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Evaluation and Optimization of Agro-industrial Wastes for Conidial Production of *Metarhizium* and *Beauveria* Isolates under Solid State Fermentation

BY

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Abbreviations

AAU = Addis Ababa University

AUBI1 = Addis Ababa University *Beauveria* isolate one

AUBI2 = Addis Ababa University *Beauveria* isolate two

AUMI1 = Addis Ababa University *Metarhizium* isolate one

AUMI2 = Addis Ababa University *Metarhizium* isolate two

AUMI3 = Addis Ababa University *Metarhizium* isolate three

DMCMB = Department of Microbial Cellular and Molecular Biology

LUBILOSA = Lutte Biologique contre les Locustes et sauteriaux

SSF = Solid state fermentation

SDA = Sabouraud dextrose agar

ULV= Ultra – low - Volume

USSR = Union of Soviet Socialist Republics

UV = Ultraviolet

Abstract

Two isolates of Beauveria and three isolates of Metarhizium were grown on different agricultural wastes to evaluate their conidia production potential under SSF system. Coffee husk, Tea waste, Wheat bran and Vegetable wastes were used as substrates to determine maximum conidiation of the isolates. Among these vegetable wastes were the best medium to yield 5.77±1.53 (10^7), 5.80±0.72 (10^7), 4.44 ± 0.55(10^7) and 5.58 ± 0.66 (10^7) conidia/gram of substrate under quantitative assessment for isolates (AUBI2, AUMI1, AUMI2 and AUMI3 respectively) at 60% moisture content. The highest conidia count record of AUBI1 6.34 ± 2.27 (10^7) conidia/gram was obtained at 60% moisture content on wheat bran. It has been observed that two sample t-Test statistical analyses of conidial yield of vegetable wastes was significantly different from 2mm and 4mm particle size of coffee husk used as substrates. The optimization for temperature indicated that all substrates supported maximum conidia yield with-in the range of temperature from 27 to 30^0_C. Beauveria isolate AUBI1 was produced 11.63 ± 8.34 (10^7) conidia/gram of substrate at coffee husk when treated at 3.5 pH value. The pH values used in the present study for optimization best favored only for coffee husk as substrate. The high conidia yielding substrates were best productive at their own natural at 6.29 for Vegetable wastes, 6.63 for Wheat bran and 5.4 for Tea waste pH values. Vegetable wastes supported AUBI1, AUBI2, AUMI1, AUMI2 and AUMI3 to produce high conidia yield of 4.31 ± 1.18 (10^7), 5.07 ± 0.76 (10^7), 3.43 ± 0.30 (10^7), 1.86 ± 0.16 (10^7) and 5.17 ± 0.61 (10^7) conidia/gram of substrate at optimum incubation period of 21 days. The optimum incubation period of the fungal isolates on wheat bran is 3 - 4 weeks under sufficient exposure to light. AUBI1, AUBI2 and AUMI1 produce high conidia under exposure to light but AUMI2 and AUMI3 at dark condition when cultivated on vegetable wastes. Therefore, as successful microbial control of insect pests depends on cheap and large scale industrial productivity, entomopathogens cultivation on vegetable wastes and wheat bran can be attained by SSF system.

Key words: *Beauveria, Metarhizium, Inoculation, Optimization and Conidia*

1. Introduction:

Entomopathogenic fungi are often reported as causing high levels of epizootics in nature and are the most versatile biological control agents, and are environmentally safe. These fungi subsume a heterogeneous group of over 100 genera with approximately 750 species, notified from different insects. Many of these are proved to be highly potential in insect pest management. The most considerable fungal species are *Metarhizium spp., Beauveria spp., Nomuraea rileyi, Verticillium lecanii* and *Hirsutella spp* (Jitendra *et al.,*) (2012).

The origins of microbial pest control dated back to the early 19th century, when the Italian scientist Agostino Bassi studied white muscardine disease in silkworms (*Bombyx mori* L.). He identified *Beauveria bassiana* as the cause of the disease (Yadav Seema and Neeraj 2012; Chinnadurai and Ganesh 2013). His discovery laid the foundation for microbial pest control and triggers using entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae* as highly active insect pests (Chinnadurai and Ganesh) (2013).

The first attempt to control a pest with a fungal agent was carried out in Russia in 1888, when the fungus now known as *Metarhizium anisopliae* was mass produced on beer mash and sprayed in the field for control of the beet weevil *Cleonus punctiventris* (Lord 2005). Boverin, a *Beauveria bassiana*-based mycoinsecticide for control of the Colorado potato beetle and codling moth was developed in 1965 in the former USSR, (Marcos and Wraight, 2007).

In the last years Entomopathogenic fungi biology and ecology have been studied more and the attention of scientists was focused mainly on fungi genus *Beauveria* and *Metarhizium* since they attack the broad spectrum of insects (Mudroncekova *et al.,* 2013). Their natural environment is the soil as a reservoir of their resistant spores. They are able to infect their hosts waiting in natural habitat.

Beauveria bassiana have the broadest potential as a viable insect control agent. Its potential to control more than 700 insect pests Pham *et al.,* (2009).
Metarhizium anisoplae is the second most widely exploited entomopathogenic fungus in biocontrol trials. It is known to attack over 200 species of insects belonging to orders *Coleoptera*, *Dermoptera*, *Homoptera*, *Lepidoptera* and *Orthoptera* Jitendra *et al.,* (2012). This has been responsible for a substantial increase in interest for large scale production of good quality inoculums of the fungus for field applications sake of the biocontrol program.

The worldwide Food, Agricultural and Forestry industries produces a tremendous amount of wastes annually, which causes a serious disposal problem Orzua *et al.,* (2009). Some of these wastes include the bagasse and peels generated in the beverages and juice industries, coffee pulp obtained from coffee industries, and husks from cereals. Therefore, utilization of these products for large scale production of mycoinsecticide conidia would be economically viable despite of its role in controlling environmental pollution.

Different studies have been conducted so far for mass production of conidia and secondary metabolites using agricultural wastes under solid state and submerged fermentation. Wheat straw, tea waste, Vegetable waste, Coffee husk and barley bran have been evaluated to determine optimal conditions for conidia production of *Trichoderma* (Zuriash Mamo and Tesfaye Alemu, 2012). Sugarcane baggase, orange peel, wheat straw, cotton seeds, castor been and rice husk were used alone as carbon source for the production of single cell protein Khan and Dahot (2010) and Kitchen wastes are used for bio pesticide production under solid state fermentation Zhang *et al.,* (2013).

According to Jianhui Wu *et al.,* (2010) germination, spore production of the different isolates and their virulence is associated with appropriate concentrations of nitrogen and carbon in the culture medium. The nutritional requirements of entomopathogenic fungi vary with the fungal species and even the fungal strains under consideration. Generally

fungi require oxygen, water, source of carbon, inorganic or organic nitrogen besides minerals that play a major role in growth and their pathogenicity including novel metabolite production. Therefore, the success of microbial control of insect pests depends not only on the isolation, characterization and pathogenicity, but also on the easy and cheap mass production of the microbial agents in the laboratory Sahayaraj and Namasivayam (2008).

Studies on entomopathogenic fungi *B. bassiana* and *Metarhizium anisoplia* are conducted to isolate, characterize and demonstrated the effectiveness of fungal pathogens to control the target organism and to evaluate different substrates for mass production of the fungi and its secondary metabolites (Sahayaraj and Namasivayam 2008, Yadav Seema *et al.,* 2013). However, the production of locally isolated entomopathogenic fungi in suitable media for large scale application has not yet been studied. Therefore, in the present study isolates of *Beauveria* and *Metarhizium* were screened for mass production of conidia under Laboratory scale solid state fermentation using different cost effective substrates.

This study was initiated to evaluate and optimize agricultural wastes (wheat bran, coffee husk, tea waste and vegetable wastes) using *Metarhizium* and *Beauveria* isolates at different growth conditions of temperature, pH, moisture content, and incubation period, Inoculum size, and concentration and effect of light for maximum conidia production under solid state fermentation.

2. Objectives

General Objective: To evaluate and optimize *Beauveria* and *Metarhizium* isolates on agricultural wastes at different growth conditions for maximum conidia production under solid state fermentation.

Specific Objectives:

- To evaluate and determine optimal growth conditions for maximum conidia production using fungal isolates under solid state fermentation.

- To evaluate nutritional value of different agricultural wastes for maximum conidial production under solid state fermentation.

3. Literature review

The benefits of biological control

A compelling motivation for adoption of biological control is reduced ongoing expenditure for pesticides, labor, specialized equipment, and – potentially – a permanent return to ecological conditions more similar to those seen before the arrival of the pest. Economic analyses indicate that **benefit: cost** ratios for successful biological control of arthropod pests are high, and can exceed 145:1 (Norgaard 1988, Pickett *et al.,* 1996) and potential benefit:cost ratios overwhelmingly favor support for biological control programs as an option for pest control Gutierrez *et al.,* (1999).

Estimates of economic benefits from successful biological control programs tend to be conservative and profits continue to accrue annually with little or no additional management of the system Norgaard (1988). Comparisons of costs for biological control programs indicate that benefits amassed from successful projects outweigh the combined costs of unsuccessful projects, even though failures are more numerous. For example, just 10% of arthropod biological control programs have provided full control of the target pest Gurr *et al.,* (2000), and for weed programs, less than 30% of projects have resulted in either total or partial control of the target Syrett *et al*., (2000) cited in(Hoddle) although in some instances evaluations may have been conducted too early to determine final outcomes McFadyen, 1998 in (Hoddle). Projects sponsored by the Australian Center for International Agricultural Research had a cost:benefit ratio of 13.4:1 for ten projects that spanned 1983-1996, even though just four of these projects were documented successes Lubulwa and McMeniman, 1998 cited in(Hoddle).

Biological control of agricultural pests can indirectly benefit native wildlife through the reduction of pesticides released into the environment because of natural enemy suppression of economically important targets. The acute impact of insecticides on wildlife because of aerosol drift from agricultural areas, run off into waterways, food chain accumulation, or indiscriminant application was first brought to public attention by Rachel Carson (1962).

An insidious, chronic side effect from pesticide use that has been recently postulated is the potential ability of synthetic chemical pollutants in the environment to accumulate in the bodies of vertebrates, and for these sequestered compounds to mimic or block the actions of endogenous hormones Colborn *et al*., 1997 cited in (Hoddle). According to Hoddle (2002) the environmental endocrine hypothesis has been used as a unifying theory linking wildlife declines, reproductive ailments, behavioral abnormalities (e.g., reproductive and anti-predator), and gross physical deformities with agricultural pesticides, pharmaceuticals, and other industrial chemicals that mimic or obstruct hormonal activity in animals and humans.

Microbial insecticides:

The increasing costs of insecticide development, the dwindling rate of commercialization of new materials, and the demonstration of cross or multiple resistance to new classes of insecticides almost before they are fully commercialized contribute to pest resistance which is becoming the greatest problem facing applied entomology (Glazer and Nikaido, 2007). The only reasonable hope of delaying or avoiding pest resistance lies in integrated pest management programs that decrease the frequency and intensity of genetic selection by insects exposed to insecticides. These includes, multiple interventions in insect population control by natural enemies, insect diseases, cultural manipulations, and host plant resistance (Glazer and Nikaido, 2007).

Like all living things, insets are susceptible to infection by pathogenic microorganisms (bacteria, fungi, and protozoa) and viruses. Many of these biological agents have a narrow host range and consequently do not harm non-target beneficial insects and are not toxic to vertebrates. In spite of this very attractive feature, microbial pest control agents represent less than 1% of total insecticide sales (Glazer and Nikaido, 2007). One of the candidates which have been classified as microbial insecticide is fungi.

Rational utilization of pathogens, whether from bacterial, fungal, nematode or arthropod origin, to maintain pest maintenance at a non-economic level, with reduced environment aggression, has been adopted worldwide in biological control programs (Diogo *et al.,*

2009). The use of microbial agents for biological control of pests and plant diseases is an important strategy to minimize synthetic chemical pesticides and their effects on humans, animals and the environment (Diogo *et al.,* 2009).

All the four major groups of fungi, *Phycomycetes, Ascomycetes, fungi imperfecti* and *Basidiomycetes* contain pathogens of insects. Fungi are microorganisms that hold the highest potential for biological control and they have a limited host range, with minimal impact on non-target species (Diogo *et al.,* 2009). Although thousands of fungal species infect insects, few have received serious consideration as potential commercial candidates (Pham *et al.,* 2010).

The great difficulty with using fungi for biological control is that environmental conditions including temperature and humidity must be adequate for spore germination and insect cuticle penetration by the hyphae (MUHAMMAD *et al.,* 2010, Okafor 2007).

Entomopathogenic fungi:

Entomopathogenic fungi are often reported as causing high levels of epizootics in nature they are the most versatile biological control agents, and are environmentally safe. An attractive feature of these fungi is that the virulence caused by contact and the action is through penetration (Nadau, *et al*., 1996 cited in Jitendra *et al.,* 2012) Fungal cell attachment to the cuticle may involve specific receptor-ligand and/or nonspecific hydrophobic and electrostatic mechanisms (Diane and Nemat 2005).

These fungi subsume a heterogeneous group of over 100 genera of which approximately 750 species, were isolated from different insects. Many of these are proved to be highly potential in pest management. The most considerable fungal species are *Metarhizium spp., Beauveria spp., Nomuraea rileyi, Verticillium lecanii* and *Hirsutella spp* (Jitendra *et al., 2012).*

In 1883, Metchnikoff commenced mass culturing of fungus and carried out the first experiment with two beetle pests. Under intensive study for use as a biopesticide, the entomopathogenic fungus *Beauveria bassiana* displays a broad host range and is able to

target a number of diverse arthropod species. *Beauveria bassiana* have the broadest potential as a viable insect control agent. Strains of *B. bassiana* have been selected for control of insects and other arthropods that act as disease vectors, including mosquitoes and ticks; crop pests, such as whiteflies, caterpillars, grasshoppers, and borers; and even ecologically hazardous, invading pests, such as fire ants and termites (Holder *et al*., 2005).

Metarhizium anisoplae is the second most widely exploited entomopathogenic fungus in bio control trials. It is known to attack over 200 species of insects belonging to orders *Coleoptera*, *Dermoptera*, *Homoptera*, *Lepidoptera* and *Orthoptera* Jitendra *et al.,* (2012).

Development of fungal spore formulations as mycopesticides

Indigenous Deuteromycete fungi have considerable potential as microbial control agents because they are genetically stable and can be produced cheaply in large quantities. Formulating the spores in oil should avoid the normal requirement of such fungi for high humidity during the infection process, and, as the infection penetrates the cuticle, the formulation can be used as a contact insecticide. These considerations formed the conceptual basis for the project Lutte Biologique contre les Locustes et Sauteriaux (LUBILOSA). The LUBILOSA project brought together a multidisciplinary team, which was successful in developing the first effective mycopesticide for locust control (Lomer *et al.,* 2001).

