Use of Solid-State Fermentation on Selected Agricultural wastes for Mass Production of *Beauveria bassiana*

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Abstract

Two isolates of Beauveria bassiana were grown on different agricultural waste mediums to evaluate their conidia production potential under Solid State Fermentation (SSF). Coffee husk, wheat bran, tea and vegetable wastes were used as substrates. Among the substrates, vegetable wastes best favored to isolate II (AUBI2) for 5.77±1.53 (10^7), conidia/gram yield. Whereas, the highest conidia count record 6.34 ± 2.27 (10^7) conidia/gram of AUBI1 was obtained at 60% moisture content on wheat bran. All substrates supported maximum conidia yield at 30^0C. At pH 3.5 AUBI1 produced 11.63 ± 8.34 (10^7) conidia/gram on coffee husk but the high conidia yielding substrates were best favored at their natural PH values. Vegetable wastes supported AUBI1, AUBI2, to produce high conidia yield of 4.31 ± 1.18 (10^7), 5.07 ± 0.76 (10^7) conidia/gram of substrate at 21 days incubation period respectively. The optimum incubation period on wheat bran was 4 weeks under sufficient exposure to light. Successful biological control depends on cheap availability of the agent. Therefore, large scale industrial production on vegetable waste and wheat bran can be the plausible solution.

Keywords: *Beauveria bassiana, Enthomopathogen, Solid state fermentation, substrate and Conidia*

Introduction

Beauveria bassiana is one of the most important entomopathogenic fungi used in insect pest control. In the past biology and ecology of entomopathogens have been studied more and the attention of scientists was mainly on the fungi genus *Beauveria* and *Metarhizium* since they have a potential to attack broad spectrum of insects (*Mudroňeková, et al. 2013*). They are often reported as causing high levels of epizootics in nature and are the most versatile and eco-friendly biological control agents. Pest control using entomopathogenic fungi have a paramount importance to preserve ecological balance, minimize adverse effects on non-target organisms and ensure the safety of animals and human health (*Ambethgar, 2009*). As the modern agriculture is looking for environmentally benign practices in biodiversity conservation and pest control management, biological methods caught up global attention in the last few decades (*Bhadauria, et al. 2012*). Without efficient pest management, about two-third of world crop product would be lost (*Ambethgar, 2009*). One of the most

essential means to protect this is to minimize pest associated loss. Therefore, there is a resurgence of entomopathogenic fungi to form biologically compatible insect pest control.

Unlike synthetic insecticides, the advantage of using microbial pest control agents is their relentless potential to reproduce and persistent suppression of insect population in the environment (*Subramanian and Punamalai 2013*). *B. bassiana* has broadest high potential as a viable insect control agent, with documented potential to control more than 700 insect species (*Pham, et al. 2009*). The fungus is abundant in soil environment under different climatic conditions throughout the world and reproduces both sexually and asexually (*Sergio et al. 2002 and Yi qin et al. 2010*) *B. bassiana* infects its hosts up on direct contact. The conidia adhere to the insect cuticle and penetrate the body and germinate to flourish into blastospore by destroying the internal structure, causing morbidity within 36-72 hours (*Chinnadurai and Ganesh, 2013*). Larva and adult stages of hosts can also be attacked by *B.bassiana.*

It is a potential alternative to harvest the infective stage conidial biomass of biological agents on a highly productive, cheap and economically viable agricultural waste substrates. As the annually produced waste from food, agricultural and forestry industries are causing a serious environmental, health and disposal management problems (*Orzua, et al. 2009; Zuriash Mamo and Tesfaye Alemu 2012*). Utilization of wastes for large scale conidia production would be economically viable and environmentally friendly.

Material and Methods

Beauveria isolates and cultivation

Two *B. bassiana* isolates were obtained from Addis Ababa University Microbial, Cellular and Molecular Biology (MCMB) Department, mycology laboratory culture collection. All the isolates of *B. bassiana* used in this study were originally isolated from insect cadavers of grasshoppers, beetles, and locusts from south western parts of Ethiopia.

The fungal isolates were first grown on Sabouraud dextrose agar (SDA) medium. Medium was sterilized at 121^0_C for 15 minutes, plated inside a bio-safety hood and inoculated with pure culture of the fungal isolates under aseptic conditions. Inoculation plates were allowed to incubate at 27^0_C for 14 days (*Holder and Keyhani 2005; Masoud, et al. 2013).* Pure sporulated isolate cultures were kept in a refrigerator at 4^0_C until used.

