BIBLIOGRAPHY

AGLUYA, CRESCINTI P. APRIL 2012. Isolation and Characterization of Entomopathogenic Microorganism Affecting Insect Pests in Organic Farms in La Trinidad Benguet.Benguet State University, La Trinidad, Benguet.

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ABSTRACT

The study was conducted to identify pathogens infecting insect pest in organic vegetable farms and to characterized the pathogens and symptoms exhibited by the host insets. The study was conducted at the Plant Pathology Laboratory Benguet State University from November to March 2012. The growth of microorganisms isolated from white grubs (*Phyllophagaspp)* (*B. bassiana* and *N. rileyi*) and semi looper*(Trichoplusiani*) (*Trichoderma, Aspergillus* and *Penicillium*and the bacteria coded as S4, S6, S8 and S10)in three different media (Sabraud Dextrose Agar, Malt Extract Agar and Potato Carrot Agar) were evaluated. Their efficacy was tested in their host insect.

Two fungal species were isolated from infected white grubs (*Phyllophagaspp)* while three fungal species from semi looper*(Trichoplusiani)*. These fungi were *Beuvariabassiana, Nomuraearileyi* in white grubs and *Trichoderma sp., Aspergillus sp*. and *Penicillium sp*. from semi looper. Four bacterial pathogens were likewise isolated from semi looper.

The four bacterial isolates have similar colony form, edge and catalase test in the three different media but differ in color of the colony, *B. bassiana* has cottony growth in SDA and no growth observed in MEA and PCA, *Trichoderma* has a whitish to graycolor on the bottom and

green spores on the top view in MEA and PCA while in SDA it is color orange on the bottom and yellow green on top view, *Penicillium*has green spores in MEA and PCA while in SDA it is creamy white to yellow green while *Aspergillus* has a black color on the top and bottom view in MEA and PCA while in SDA it is yellow to dark green on the top view.

Diamond back moth exposed to *Trichoderma*, *Aspergillus* and *Penicillium*become sluggish unequal in size, rapidly when disturbed and transfer from one place to another, while diamond back moth exposed in bacterial isolates (S4, S6, S8 and S10) were observed to have unequal in size, discoloration and decayed if the infection is severe.

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INTRODUCTION

Vegetables grown without pesticides are natural foods that are grown with consciousness for the earth and the environment. Studies have proven that organic foods have a much higher nutritional quality than other fruits and vegetables, and they minimize the risk of exposing your family or even other people to chemical that could harm our health (Bolling, 2010).

Organic farming is a system of agriculture that uses environmentally sound materials and techniques in raising crops or even livestock that are free from growth hormones and antibiotics. Organic farmers rely on pesticides and fertilizers derived from plants, animal waste and minerals. They incorporate use of biological organism for pest management. These methods used in organic farming seek to increase soil fertility, balance insect population and reduce air, soil and water pollution (Hynes, 2007).

Organic agriculture as defined by International Federation of Organic Agriculture Movement (IFOAM, 2008) is a sustainable farming system that embraces environmentally, socially and economically sound production methods for food , fibber and livestock, recycling nutrient and strengthening natural processes that help to control pests and diseases, and maintain long term soil fertility to ensure successful production.

Organically grown vegetables are in demand due to the increasing awareness of hazards of pesticides to the environment, animals and human health (Tad-awan*et al.*, 2007).Surveys show that organically grown vegetable demand higher price than that conventionally produced.

Cotthem (2009) stated that organic vegetables are important because crops grown organically have fifty percent more nutrient and vitamins compared to crops produced by conventional farming methods.

Organically produced food are fairly easy to find. But it can be more expensive compared to conventionally produced food because growing organic food means using organic garden fertilizers, organic pesticide and organic soil that may cause more produce (Douglas, and Anderson, 2010).

Entomopathogens (fungi, bacteria, nematode and viruses) are widespread in the natural environment and cause infection in many pest species. Entomopathogens are safe and selective insecticides, asit only kills its host insect (Steinnhaus, 1946).

Bacillus thuringensis (Bt) is one of the most widely used pathogen for biological control of insects. It is a naturally occurring soil bacterium that causes disease in insect pests (pan Germany, OISAT, 2005) and is the so called insecticide that is acceptable for managementof insects in organic farming systems (Steinhaus, 1963). It is accepted as an alternative in organic farming and is considered ideal for pest management because it is host specific and is non-toxic on natural enemies and on humans (Pan Germany, OISAT, 2005).

The result of this experiment would provide alternatives to the farmers that can lower overall unit cost of production and allow the farmer to be more competitive.

The study was conducted to:

1. Identify pathogens infecting insect pest in organic vegetable farms, and
2. Characterize the pathogens and symptoms exhibited by the host insects.

The experiment was conducted at the College of Agriculture, Plant Pathology Laboratory, Benguet State University, La Trinidad, Benguetfrom November 2011 to April 2012.

REVIEW OF LITERATURE

Organic Agriculture

Organic agriculture is the integration of our responsibilities to the present and future generations in the way we produce the food and fiber we all require and our duties to enhance and maintain the natural environment which is both our resource based and our personal setting. It extends beyond the farm gate to the community, local and global (Bolling, 2010).

Entomopathogens/ Biopesticides

Much have been said and done about chemical approach to disease and insect control and the problems associated with the use of hazardous chemicals for pest control. There is considerable pressure on growers to reduce or eliminate the use of pesticide residues on human health and environment an alternative to chemical control is biological control with entomopathogens (Koul and Cuperus, 2007).

Biopesticides are pest management tools that are based on beneficial microorganism (fungi, bacteria, viruses and protozoan), beneficial nematodes or other safe, biologically based active ingredients. They can be specific to disease, weeds and arthropods pest. Biopesticides are usually inherently less toxic than conventional pesticides and it degrades rapidly in the environment. They are effective in very small quantities and often decomposed quickly, thereby resulting in lower exposure and largely avoiding the problem caused by environment pesticides (Steinhaus, 1946).

Entomopathogens are microorganisms that caused disease on arthropods, particularly insects and mites. Entomopathogens are used for microbial control have range of desirable characteristics including safety to people, compatibility with other

natural enemies and a lack of toxic residues. They also offer the possibility of providing persistent control by multiplying in the pest population (Chandler, 2005).