Mass Production:

In industrial terms, liquid fermentation of microbial control agents is more advanced than solid-state fermentation and has the potential for much lower cost production (Lomer *et al.,* 2001). However, the conidia of the hyphomycete fungi are the only stage with good environmental persistence; the conidiospores of *Metarhizium* and *Beauveria* are hydrophobic and are only produced at an interface between substrate and air.

A spore production pilot plant was therefore constructed at the International Institute of Tropical Agriculture (IITA) in Cotonou, Benin. The production method was adapted

from similar plants in China and Brazil and involved an initial liquid phase in sucrose/brewer‟s yeast broth, followed by a solid phase on sterilized rice in polypropylene bags (Lomer *et al.,* 2001).

Although the pilot plant was uneconomic to run on a large scale, it was an essential research tool without which neither optimization of fermentation process characteristics nor field testing would have been possible. For instance, a prolonged conditioning period of 2 weeks during which the spores dry on the rice substrate was found to be essential to produce spores with good storage characteristics. Spore extraction was another important step in developing a refined product (Lomer *et al.,* 2001).

Normally, spores are separated from their substrate by simple mechanical sieving. However, for a product intended for ultra-low-volume (ULV) application, a uniform particle size is essential. In particular, even substrate particles the same size as the spores must be excluded, because these particles may subsequently absorb moisture and swell. To achieve the uniform particle sizes needed for ULV, a cyclone air-stream device was developed, which is now available commercially.

The mass production of *B. bassiana* is well advanced and, in particular, the production units of Mycotech Inc. in Butte, Montana, are able to produce spores in high volume at low cost (Lomer *et al.,* 2001). Investigations on production of *Metarhizium* in liquid fermentation are proceeding; both blastospores and aqueous conidia are produced in this way (Lomer *et al.,* 2001). Stephan *et al.,* (1997) cited in (Lomer *et al.,* 2001) described a low-cost blastospore production system based on waste from poultry production and a method of freeze-drying the formulation.

Control of Pine Moth with *Beauveria bassiana:*

The application of *B.bassiana* for pine moth caterpillars, *Dendrolimus* spp., in the People‟s Republic of China, probably represents the largest use of a biocontrol agent. It illustrates several of the features that exemplify a currently successful microbial control

program. There are large and diverse research and application communities in china that have had years of sustained effort that was not impeded by commercial profit demands.

The programs began in the 1950s and expanded in the 1960s when they benefited from a low-cost labor pool that allowed initial production and application without a large capital investment. The early conidia production was in pits and baskets where contamination was minimized by heavy inoculation. Since then, the production has evolved into sophisticated closed systems. In 1980, there were 60 factories in Hunan Province alone, and 85% of the biological control efforts were with *B. bassiana* (McFadden *et al*., 1981 cited in Lord, 2005). At least one million hectares of pine forests are now treated with locally propagated, fungus on cheap bran or peat substrate, and is applied by air or ground equipment, as a spray or dust. Initially, during the 1970s, „mortar bombs" containing firecrackers were used for dispersal of the fungus to control the pine moth in tall trees on plantations.

The technique proved to be effective, but was abandoned in the 1980s because the price of firecrackers made it too expensive to use and the regulation of such goods as firecrackers and fireworks became stricter. It was also a potentially dangerous means of dispersing the fungus even though there were no reports of accidents involving this method. Applications are usually only needed at 3 year intervals(Lord, 2005).

Metarhizium on Field Trials in Africa

The central problem of field trials of slow-acting control agents against migratory pests is always that the treated insects have the opportunity to disperse before the control agent has a measurable field impact. Use of large treatment plots is the obvious solution, but in the early development stages of a biopesticide, only small quantities of active agent may be available and the development of alternative experimental formats becomes important (Lomer *et al.,* 2001).

Field Trials in West Africa: The rice grasshopper, *H. daganensis*, is relatively immobile, and plots of 4 ha were large enough to demonstrate a field impact for

*Metarhizium (*Lomer *et al.,* 1997). Similar-sized plots were used to demonstrate an effect on mixed young nymph populations in Mali (Lomer *et al.,* 2001). In a comparison with a toxic chemical standard, fenitrothion, in 1995, *Metarhizium* outperformed the chemical in every respect except speed of kill. Fenitrothion killed grasshoppers immediately, but after 10 days, insects reinvaded the plots.

Meanwhile, in the *Metarhizium*-treated plots the counts were declining steadily, so that after 10 days, counts in fenitrothion and *Metarhizium* plots were more or less equal. After 10 days, the counts in the fenitrothion plots continued to recover, whereas counts in the *Metarhizium* plots continued to decline. Because the field season lasts only 2–3 months, a single application of mycopesticide provided season-long control, whereas repeat applications of fenitrothion would have been necessary to achieve such control. This result was confirmed in 1998 trials with aerial application on 800-ha plots (Langewald *et al.,* 1999).

Field Trials with Other *Metarhizium Strains* Outside the African Mainland

Australia: In Australia, trials with *M. anisopliae* var. *acridum* FI984 followed a pattern similar to the trials in Africa. An indigenous isolate, FI 984 isolated from *Austracris guttulosa* was found to be pathogenic to target species in laboratory tests (Milner and Prior, 1994) . In the beginning, working with the wingless grasshopper *Phaulacridium vittatum* (Sj¨ostedt), a less mobile species, field trials demonstrated population reductions (Milner *et al.,* 1994). When Australian plague locust populations became available, trials were conducted and were also successful against this species (Lomer *et al.,* 2001). Trials have now progressed to 50-ha plots and the FI 984 mycopesticide have been registered.

Madagascar: In Madagascar, the indigenous *M. anisopliae* var. *acridum* isolate SP9 gave promising results against *L. migratoria* in the laboratory and on 10-ha plots (Lomer *et al.,* 2001) it has now been registered.

Brazil: In Brazil, the indigenous isolate CG423 was found to be virulent to the pest species *Rhammatocerus schistocercoides* and it subsequently has been field tested (Magalh˜aes *et al.,* 2000), with encouraging results.

Factors affecting the distribution of Enthomopathogens and insect hostes

Annually cropped agro-ecosystems are highly disturbed mostly due to tillage regimes and this affects the populations of natural enemies of crop pests. The communities of entomopathogenic fungi in the arable soil environments are different from communities of less disturbed habitats (Meyling *et al.,* 2006) and less disturbance in the cropping system also affect the populations of the fungi. In corn fields in the US, soil densities of B. bassiana (as measured by colony forming units per g of soil) under different tillage regimes were very variable between years, but were seemingly higher in no-tillage systems compared to systems subjected to ploughing and chiseling (Meyling and Eilenberg 2007).

Exposed fungal inoculum is usually inactivated by the UV-components of solar radiation (Fargues *et al.,* 1996). Other abiotic factors affecting entomopathogenic fungi include temperature (Meyling and Eilenberg, 2007) with strains exhibiting different temperature optima for growth. Indeed, temperature, moisture and UV-radiation seem to be most important for B. bassiana survival (Meikle *et al.,* 2003). Persistence of applied fungus material in soils has been studied for several isolates of different species but the complexity of the soil environment makes it difficult to evaluate single factors determining survival (Meyling and Eilenberg, 2007). Factors such as soil texture, pH values and moisture contents have been explored and are thoroughly reviewed in different studies (Meyling and Eilenberg, 2007).

Population increase and infections of hosts Entomopathogenic fungi rely on arthropod hosts to build up population levels of infective stages (mitosporic conidia). During the cropping season outbreaks of diseases can regularly be observed in insect populations in the field, referred to as epizootics. Generally, the development of epizootics rely on host

population dynamics, the number of infective stages in the pathogen population and the viability of these, infection efficiency and development and a complex set of environmental factors and timing (Meyling and Eilenberg, 2007). Considerable information on the biology of the organisms as well as specific environmental parameters (in time and space) is necessary to understand and predict the development of epizootics. Key components of population dynamics of the entomopathogenic fungi are the buildup of the population, the infection of hosts, and the survival and dispersal in the environment (Meyling and Eilenberg, 2007).

However, the persistence and the effectiveness of infection of conidia are also critical properties for the ecological fitness of entomopathogenic fungi. Most conidia are likely to disintegrate quickly in the environment and only minimal proportions will presumably succeed in infecting new hosts. Infection success is density dependent and the number of conidia must exceed a critical threshold level or minimal viable population size (Meyling and Eilenberg, 2007). Thus the acquisition of the threshold of infective conidia by a susceptible host is necessary for the continued survival of the fungus.

Agricultural wastes used as substrates:

The major organic materials available in nature are polymeric such as polysaccharides (cellulose, hemicellulose, pectin, and starch etc.), lignin and protein, which can be metabolized by different microorganisms as a source of energy (Muhammad *et al.,* 2012). Agricultural wastes which are abundant in several industries can be used as raw materials for developing biotechnological process of industrial interest as they are easily assimilated by microorganisms (Orzua *et al.,* 2009). These substrates degrade under certain optimum cultural and nutritional conditions by the microorganisms. Substrates which are insoluble in water absorb water onto their matrix, which provides required moisture for the growth and metabolic activities of microorganisms to allow high rates of biochemical processes; (Khan M. Yakoub and Dahot M. Umar, 2010).

Bacterial and yeast cultures grow on the surface of substrate fibrils and particles while fungal mycelia penetrate into the particles of substrate for nutrition. The solid phase in SSF provides a rich and complex source of nutrients that may be sufficient with respect to the overall nutritional requirements of that particular microorganism. As the microorganisms in solid state fermentation (SSF) grow under conditions closer to their natural habitats, they may get conducive environment for production of certain enzymes and metabolites which otherwise will not be produced with considerable yield in a submerged culture (Luiza, 2000).

The constituents in the agricultural solids are carbohydrates, proteins, lipids, various elements and ash. The solid substrates generally contain some small carbon compounds whereas the bulk of total dry weight is a complex polymer. The polymeric forms require enzymatic hydrolysis for their mineralization as carbon-energy sources for microbial metabolism (Nigam and Pandey, 2009).

Solid-State Fermentation (SSF)

Solid state fermentation (SSF) involves the growth of microorganisms on moist solid substrates in the absence of visible water between the substrate particles (Pandey et al., 2001). In contrast to Submerged (liquid state) Fermentation, Solid State Fermentation (SSF) is the growth of micro organisms under controlled conditions in the absence of free water for the production of desired products of interest. The application of modern biotechnical knowledge and process control technologies can extremely lead to significant productivity increases from this ancient process. Comparative studies between submerged liquid fermentation (SLF) and SSF have proved higher yields and other advantages for products made by SSF (Mienda *et al*., 2011).

- The low availability of water reduces the possibilities of contamination by bacteria and yeast. This allows working in aseptic conditions in some cases.
- Higher levels of aeration, especially adequate in those processes demanding an intensive oxidative metabolism.

- Similar environment conditions to those of the natural habitats for fungi, which constitute the main group of microorganisms used in SSF.
- The inoculation with spores (in those processes that involve fungi) facilitates its uniform dispersion through the medium.
- The substrate usually provides all the nutrients necessary for growth. Therefore culture medium composition is often quite simple.
- Reactors with simple design and few spatial requirements can be used due to the concentrated nature of the substrates.
- SSF is in most cases characterized with low energy requirement which may likely reduces the production cost at industrial level as autoclaving or vapor treatment, mechanical agitation and aeration are not often necessary in some cases.
- Polluting effluents volumes are generally small. Fewer requirements of dissolvent is evident for product extraction due to their high concentration.
- Some SSF bioreactors have easier downstream processing

Despite the mentioned advantages of SSF over SLF, SSF is beset with following disadvantages as reported by

- The substrate in most cases requires pretreatment which include size reduction by grinding, physical or chemical and enzymatic hydrolysis, cooking or vapor treatment.
- Microorganisms like bacteria which may require high moisture levels can perform poorly in SSF. Therefore fungi perform better than bacteria, because of its low moisture requirement.
- Difficulties are usually encountered in biomass determination.
- Monitoring of process parameter such as pH, moisture content, substrate, oxygen and biomass concentration becomes a problem because of solid nature of the substrate.
- Static condition is mostly preferred as agitation most often proved to be very difficult.
- Spores needs to be germinated as they usually have longer lag phases, so cultivation times are longer than in SLF.

Despite the disadvantages of SSF, scientist still believes that is going to solve many of the present industrial production and some of the environmental plights.

SSF utilizes solid substrates, like bran, bagasse, and paper pulp. The main advantage of using these substrates is that nutrient-rich waste materials can be easily recycled as substrates. In this fermentation technique, the substrates are utilized very slowly and steadily, so the same substrate can be used for long fermentation periods. Hence, this technique supports controlled release of nutrients. SSF is best suited for fermentation techniques involving fungi and microorganisms that require less moisture content. However, it cannot be used in fermentation processes involving organisms that require high a_w (water activity), such as bacteria (Subramaniyam and Vimala, 2012).

Substrates used for fermentation

The outcome of fermentation highly varies for each substrate; hence, it is extremely important to choose the right substrate. Fermentation techniques have to be optimized for each substrate. This is primarily due to the reason that an organism reacts differently to each substrate. The rates of utilization of various nutrients differ in each substrate, and so does productivity Subramaniyam and Vimala (2012). Some of the common substrates used in solid state fermentation are wheat bran, rice and rice straw, hay, fruit and vegetable waste, paper pulp, bagasse, coconut coir, and synthetic media Pandey *et al.*, (1999).

Ecological and Economic benefits of biological control emphasizing the positive:

The reaction of the biological control community regarding the non-target impacts by biological control agents and subsequent criticism of the use of this technology by reputable biologists has been largely defensive. Two recent books Nontarget Effects of Biological Control (Follet and Duan, 2000) and Evaluating Indirect Ecological Effects of Biological Control (Wajnberg, Scott, and Quimby, 2000) advocate improved host specificity testing procedures, more rigorous field assessments of potentially rogue agents with food web analyses, greater legislative guidance, more use of theoretical community assemblage studies, and ownership of past mistakes with a vision to strive towards improved safety and greater efficacy. Retrospective analyses of data sets that have well

quantified non-target impacts may be invaluable in guiding studies and legislation that aim to improve the safety of biological control programs Louda *et al.*, (2003).

A major publication by respected biological control scientists that compiles and documents the positive ecological and economic benefits derived from classical biological control programs is needed to temper some of the current criticism directed at the use of natural enemies for pest suppression. These case studies need not be limited to terrestrial arthropods and weeds and the scope should be expanded to include new projects focusing on the biological control of "non-traditional" targets. New targets being evaluated for biological control include marine algae, green crabs, zebra mussels, brown tree snakes, and vertebrates with sterilizing viruses.