Preparation of conidia suspension

Conidia inoculums for Solid State Fermentation (SSF) were obtained from 14 days old sporulated cultures on SDA. It was harvested by scrapping with hockey glass stick of flooded plate with 0.02% tween 80 solution to remove conidia from the mycelial mat of the culture. Conidial suspension was collected by filtering through three layers of cheese cloth and conidial concentration determined by direct counting using heamocytometer under compound Microscope.

Solid state fermentation using waste substrates

Agricultural wastes used in this study include: wheat bran, coffee husk, tea and vegetable wastes. The wastes were initially cleaned to remove debris and washed with water followed by drying under shade condition. Subsequently, all the wastes were ground in to powder using a Hammer beater mill (*Muhammad Irfan, et al. 2012*). The powder form of each waste was stored in plastic bags until used.

Ten grams of agricultural waste powder was used for a single inoculum experiment and autoclaved at 121^0_C for 15 minutes in a heat resistant plastic bag (*Pham, et al. 2010*). The substrates were transferred to sterilized ($20x30cm^2$ sized) plastic bags under safety hood. One milliliter of suspension containing 1.0 x 10^5 conidia/ml seeding inoculums was sprayed into each bag using hand sprayer. Samples were incubated in light transparent incubator at 27^0_C for two weeks. All treatments were replicated three times.

Conidia harvesting from the substrate

One gram of conidiated culture was taken from each substrate and suspended in 20ml of 0.05% Tween 80 solution. The mixture was vigorously agitated for 3-5 minutes to dissociate conidia clumps. The suspension was filtered through three layers of cheese close and adjusted to the required conidia concentration using a heamocytometer.

Conidia counting

To estimate the amount of conidia suspended in the bottle the mixture was vigorously agitated by hand to uniformly distribute spores over the solution. Serial dilution was prepared from the original suspension by taking 1 ml of conidia concentrated solution from the original suspension and poured in to a test tube containing 9ml distilled water to dilute the conidia concentration by a factor of 10. The process was repeated until countable number of spores found over the grids of the heamocytometer. Using a micro pipette 20 µl of spore suspension was loaded to a clean cover slip affixed heamocytometer. Chambers of the heamocytometer were allowed to fill carefully via capillary action. Stock conidia concentration per milliliter and conidia concentration per gram of substrate were arithmetically calculated.

Spore Viability assay

Spore viability was determined by spread plating of 1ml of conidial suspension (titrated to $1x10^3$ conidia/ ml) on SDA at 27^0_C Culture was examined by halting germination using separate drops of lactophenol cotton blue after 12-18 hours. The proportions of viable conidia were determined by examining 100 spores in each of three stained fields of view at 400X magnification with a compound microscope. The proportion of germinated spores was determined by counting spores that possessed a distinct germ tube.

Optimization of moisture content for conidia production

Moisture contents of the agricultural wastes were determined by oven drying method (*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. The bottles were weighed and recorded at

room temperature with cover lids put on. Ten grams of substrate were added to each glass bottles and a note of the new weight was taken for wet weight of the substrates with cover lids.

The substrate containing bottles were placed back in to the oven at 60^0_C for six hours until constant mass of the dry weight was recorded. Finally moisture content of the substrates was calculated based on wet-weight basis and expressed as a percentage, using the following formula.

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

Moisture content of each agricultural waste was adjusted to 35%, 45%, 60% and 80%, respectively.

Temperature optimization for maximum conidia production

Substrates at their natural pH inoculated with *B. bassiana* isolates and incubated under light transparent incubator at 24^0_C, 27^0_C and 30^0_C. Each fermenting plastic bag was sprayed with 1ml of $1x10^5$ conidia concentration and tied up with rubber band to prevent air blow and contamination. Aeration was allowed by two 1cm sized holes at the top of the bag. The culture was incubated for 14 days before spore sample taking to count under Heamocytometer (*Pham, et al. 2010*).

PH Optimization for conidia production

Ten grams of each substrate was weighed to examine under different pH levels. The pH values were adjusted to 3.5, 4.5 and 5.5 using 1N of HCL and NaOH. 1ml of $1x10^5$ conidia concentration of each isolate was sprayed to their respective autoclaved substrate bags at 60% moisture content followed by incubation at 27^0_C. After 14 days' conidial productivity was studied under heamocytometre by suspending 1g of conidiated substrate in 20 ml of distilled and sterile water.