Diseases of Insect Pest

Insects suffering from bacterial diseases exhibit lack of motility, a diminished appetite, and rectal and oral discharges. In most cases, the infecting bacterium eventually invades the body cavity of the insects, and infection ends in septicemia. After death, the body (of the larvae specially) darkens rapidly to a brown to black color. It is usually very soft and becomes more or less shapeless. The internal tissues may breakdown to a viscid consistency, sometimes accompanied by odor, but ordinarily they do not "melt" or liquefy to the extent characteristics of certain virus infections. Usually the insect dries and become shriveled, with the integument remaining intact. One of the factors that affects the effectiveness of biopesticides is microbes enters the body of the insects. Disease producing microorganisms frequently have some special part of the body that afford them ready entrance into the insects body. This vulnerable point may be the integument, broken or intact, the intestinal tract, and the spiracle of their body openings. The portal of the entry may, in general, vary also according to the group of microorganism concerned. Whereas, most fungi enter the body cavity by penetrating the integument of body wall of the insect, the spore of the fungus developing in a diseased insect are disseminated in such a way that they come into direct contact with the integument of a healthy specimen which is the invaded (Steinhaus, 1946).

Norris *et al*. (2003) stated that pathogenic microorganisms, primarily the fungi, bacteria and viruses, disrupt the normal function of their host, resulting in reduced growth or even death. Pathogens that use insect pest as hosts, therefore they have the potential to

provide biological control of their hosts. Several different types of parasitic pathogen infect arthropods and can provide substantial level of biological control. An epidemic of pathogen against arthropods is referred to as an epizootic. Under favorable conditions, an epizootic can kill all susceptible individuals in a population, resulting in a population crash of the target insect pest.

Entomopathogenic Microorganisms

Most of the bacteria pathogenic to insects are confined to the family: Bacillaceae, *Enterobacteriaceae, Bacteriaceae, Lactobacteriaceae, Micrococcaceae,* and *Pseudomona-ceae*. This group consist of simple undifferentiated cells, where many of the species are pleomorphic, one-celled plant-like organisms that multiply by fission (Steinhaus, 1963).

Entomopathogenic viruses are either DNA or RNA infective unit surrounded by occlusion body (OB). Viruses have been used for classical and augmentative biological control for insect pest they are specific and environmentally safe. The transfer of a virus to a host usually requires the virus to survive in soil litter and on the plant surfaces, before they are moved passively by biotic or biotic agents. Additionally, the efficacy of many viruses as biological control is adversely affected by direct sunlight (Rechcigl and Rechigl, 2000).

Fungi known to cause infection and disease in insects are Phycomycetes, Ascomycetes, Basidiomycetes and Deoteromycetes. Some fungi infecting insects are obligate parasites, others were semi-parasitic and others are common saprophytic species which under certain conditions, are able to cause frank infection in susceptible insects. Rechcigl and Rechigl (2000) stated that fungi differ from most of the other types of insect

pathogens in that they do not have to be ingested in order to invade their host. Fungi can enter their host through natural openings in the insect cuticle and spread to the homocoel. Because fungi infect insects by penetrating the cuticle, direct contact between the fungi and the insect host is necessary. The ability of fungi to infect the insect external integument makes them good candidates for controlling piercing/ sucking herbivores, which are usually immune to other pathogens due to their feeding behavior (Koul and Cuperus, 2007).

Metarhiziumanisopliae

*Metarhiziumanisopliae*s also known as*Entomophoraanosplia* in the early1990s named after the insect species it was originally isolated from the beetle,*Anisopliaaustriaca* (Steinhaus, 1963).

The disease caused by this fungus is called green muscardine disease because of the green color of its spores. When the sexual spore of the fungus come in contact with the body of an insect host, they germinate and the hyphae that emerge penetrate the cuticle. The fungus develops inside the body eventually killing the insect. The cuticles of the cadaver often become red. If the ambient humidity is high enough, a white mold then grows on the cadaver which soon turns green as spores are produced.

Metarhiziumaniopliae is known to have worldwide distribution and is capable of infecting more than one hundred different specie belonging to a variety of insect orders (Chandler, 2005).

Nomureaerileyi (Farlow) Samson

Nomureaerileyi hasa pale green conidiophores ona white basal felt of mycelium. The conidiaare broadly ellipsoidand in fry chain. They are 3.5-4.5 x 2-3micrometer long. The conidiophores have branches contains 2-5 phialidesor conidial chains.

The germ tube passes directly into the spicuticle. There is also evidence of direct penetration of the cuticle. Points of entry darken indicating lysis, presumably due to enzymatic action. Hyphalbodies formby budding from pre-existinghyphae and are distinctly nucleated. At the death of the host, hyphae began to grow outward. Mohamed and Mohamed (1978) also reported that in vitro enzymatic tests the fungus secrets chitinase, protease and lipase.

Nomuraearileyi attacks larvae of rice insect such as leaf folder, stem borer, green caterpillar army worm and case worm (Rechigl and Rechigl, 2000).

Beauvariabassiana

Beauvariabassiana (Balsamo) Vuillemin, white muscardinefungus, was the first microorganism to be recognized as a disease agent.

Beauvariabassiana is a naturally occurring fungi, it generally infects throughtheintegument.Mahr(1997)explainedthat under favorable environment and moistureconditions, a conidium adhere into the host cuticle will germinate. The hypha growing from the spore secrets enzymes which attack and dissolve the cuticle allowing it to penetrate the skin and grow into the insect body. Once inside the host, it produces toxin called beauvarian that weakens the host immune system. After the insect dies, anantibiotic (oosperin) is produced that enables the fungus mass. When conditions are

favorablethey will growth through the softer parts ofthe insect bodyproducing thecharacteristics “white bloom”. It was further found out that it infects the highest number of potato weevil (Mayapit, 2007).

Beauvariabassiana occurs worldwide. It has an extensive host list that include such important pest as white flies, aphids, grass hopper, termites, Colorado beetle, Mexican bean beetle, Japanese beetle, ball weevil, cereal leaf beetle, bark beetles, lygus bugs, cinch bug, fire ant, European corn borers, codling moth and douglas fir cassock moth (Mahr, 1997).

Other fungi that attacks insects belong to species in the genus Entomophthora and are the bestknown that provide very high levels of control of many insects, such as aphids, lepidopterous caterpillars, and grasshoppers (Steinhaus, 1963). Other species belong to *Penicillium*and *Trichoderma*(Sullivan, 2004).

Nuclear Polyhedrosis Virus

Nuclear Polyhedrosis Virus (NPV) belongs to family Baculviridea. These are double strand-DNA virus (ds-DNA) with rod-shaped nucleocapsids. This enables the virus to infect cells more easily, and aids in reproduction of the virus.

When the insect eats the virus cell (occlusion body), its protein is degraded by gut proteases, freeing virions, which penetrate the midgut cells of theinsect. The virions invade the cell nucleus, and multiplying causing cell rapture. Virions are then passed into the haemocoel, invades and form OBs in additional susceptible tissues including the tracheal matrix, fat body and hypodermis causes rapture of the intersegmental membrane and oozing of the larval contents to the exterior of the insect, once this fracture begins,

the insect melts and contents containing OBs that will be dispersed by abiotic and biotic agents (Rechigl and Rechigl, 2000).