Economic and Political issues

In attempting to draw up a concise overview of the political and economic issues associated with cereal pest attacks and the economic benefits of different treatment options, Lomer *et al.*, (2001) gives the following points which needs to be considered:

1. Locust plagues are essentially unpredictable; the locust swarm may fly across desert areas into the sea, attack subsistence millet fields, or invade high-value crops. Therefore, not only is the development and movement of the swarm population unpredictable, but also the value of the damage is even more unpredictable and difficult to estimate. Nevertheless, the unpredictable risk could be reduced by containing the pest outbreak at its source.
2. The total cash value of the crop damage may be modest by most standards, but even modest amounts of total damage may be very severe in some localities and cause disruptions in the local economy. In farming systems where >90% of the crops are produced for the personal subsistence of farmers, the use of simple cost-benefit ratios based on the market value of the crops can be questioned Lomer *et al.*, (2001). Food

aid has sometimes been proposed as an alternative to treatments, but such aid brings its own external costs in terms of the disruption of local food-production systems.

3. Most entomological cost-benefit analysis formats rely on the benefit that accrues to the farmer carrying out the control treatments. This formula can be difficult to apply in the case of a migratory pest. Indeed, for highly mobile species such as the desert or brown locust, the burden of control may fall on one country whereas the benefits may be accrued somewhere else completely.
4. More than half of pest controls costs are usually met by external donors‟ who may have their own objectives. In addition, the nature of donor funding for locust control is such that funds are normally considered as one-off disaster funds, released for plague treatment, but not for preventive treatments. This can lead countries to exaggerate infestations so that emergency funds can be used for routine control.
5. Because of public pressure or confounded interests of decision makers, governments may conduct control operations irrespective of any expected economic benefit. The environmental impact of such treatments is also high.
6. Even where control is justified and necessary, local environmental and economic factors may place restrictions on the type and nature of control operations. For example, use of conventional chemical insecticides may be inappropriate in areas of organic livestock production and national parks and may create pressure for alternative technologies.

Recent studies by the World Bank in Madagascar address some of these concerns. Wright estimated a benefit:cost ratio of between 23:1 and 29:1 for chemical control operations against the Australian plague locust, *Chortoicetes terminifera* Walker.

It can be concluded from these observations that locusts will be controlled, irrespective of the costs and benefits. Furthermore, (*a*) preventive locust control is cheaper and less environmentally damaging than plague control; (*b*) accurate estimates of the benefits of control are unlikely to be possible in Africa; (*c*) preventive control will reduce risks of outbreaks and concomitant plague control costs but continuous low-level donor support is needed, even when locust populations are low and the immediate risks minimal. The difficulty in maintaining donor and government support for long-term preventive control

programs (and hence maintaining the skilled knowledge base and infrastructure that this brings) is one of the factors that lead to the exacerbation of problems in outbreak situations.

Environmental issues

Environmental issues arising from the standard use of chemical pesticides against locusts and grasshoppers include the impact on operators, other people, livestock, birds, other terrestrial vertebrates (especially lizards), aquatic organisms (fish and invertebrates), and terrestrial arthropods, including the natural enemies of locusts and grasshoppers, as well as pollution issues, contamination of groundwater and wells, and disposal of surplus pesticide stocks Lomer *et al.,* (2001). Several publications deal with the state of our knowledge when the alarm was first raised and much useful research has been conducted since then. Murphy et al cited in Lomer *et al.,* (2001) reviewed the toxicities of commonly used pesticides and found that in 45%–55% of the records, the chemicals gave mortality rates >90% in nontarget species. Initially, desert environments were viewed as fragile per se more recently; attempts have been made to define which particular environments are most at risk. In general, ephemeral aquatic habitats are especially vulnerable, particularly if used by migratory birds as feeding and resting areas.

Few attempts have been made to quantify the external costs associated with grasshopper and locust control; Houndekon and DeGroote 1998 cited in Lomer *et al.,* (2001) estimated veterinary, health, and disposal costs and found a small but significant value for these externalities. Numerous studies relate the negative impacts of chemical pesticides on nontarget organisms. Peveling *et al.,* 1997 cited in Lomer *et al.,* (2001) showed that in Madagascar even single applications of fenitrothion, which has shorter environmental persistence, caused long-lasting population declines among epigeal collembollans. Van der Valk H *et al.,* (1999) summarized the impact of insecticide treatments on terrestrial arthropods, particularly natural enemies of acridids, and discussed the possibility that insecticide applications may have increased the impact of grasshoppers in the Sahel.

With the available information on the selectivity of chemical pesticides and the areas particularly at risk, it should be possible to design pesticide use so that environmental damage is minimized while control objectives are achieved. For instance, Martin PA *et al*., (1998) investigated the impact of treating grassland with the pyrethroid deltamethrin to control grasshoppers on the availability of insects, primarily grasshoppers, as a food source for grassland songbirds.

Levels of control of 90% were economically effective while allowing persistence of sufficient numbers of grasshoppers to allow survival of nestlings and successful fledging. Similarly, the use of low levels of insecticides with known low avian toxicity, such as carbaryl in wheat bran bait has been shown to result in reductions of 70%, which allows survival of post treatment populations of 1–5 grasshoppers per square meter. Such levels are well below the economic threshold, but are above the levels required for survival of insectivorous grassland birds.

A slightly different approach that focuses on reduction of the total area treated is proposed by Schell and Lockwood 1997 cited in Lomer *et al.,* (2001). The long persistence of both insect growth regulator chemicals and fipronil means that they can be used to treat barrier strips in much the same way as dieldrin was used. In this way, the total dose per hectare is much reduced, as is the impact on nontarget organisms Lomer *et al.,* (2001). Despite these advances, there remains scope for the use of biological control.

The future of Microbial control:

Reviews of the prospects for microbial control are not always optimistic, and there is little doubt that some of the familiar approaches will fade away. What will drive microbial control in the future? It is apparent that utilization of pathogen genes, especially for toxins, will continue to be a productive major thrust. It is unlikely that before World War II anyone could have foreseen the agricultural revolution that would develop from the implantation of bacterial genes into plants. No doubt, there are useful discoveries to be made as the genomes of less understood pathogens become sequenced

and studied. Certainly, the revolution in genetic manipulation will have a profound and unpredictable impact on the future on microbial control Lord (2005).

According to Lord 2005 certain past trends are likely to continue. Microbial control has had many of its greatest successes where chemical insecticides are not practical or acceptable, such as in forestry. There is an ongoing shift in the venues of inundative applications of mass-producible pathogens that is likely to continue. The less developed countries offer advantages for low-cost production and manual application, which allows the necessary contact delivery. One of the most difficult barriers to commercial microbial pesticides has been the gauntlet of regulations for individual national and regional governments. Less developed nations have less onerous regulatory constraints. In the developed countries, the organic farming movement and anti-chemical sentiment will continue to offer opportunities for microbial control for the foreseeable future.

Several forces are at work that offers encouragement for the future of microbial control. While the progress to date has been gradual, there is promise for improved regulatory harmonization, significantly reducing development costs. As more is learned about the biology of pathogens, not only can they be used more efficiently, but government regulators will be better able to judge and document pathogen safety. Aside from the regulation of microbial control itself, the current regulatory climate offers an opportunity for pathogens in the loss or restriction of many traditional chemicals Lord (2005).

No doubt, there are unpredicted opportunities for the conservation strategy to enhance natural control. The successful, if unintentional, introductions that have occurred in the past and ever improving detection methods argue for continued pursuit of that approach. In the past, much of the success in classical biological control by introduction of microbes has been due to fortunate discoveries. With advances in sampling and detection technology and in our knowledge of pest and natural enemy ecology, selection and implementation of agents and strategies will improve. However, the barriers to importation of beneficial species will have to be made less problematic.

4. Material and Methods

Source of *Metarhizium* and *Beauveria* isolates

Two *Beauveria* and three *Metarhizium* isolates were obtained from the Mycology Laboratory, Department of, Microbial, Cellular and Molecular Biology, Addis Ababa University (AAU), Culture collection. All the isolates of *Beauveria* and *Metarhizium* used in this study were isolated from insect cadavers of grasshoppers, beetles, and locusts from soil samples of south wastern parts of Ethiopia. The acronyms given to the isolates were derived from Initial letters of the Universities name and the genus name of the isolate followed by the letter "I" to represent isolate and number to differentiate which isolate number is it.

Table 1. Designation and places of fungal isolates

Fungal isolates	Designation	Place of Isolation
Addis Ababa University *Beauveria* isolate 1	AUBI 1	Jimma Sekachokorssa
Addis Ababa University *Beauveria* isolate 2	AUBI 2	Jimma Sekachokorssa
Addis Ababa University *Metarhizium* isolate 1	AUMI 1	Jimma Sekachokorssa
Addis Ababa University *Metarhizium* isolate 2	AUMI 2	Jimma Sekachokorssa
Addis Ababa University *Metarhizium* isolate 3	AUMI 3	Jimma Sekachokorssa

For laboratory study the Fungal isolates was first grown on Sabouraud dextrose agar (SDA) medium using standard methods (Holder and Keyhani 2005, Masoud Latifian *et al.,* 2013). After complete growth of the fungal isolates the media were kept in refrigeration temperature (4^0_C) for further study.

Preparation of conidia suspension:

Conidia inoculum from 14 days old culture was harvested using sterile 0.02% (v/v) Tween 80 solution by scrapping spores from the mycelial mat using hockey glass stick. The spores of *Metarhizium* and *Beauveria* were collected in to conical flasks. The conidial suspensions of the isolates were filtered through three layers of cheese cloth and their concentrations were determined by direct counting using heamocytometer under phase contrast microscopy (Wang *et al.*, 2002).

Procurement of Agricultural wastes for conidial production: The agricultural wastes were collected to use in this study was collected from different areas in Addis Ababa. Wheat bran was collected from Misrak flour and sweet food factory located around Gottera of Addis Ababa city administration. The Coffee husk was collected from Mullegea private limited company which is one of the exporters of coffee Arabica from Ethiopia, and the tea and vegetable wastes were collected from Addis Ababa University students cafeteria. All wastes were initially clean to remove debris and washed with water followed by drying under shade condition. Subsequently, all the wastes were ground in to 1mm, 2mm, and 4mm size powder using a Hammer beater mill. The powdered form of each waste was stored in plastic bags for subsequent study.

Solid state fermentation using agricultural substrate for conidia production: Ten grams (10g) of each of the agricultural waste were used for a single inoculum experiment in a sterilized heat resistant 20x30cm^2 sized plastic bags to which 1ml of seeding inoculum with conidial concentration of 1.0 x 10^5 conidia/ml was sprayed using hand sprayer. The samples were then incubated in light transparent incubator at the appropriate temperature 27^0C for two weeks (Pham *et al.*, 2010).

Within this form of solid state fermentation technique all high conidial biomass producing wastes were evaluated to optimize conditions such as temperature, pH, moisture content, incubation period, inoculum concentration, inoculum size and the effect of light for maximum conidial productivity. All experiments in this research finding were replicated three times.

Conidia harvesting from the substrates medium: From each substrate medium 1g of 14days old conidiated culture was suspended in 20ml of 0.05%Tween 80solution in beaker. The content was then vigorously agitated by hand for 3-5 minutes to dissociate conidia clumps and filtered through three layered cheese close. The conidial number was counted after making serial dilution until countable number of spors over the grids.

Conidia counting:

Using a micro pipette 20 microliters of spore suspension were sucked and loaded to a clean cover slip affixed heamocytometer with a pipette tip. Chambers of the heamocytometer were allowed to fill via capillary action carefully not to overfill or underfill it. There are four big squares in the heamocytometere with 16 smaller squares containing each, all the cells in the four corner squares of each big square were counted by placing the heamocytometer chamber on the microscope stage. Numbers of conidia count on the four outer squares in the grids were recorded. Calculating cell concentration per milliliter was conducted using the following formula (Bastidas www.celeromics.com).

Cell concentration/ml = average number of one large square X dilution factor X 10^4

NB. 10^4= conversion factor to convert 10^{-4}ml to 1ml

Or

$$\textbf{Cell } concentration/ml = \frac{Number\ of\ cells * 10000}{number\ of\ squares * dilution\ factor}$$ (Bastidas www.celeromics.com).

Stock cell concentration per milliliter and conidial concentration per gram of substrate were arithmetically calculated respectively.

Spore viability: The viability of conidia was determined by spread plating. According to (Fatima *et al.,* 2013) 1ml of conidial suspension was titrated to $1x10^3$ conidia/ml on (8cm) sized Sabouraud dextrose agar plates and incubated at 27^0C for 12-18 hours. Spore germination was held by placing 3-separate drops of lactophenol cotton blue and then sterile microscopic cover slips were placed over the stained droplets. The proportion of viable conidia were determined by examining 100 spores in each of the three different fields of view at 400X magnification with a compound microscope and determining the proportion of spores that possessed a distinct germ tube, as defined by germ tube lengths that are two times the diameter of the spore as stated on (Philip *et al.,* 2011).

Optimization of moisture content for conidia production: The moisture content for different agricultural wastes was determined by oven drying method as stated on Rao et al., (2006). Labeled small glass bottles were placed in an oven at approximately 80^0_C for about two hours to ensure that they were completely dry. Ten (10 g) gram of substrate from each agro-industrial waste was added to their respective glass bottles and make a note of the new weight for wet weight of the substrates by replacing the cover lids.

The container bottles with substrates were placed back in to the oven at 60^0C for one to six hours until constant weigh. Finally moisture content of the substrates was calculated based on wet-weight basis and express it as a percentage to one decimal place, using the following formula Rao *et al.,* (2006).

$$\text{Moisture content (\% wb)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} * \mathbf{100}$$

Based on the above stated formula moisture content of different agricultural wastes were adjusted to 35%, 45%, 60% and 80% respectively to determine the maximum conidial production capability of *Metarhizium* and *Beauveria* isolates under solid state fermentation (SSF) technique.

Optimization of temperature for maximum conidia production: The isolates were inoculated in to their respective substrate as before and incubated in plastic bags at 24^0C, 27^0C and 30^0C for 14 days to study the effect of temperature on maximum conidial production. Each of the 10g substrate containing plastic bags were sprayed with 1ml of $1x10^5$ conidia concentration and tied up with rubber band to prevent air blow and contamination in the incubator. Aeration was allowed to the culture by providing two 1cm sized holes at the top of the plastic container. (Pham *et al.,* 2010).

Optimization of pH for conidia production: Ten (10g) gram of substrates from each agricultural waste was weighed to examine under different pH levels. The pH were adjusted to 3.5, 4.5, and 5.5 using 1N of HCL (hydrochloric acid) and NaOH (sodium hydroxide) to determine the optimum pH for maximum conidia production of the isolates. The media was autoclaved at 121^0C for 15min and transferred to their respective plastic bags under aseptically safe conditions. After the substrates were allowed to cool 1ml of $1x10^5$ conidia concentration of each isolate was sprayed to their respective plastic bags followed by incubation at 27^0C. After 14 days conidial productivity was studied by suspending 1g of conidiated substrate in 20ml of distilled and sterile water. The conidia yield was determined by heamocytometer count after filtering the suspension by three layers of cheese cloth.

