc}	0.0^{d}	2.160^{bc}
Corn grits + coconut milk (CgCm)	1.465^{ab}	0.885^{abc}	0.738^{cd}	2.435^{def}	0.602^{c}	0.0^{d}	4.650^{b}
Palay + coconut milk (PCm)	0.725^{b}	0.310^{c}	0.398^{d}	1.238^{f}	0.954^{bc}	0.381^{d}	0.829^{c}

Legend: Ma - Metarhizium anisopliae
Bb - Beauveria bassiana.
Vl - Verticillium lecanii
Aa - Aphanocladium album

Source of isolates:
b- ARSEF
c- Cebu
d- Leyte

Moisture is an important factor in the growth of the fungus. Results showed that addition of coconut milk enhanced good spore production comparable with water on Metarhizium anisopliae but not on other isolates. The use of plastic bags in mass production was efficient and economical and likewise, uniform distribution of the inoculum was achieved by crushing the bag to avoid aggregation of the substrates providing more surface area for the development of the fungus.

In this study, the use of rice, corn and palay has enhanced conidial germination on the viability of fungal conidia (E. Francisco **et al.** 2004). Conidia germination at 12 hrs after incubation was generally high, ranging from 85 to 99.44% among isolates and substrates used indicating a good germination (Table 2).

Table 2. Percent viability of fungal spores grown on different substrates in plastic (polypropylene) bags

Substrate	Fungal Isolates Tested						
	Ma[c]	*Ma*[d]	*Ma*[b]	*Bb*[d]	*Bb*[b]	*Vl*[d]	*Aa*[b]
Rice whole + water (RwW)	98.89	85.78	98.00	97.78	89.78	91.00	97.44
Rice broken + water (RbW)	98.78	97.00	97.89	99.22	88.33	92.00	96.67
Corn cracked + water (CcW)	99.44	95.89	99.11	98.33	86.00	93.00	91.67
Corn grits + water (CgW)	97.89	97.78	99.00	98.89	86.67	85.00	91.00
Palay + water (PW)	97.89	98.33	98.44	96.89	92.67	94.00	94.00
Rice whole + coconut milk (RwCm)	98.11	97.67	97.45	97.00	92.56	90.56	94.89
Rice broken + coconut milk (RbCm)	97.56	96.67	97.67	96.00	90.78	88.55	90.67
Corn cracked + coconut milk (CcCm)	97.89	95.67	98.44	97.67	95.11	..	93.11
Corn grits + coconut milk (CgCm)	98.11	96.44	98.33	99.22	89.89	..	97.11
Palay + coconut milk (PCm)	98.11	98.22	98.55	96.44	91.33	95.89	85.78

Legend: Ma - Metarhizium anisopliae
Bb - Beauveria bassiana.
Vl - Verticillium lecanii
Aa - Aphanocladium album

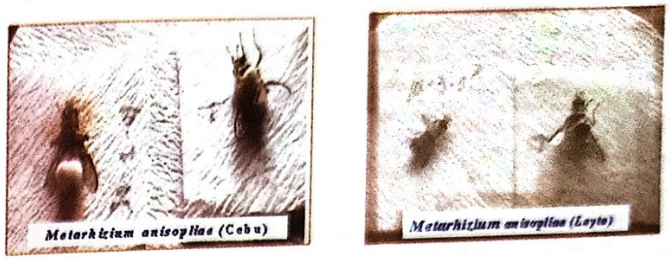
Source of isolates:
b- ARSEF
c- Cebu
d- Leyte

4. Bioefficacy test of different entomopathogenic fungi

Laboratory trials

a. General test - The mean fruitfly adult mortality observed ten days after treatment of Metarhizium anisopliae varied between the two isolates tested. For Ma Cebu, 100% mortality was observed at 5-7 days after inoculation by dipping while, on Ma Leyte at 5 - 9 days, with mean mortality of 75%. Mycosis was 100% for Ma Cebu and 83.33 % for Ma Leyte. Evidence of infection was observed after 2-3 days with profused sporulation of the fungus on the insect cadaver. The use of Ma Cebu for specific test on 2 species of fruit flies was therefore considered.