Nuclear polyhedrosis virus is naturally occurring virus that infects many caterpillar pests, alfalfa looper, corn earworm, imported cabbageworm, cabbage lopper, cotton bollworm, cotton leafworm, tobacco budworm, armyworms, European corn borer, almond moth, spruce budworm, Douglas fir tussock moth, pine sawfly andgypsy moth. Preparations of granulosis virus has been isolatedfrom several caterpillar species, including imported cabbageworm, cabbage looper, armyworm, fall webworm, and mosquitoes, among many others (Rechigl and Rechilg, 2000).

MATERIALS AND METHODS

Collection of Diseased Insects in Organic Farm

Organic farms (BSU Organic Farm, BSU Experimental Area and Swamp Experimental Area) in La Trinidad were visited. Diseased insects belonging to the order Lepidoptera and Coleoptera were collected. Infected larvae that were usually sluggish, hanging on the leaves, or present on the upper surface of the leaves were collected. In addition dead insects that wereattachedon the leavesand larvaewhich were smelly, discolored and flaccidwere likewise collected randomly and were brought to the laboratory for microscopy of microbial growth in the gut.

Culture of Potential Microorganisms

Diseased insects collected from different organic farms were incubated at room temperature and were initially isolated in Nutrient Agar (NA) for bacteria and water Agar (WA) for fungi. The pure culture for bacteria was obtained using NA for bacteria and PDA for fungi.

Characterization of Microorganisms

Microorganisms isolated from the different diseased insects were grown in different media (SDA, MEA and PCA) characterization was based on their structures, for fungi color and shape of the spores and hyphae and for bacteria shape of the cell catalase test and the gram stain reaction.

Growth in Different Medium

Pure culture of the microorganisms were cut using a cork borer or blade and were grown in three different culture media (Sabraud Dextrose Agar, Malt Extract Agar and Potato Carrot Agar). Characterization of growth was done.

Preparation of Fungal Suspension
For Bioassay Tests

The seven-day old pure cultures of fungal isolates were used as treatments for the bioassay test. In preparing the suspensions, the plates containing pure culture of the fungus were scraped with sterilized wire loop and dispensed in Sterilized Distilled Water (SDW) on test tubes. The spore count (Table 1) was standardized by counting the number of the spores per ml using the haemocytometer.

Preparation of Bacterial Suspension
For Bioassay Test

Twenty ml SDW were poured on the plates containing pure culture of bacteria. A wire loop was used to scrape the bacterial growth on the NA and poured into the new sterilized Petri plates ready for the bioassay test.

Table 1. Spore count of the fungal isolates used in bioassay tests

FUNGAL ISOLATES	SPORE COUNT PER ML
Beauvariabassiana	3.56×10^6
Nomuraearileyi	4.45×10^6
Trichoderma sp.	5.40×10^6
Aspergillus sp.	5.32×10^6
Penicillium sp.	5.08×10^6

Mass Production of Entomopathogenic Microorganism
In Target Insects

White Grub (*Phyllophaga* spp)

Healthy white grubs were collected from infested sweetpotato roots at the Mountain Province Provincial Nursery Area located at OtucanBauko Mountain Province. These were placed in sterile plastic container with sterile soil and roots of sweet potato, ten (10) larvae were placed in each container.Fifteen ml of the fungal organism (*Beauvariabassiana* and *Nomuraearileyi*) weresprayed in each container. A separate set-up without inoculum was designated as control. Appearance of symptoms was observed at different duration of time (48, 72, 120,168 and 240 hours).

Diamond back moth (*Plutellaxylostella)*

Healthy diamond back moth were collected in the field. After gathering sufficient semi lopper, ten (10) larvae were introduced in sterilized plastic container containing three leaves of Chinese cabbage that was dipped on the fungal suspension (*Penicillium sp. Aspergillussp*. and *Trichoderma sp*.) and bacterial suspension (S4, S6, S8 and S10). Addition of three leaves daily was done to ensure enough supply of food likewise, the tissue paper was replaced regularly during the gathering of larval mortality at 24, 48,72, 120, 168 and 240 hours (method1). Likewise one Chinese cabbage leaf was dipped on the residual fungal suspension used in method 1 and one larva was introduced (method 2). The number of dead larvae was recordedafter 24, 48, 72, 120, 168 and 240 hours.

A.  B. 

Figure1. Bioassay testsset-up. A. Bioassay ofmicroorganism on semi diamond back moth larvae B. Bioassay of microorganism on white grub

Data Gathered

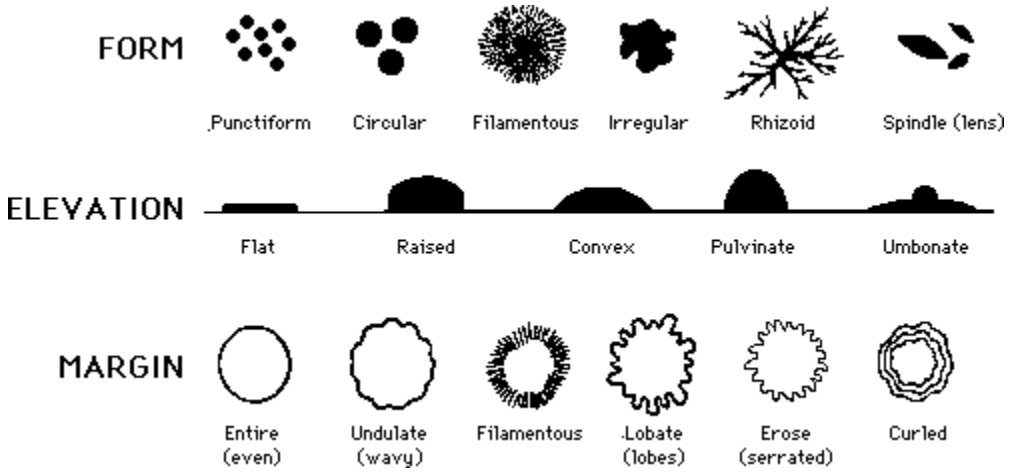
1.Identification and Characterization of Pathogen

a. Identification of pathogen. Thepure culture of the microorganism grown in PDA or NA will be identified using morphological, culture characteristics and gram stain (for bacteria only)

b. Characteristics of the Pathogen

b.1. Pure culture of the isolates will be transferred in three different media (Sabraud Dextrose Agar, Malt Extract Agar and Potato Carrot Agar) to characterize their growth on what medium they prefer.

b.2. Morphological characteristics of the pathogen in term of fruiting body, mycelia branching (for fungus), reaction to stain and shape of the cells (for bacteria).



c. Cultural characteristics of the pathogen in term of production of pigments.

2.Description of Diseased Insects. This was done by observing and listing the appearance of the collected insects from organic farm that are showing positive infection (this will be based on the review of literature).