Optimization of incubation period for conidia production: The effects of incubation period for maximum conidial production on sterilized agricultural wastes were evaluated by adjusting the incubation period to 14, 21, and 28 days respectively. Substrates of coffee husk, wheat bran, vegetable waste and tea waste were sterilized at 121^0_C for 15 min and transferred to their respective plastic bags aseptically with moisture content adjustment according to their high productivity as it was indicated in the above

experiment. All the samples were inoculated with 1x 10^5 conidia concentration and incubated at 27^0C for the required period of time interval. After 14, 21, and 28 days of cultivation conidial productivity per gram of substrate was filtered through three layers of cheese cloth and enumerated by heamocytometer count.

Optimization of inoculum size for conidia production: Ten gram of each sterilized substrates of coffee husk, wheat bran, vegetable waste, and tea waste were inoculated with 1ml, 2ml, and 4ml inoculum size of $1x10^5$ conidial concentrations per milliliter in to their respective plastic containers. Moisture content of each experimental substrate was adjusted according their results of high productivity during optimization of moisture. The inoculated substrates were allowed to incubate at 27^0C in light transparent incubator for two weeks. After 14 days of successive incubation period 1g of conidiated substrate were suspended in 20ml of sterilized water and filtered by three layers of cheese close. Numeration of conidial productivity was held by heamocytometer and the maximum conidial count was considered as the best standard inoculum size for the substrate.

Optimization of inoculum concentration for conidia production: The sterilized substrates in the plastic bags were adjusted at the best moisture level. Conidial concentrations per gram of each substrate were known after numerated by heamocytometer for stock concentration preparation. Stock concentration of each substrate was prepared to adjust different conidial concentrations of $1x10^3$, $1x10^4$, and $1x10^5$ in different flasks used to conduct the experiment. Conidial concentration preparation was conducted using the following formula as indicate in the Insect Pathology manual.

$$X = \frac{\text{Required concentration} * \text{Final volume Needed}}{\text{Counted concentration}}$$

Where

X= *number of ml of spores from original suspension to be added to the distilled water*

The serial dilutions of conidial concentrations prepared were sprayed in to their respective substrates arranged and replicated three times. Each of the agricultural waste substrates was evaluated by all the prepared concentration levels with 1ml of inoculum size incubated for 14 days. Conidial harvest and numeration was conducted by taking 1g of conidiated substrate from each experimental plastic bag making a suspension using 0.05% Tween 80 solution containing distilled water. Conidia productivity was determined by numerating under heamocytometer after the suspension was filtered through three layers of cheese close.

Effect of light on conidia production: Ten (10g) gram of agricultural substrates were weighed for each plastic bag in sufficient number and autoclaved at 121^0C for 15 min. sterilized substrates were transferred to plastic bags under aseptic technique and inoculated with 1ml of $1x10^5$conidia/ml. Inoculated substrates were placed in to incubator in two types of plastic bags in order to determine the effect of light on conidia production at 27^0C. One of the plastic bags were transparent to light and the control was opaque both with aeration pores at the top as stated on (Zuriash Mamo and Tesfaye Alemu 2012). The cultures were incubated for 14 days period of time in order to harvest matured conidial yield. Finally 1g of conidial yield containing substrate was suspended from each plastic bag by 0.05% Tween 80 solution containing distilled water and numerated by heamocytometer after filtered through three layers of cheese close.

Data analysis: All the data were collected and analyzed using SPSS (version 20).conidia production potential of each substrate with respect to each isolate were analyzed by Mean±SEM (standard error of mean) and Mean difference of each isolates conidia yield concentration on different substrates were computed using two sample t-Test.

5. Results

Evaluation of agricultural wastes for conidia production: The effect of substrate and substrate size on conidia production of entomopathogenic fungi *Beauveria* and *Metarhizium* isolates is shown on Table 2. Accordingly Vegetable wastes supported high conidial productivity of AUBI2, AUMI1, AUMI2 and AUMI3 with ($3.82\pm0.63x10^7$ conidia/gram of substrate, $4.90\pm0.33x10^7$ conidia/gram of substrate, $3.74\pm0.57x10^7$ conidia/gram of substrate and $3.62\pm0.44x10^7$ conidia/gram of substrate respectively).

However, AUBI1 was supported by Wheat bran which was recorded $4.18\pm0.84x10^7$ conidia/gram followed by the Vegetable waste ($3.75\pm0.53x10^7$ conidia/gram of substrate). Tea waste substrate produces the second highest conidial yield of isolates AUBI2, AUMI1 and AUMI2 (2.26±0.40 conidia/gram, 1.41±0.16 conidia/gram and 1.50±0.14 conidia/gram respectively). The second maximum conidial yield for the isolate AUBI1 and AUMI3 was harvested from vegetable waste and wheat bran respectively.

Table 2 illustrates the overall conidial productivity of each substrate under solid state fermentation technique.

Table 2: Conidia yields of *Beauveria* and *Metarhizium* isolates on different agricultural wastes

Substrates	Mean±SEM of conidia count of substrates at ($x10^7$ conidia/gram) after 14 days of incubation				
	Beauveria and *Metarhizium* isolates				
	AUBI1	AUBI2	AUMI1	AUMI2	AUMI3
1mm Coffee husk	1.27±0.13	1.22±0.08	0.83±0.08	1.06±0.18	0.60±0.07
2mm Coffee husk	0.33±0.03(b)	0.33±0.03(b)	0.20±0.02(b)	0.46±0.05	0.33±0.04(b)
4mm Coffee husk	0.65±0.08	0.46±0.12	0.62±0.15	0.36±0.07(b)	0.42±0.08
Tea waste	2.08±0.17	2.26±0.40	1.41±0.16	1.50±0.14	0.89±0.10
Wheat bran	4.18±0.84(a)	1.20±0.22	1.14±0.13	1.38±0.15	1.09±0.10
Vegetable waste	3.75±0.53	3.82±0.63(a)	4.90±0.33(a)	3.74±0.57(a)	3.61±0.44(a)

AUBI1= Addis Ababa University *Beauveria* Isolate one AUBI2 = Addis Ababa University *Beauveria* Isolate two

AUMI1 = Addis Ababa University *Metarhizium* Isolate one AUMI2 = Addis Ababa University *Metarhizium* Isolate two

AUMI3 = Addis Ababa University *Metarhizium* Isolate three

a= maximum conidia yield b= list conidia yield

Mean calculated from three replications, all the isolate cultures on each substrate was incubated at 27^oC for 14 days.

Results of Table 2 has indicated that vegetable wastes favored maximum conidial productivity of both *Beauveria* and *Metarhizium* isolates when the fungal isolates were

treated at 27^0C for 14 days of incubation under solid state fermentation. The lowest conidial productivity was overall recorded on coffee husk ($0.2 – 1.27x10^7$) conidia/gram.

AUBI1 showed maximum conidia yield on wheat bran for *Beauveria Isolates*, whereas AUMI1 showed the maximum conidial yield on vegetable waste from *Metarhizium* isolates. The lowest conidia yield was recorded by AUBI2 on 2mm size coffee husk from *Beauveria* isolate and AUMI1 on 2mm size coffee husk from *Metarhizium* isolates. It is clearly shown on (Fig 1), that the conidia yield of all isolates on 2mm size coffee husk was not significantly different but conidia yield of AUBI1 on wheat bran was significantly different from the rest of the isolates of both *Beauveria* and *Metarhizium* isolates.

Based on two sample of t-test statistical analysis of the agro industrial wastes taking the highest and the lowest conidia yielding substrates "The mean conidia productivity of AUBI1 on wheat bran was significantly different from 2mm size coffee husk, $t(11.04) =3.84$, $p = 0.001$."

"The mean conidia productivity of AUBI2 on vegetable waste was significantly different from 2mm size coffee husk, $t(9.06) = 3.49*10^7$, $p = 0$."
The same was true for AUMI1 on vegetable waste and 2mm size coffee husk, AUMI2 on vegetable waste and 4mm size coffee husk and AUMI3 on vegetable waste and 2mm size coffee husk respectively. "Mean conidia yield of AUMI1 was significantly different on vegetable waste than 2mm size coffee husk, $t(11.06) = 4.70*10^7$, $p = 0$."

Comparing two means using two sample t-Test revealed that "The mean conidia yield of AUMI2 and AUMI3 on vegetable wastes were significantly different form conidia yields on 4mm and 2mm size coffee husk, with $t(11.35) = 3.38*10^7$, $p = 0$ and $t(11.23) = 3.28*10^7$, $p =0$ respectively."

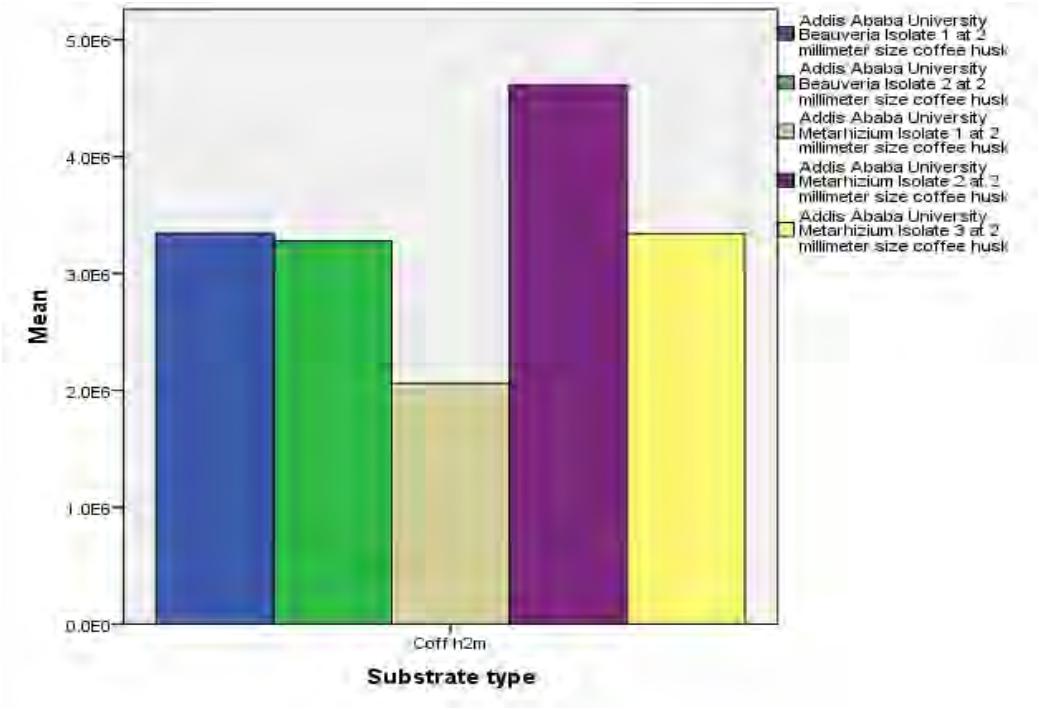


Fig 1: Conidia yield of isolates on 2mm size coffee husk

Mean calculated form three replications, each isolate was cultured at 27^{o}_{C} for 14 days at moisture content of 35% for *Beauveria* isolates and at 45% for AUMI1, AUMI3, and 60% for AUMI2.

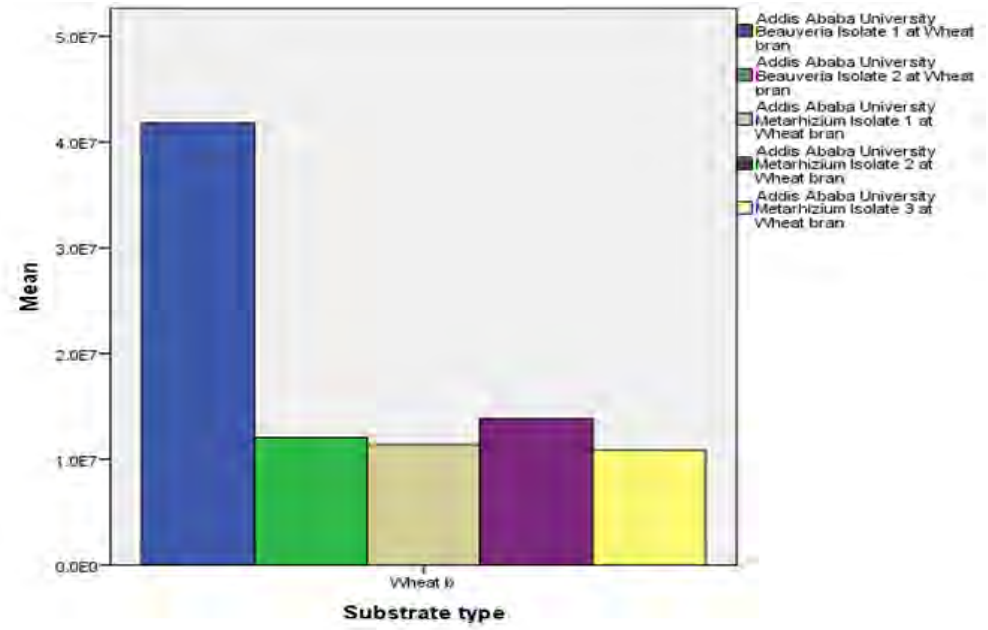


Fig 2: Conidia yield of isolates on wheat bran

Mean of the conidia yield was calculated from three replicates and each isolate was cultivated at 27^{0}_{C} for 14 days under sufficient light exposure. Moisture content was adjusted at 60% for all isolates.

Effect of Moisture content on conidia production of *Beauveria* and *Metarhizium* isolates: To study the effect of moisture content on conidia productivity of each substrate type, isolates were incubated at 35%, 45%, 60% and 80% of moisture content of each substrate for 14 days of incubation at 27^0C. The maximum conidia harvest favored moisture content was taken as optimum. It was observed that the optimum moisture content for conidia production of each isolate vary with in the same substrate type and among the substrates (Table 3). The results in table 3 show that 35% of moisture content was the optimum for isolate AUMI1 and AUMI2 which was given (0.96±0.12 conidia/gram of substrate and 1.62±0.44 conidia/gram of substrate) in 1mm size coffee husk. Whereas, 45% moisture content was the optimum for AUBI1 and AUMI3 with conidia count (1.86±0.26 and 0.82±0.07 conidia/gram of substrate respectively) except that 80% is the optimum for AUBI2 in 1mm size coffee husk.

At substrate 2mm size coffee husk 35% of moisture content were favored the maximum conidia harvest for isolate AUBI1 and AUBI2 with 0.42±0.06 and 0.47±0.05 conidia/gram of substrate respectively. The isolates AUMI1 and AUMI3 produces high conidial biomass of 0.26±0.02 and 0.49±0.10 conidia/gram of substrate respectively at 45% moisture content of 2mm size coffee husk while AUMI2 give (0.57±0.10 conidia/gram of substrate) at 60% of moisture content.

The optimum moisture content for all isolates in the substrate of vegetable waste was 60% and the conidia count recorded for AUBI2, AUMI1, AUMI2, and AUMI3 is significantly high (5.77±1.53, 5.80±0.72, 4.44±0.55 and 5.58±0.66 conidia/gram of substrate respectively) among the rest of the substrates. Except that the highest conidia count for AUBI1 was recorded on 60% of moisture content at wheat bran (6.34±2.27 conidia/gram of substrate). Vegetable waste scored the second largest conidia harvest of AUBI1 (5.19±1.04 conidia/gram of substrate) at 60% moisture content.