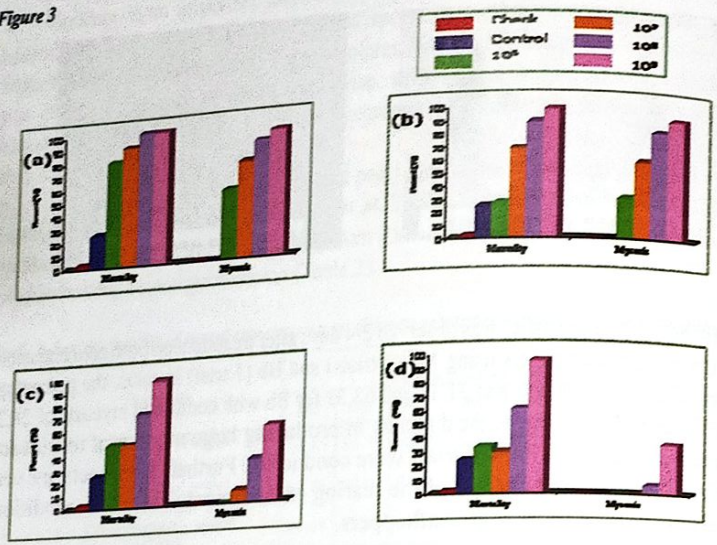
Fig.2. Fungal growth in infected cadaver of fruit flies



For mirid bug, 100% mortality was observed 2-4 days after treatment on both concentrations used. On mango leafhoppers using Ma (2 trials) and Bb (1 trial) isolates, the percentage cumulative mortality on Ma was 31.11 and 63.33 for Bb with confirmed mycosis of 28.25 and 8.0%, respectively. Due to the difficulty in producing large number of test insects to be used in the study, limited trials were conducted. Further, the insects are very sensitive to handle and change of the rearing environment. The same conditions were also observed for mango leafhoppers.

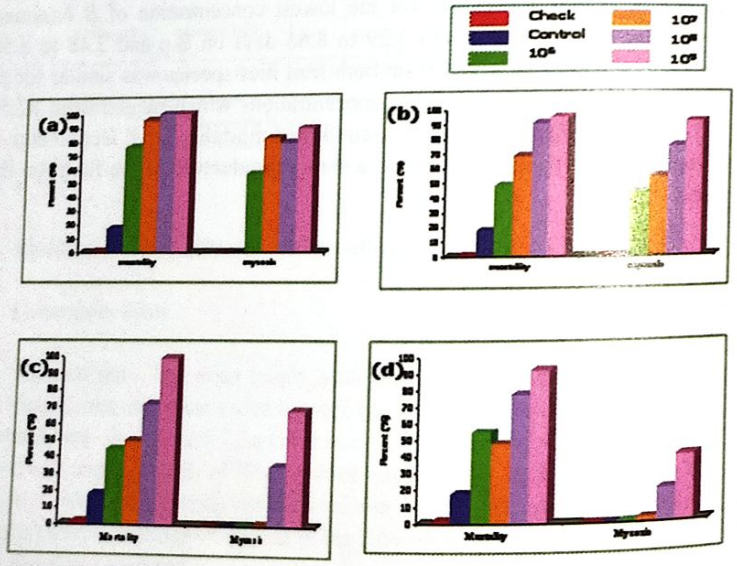
b. Specific test - The response of adult fruit flies, *Bactrocera philippinensis* and *B. occipitalis* varied among the four fungal isolates and concentrations used is presented in Figure 3. *B. philippinensis* responded similarly to different concentrations of *B. bassiana, M. anisopliae* at 1 x 109 and 1 x 108 conidia ml-1 and at highest concentrations of *V. leccanii* and *A. album*. The same result was obtained for *B. occipitalis* except for the lowest concentration of *B bassiana* (Figure 4). The LT50 ranged from 3.29 to 8.66 days on B.p and 3.48 to 8.58 days on B.o. Incidence of mycosis on both fruit flies species was similar for *B. bassiana* and *M. anisopliae* at higher concentrations which ranged from 82.50 to 93.33%. On the other hand, mycosis-confirmed mortality for *V. lecanii* and *A. album* was significantly low indicating a slow reproduction of the fungi on the test insects.