RESULTS AND DISCUSSION

Microorganisms Isolated from White Grub and Semi looper

Fungi and Bacteria were isolated from white grub and semi lopper. The fungal isolates include *Beauvariabassiana, Numoraearileyi, Penicillium sp., Trichoderma sp*., and bacteria coded as S4, S6, S8 and S10 were likewise isolated.

Bacteria isolated from Semi looper.The cultural and morphological characteristics of the different isolated bacteria are summarized in Table 1 and Table 2 respectively.

The four bacterial isolates have similar colony form, edge and surface in the three different media used (Sabraud Dextrose Agar, Malt Extract Agar and Potato Carrot Agar).

Bacterial cells of the four isolates have the same form, catalase test and staining reaction (Plate 2, 3, 4, and 5). In terms of cell arrangement isolates S4, S6, and S8 are single or in pair while isolates S10 are in chain. However, regarding the cell size the diameter of the four isolatesis ranging from 1-2 u in diameter.

Fungi isolated from white grub and semi looper. The fungi that were isolated in white grubs were *Beauvariabasiana*and *Nomuraearileyi* while *Trichoderma*, *Penicillium* and *Aspergillus*were isolated in the semi looper. The different genera of fungi are shown in Figures 6, 7, 8 and 9 respectively.

Beauvariabassiana is a slow-growing fungus and is difficult to grow in agar. Mycelium appears white in culture and bears masses of powdery spores. It exhibits cottony growth in Sabraud Dextrose Agar (Figure 6) while in Malt Extract Agar and

Potato Carrot Agar no growth was recorded. The one-celled colorless conidia are born along a thin filament in a zigzag fashion as the conidia are produced. Conidiogenous cells are flask-shaped, rachiform, proliferating sympodialy aggregated into sporodochia. Conidia are hyaline and globose in shape and are easily detached, a condition leading to scattered colonies (Figure 6).

Table 2. Cultural characteristics of bacterial isolates in different media

TREATMENTS/ ORGANISMS	CULURAL CHARACTERISTICS				
	Form	Surface	Edge	Elevation	Color
S4					
SDA	Circular	Slimy, glistening	Entire	Raised	Light yellow
MEA	Circular	Slimy, glistening	Entire	Raised	Light yellow
PCA	Circular	Slimy, glistening	Entire	Raised	Light yellow
S6					
SDA	Circular	Slimy, glistening	Entire	Raised	Yellow
MEA	Circular	Slimy, glistening	Entire	Convex	Yellow
PCA	Circular	Powdery, dry	Entire	Flat	Yellow
S8					
SDA	Circular	Slimy, glistening	Entire	Raised	Creamy white
MEA	Circular	Slimy, glistening	Entire	Raised	Creamy white
PCA	Circular	Slimy, glistening	Entire	Convex	Creamy white
S10					
SDA	Circular	Slimy, glistening	Entire	Raised	White
MEA	Circular	Slimy, glistening	Entire	Flat	White
PCA	Circular	Slimy, glistening	Wavy	Flat	White

Nomuraearileyi is a fast growing fungus and is easily to grown in Sabraud Dextrose Agar the species produces green spores while in Potato Carrot Agar and Malt

Extract Agar no growth was observed. A young culture has white mycelia which turns green as the culture ages, (Figure 6). *Nomuraearileyi* is composed of pale green to gray conidiophores on a white basal felt mycelium. The conidia are broadly ellipsoid and in dry chains. Size ranges from 3.5-4.5 x 2.3 um long. The conidiophores have branches were each branch contains phialide spores, (Figure 6).

Table 3. Morphological characteristics of bacterial isolates in different media

	MORPHOLOGICAL CHARACTERISTICS			
TREATMENTS/ ORGANISMS	Form	Arrangement	Staining Reaction	Catalase test
S4				
SDA	Coccus	Single/ pair	Negative	Positive
MEA	Coccus	Single/ pair	Negative	Positive
PCA	Coccus	Single/ pair	Negative	Positive
S6				
SDA	Coccus	Single/ pair	Negative	Positive
MEA	Coccus	Single/ pair	Negative	Positive
PCA	Coccus	Single/ pair	Negative	Positive
S8				
SDA	Coccus	Single/ pair	Negative	Positive
MEA	Coccus	Single/ pair	Negative	Positive
PCA	Coccus	Single/ pair	Negative	Positive
S10				
SDA	Coccus	Chain	Negative	Positive
MEA	Coccus	Chain	Negative	Positive
PCA	Coccus	Chain	Negative	Positive

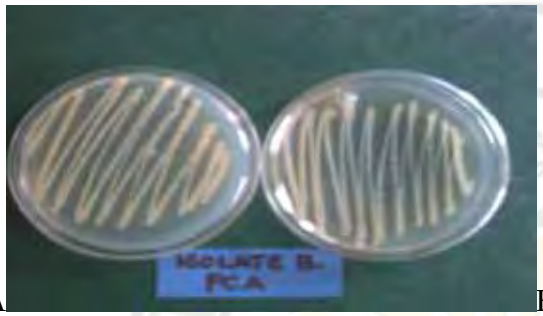
A 

B 

C 

D 

Figure 2. Bacterial isolate S4. Isolated from semi lopper. (a) Colony growth in PCA (b) colony growth in MEA (c) colony growth in SDA and (d) gram staining

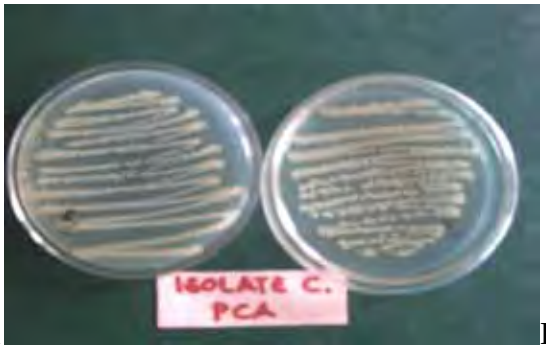
A 

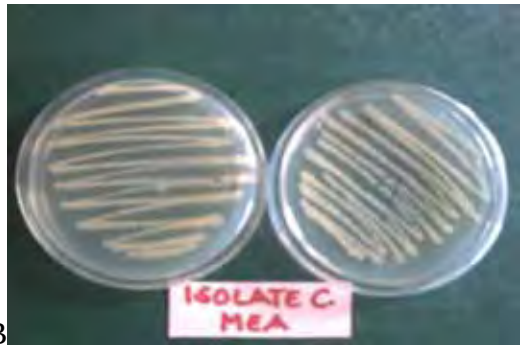
B 

C 

D 

Figure 3. Bacterial isolate S6. Isolated from semi lopper. (a) Colony growth in PCA (b) colony growth in MEA (c) colony growth in SDA and (d) gram staining