The result obtained in Table 3, reveals that the total conidia harvest of each isolate with respect to moisture contents on substrates of 2mm and 4mm size coffee husks was lower than any other substrate used in this study. AUBI2, AUMI2, AUMI1, and AUBI1and

AUMI3 gives their maximum conidia productivity (0.63±0.43, 0.40±0.21, 1.01±0.52, and 0.74±0.02, 0.57±0.17 conidia/gram of substrate) at 35%, 45%, 60% and 80% moisture content of 4mm size coffee husk respectively.

Table 3. The effect of different levels of moisture on conidia yield of *Beauveria* and *Metarhizium* isolates after 14 days of incubation at 27^0C

Substrates	Mean ±SEM of conidia count at ($x10^7$ conidia/gram) for moisture content optimization after 14 days of incubation																			
	AUBI1				AUBI2				AUMI1				AUMI2				AUMI3			
	35%	45%	60%	80%	35%	45%	60%	80%	35%	45%	60%	80%	35%	45%	60%	80%	35%	45%	60%	80%
1mm size coffee husk	1.13±0.16	1.86±0.26	0.98±0.15	1.09±0.17	1.30±0.12	0.93±0.10	1.29±0.12	1.36±0.25	0.96±0.12	0.95±0.09	0.95±0.18	0.46±0.06	1.62±0.44	1.39±0.20	0.69±0.18	0.55±0.08	0.63±0.03	0.82±0.07	0.68±0.15	0.27±0.04
2mm size coffee husk	0.42±0.06 (b)	0.29±0.05	0.36±0.11	0.27±0.02	0.47±0.05 (b)	0.36±0.03	0.21±0.02	0.27±0.06	0.18±0.04	0.26±002 (b)	0.24±0.02	0.14±0.02	0.53±0.10	0.39±0.09	0.57±0.10	0.34±0.03	0.24±0.06	0.49±0.10 (b)	0.40±0.02	0.20±0.05
4mm size coffee husk	0.93±0.20	0.32±0.04	0.63±0.07	0.74±0.02	0.63±0.43	0.39±0.23	0.40±0.18	0.42±0.17	0.37±0.18	0.65±0.19	1.01±0.52	0.46±0.19	0.37±0.20	0.40±0.21 (b)	0.30±0.09	0.38±0.14	0.33±0.14	0.46±0.22	0.33±0.10	0.57±0.17
Tea waste	1.90±0.22	2.03±0.24	2.53±0.50	1.86±0.37	1.34±0.45	1.52±0.18	2.31±0.64	3.62±0.92	1.07±0.07	0.96±0.18	1.55±0.37	2.05±0.10	0.94±0.07	1.27±0.14	1.89±0.12	1.90±0.23	0.78±0.06	1.32±0.15	0.83±0.24	0.63±0.05
Wheat bran	2.36±0.27	2.03±0.04	6.34±2.27 (a)	5.98±1.59	2.00±0.49	0.91±0.39	1.13±0.37	0.79±0.32	1.25±0.34	1.46±0.09	1.01±0.26	0.82±0.20	1.56±0.23	1.59±0.10	1.43±0.56	0.95±0.08	1.19±0.34	1.07±0.27	1.10±0.09	0.99±0.13
Vegetable waste	1.87±0.27	2.96±0.42	5.19±1.04	4.96±1.06	2.21±0.05	2.88±0.30	5.77±1.53 (a)	4.24±0.52	4.34±1.08	4.56±0.09	5.80±0.72 (a)	4.92±0.40	3.53±2.23	3.80±0.59 (a)	4.44±0.55	3.19±0.95	2.98±0.46	2.38±0.78	5.58±0.66 (a)	3.52±0.34

AUBI1= Addis Ababa University *Beauveria* Isolate one AUBI2 = Addis Ababa University *Beauveria* Isolate two

AUMI1 = Addis Ababa University *Metarhizium* Isolate one AUMI2 = Addis Ababa University *Metarhizium* Isolate two

AUMI3 = Addis Ababa University *Metarhizium* Isolate three

a= maximum conidia yield b= list conidia yield

Mean calculated from three replications, all the isolate cultures on each substrate was incubated at 27^o_C for 14 days.

There was a gradual increment in the conidia count record of AUBI2, AUMI1 and AUMI2 as the effect of moisture content increases from 35 to 80% using tea waste as substrate. However, conidia count result of AUBI1 and AUMI3 at tea waste is higher around 60% and 45% of moisture content. Therefore, tea waste specially provides its highest conidia count record at 80% of moisture content for three isolates out of the total five next to vegetable wastes which provides the highest conidial count record for all isolates of both *Beauveria* and *Metarhizium* at 60% of moisture content (Table 3).

Wheat bran give the maximum conidia yield of AUBI1 isolates at 60% moisture content (6.34±2.27conidia/gram of substrate) whereas the rest AUBI2, AUMI3 and AUMI1, AUMI2 (2.00±0.49, 1.19±0.34 and 1.46±0.09, 1.59±0.10 conidia/gram of substrate) highest conidia yield record falls within 35% and 45% of moisture content respectively.

According to two sample t-Test statistical analysis "the mean conidia yield of AUBI1 at wheat bran ($M = 6.34*10^7$, $SD = 3.94*10^7$, $N = 3$) was not significantly different from 2mm size coffee husk ($M = 4.18*10^6$, $SD = 1.08*10^6$, $N = 3$), $t(2) = 5.92*10^7$, $p = 0.12$."
The mean conidia yield of AUBI2 at vegetable waste ($M = 5.77*10^7$, $SD = 2.65*10^7$, $N = 3$) was significantly different from 2mm size coffee husk ($M = 4.68*10^6$, $SD = 9.41*10^5$, $N = 3$), $t(4) = 5.30*10^7$, $p = 0.03$ whenever equal variances are assumed but, if equal variances not assumed the mean conidia yield of vegetable waste ($M = 5.77*10^7$, $SD = 2.65*10^7$, $N = 3$) was not significantly different from 2 mm size coffee husk ($M = 4.68*10^6$, $SD = 9.41*10^5$, $N = 3$), $t(2) = 5.30*10^7$, $p = 0.07$.
The mean conidia yield of AUMI1 at vegetable waste ($M = 5.81*10^7$, $SD = 1.25*10^7$, $N = 3$) was significantly different from 2mm size coffee husk ($M = 2.57*10^6$, $SD = 3.79*10^5$, $N = 3$) $t(4) = 5.55*10^7$, $p = 0.002$.
The mean conidia yield of AUMI2 at vegetable waste ($M = 4.45*10^7$, $SD = 9.47*10^6$, $N = 3$) was significantly different from 4mmsize coffee husk ($M = 4.05*10^6$, $SD = 3.60*10^6$, $N = 3$) $t(2.57) = 4.04*10^7$, $p = 0.01$ when equal variances are not assumed.
The mean conidia yield of AUMI3 at vegetable waste ($M = 5.59*10^7$, $SD = 1.15*10^7$, $N = 3$) was significantly different from 2mm size coffee husk ($M = 3.63*10^6$, $SD = 5.80*10^5$, $N = 3$) $t(4) = 5.22*10^7$, $p = 0.001$.

N.B: M = mean of the conidia yield , SD= standard deviation of the group
t = t-Test for equality of means, P = P- value

Effect of temperature on conidia production of *Beauveria* and *Metarhizium* isolates:- Incubation temperature markedly affects conidia yield as it can be noticed in Table 4. The majority of the isolates in all substrate types the optimal temperature for high conidia productivity was 30^0C after 14 days of incubation. AUBI1, AUBI2, AUMI1 and AUMI2 got their maximum conidia yield of (1.78±0.16, 2.75±0.24, 2.09±0.25 and 1.28±0.27conidia/gram of substrate respectively) when incubated for 14 days at 30^0_C using coffee husk as a substrate. Whereas the optimum conidia yield for AUMI3 in coffee husk was found at 24^0C after 14 days of incubation.

Among the substrate types examined wheat bran for AUBI2 (6.25±0.84 conidia/gram of substrate) and vegetable waste for AUMI2 and AUMI3 (2.97±0.16 and 4.60±0.94 conidia/gram of substrate respectively) supported for maximum conidia yield at 30^0C optimum temperature. However, vegetable waste also supported AUBI1 (6.40±0.63 conidia/gram of substrate) and AUMI1 (5.05±0.43conidia/gram of substrate) for maximum conidia yield at 27^0C optimum temperature. The optimum temperature for AUBI1, AUMI1 and AUMI2 grown at tea waste substrate was 30^0C with a result of 0.91±0.22, 1.10±0.04 and 0.81±0.10 conidia/gram of substrate respectively. Among the isolates tea waste at 27^0C optimum temperature favored maximum conidia yield for AUBI2 and AUMI3 (1.71±0.22 and 1.43±0.28 conidia/gram of substrate) respectively.

Compering two means based on two sample t-Test the mean conidia yield of AUBI1 at vegetable waste ($M = 6.41*10^7$, $SD = 1.09*10^7$, $N = 3$) was significantly different from tea waste ($M = 9.07*10^6$, $SD = 3.79*10^6$, $N = 3$), t (2.48) = $5.50*10^7$, p = 0.007 when equal variances are not assumed.
The mean conidia yield of AUBI2 at wheat bran ($M = 6.25*10^7$, $SD = 1.45*10^7$, $N = 3$) was significantly different from tea waste ($M = 1.72*10^7$, $SD = 3.79*10^6$, $N = 3$) t(4)= $4.53*10^7$, p = 0.006.

The mean conidia yield of AUMI1 at vegetable waste ($M = 5.05*10^7$, $SD = 7.41*10^6$, $N= 3$) was significantly different from tea waste ($M = 1.10*10^7$, $SD = 7.01*10^5$, $N = 3$), $t(4) = 3.95*10^7$, $p = 0.001$.

The mean conidia yield of AUMI2 at vegetable waste ($M = 2.97*10^7$, $SD = 2.71*10^6$, $N = 3$) was significantly different from tea waste ($M = 8.07*10^6$, $SD = 1.19*10^6$, $N = 3$), $t(2.74) = 2.17*10^7$, $p = 0.002$ when equal variances are not assumed.

The mean conidia yield of AUMI3 at vegetable waste ($M = 4.60*10^7$, $SD = 1.63*10^7$, $N = 3$) was significantly different from coffee husk ($M = 1.22*10^7$, $SD = 1.12*10^6$, $N = 3$) $t(4) = 3.39*10^7$, $p = 0.02$.

Table 4: Effect of temperature on conidia yield of *Beauveria* and *Metarhizium* isolates grown at different substrates conditions

Substrate	Mean ± SEM of conidia count (x 10^7 conidia /gram) for temperature optimization after 14 days of incubation														
	Beauveria and Metarhizium Isolates														
	AUBI1			AUBI2			AUMI1			AUMI2			AUMI3		
	24°C	27°C	30°C	24°C	27°C	30°C	24°C	27°C	30°C	24°C	27°C	30°C	24°C	27°C	30°C
Coffee husk (1mm)	1.08±0.19	0.95±0.29	1.78±0.16	1.84±0.01	0.63±0.25	2.75±0.24	1.14±0.03	1.15±0.24	2.09±0.25	0.97±0.13	0.81±0.06	1.28±0.27	.22±0.06 (b	0.96±0.18	1.19±0.04
Tea waste	0.21±0.06	0.35±0.04	.91±0.22 (b	0.70±0.17	.71±0.22 (b	1.43±0.14	0.48±0.03	0.70±0.12	.10±0.04 (b	0.76±0.04	.81±0.07 (b	0.81±0.10	0.76±0.28	1.43±.0.28	0.82±0.05
Wheat bran	3.73±0.39	1.47±0.04	5.77±1.08	3.94±0.37	1.26±0.09	.25±0.84 (a	2.04±0.24	0.57±0.33	3.23±0.05	1.73±0.28	1.22±0.13	1.79±0.30	2.86±0.31	1.10±0.33	3.04±0.91
Vegetable waste	3.13±0.74	.40±0.63 (a	2.27±0.11	3.24±0.33	1.72±0.71	5.28±0.88	4.03±0.40	.05±0.43 (a	3.79±0.48	2.29±0.11	2.67±0.23	.97±0.16 (a	2.94±0.39	3.69±0.41	.60±0.94 (a

AUBI1= Addis Ababa University *Beauveria* Isolate one AUBI2 = Addis Ababa University *Beauveria* Isolate two

AUMI1 = Addis Ababa University *Metarhizium* Isolate one AUMI2 = Addis Ababa University *Metarhizium* Isolate two

AUMI3 = Addis Ababa University *Metarhizium* Isolate three

a= maximum conidia yield b= list conidia yield

Effect of pH levels on conidia production of *Beauveria* and *Metarhizium* isolates:- The result of (Table 5) indicated that the pH level significantly affected conidia yield of AUBI1 (11.63±8.34 conidia/gram of substrate) at 3.5 pH level on coffee husk. The optimum pH concentration for AUBI2, AUMI1 and AUMI3 at coffee husk using as substrate was 4.5 with 3.53±0.08, 2.77±0.23, and 3.35±0.23 conidia/gram of substrate harvest respectively. AUMI2 gave its maximum conidia yield of 3.18±0.34 conidia/gram of substrate when treated on 3.5 pH adjusted coffee husk. The highest mean of conidial harvest on tea waste was recorded by AUBI2 and AUMI1 with 1.37±0.03 and 1.14±0.11 conidia/gram of substrate respectively.

The overall conidia yield result record on AUBI1, AUMI2 and AUMI3 are not significantly varied among the tested pH levels (Table 5). The optimum pH concentration for AUBI1and AUMI2 was at pH5.5 when treated on wheat bran. Their highest mean of conidial harvest was 2.23±0.30 and 1.49±0.30 conidia/gram of substrate, whereas AUBI2, AUMI1 and AUMI3 give 2.29±0.15, 1.19±0.39 and 2.26±.37 conidia/ gram of substrate at 3.5 pH concentration respectively.

The pH concentration test on vegetable wastes reduces the total conidial harvest record of all isolates from the records on moisture content and temperature determination. The optimal pH for conidia production of all fungal isolates was between 3.5 and 4.5 (Table. 5). Isolate AUBI1 and AUMI1 produced maximum conidia (0.53±0.19 and 1.31±0.99 conidia/gram of substrate respectively) at pH 3.5 isolates AUBI2, AUMI2 and AUMI3 produced 2.02±0.85, 1.72±0.31 and 2.83±0.61 conidia/gram of substrate respectively at pH 4.5. Most of the substrates used in this study gave the lowest conidia yield record in all ranges of the pH tested except on coffee husk. Coffee husk conidia productivity of all fungal isolates was increased when treated under pH concentrations and the records of all treated pH levels was higher than the results treated on natural pH of the substrate. The rest substrates were produced high conidial yield at their natural pH concentrations.