Figure 3



Legend. a- *Beauveria bassiana* c- *Verticillium lecanii*
b- *Metarhizium anisopliae* d- Aphanocladiumalbum

Figure 4



Legend : a- *Beauveria bassiana* c- *Verticillium lecanii*
b- *Metarhizium anisopliae* d- Aphanocladiumalbum

Field Trial

Field efficacy test of *Metarhizium anisopliae* (1 trial) and *Beauveria bassiana* (Leyte) (2 trials) at 1 x 1012 conidia ml-1 against mango leafhopper were conducted. Nine trees were induced to flower and in each tree five panicles were enclosed in cloth bags (Figure 5). Forty mango leafhoppers (adults and nymphs) were introduced in each bag. Fungal suspension was applied at 200 ml per panicle, done late in the afternoon at three days interval starting from 10 days after flower induction (DAFI). Treatment application was terminated at 26 DAFI. The cumulative mortality obtained 17 days after 1st treatment was 50.25% for Beauveria bassiana and 42.83% for *Metarhizium anisopliae*. Mycosis observed ranged from 0.83 to 7.67% of *Beauveria bassiana* treated insects with no mycosis on *Metarhizium anisopliae*.

The germination of spores on both isolates 12 hours field exposure was 77.22 for Ma and 76.44% for Bb.

The very windy and high temperature recorded (27-32.300C) during the conduct of the experiment could have affected the viability of the fungus that resulted to the low incidence of mycosis on dead insects collected. Furthermore, the application of the entomopathogenic fungi (EF) alone, did not give a good control for mango leafhoppers in the field. Surviving adults were able to reproduced resulting to higher nymphal population, which have damaged the flowers. It is therefore recommended that the use of EF should be combined with other strategies for effective control.

Fig. 5. Set-up for field efficacy trial of Beauveria bassiana and Metarhizium anisopliae against mango leafhoppers.

Setting of cloth bags with test insects

Treated mango trees panicles

Collection of dead test insects

CONCLUSION

The study revealed naturally occurring entomopathogenic fungi (*Hirsutella versicolor* & *Verticillium spp.*) were present in the field. However, *Hirsutella* was not tested because it is slow growing and does not produce conidia abundantly.

B. bassiana, M. anisopliae, V. lecanii and *A. album* are potential biocontrol agents for important mango pests. The four fungi were successfully mass produced on locally available substrates like rice in water or coconut milk. Corn is as good as rice as substrate for mass production.

Efficacy tests conducted under laboratory condition resulted in high mortality and mycosis of adult fruit flies treated with either *B. bassiana* and *M. anisopliae* at 1 x 109 and 1 x 108 conidia ml-1. However under field condition, efficacy of both isolates against mango leafhoppers required a higher conidial concentration of 1 x 1012 per ml. On the other hand, *M. anisopliae* failed to cause mycosis even at higher concentration of 1 x 1012 conidia ml-1.

At higher insect population, application of entomopathogenic fungi alone did not show good control.

RECOMMENDATIONS

Mass production of entomopathogenic fungi using rice in combination of water or coconut milk is recommended for maximum conidial production.

Laboratory efficacy of *B. bassiana* and *M. anisopliae* was proven effective against adult fruit flies at concentrations of 1x 108 and 1x109 conidia ml-1. However, more tests are needed on other major insect pests of mango.

Field efficacy of *B. bassiana* and *M. anisopliae* at 1 X 1012 conidia ml-1 against mango leafhoppers at lower insect population was effective. However, further field trial should be done at higher insect population in combination with other control strategies and factors affecting the virulence of the fungus should be considered. On the other hand, field efficacy of isolates against fruit flies and other major insects attacking mango should be done.

IMPACT OF THE STUDY

1. Able to identify cheap and locally available substrates for mass producing entomopathogenic fungi.

2. Able to find an alternative biological control for mango insect pests and other agricultural crops

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FINANCIAL REPORT

Breakdown	Budget Released	Total Obligation	Un obligated Balance
I. Personal Services	₱230,400.00	₱ 230,400.00	0.00
a. Research Assistant	158,400.00	158,400.00	0.00
b. Honoraria	72,000.00	72,000.00	0.00
II. MOOE	₱260,000.00	₱ 260,000.00	0.00
a. Travel	70,000.000	70,000.000	0.00
b. Supplies	120,000.00	120,000.00	0.00
c. Communication	20,000.000	20,000.000	0.00
d. Other Services	50,000.00	50,000.00	0.00
III. Administrative Cost	₱ 9,600.00	₱ 9,600.00	0.00
Total	**₱ 500,000.00**	**₱ 500,000.00**	**0.00**

APPENDIX

A. Analysis of variance and Duncan's multiple range test of fungal isolates; *Beauveria bassiana* (Bb), *Metarhizium anisopliae* (Ma), *Verticillium lecanii* (Vl) and *Aphanocladium album* (Aa) on mortality of fruit fly, *Bactrocera occipitalis* (Bo).