A 

B 

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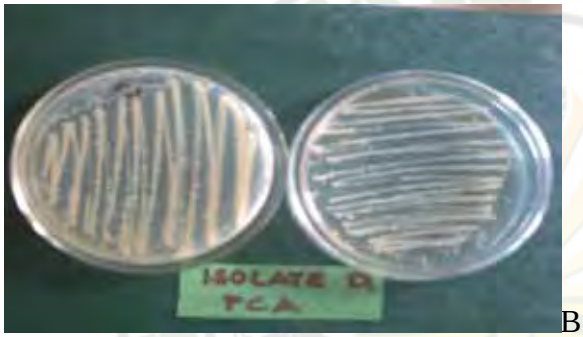
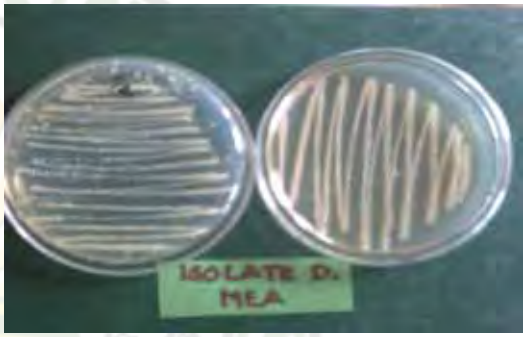
D 

Figure 4. Bacterial isolate S8. Isolated from semi lopper. (a) Colony growth in PCA (b) colony growth in MEA (c) colony growth in SDA and (d) gram staining

A 

B 

C 

Figure 5. Bacterial isolate S10. Isolated from semi lopper. (a) Colony growth in PCA (b) colony growth in MEA (c) gram staining

Trichoderma is a fast growing fungus and easily grown in agar media. It has a whitish to gray color on the bottom and green spores on the top view in MEA and PCA while in SDA it is color orange on the bottom and yellow green on top view (Plate3).

Penicillium is a fast growing fungus and easily grown in agar media. It has green spores in MEA and PCA while in SDA it is creamy white to yellow green.*Penicillium* species are fast growing fungi and have aconidial structures that resembles brushes (Plate8.d).

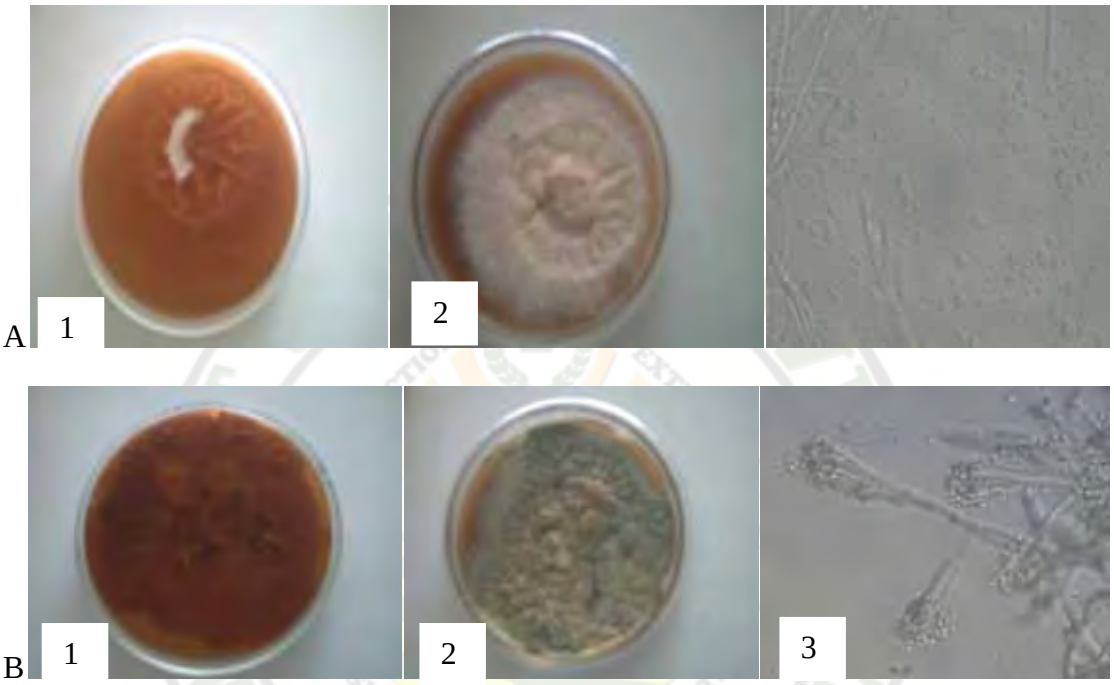
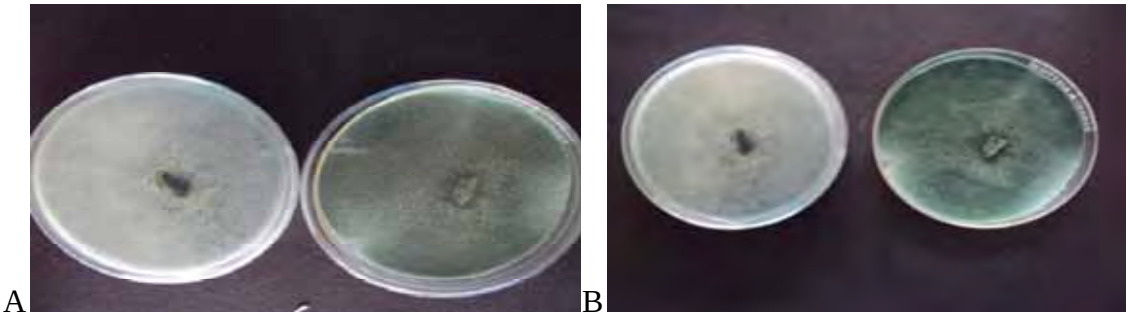
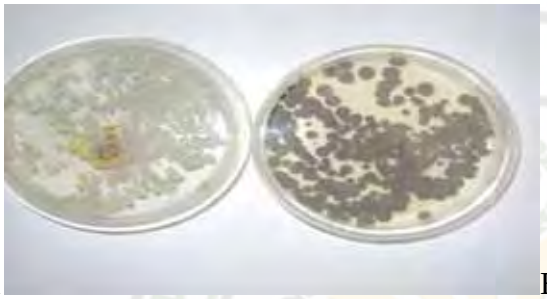
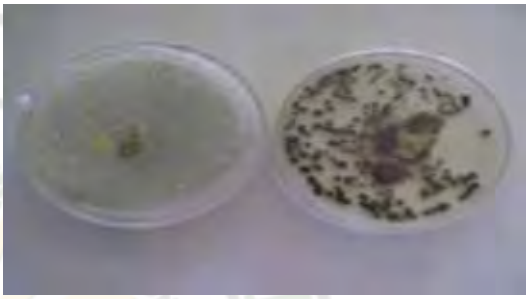