- Two sample t-Test revealed that the mean conidia yield of AUBI1 at coffee husk ($M = 1.16*10^8$, $SD = 1.44*10^8$, $N = 3$) was not significantly different from mean conidia

yield of vegetable waste ($M = 5.27*10^6$, $SD = 3.33*10^6$, $N = 3$) $t(2) = 1.11*10^8$, $p = 0.32$ when equal variances are not assumed.

- The mean conidia yield of AUBI2 at coffee husk ($M = 3.53*10^7$, $SD = 1.33*10^6$, $N = 3$) was significantly different from mean conidia yield of tea waste ($M = 1.37*10^7$, $SD = 5.29*10^5$, $N = 3$) $t(2.62) = 2.16*10^7$, $p = 0$.
- The mean conidia yield of AUMI1 at coffee husk ($M = 2.77*10^7$, $SD = 4.32*10^6$, $N = 3$) was significantly different from mean conidia yield at tea waste ($M = 1.16*10^7$, $SD = 1.93*10^6$, $N = 3$) $t(2.77) = 1.62*10^7$, $p = 0.012$ when equal variances are not assumed.
- The mean conidia yield of AUMI2 at coffee husk ($M = 3.18*10^7$, $SD = 5.86*10^6$, $N = 3$) was significantly different from mean conidia yield at tea waste ($M = 9.23*10^6$, $SD = 2.11*10^6$, $N = 3$) $t(2.51) = 2.27*10^7$, $p = 0.014$.
- The mean conidia yield of AUMI3 at coffee husk ($M = 3.35*10^7$, $SD = 3.97*10^6$, $N = 3$) was significantly different from mean conidia yield at tea waste ($M = 7.70*10^6$, $SD = 2.36*10^6$, $N = 3$) $t(3.26) = 2.58*10^7$, $p = 0.002$.

Table 5. Effect of different pH levels on the conidia yield of *Beauveria* and *Metarhizium* isolates

Coffee husk	3.5	11.63±8.34 (**a**)	3.17±1.08	2.55±0.41	3.18±0.34 (**a**)	2.59±0.21
	4.5	3.46±0.33	3.53±0.08 (**a**)	2.77±0.23 (**a**)	2.90±0.16	3.35±0.23 (**a**)
	5.5	4.25±1.32	2.88±2.28	2.41±0.33	2.03±0.20	2.97±0.20
Tea wate	3.5	0.25±0.03	0.87±0.10	0.67±0.04	0.59±0.17	0.77±0.14 (**b**)
	4.5	0.63±0.12	1.37±0.03 (**b**)	1.14±0.11 (**b**)	0.88±0.05	0.76±0.08
	5.5	0.59±0.16	0.52±0.11	0.89±0.10	0.92±0.12 (**b**)	0.69±011
Wheat bran	3.5	1.73±0.37	2.29±0.15	1.19±0.39	0.87±0.21	2.26±0.37
	4.5	1.59±0.29	1.55±0.22	0.74±0.09	0.98±0.35	1.98±0.13
	5.5	2.23±0.30	1.73±0.48	0.96±0.05	1.49±0.30	1.29±0.32
Vegetable waste	3.5	0.53±0.19 (**b**)	0.78±0.33	1.31±0.99	0.98±0.23	1.49±0.85
	4.5	0.47±0.002	2.02±0.85	0.44±0.19	1.72±0.31	2.87±0.61
	5.5	0.36±0.06	0.45±0.07	0.33±0.02	0.19±0.02	0.62±0.51

Effect of incubation period on conidia production of *Beauveria* and *Metarhizium* isolates:- The profile of conidia produced at different incubation periods was evaluated using four agricultural wastes as substrates. The maximum conidial harvest of all isolates was recorded on vegetable waste with its optimum incubation period of 21 days. AUBI1,

AUBI2, AUMI1, AUMI2 and AUMI3 were favored to produce high conidia (4.31±1.18, 5.07±0.76, 3.43±0.30, 1.86±0.16 and 5.17±0.61 conidia/gram of substrate respectively) yield on vegetable wastes with respect to other substrates used in the study. The optimum incubation period for majority of the isolates tested in coffee husk and wheat bran was 28 days of incubation. Four of the isolates tested on coffee husk and three of the isolates tested on wheat bran were produced their maximum conidia yield after 28 days of incubation at 27^0_C (Table 6).

Table 6. Effect of different incubation period on conidia yield of *Beauveria* and *Metarhizium* isolates conditions

Substrate	Incubation period	Mean ± SEM of conidia count at (x107 conidia/gram) for Incubation period optimization				
		Beauveria and Metarhizium Isolates				
		AUBI1	AUBI2	AUMI1	AUMI2	AUMI3
Coffee husk	14 days	1.19±0.01	1.23±0.17	1.36±0.11 **(b)**	1.28±0.26	1.55±0.13
	21 days	1.06±.19	0.99±0.13	1.04±0.18	0.85±0.06	1.15±0.12
	28 days	1.61±0.14	1.32±0.13 **(b)**	1.32±0.06	1.35±0.09	1.71±0.10
Tea waste	14 days	1.17±0.20 **(b)**	1.37±0.48	0.89±0.12	0.91±0.06	0.87±0.12 **(b)**
	21 days	0.99±0.06	1.71±0.06	1.24±0.27	1.23±0.18 **(b)**	0.65±0.09
	28 days	1.13±0.28	2.07±0.37	1.52±0.12	1.18±0.08	0.74±0.24
Wheat bran	14 days	1.47±0.04	1.26±0.09	1.43±0.58	0.79±0.15	1.26±0.18
	21 days	1.57±0.23	1.41±0.23	1.60±0.16	1.15±0.23	1.67±0.41
	28 days	3.84±0.65	2.84±0.20	1.25±0.36	1.26±0.17	1.56±0.50
Vege waste	14 days	0.50±0.13	0.53±0.17	0.29±0.06	0.18±0.04	0.37±0.08
	21 days	4.31±1.18 **(a)**	5.07±0.76 **(a)**	3.43±0.30 **(a)**	1.86±0.16 **(a)**	5.17±0.61 **(a)**
	28 days	0.90±0.30	0.50±0.05	0.64±0.03	0.31±0.05	0.55±0.04

The optimum incubation period for isolates tested on wheat bran falls between 21 and 28 days of incubation. It has been observed that the isolate of AUBI1, AUBI2 and AUMI2 was recorded high conidia count of 3.84±0.65, 2.84±0.20 and 1.26±0.17 conidia/gram of substrate respectively after 28 days of incubation isolate AUMI1(1.60±0.16 conidia/gram of substrate) and AUMI3 (1.67±0.41conidia/gram of substrate) were also recorded maximum conidia count after 21 days of incubation. The optimum incubation period for conidia production on tea waste significantly varied among the isolates tested (Table 6). The optimum incubation period for isolates AUBI1 and AUMI3 were 14days and 28 days was for AUBI2 and AUMI1 But, the optimum incubation period record for isolate AUMI2 was 21days.

Similarly the two sample t-Test statistical analysis was also used on incubation period optimization to see how statistically the highest conidia productive substrate with the low productive substrate was different on each isolate type.

- The mean conidia yield of AUBI1 at vegetable waste ($M = 4.31*10^7$, $SD = 2.05*10^7$, $N = 3$) was not significantly different from mean conidia yield at tea waste ($M = 1.17*10^7$, $SD = 3.50*10^6$, $N = 3$) t (2.12) = $3.14*10^7$, p= 0.114.
- The mean conidia yield of AUBI2 at vegetable waste ($M = 5.07*10^7$, $SD = 1.32*10^7$, $N = 3$) was not significantly different from mean conidia yield at coffee husk ($M = 1.92*10^7$, $SD = 2.22*10^6$, $N = 3$) t (2.11) = $3.15*10^7$, p = 0.05.
- The mean conidia yield ofAUMI1at vegetable waste ($M = 3.43*10^7$, $SD = 5.23*10^6$, $N = 3$) was significantly different from mean conidia yield at coffee husk ($M = 1.36*10^7$, $SD = 1.93*10^6$, $N = 3$) t (2.53) = $2.07*10^7$, p = 0.012.
- The mean conidia yield of AUMI2 at vegetable waste ($M= 1.86*10^7$, $SD = 2.81*10^6$, $N = 3$) was not significantly different from mean conidia yield at tea waste ($M = 1.23*10^7$, $SD = 3.06*10^6$, $N = 3$) t (4) = $6.33*10^6$, p = 0.06.
- The mean conidia yield of AUMI3 at vegetable waste ($M = 5.18*10^7$, $SD = 1.06*10^7$, $N = 3$) was significantly different from conidia yield at tea waste ($M = 8.67*10^7$, $SD= 2.05*10^6$, $N = 3$) t (4) = $4.31*10^7$, p = 0.002.

Effect of inoculum concentration on conidia production of *Beauveria* and *Metarhizium* isolates:- The results shown in Table 7 indicated that the highest level of conidia yield of AUBI1 (2.21±0.11 conidia/gram) at coffee husk, AUBI2 and AUMI1 (6.03±0.02 and 2.47±0.17 conidia/gram of substrate) at wheat bran was achieved using inoculum concentration of $1x10^3$ conidia/ml. The optimum conidia count of AUMI2 and AUMI3 was recorded at $1x10^4$ and $1x10^5$ inoculum concentrations respectively on wheat bran. The maximum conidia count per inoculum concentration was harvested at $1x10^5$ conidia/ml of inoculum on coffee husk except at $1x10^3$ conidia/ml of inoculum on AUBI1. The conidia count record with regard to inoculum concentration in all the agricultural waste substrates was not enable us to dichotomize isolates since the results among treatments within the same substrate were not significantly different. Conidia count record of the fungal isolates on wheat bran and vegetable wastes treated in all the

inoculum concentrations were significantly lower than the records on optimization of moisture content and temperature. The optimum inoculum concentrations for Metarhizium isolates on vegetable waste were recorded at $1x10^4$ conidia/ml whereas $1x10^3$ and $1x10^5$ conidia/ml for *Beauveria* isolate AUBI1 and AUBI2 respectively. Inoculum concentration optimization test conducted on tea waste were also have different optimum concentrations for each isolate. AUBI1 and AUMI1 were favored for maximum conidia productivity at $1x10^4$ conidia/ml of inoculum concentration. The conidia count result 1.19±0.11 conidia/gram of substrate and 0.40±0.18 conidia/gram of substrate were recorded for AUMI2 and AUMI3 respectively at $1x10^3$conidia/ml and AUBI2 was produced its maximum conidia yield when inoculated with $1x10^5$ conidia/ml (Table 7).

Table 7. Effect of different inoculum concentrations on conidia yield of *Beauveria* and *Metarhizium* isolates conditions

Substrate	Inoculum concentration	Mean ± SEM of conidia count at (x107 conidia/gram) for Inoculum concentration optimization				
		Beauveria and Metarhizium Isolates				
		AUBI1	AUBI2	AUMI1	AUMI2	AUMI3
Coffee husk	1*103	2.21±0.11	0.70±0.17	1.30±0.17	0.75±0.18	1.14±0.02
	1*104	1.76±0.30	1.10±0.21	0.83±0.15	0.95±0.04	1.06±0.40
	1*105	1.59±0.36	1.36±0.33	1.35±0.06	1.32±0.02	1.50±0.51
Tea waste	1*103	0.45±0.30	0.72±0.21	0.64±0.12	1.19±0.11	0.40±0.18
	1*104	1.09±0.74	0.29±0.01	0.88±0.005	0.91±0.13	0.20±0.03
	1*105	0.50±0.02	0.73±0.25	0.63±0.19	0.74±0.02	0.26±0.06
Wheat bran	1*103	1.46±0.45	6.03±0.02	2.47±0.17	0.89±0.01	1.29±0.42
	1*104	1.12±0.61	3.43±0.74	1.93±0.30	1.47±0.33	2.00±0.45
	1*105	0.77±0.19	2.50±0.31	1.50±0.20	1.14±0.34	3.35±1.74
Vege waste	1*103	0.76±0.02	0.52±0.03	0.55±0.20	0.79±0.37	0.82±0.03
	1*104	0.71±0.17	0.38±0.11	0.79±0.07	0.88±0.06	1.07±0.14
	1*105	0.69±0.08	0.79±0.06	0.73±0.05	0.70±0.13	0.38±0.24

Effect of inoculum size on conidia production of *Beauveria* and *Metarhizium* isolates: In order to determine standard inoculum size for exponential conidia productivity of *Beauveria* and *Metarhizium* isolates. Different agricultural waste substrates were tested at different inoculum sizes for each fungal isolate. The optimum inoculum size for solid state fermentation technique of each isolate on agricultural wastes was determined by quantitative assay of conidia yield. Coffee husk favored AUMI1 to produce the highest conidia at 1ml inoculum size. Its conidia harvest at 1ml inoculum size was significantly different from the rest testes evaluated in the study. 2ml inoculum

size tested on coffee husk was best favored for *Beauveria* isolates as clearly illustrates on (Fig 3). The optimum inoculum size for *Metarhizium* isolates was 1ml but, the conidia count record was not significantly different from the conidia yield evaluated on 2ml inoculum size except on AUMI1.

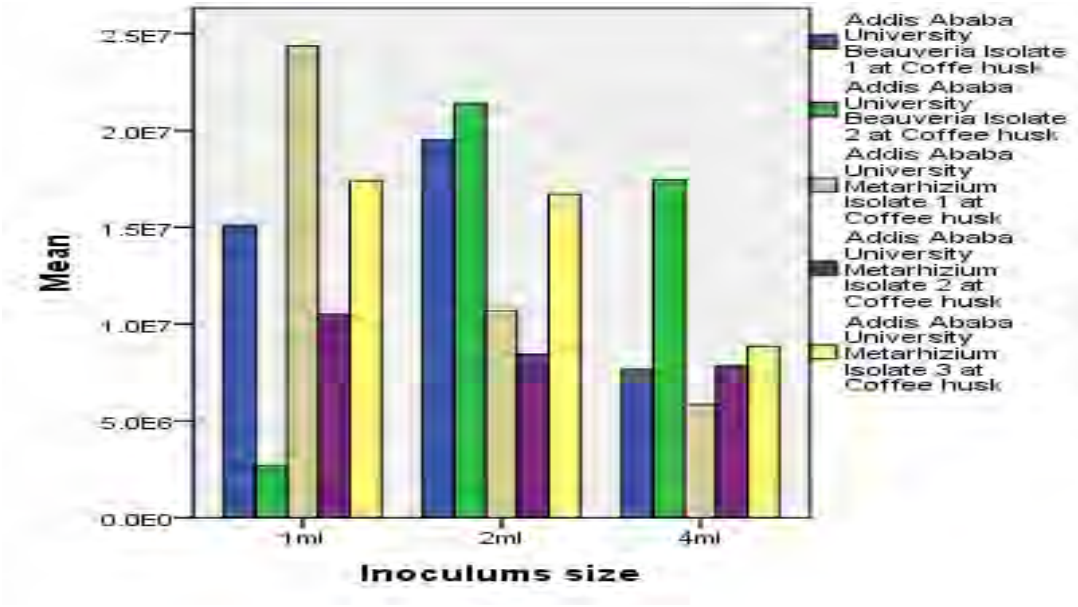


Fig 3. Effect of inoculum size on the conidia yield grown on coffee husk

Tea waste produced maximum conidia yield at 4ml of inoculum size for isolates AUBI1, AUBI2 and AUMI1 (Fig 4). Whereas, the highest conidia productivity of AUMI2 and AUMI3 at tea waste were fall between 1ml and 2ml inoculum sizes respectively.