Mortality

Analysis of Variance

Sources of Variance	Degree of Freedom	Sum Square	Mean Square	F
Trt	15	19912.69	1327.513	23.55791
Block	2	235.4688	117.7344	2.089303
Error	30	1690.531	56.35104	

Coeff. of variation (%) = 9.881937

Duncan's Multiple Range Test

Isolate	Treatment concentration	Mean	Std. Deviation	Statistical significance
Bb	1×10^9	100	0	a
	1×10^8	100	0	a
	1×10^7	95.9	2.640304	a
	1×10^6	76.58667	6.106607	b
Ma	1×10^9	95.25666	4.447633	a
	1×10^8	91.06	5.711942	a
	1×10^7	68.74667	3.795738	b
	1×10^6	48.84334	13.93106	b
Vl	1×10^9	99.27334	1.258624	a
	1×10^8	71.33667	10.39811	b
	1×10^7	49.83333	3.083382	c
	1×10^6	45.10667	2.80402	c
Aa	1×10^9	92.59668	7.711564	a
	1×10^8	77.75666	9.716158	b
	1×10^7	47.93334	19.10886	c
	1×10^6	55.19667	3.429306	c

Means with the same letter are not significantly different at significance level of 5%

B. Analysis of variance and Duncan's multiple range test of fungal isolates; *Beauveria bassiana* (Bb), *Metarhizium anisopliae* (Ma), *Verticillium lecanii* (Vl) and *Aphanocladium album* (Aa) on mortality of fruit fly, *Bactrocera philippinensis* (Bp).

Mortality

Analysis of Variance

Sources of Variance	Degree of Freedom	Sum Square	Mean Square	F
Trt	15	32451.1	2163.406	17.22757
Block	2	29.53125	14.76563	.1175812
Error	30	3767.344	125.5781	

Coeff. of variation (%) = 15.87754

Duncan's Multiple Range Test

Isolate	Treatment concentration	Mean	Std. Deviation	Statistical significance
Bb	1×10^9	99.12333	1.51843	a
	1×10^8	99.07333	1.605033	a
	1×10^7	89.72332	6.222718	ab
	1×10^6	79.59001	13.18809	abc
Ma	1×10^9	98.88999	1.922577	a
	1×10^8	89.04667	7.205236	ab
	1×10^7	68.73001	12.72458	c
	1×10^6	27.93334	7.973207	e
Vl	1×10^9	96.79666	2.811234	a
	1×10^8	71.45333	12.24227	bc
	1×10^7	48.61333	1.325041	de
	1×10^6	40.98	14.57778	de
Aa	1×10^9	95.21999	5.714883	a
	1×10^8	60.55334	18.41211	cd
	1×10^7	29.44667	24.46164	e
	1×10^6	34.08667	7.596226	e

Means with the same letter are not significantly different at significance level of 5%

C. Analysis of variance and Duncan's multiple range test of fungal isolates; *Beauveria bassiana* (Bb), *Metarhizium anisopliae* (Ma), *Verticillium lecanii* (Vl) and *Aphanocladium album* (Aa) on mycosis of fruit fly, *Bactrocera occipitalis*

Mycosis

Analysis of Variance

Sources of Variance	Degree of Freedom	Sum Square	Mean Square	F
Trt	15	50149.48	3343	53.34452
Block	2	82.28906	41.14453	.656488
Error	30	1880.211	62.6737	

Coeff. of variation (%) = 17.15577

Duncan's Multiple Range Test

Isolate	Treatment concentration	Mean	Std. Deviation	Statistical significance
Bb	1×10^9	88.33334	8.779709	ab
	1×10^8	78.33334	8.779709	abc
	1×10^7	81.66666	1.443376	abc
	1×10^6	56.66667	2.886752	de
Ma	1×10^9	92.5	6.614378	A
	1×10^8	75	6.614378	bc
	1×10^7	54.16667	8.036375	e
	1×10^6	44.16667	14.43376	ef
Vl	1×10^9	68.33334	12.33221	cd
	1×10^8	35	12.99038	f
	1×10^7	.8333333	1.443376	h
	1×10^6	0	0	h
Aa	1×10^9	40	10.89725	f
	1×10^8	20	2.5	g
	1×10^7	2.5	2.5	h
	1×10^6	.8333333	1.443376	h

Means with the same letter are not significantly different at significance level of 5%

D. Analysis of variance and Duncan's multiple range test of fungal isolates; *Beauveria bassiana* (Bb), *Metarhizium anisopliae* (Ma), *Verticillium lecanii* (Vl) and *Aphanocladium album* (Aa) on mycosis of fruit fly, *Bactrocera philippinensis* (Bp).