Figure 6. Bottom view(1), and top view (2), conidiopore and chain of spore (3) of the different fungal genera. A. *Beauvaria bassiana*, B. *Nomuraea rileyi*



C  D 

Figure 7.*Trichoderma sp*. Isolated from semi lopper. (a) Colony growth in PCA (b) Colony growth in MEA (c) colony growth in SDA and (d) morphological structure

A  B 

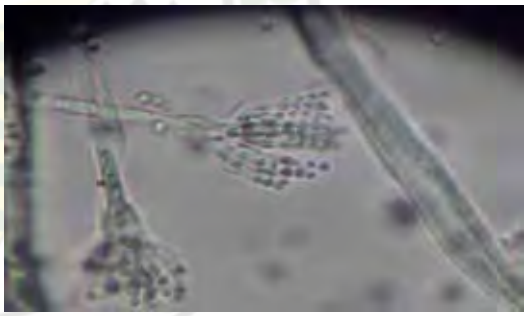
C  D 

Figure 8.*Penicillum sp*. Isolated from semi lopper. (a) Colony growth in PCA (b) colony growth in MEA (c) colony growth in SDA and (d) morphological structure

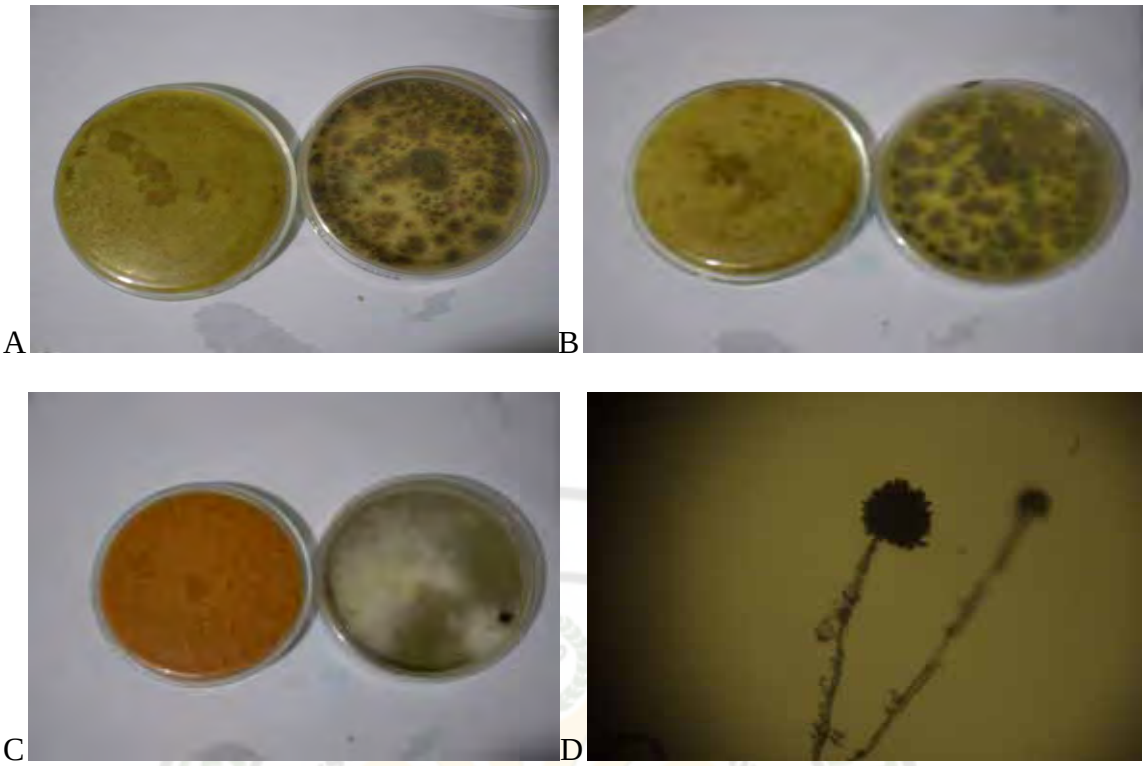


Figure 9.*Aspergillus sp*. Isolated from semi lopper. (a) Colony growth in PCA (b) colony growth in MEA (c) colony growth in SDA and (d) morphological structure

Mortality of white grubs larvae exposed to Beauvariabassiana and Nomureaerileyi

Figure 10 shows that mortality of white grubs exposed to *B. bassiana* and *N. rileyi.Beauvariabassiana* infects the highest number of white grubs in 168 hrs and 240 hrs as compared to *Nomuraearileyi.*

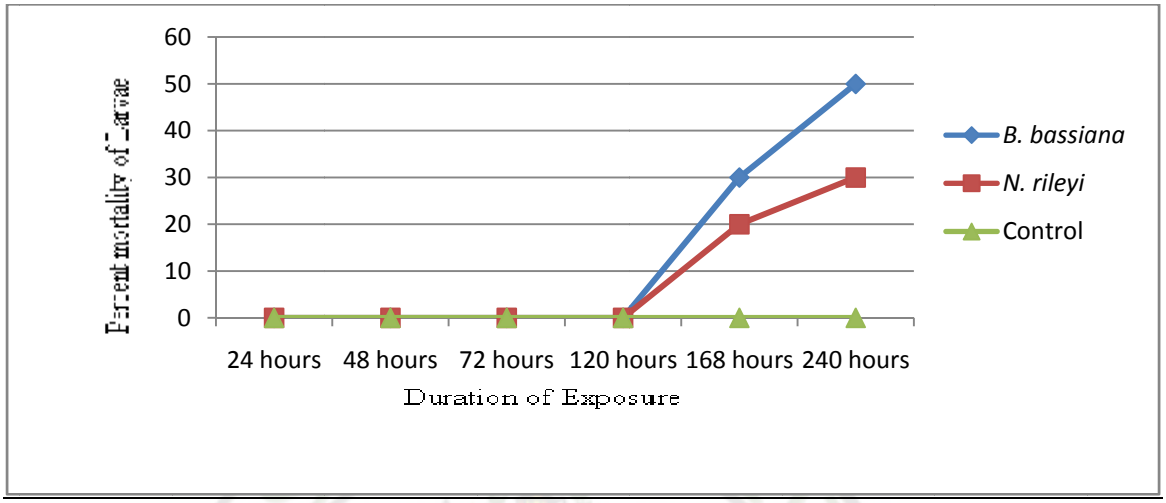


Figure10. Percent mortality of White grubs larvae treated with fungal isolates at different duration of time (hours)

Mortality of diamond back moth larvae exposed to *Penicilium*,*Aspergillus*and *Trichoderma*

Figure 11 shows the percent mortality of larvae that fed on Chinese cabbage leaves dipped in fungal suspension. The result showed that *Penicillium, Aspergillus* and

Trichoderma caused infection to the larvae. The result further showed that microorganisms are more host specific in causing fatalities to other organisms.