Productivity of conidia for isolates AUBI1, AUBI2, AUMI1 and AUMI3 using wheat bran as substrate were higher at 1ml inoculum size except for AUMI2 in which its highest conidial harvest was recorded on 4ml with the mean of $8.2x10^7$conidia/gram of substrate. The maximum conidia yield of AUMI1 with the mean $1.21x10^8$ conidia/gram of substrate was recorded as the maximum and $3.21x10^7$conidia/gram of substrate for AUMI3 as minimum at 1ml inoculum size was harvested among the isolates tested for inoculum size optimization on wheat bran.

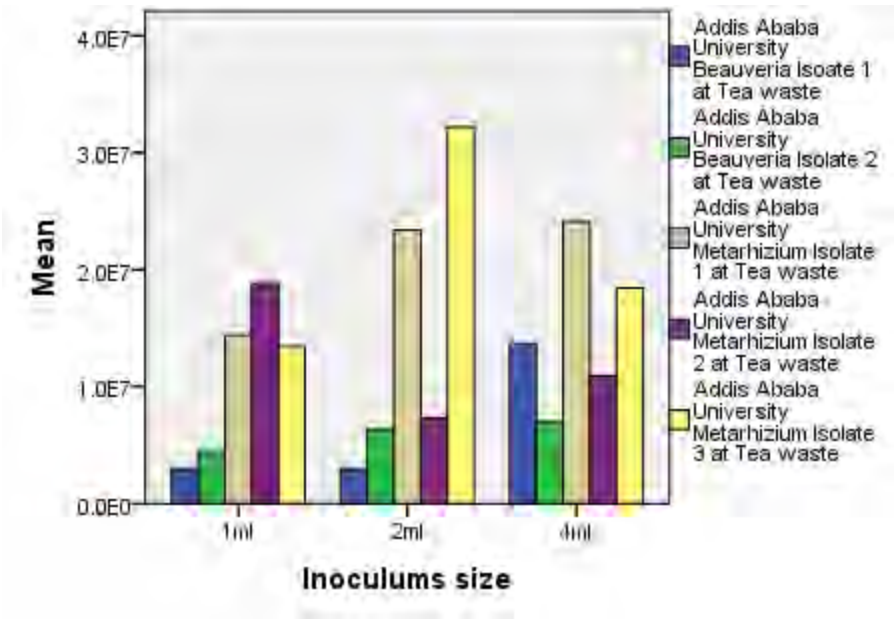


Fig 4. Effect of inoculum size on the conidia yield grown on tea waste

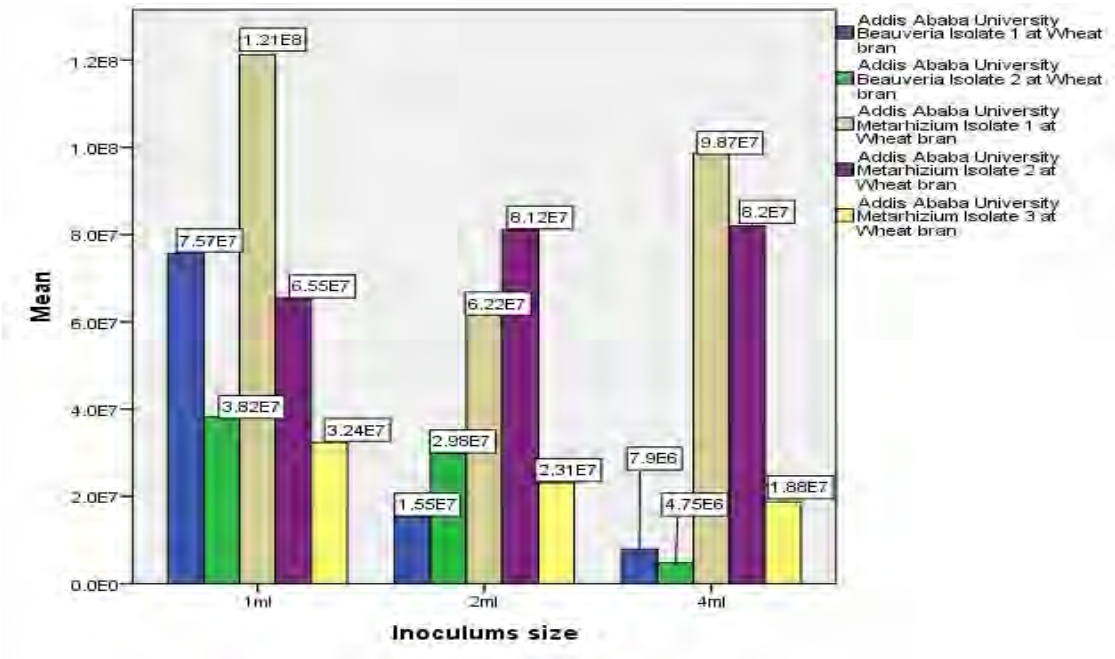


Fig 5. Effect of inoculum size on the conidia yield grown on Wheat bran

The optimum inoculum size for AUMI2 and AUMI3 are recorded at 1ml of inoculum when cultivated on vegetable waste. The highest conidia count result for AUBI2 and AUMI1 on vegetable waste was also found at the same inoculum size but, the conidia

yield records are not significantly different with the results on 2ml inoculum size and 4ml inoculum size as well. The result (Fig-6) indicated that when inoculum size increased on vegetable waste conidia yield of AUMI2 and AUMI3 were decreased.

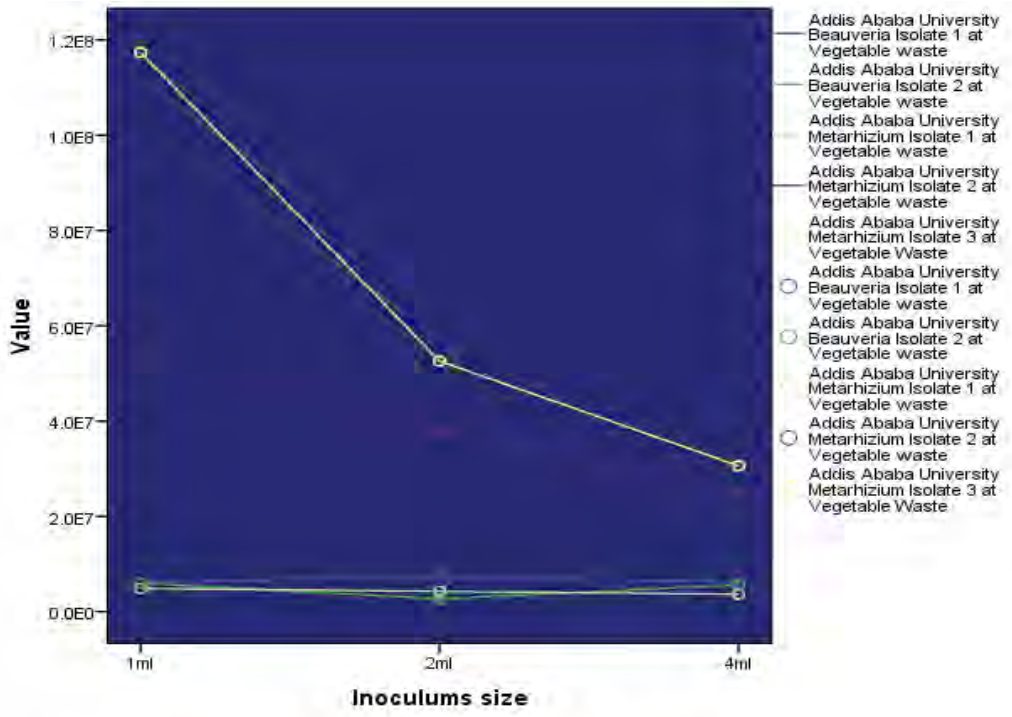


Fig 6. Effect of inoculum size on the conidia yield grown on vegetable waste

Effect of light on conidia production of *Beauveria* and *Metarhizium* isolates:- The conidia yields of AUBI1, AUBI2, AUMI1 and AUMI3 in the absence of light were larger than in the presence of light at coffee husk. The substrate afforded comparatively higher conidia growth for AUMI1 and AUMI3 in opaque condition whereas in the presence of light for AUMI2. Conidia yield records of AUBI1 both in light and opaque conditions were not significantly different as clearly indicated in Fig - 7. Excellent growths of all Fungal isolates were afforded by the substrate tea waste at dark condition. It has been observed that the isolates, AUMI1 and AUMI2 was also produced higher conidium in the presence of light but, the maximum conidia yield was observed on cultures in the absence of light (Fig 8). Therefore, conidia production of the fungal isolates on tea waste was light independent when compared with wheat bran in which conidia productivity of all the fungal isolates were light intensive. Opaque condition significantly affects the conidia productivity of AUBI1, AUMI2 and AUMI3 on wheat bran (Fig-9). Growth of AUBI2

and AUMI1 didn‟t show a very significant difference between cultures on opaque and light conditions except that the maximum conidia yield belongs to light induced condition. The conidia production potential of vegetable waste under the presence and absence of light was evaluated and the results (Fig. 10). The conidia production of isolate AUBI1 and AUBI2 was highly light dependent followed by AUMI1. Opaque condition was favored for AUMI2 and AUMI3 to produce the highest conidia yield at vegetable wastes.

Fig. 7. Conidia yield of the effect of light on coffee husk

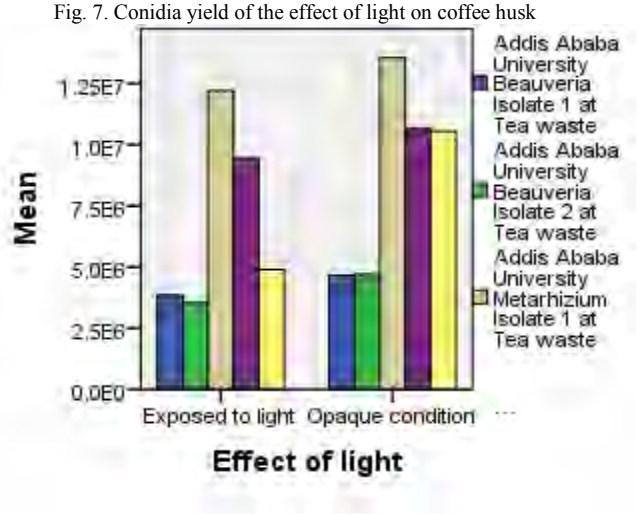
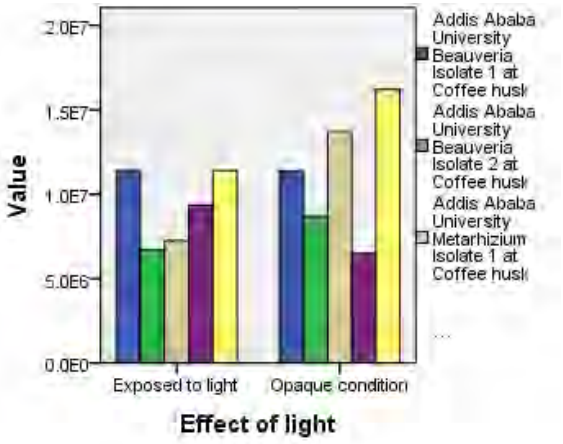


Fig. 8. Conidia yield of the effect of light on tea waste



Mean of the conidia yield was calculated from three replicates and each isolate was grown at 27^0C for 14 days under sufficient light exposure and complete opaque condition as required.

- Mean conidia yield on the effect of light for AUBI1
- Mean conidia yield on the effect of light for AUBI2
- Mean conidia yield on the effect of light for AUMI1
- Mean conidia yield on the effect of light for AUMI2
- Mean conidia yield on the effect of light for AUMI3

Fig. 9. Conidia yield of the effect of light on wheat bran

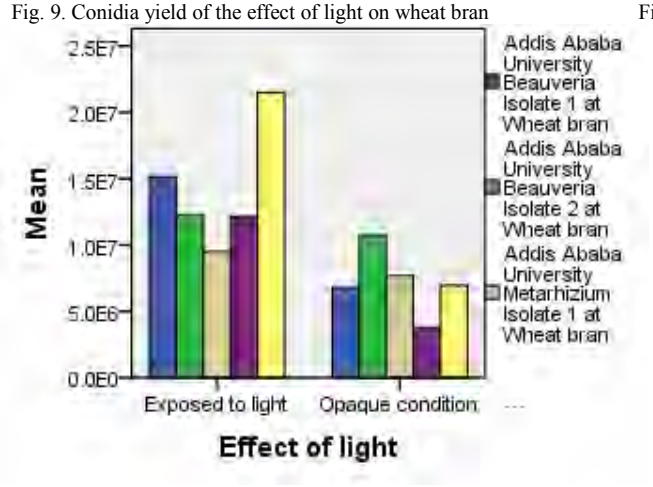
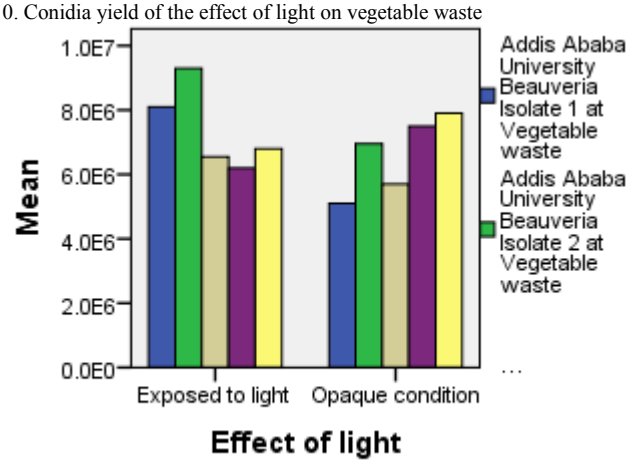


Fig. 10. Conidia yield of the effect of light on vegetable waste



Mean of the conidia yield was calculated from three replicates and each isolate was cultivated at 27^0C for 14 days under sufficient light exposure and complete opaque condition as required.

- Mean conidia yield on the effect of light for AUBI1
- Mean conidia yield on the effect of light for AUBI2
- Mean conidia yield on the effect of light for AUMI1
- Mean conidia yield on the effect of light for AUMI2
- Mean conidia yield on the effect of light for AUMI3

6. Discussion

Biological pest management using entomopathogenic fungi as microbial agents primarily requires mass multiplication of the microbial candidate on cheap cultivation media (Mohammed 2006, Masoud *et al.,* 2013). Different agricultural wastes were evaluated in this study as cheap potential substrates for conidia production of *Beauveria* and *Metarhizium* isolates.