Mycosis

Analysis of Variance

Sources of Variance	Degree of Freedom	Sum Square	Mean Square	F
Trt	15	55038.45	3669.23	55.6069
Block	2	4.289063	2.144531	3.250021
Error	30	1979.555	65.98516	

Coeff. of variation (%) = 18.59672

Duncan's Multiple Range Test

Isolate	Treatment concentration	Mean	Std. Deviation	Statistical significance
Bb	1×10^9	93.33334	5.204166	a
	1×10^8	86.66666	10.10363	a
	1×10^7	71.66666	14.64866	bc
	1×10^6	51.66667	11.547	d
Ma	1×10^9	91.66666	8.036375	a
	1×10^8	82.5	4.330127	ab
	1×10^7	59.16667	7.637625	cd
	1×10^6	31.66667	3.818813	e
Vl	1×10^9	57.5	6.614378	cd
	1×10^8	32.5	2.5	e
	1×10^7	9.166667	2.886751	f
	1×10^6	0	0	f
Aa	1×10^9	25.55334	17.08595	f
	1×10^8	5.833334	2.886751	f
	1×10^7	0	0	f
	1×10^6	0	0	f

Means with the same letter are not significantly different at significance level of 5%

E. Bioefficacy test of entomopathogenic fungi (laboratory condition).

Isolate	Concentration	Fruit fly					
		Bactrocera philippinensis			*Bactrocera occipitalis*		
		% mortality (Abbott's)	% mycosis	LT_{50}	% mortality (Abbott's)	% mycosis	LT_{50}
Beauveria bassiana	10^9	99.22^a	93.33^a	3.29	100.00^a	88.33^{ab}	3.48
	10^8	99.07^a	86.67^a	4.19	100.00^a	78.33^{abc}	4.26
	10^7	89.72^{ab}	71.67^{bc}	4.73	95.50^a	81.67^{abc}	4.31
	10^6	79.59^{abc}	51.67^d	5.26	76.59^b	56.67^{dc}	5.42
Metarrhizium anisopliae	10^9	98.89^a	91.67^a	4.13	95.26^a	92.50^a	5.00
	10^8	89.05^{ab}	82.50^{ab}	6.17	91.06^a	75.00^{bc}	5.17
	10^7	68.73^c	59.17^{cd}	7.57	68.75^b	54.17^e	7.00
	10^6	27.93^e	31.67^e	*	48.84^c	44.17^{ef}	*
Verticillium lecanii	10^9	96.80^a	57.50^{cd}	4.58	99.27^a	68.33^{cd}	4.83
	10^8	71.45^{bc}	32.50^e	6.75	71.34^b	35.00^f	6.47
	10^7	48.61^{dc}	9.17^f	*	49.83^c	0.83^h	*
	10^6	48.98^{dc}	0.00^f	*	45.11^c	0.00^h	*
Aphanocladium album	10^9	95.22^a	35.83^e	4.53	92.60^a	40.03^f	4.34
	10^8	60.55^{cd}	5.83^f	8.66	77.76^b	20.00^g	4.99
	10^7	29.45^e	0.00^f	*	47.93^c	2.50^h	*
	10^6	34.09^e	0.00^f	*	55.20^c	0.83^h	8.58
Control		24.74	0.00	*	17.96	0.00	*
Check		2.20	0.00	*	1.30	0.00	*

F. Cumulative percent mortality and percent mycosis of mango leafhoppers treated with *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* (Ma) in field condition.

Schedule of treatment application	DAFI	@ conc. 1×10^{12}			
		Bb		Ma	
		Cumulative %mortality	% mycosis	Cumulative % mortality	% mycosis
1^{st}	10	16.67	0.00	16.33	0.00
2^{nd}	14	27.00	0.00	25.00	0.00
3^{rd}	18	44.17	0.83	32.83	0.00
4^{th}	22	52.33	4.50	41.00	0.00
5^{th}	26	56.50	7.67	42.83	0.00