The result shows that semi looper mortality increases as the duration of exposure to the treated leaves up to 168 hrs of exposure except for *Trichoderma* were mortality decreased on 168 hrs of exposure. This showed that the virulence or of the microorganisms decreases as exposure of time increases.

The mode of entry of the fungi could also affect the mortality rate of the larvae. The fungi needs to penetrate the insect cuticle before it produces toxins that cause the mortality of larvae. The low population of dead larvae could be attributed to the virulence of the microorganisms.

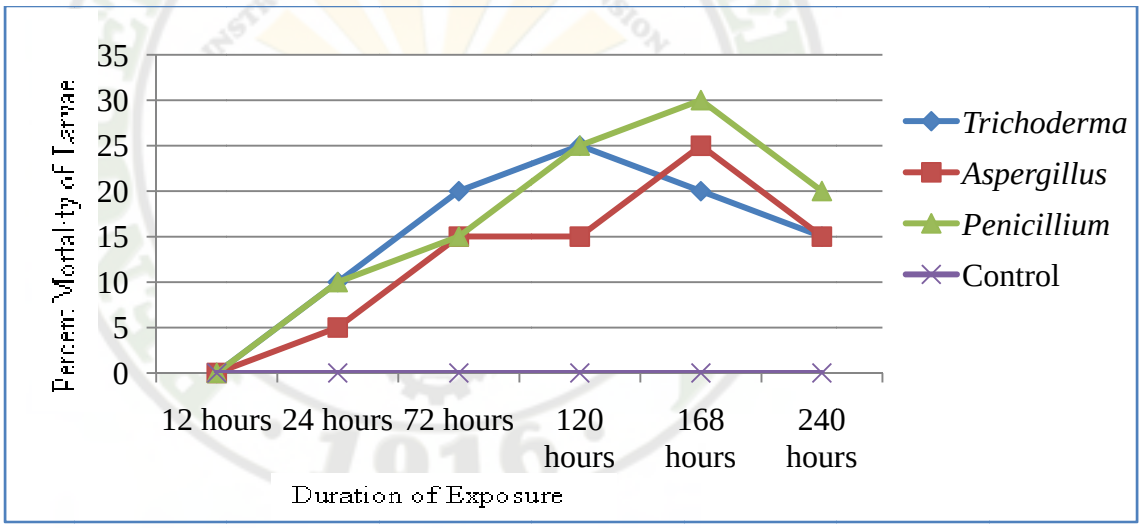


Figure 11. Percent mortality of Diamond back moth larvae treated with fungal isolates at different duration of time

The larvae become sluggish after 24 hours and respond less rapidly when disturbed. The larvae ceases to eat. Likewise, the infected larvae show migration from one position to another while other infected larvae climbed to the top of the container or highest tip of leavesand they hang their pro-legs and die (Stainhuas, 1946) then molds were observed growing on the insect cuticle after a day. The dead larva then was covered

with mycelium and later turns green due to accumulation of green spores (Shepard et. al, 1999)

Figure 12 shows the feeding and dead larvae after 24 hour of exposure to the left over fungal suspension (Method 2) bioassay. The larvae consume a small portion of the leaves that were inoculated with fungal and bacterial isolates. It was also observed that larvae excrete feces and regurgitate yellow substances just after feeding on the leaves.

A

B

Exposed to Aspergillus isolates

Exposed to Aspergillus isolates

A

B

Exposed to Penicillium isolates

Exposed to Penicillium isolates

A

Exposed to Trichoderma isolates

B

Exposed to Trichoderma isolates

Plate12. Dead larvae after 24 hours of exposure to fungal suspensionA method 1 B. Method 2

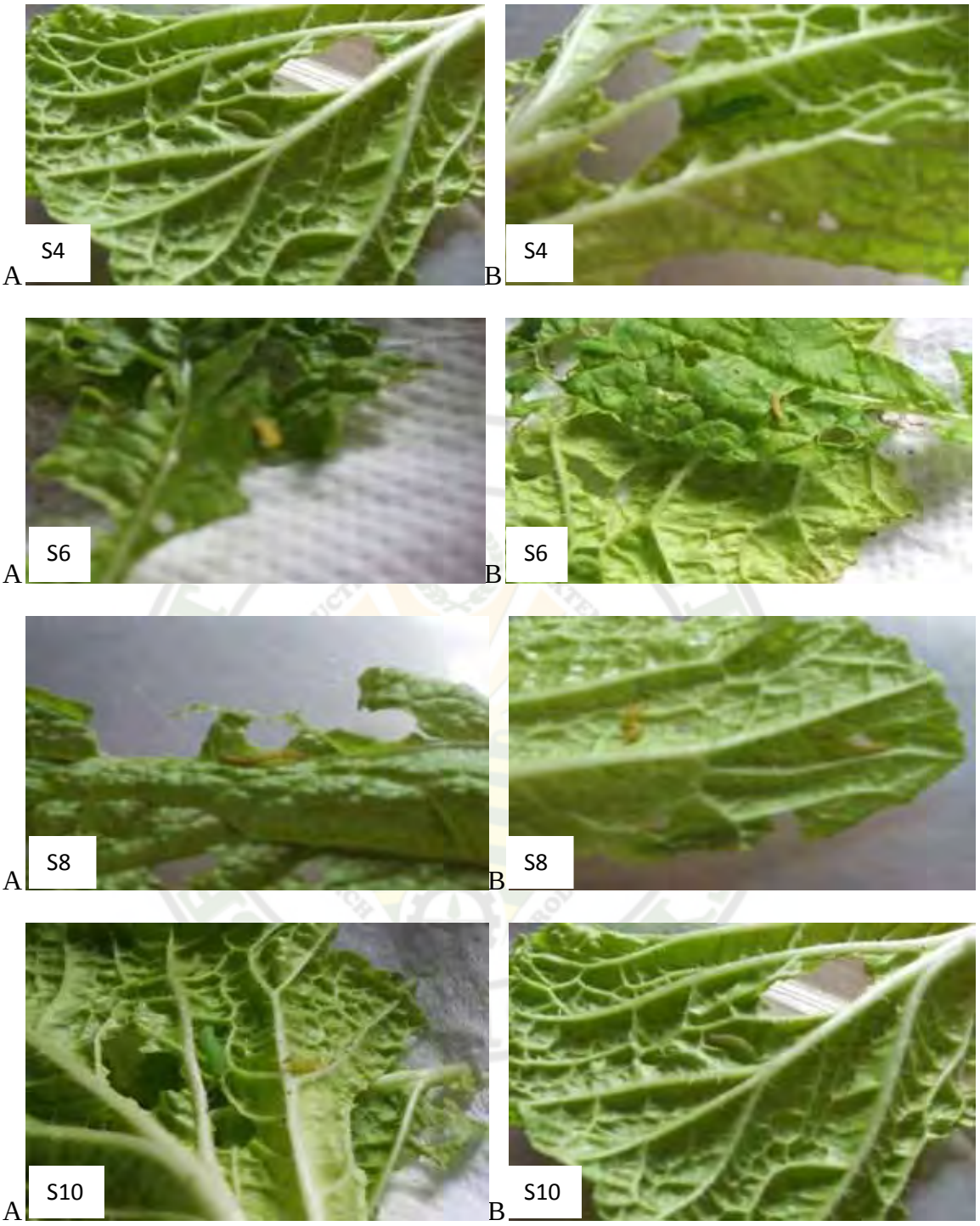


Figure 13. Dead larvae after 24 hours exposure to bacterial isolate A. method 1 B. Method 2.