All agricultural substrates were potential producers for high number of conidia even though their differences in number were recorded among substrates. *Metarhizium* isolate (AUMI1, AUMI2 and AUMI3) was able to produce significantly high number of conidia ($4.90x10^7$, $3.74x10^7$ and $3.61x10^7$ conidia/gram of substrate respectively) on vegetable wastes than their respective less productive substrates.

AUBI2 was also best favored by vegetable waste for its maximum conidia yield of $3.82x10^7$ conidia/gram of substrate and this was in agreement with Chaudhari et al., (2011) vegetable wastes recorded for maximum spore production of *Trichoderma viride* which is $31.2x10^8$ CFU on solid state fermentation. Different vegetables contain different carbon and nitrogen source nutrients and the combination of them increases nutritional value of the substrate. Therefore, vegetable wastes high productivity could be due to large availability and high macromolecular composition of lignocellulosic nutrients which provides the necessary carbon and nitrogen source. Similarly, Herta Stutz Dalla Santa *et al.,* (2005) and Gao (2011) have indicated that combination of different carbon and nitrogen source resulted in high spore production.

In the present study, the effect of various initial moisture contents were evaluated for coffee husk, tea waste, wheat bran and vegetable wastes. The best result was obtained at 60% moisture content on vegetable waste for all isolates of *Beauveria* and *Metarhizium*, and a comparable result of conidia count was also obtained at 80% moisture content on vegetable wastes. Therefore, the optimum moisture content 60% was obtained to grow isolates of *Beauveria* and *Metarhizium* on vegetable wastes. Despite that, the optimum moisture content of other substrates required to conidiate each isolate varies. The result

shown in table 3 indicated that 35% moisture content of 1mm size coffee husk favored AUMI1 and AUMI2 for maximum conidia harvest (0.96±0.12 conidia/gram and 1.62±0.44 conidia/gram respectively) on the substrate. Whereas 45% of the same substrate supports AUBI1 and AUMI3 and 80% moisture content for AUBI2. These results revealed that 1mm size of coffee husk was efficient in conidiation than the larger in particle size 2mm and 4mm using coffee husk.

This is due large particle size coffee husk is too big to be assimilated by the enzymes secreted from the fungi. Overall coffee husks in the present study at any moisture content were less efficient for conidia production of all fungal isolates when compared to vegetable waste, wheat bran and tea wastes. Similarly, (Zuriash Mamo and Tesfaye Alemu 2012) observed that the lowest conidia count of *Trichoderma* isolates was recorded on coffee husk under SSF technique.

Wheat bran exhibited the highest conidia count population of AUBI1 (6.34±2.27 conidia/gram of substrate) at 60% of moisture content. An increasing in the moisture content to 80% strongly decreases the spore amount produced by all fungal isolates that were studied except the isolate AUBI1 which produced the second large conidia yield (5.98±1.59 conidia/ gram of substrate).

These findings were in agreement with Rosane *et al.,* (2008) have observed that the wheat bran under SSF medium was used and higher conidia yield were obtained at moisture content greater than 60% but, moisture content above 74% caused oversaturation. Oscar Nuñez-Gaona *et al.,* (2010) have also stated that conidia productivity potential of *Beauveria bassiana* on wheat bran decreases when moisture content increased from 66 to 80% with values of 1.18±0.34($x10^{10}$) conidia/g and 1.85±0.21($x10^{9}$) conidia/g respectively. The optimum moisture content of wheat bran to grow isolate AUBI1 is therefore 60% whereas the optimum moisture content for the rest AUBI2, AUMI3 (2.00±0.49, 1.19±0.34 conidia/gram) and AUMI1, AUMI2 (1.46±0.09, 1.59±0.10 conidia/gram of wheat bran respectively) are at 35% and 45% respectively.

There was a gradual increment in the conidia count record of AUBI2, AUMI1 and AUMI2 as the effect of moisture content increases from 35% to 80% on Tea waste. However, conidia count result of AUBI1 and AUMI3 on the same substrate was higher around 60% and 45% of moisture content respectively. Therefore, Tea waste specially provides its highest conidia count record at 80% of moisture content for AUBI2, AUMI1 and AUMI2 isolates. This is perhaps due to maximum water absorption capacity of the substrate. Pandey *et al.,* (2001) Have observed that the water absorption potential depends on factors such as solid matrix structure and superficial area, as well as the ability of hydrogen-bond formation sites, among others. As clearly indicated in Table-3 that the moisture content level of the substrate are not the only factors that affect conidia production, composition and structure of the substrate as well as the type of isolates that were cultivated are also determinants. Experiment that was conducted by Rosane *et al.,* (2008) for comparison of conidia yield among different strains of *Trichoderma* on different substrate under the same moisture content results with no fungi growth and spore formation this assures that moisture content is not the only factor.

Each isolate were incubated at 24, 27 and 30^0C to evaluate the effect of temperature on conidia production. Among others the substrates examined on wheat bran AUBI2 (6.25±0.84 conidia/gram of substrate) and vegetable waste AUMI2 and AUMI3 (2.97±0.16 and 4.60±0.94 conidia/gram of substrate respectively) were supported for maximum conidia yield at 30^0C optimum temperature. However, vegetable waste also supported AUBI1 (6.40±0.63 conidia/gram of substrate) and AUMI1 (5.05±0.43conidia/gram of substrate) for maximum conidia yield at 27^0C as an optimum temperature. Avanti *et al.,* (2014) have studied that the Mutants of *Beauveria bassiana* produced higher level of biomass and blastospore at 27^0C and the Ethiopian isolates of *Metarhizium spp* also attained peak conidia production at 28^0C Seneshaw *et al.,* (2003).

The high conidia productivity of wheat bran and vegetable wastes at 30^0_C for AUBI2, AUMI2 and AUMI3 isolates was obtained due to water stress at a precise growth stage since high temperature causes loss of water via desiccation. Reynaldo *et al*., (2013) have clearly stated that water stress under SSF during the maximum growth period or biomass

formation helps sporogenesis to start earlier and allows obtaining higher sporulation yield.

As the result has shown in Table (5) pH optimization test changed the conidial productivity potential of each substrate. The high conidia productive vegetable waste at natural pH levels of each substrate handovers to coffee husk. AUBI1 produced the maximum conidia yield ($11.6x10^7$ conidia/gram of substrate) at pH 3.5 on coffee husk and AUMI2 (3.18±0.34 conidia/gram). The optimum pH concentration for AUBI2, AUMI1 and AUMI3 was obtained at pH 4.5 using coffee husk as substrate yielding 3.53±008, 2.77±0.23, and 3.35±0.23 conidia/gram respectively.

Similarly, (Zuriash Mamo and Tesfaye Alemu 2012) reported that the optimum pH for conidia production of *Trichoderma* isolates was between 4.5-5.5. Except for coffee husk the overall conidia count record of the substrates on their natural pH values (Wheat bran = 6.63, Vegetable waste = 6.3, Tea waste = 5.4) was significantly higher than the initial pH value used for optimization.

Conidia production was studied at various incubation periods of 14 days, 21days and 28days. Maximum conidia production of all isolates was recorded on vegetable wastes with its optimum incubation period of 21 days. AUBI1, AUBI2, AUMI1, AUMI2 and AUMI3 were favored to produce high conidia (4.31±1.18, 5.07±0.76, 3.43±0.30, 1.86±0.16 and 5.17±0.61 conidia/gram of substrate respectively) yield after three weeks cultivation on vegetable waste. Nelson *et al.,* (1996) observed that maximum conidial yields for *Beauveria bassiana* and *Metarhizium anisolpiae* after three weeks of incubation of the fungi on rice. The optimum incubation period for the rest substrates used in the present study ranges between 21 to 28 days. Exceptionally AUMI1 on coffee husk and AUBI1 and AUMI3 at tea waste were found their optimum incubation period after 14 days. Similarly, Pham *et al.,* (2010) obtained the maximum conidia production of *Beauveria bassiana* after 14 days of cultivation on rice (4.05g conidia/100g dry white rice), a rate was 2.83 times greater than conidia yield of pre-optimization.

The highest values of conidia germination of AUBI1 (2.21±0.11 conidia/gram) on coffee husk, AUBI2 and AUMI1 (6.03±0.02 and 2.47±0.17 conidia/gram of substrate) on wheat bran was achieved using an inoculum concentration of $1x10^3$ conidia/ml with 96.4% spore viability. The optimum conidia count of AUMI2 and AUMI3 was recorded at $1x10^4$ and $1x10^5$ inoculum concentrations respectively on wheat bran. The highest conidia yield of *Beauveria bassiana* was achieved at $1x10^7$ initial inoculum concentration using rice as substrate Pham *et al.,* (2010). Under inoculum concentrations optimization wheat bran over all tended to produce more conidia on AUBI2, AUMI1, AUMI2 and AUMI3 than the rest substrates used in the study. For isolate AUBI1 conidia production on coffee husk was higher than others.

The highest conidia productivity of most isolates on substrates used falls between 1ml to 2ml of inoculum sizes. Tea waste produces its maximum conidia yield with 4ml inoculum size for majority of the isolates and wheat bran for isolate AUMI2. This is possibly due to high water absorption potential of the substrates Rosane *et al.,* (2008) clearly states that wheat bran presented the largest moisture range and has a good ability to absorb water. Low conidia productivity of the remaining substrates at 4ml inoculum size was due to the over saturation inhibits aeration across the particles. similarly (Zuriash Mamo and Tesfaye Alemu 2012) and Pham *et al.,* (2010) observed that the same optimization under SSF for conidia production of *Trichoderma* and *Beauveria bassiana* respectively.

In the present study conidiation of *Beauveria* isolates of AUBI1, AUBI2 and AUMI1, AUMI3 of the *Metarhizium* were best favored in the absence of light on coffee husk. The same was true for excellent conidia production of all isolates on tea waste when compared with wheat bran in which all the isolates were light dependent. Under exposure to light AUBI1, AUBI2 and AUMI1 were high productive on vegetable wastes while, AUMI2 and AUMI3 requires opaque condition. Therefore, it is very important to keep in mind that light and dark conditions have essential effect on conidia production of *Beauveria* and *Metarhizium* isolates under SSF system on different substrates.

7. Conclusion

- Vegetable wastes were the best substrate which supports high conidia yield under solid state fermentation. As moisture content affects mycelial growth of the isolates, 60% moisture content on vegetable wastes were suitable for conidia production of both *Beauveria* and *Metarhizium* isolates.
- The optimum temperature for conidia production of vegetable waste under SSF was best between 27^0C -30^0C.
- The pH of the media was essential for fermentation cultivation under natural value for vegetable wastes (pH 6.3) give to rise to maximum conidia harvest. The fermentation period of the isolates under SSF system preferred to be held for 21 days under exposure to light and opaque conditions to their respective strains.

- Wheat bran was also among the most effective substrates to cultivate *Beauveria* and *Metarhizium* isolates for high conidia production. *Beauveria* isolate AUBI1 was best favored by wheat bran under quantitative assessment of conidia production at 60% moisture content. Due to its high water absorption potential a comparable good conidia yield of AUBI1 can also be obtained when the moisture content even increases to 80%. The optimum temperature (30^0C) for conidiation of all isolates of *Beauveria* and *Metarhizium* cultivated on wheat bran. Natural pH of wheat bran was measured 6.63 and that was the right pH value to produce significantly high conidia yield with respect to optimum value of other factors.

- Generally, that, all *Beauveria* and *Metarhizium* isolates tested on wheat bran provides their maximum conidial yields within 3 to 4 weeks period of cultivation under sufficient light exposure.

8. Recommendation

- Further studies regarding optimum pH values of each isolate on vegetable wastes and wheat bran is necessary to enhance industrial scale production of the entomopathogensic fungi.
- The nutritional factors should be further studied for isolates AUBI2, AUMI2 and AUMI3 which endows to require high temperature to flourish on wheat bran and vegetable waste substrates under SSF.
- Further studies regarding the amount of Tween 80 solution used for suspension are required to know the exact amount of solution required to sufficiently dissociate conidial yield from substrate particles.

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10. Appendix

- The mean conidia productivity of AUBI1 on wheat bran was significantly different from 2mm size coffee husk, $t(11.04) = 3.84$, $p = 0.001$."
- The mean conidia productivity of AUBI2 on vegetable waste was significantly different from 2mm size coffee husk, $t(9.06) = 3.49 \times 10^7$, $p = 0$.
- Mean conidia yield of AUMI1 was significantly different on vegetable waste than 2mm size coffee husk, $t(11.06) = 4.70 \times 10^7$, $p = 0$.
- The mean conidia yield of AUMI2 and AUMI3 on vegetable wastes were significantly different form conidia yields on 4mm and 2mm size coffee husk., with $t(11.35) = 3.38 \times 10^7$, $p = 0$ and $t(11.23) = 3.28 \times 10^7$, $p = 0$ respectively.
- The mean conidia yield of AUMI3 at vegetable waste ($M = 5.59 \times 10^7$, $SD = 1.15 \times 10^7$, $N = 3$) was significantly different from 2mm size coffee husk ($M = 3.63 \times 10^6$, $SD = 5.80 \times 10^5$, $N = 3$) $t(4) = 5.22 \times 10^7$, $p = 0.001$.
- The mean conidia yield of AUMI2 at vegetable waste ($M = 2.97 \times 10^7$, $SD = 2.71 \times 10^6$, $N = 3$) was significantly different from tea waste ($M = 8.07 \times 10^6$, $SD = 1.19 \times 10^6$, $N = 3$), $t(2.74) = 2.17 \times 10^7$, $p = 0.002$ when equal variances are not assumed.
- Two sample t-Test revealed that the mean conidia yield of AUBI1 at coffee husk ($M = 1.16 \times 10^8$, $SD = 1.44 \times 10^8$, $N = 3$) was not significantly different from mean conidia yield of vegetable waste ($M = 5.27 \times 10^6$, $SD = 3.33 \times 10^6$, $N = 3$) $t(2) = 1.11 \times 10^8$, $p = 0.32$ when equal variances are not assumed.
- The mean conidia yield of AUBI2 at coffee husk ($M = 3.53 \times 10^7$, $SD = 1.33 \times 10^6$, $N = 3$) was significantly different from mean conidia yield of tea waste ($M = 1.37 \times 10^7$, $SD = 5.29 \times 10^5$, $N = 3$) $t(2.62) = 2.16 \times 10^7$, $p = 0$.
- The mean conidia yield of AUMI3 at coffee husk ($M = 3.35 \times 10^7$, $SD = 3.97 \times 10^6$, $N = 3$) was significantly different from mean conidia yield at tea waste ($M = 7.70 \times 10^6$, $SD = 2.36 \times 10^6$, $N = 3$) $t(3.26) = 2.58 \times 10^7$, $p = 0.002$.
- The mean conidia yield of AUMI3 at vegetable waste ($M = 5.18 \times 10^7$, $SD = 1.06 \times 10^7$, $N = 3$) was significantly different from conidia yield at tea waste ($M = 8.67 \times 10^7$, $SD = 2.05 \times 10^6$, $N = 3$) $t(4) = 4.31 \times 10^7$, $p = 0.002$.