Mortality of diamond back moth Treated with Bacterial Isolates

Figure 14 shows the mortality of larvae exposed to different bacterial isolates.The resultshowsthat all the bacterial isolates (S4, S6, S8 and S10) caused mortality to the larvae.

Larvae were observed to have discoloration and unequal sizes despite their uniformity during the start of the bioassay. Yellow discharges from the mouth and also from the anus were observed as symptoms of larvae infected with bacteria (Steinhaus, 1946).

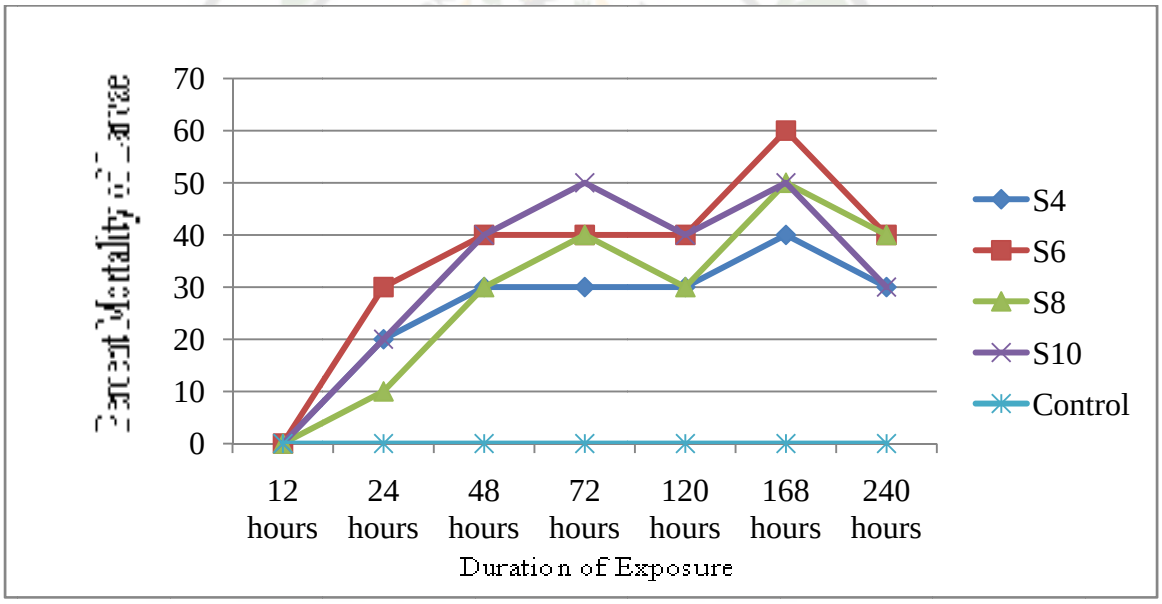


Figure 14.Percent mortality of Diamond back moth larvae treated with bacterial isolates at different duration of time(hours)

The result shows that S6, S8 and S10 caused mortality in semi looper when it is exposed up to 168 hours. However, more dead larvae were observed on the treatment S10 after 72 hours of exposure. Larvae exposed to S6 and S10 had the highest mortality at 240 hours. The mortality of larvae increased up to 168 hours in all treatments and decreased at 240 hrs.

SUMMARY, CONCLUSION AND RECOMMENDATION

Summary

The study was conducted to identify the pathogens infecting insect pests in organic vegetable farms, to characterize the pathogens isolated and symptoms exhibits by the infected white grubs and semi looper and to determine the efficacy of these microorganisms in the control of white grub and semi looper.

Based on the results, all the fungal and bacterial isolates grew on the three different media (SDA, PCA and MEA) but grew faster in SDA.

The microorganisms isolated from white grub were*Beauvariabassiana* and *Numoreaerileyi* and in semi looper include *Trichoderma, Aspergillus* and *Penicillium* and the bacterial isolates coded as S4, S6, S8 and S10. The fungal and bacterial isolates causepathologies to its host white grub (*B. bassiana* and *N. rileyi*) and semi lopper (*Trichoderma, Aspergillus, Penicillium* and the bacterial isolates S4, S6, S8, and S10).

Conclusion

The identified entomogenous fungi that can caused pathologies to white grub were *Beauvariabassiana* and *Nomuraearileyi* while in semi lopper were *Trichoderma,Aspergillus* and *Penicillium* while the entomogenous bacteria were S4, S6, S8 and S10.

The isolated entomogenous microorganisms can thrive in the three different media the Sabraud Dextrose Agar, Potato Carrot Agar and Malt Extract Agar.

Recomendation

Further studies on the identification of bacteria and fungi up to species level isrecommended. Field evaluation trial of the microorganisms must be conducted to furtherevaluate their efficacies on insects and reaction of host plants towards microorganisms.

In addition, other media including the utilization of indigenous materials for the multiplication of these microorganisms should be explored.

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APPENDICES

Appendix Table 1. Mortality of white grubs exposed to fungal isolates at different duration of time

	DURATION OF EXPOSURE					
FUNGAL ISOLATES	24 hours	48 hours	72 hours	120 hours	168 hours	240 hours
Control	0	0	0	0	0	0
B. bassiana	0	0	0	0	30	50
N. rileyi	0	0	0	0	20	30

Appendix Table 1. Mortality of diamond back moth exposed to fungal isolates at different duration of time

	DURATION OF EXPOSURE					
FUNGAL ISOLATES	12 hours	24 hours	72 hours	120 hours	168 hours	240 hours
Control	0	0	0	0	0	0
Trichoderma	0	10	20	25	20	15
Aspergillus	0	5	15	15	25	15
Penicillium	0	10	15	25	30	20

Appendix Table 3. Mortality of diamond back moth exposed to bacterial isolates at different duration of time

	DURATION OF EXPOSURE					
BACTERIAL ISOLATES	24 hours	48 hours	72 hours	120 hours	168 hours	240 hours
Control	0	0	0	0	0	0
S4	20	30	30	30	40	30
S6	30	40	40	40	60	40
S8	10	30	40	30	50	40
S10	20	40	50	40	50	30