Incubation Period Optimization for conidia production

The effects of incubation period for conidial production were evaluated by adjusting incubation period to 14, 21 and 28 days respectively. Substrates were sterilized at 121^0_C for 15 min with moisture content adjusted according to their high productivity. All the samples were incubated at 27^0_C for the required period of time interval. After 14, 21 and 28 days of cultivation conidial productivity per gram of each substrate were measured using heamocytometer.

Optimization of inoculum concentration for conidia production

Stock concentration of each isolate was prepared to adjust concentrations of $1x10^3$, $1x10^4$ and $1x10^5$ conidia/ml in different flasks used to conduct experiment. The formula used as indicated in insect pathology manual.

$$x = \frac{\text{Required concentration} * \text{Final volume Neeed}}{\text{Counted concentration}}$$

Where,

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

Each of the substrates was evaluated by all the prepared concentration levels with 1ml of inoculum size incubated for 14 days. Conidial harvest and numeration was determined by heamocytometer after filtering the suspension through three layers of cheese cloth.

Effect of light on conidia production

Autoclaved substrates were inoculated with 1ml of $1x10^5$ conidia /ml and incubated in opaque and light transparent bags to determine the effect of light on conidia production. The cultures were incubated for 14 days at 27^0_C

Data analysis

Data were statistically analyzed using SPSS (version 20). Substrates potential to support growth of *B. bassiana* isolates were statistically compared using Analysis of Variance (ANOVA) and means were separated by Tukey's honest significant difference (HSD) test. Conidia production results of each substrate with respect to isolate type were expressed as Mean ± SEM. Mean difference of two isolates conidia yield concentration on a substrate was computed using two sample t-test. For all experiments, a probability level of $p \leq 0.05$ was considered as statistically significant.

Results

Evaluation of agricultural wastes for conidia production

Substrates such as coffee husk, tea waste, wheat bran and vegetable wastes were used to evaluate their support on growing entomopathogenic fungi *B.bassiana* isolates. Conidial productivity of each substrate was examined to each isolate. Among the tested substrates, vegetable wastes supported high conidial productivity of AUBI2 ($3.82\pm0.63x10^7$ conidia/gram), and for AUBI1 wheat bran, which yielded $4.18\pm0.84x10^7$ conidia/gram, followed by vegetable waste ($3.75\pm0.53x10^7$ conidia/gram of substrate). Table 1 illustrates the overall conidial productivity of each substrate under solid state fermentation. The overall lowest conidial productivity was recorded on coffee husk, the list productive being 2mm size coffee husk.

Table 1: Conidia yield of *Beauveria bassiana* isolates on agricultural waste substrates

Substrates	Mean±SEM of conidia count at ($X10^7$ conidia/gram) after 14 days of incubation	
	Beauveria bassiana Isolates	
	AUBI1	AUBI2
1mm Coffee husk	1.27±0.13	1.22±0.08
2mm Coffee husk	0.33±0.03	0.33±0.03
4mm Coffee husk	0.65±0.08	0.46±0.12
Tea waste	2.08±0.17	2.26±0.40
Wheat bran	4.18±0.84	1.20±0.22
Vegetable waste	3.75±0.53	3.82±0.63

a) AUBI1= Addis Ababa University *Beauveria* Isolate one
b) b) AUBI2= Addis Ababa University *Beauveria* Isolate two
c) Mean calculated from three replications, all the isolate cultures on each substrate was incubated at 27^0c for 14 days.

Effect of moisture content on conidia production

To study the effect of moisture content on conidia productivity of each substrates, isolates were incubated at 35 %, 45 %, 60 % and 80 % moisture content of each substrate for 14 days at $27\ ^0_C$. It was observed that the optimum moisture content for conidia production of each isolate vary within and between the substrates (Table 2).

Table 2: The effect of Moisture on conidia yield of *Beauveria bassiana* isolates after 14 days of incubation at 27°c.

Substrates	Mean±SEM of conidial count at (x10^7 conidia/gram) for moisture content optimization after 14 days of incubation							
	AUBI1				AUBI2			
	35%	45%	60%	80%	35%	45%	60%	80%
4mm coffee	0.93±0.20$^{(a, f)}$	0.32±0.04$^{(a)}$	0.63±0.07$^{(a)}$	0.74±0.02$^{(a)}$	0.63±0.43$^{(a)}$	0.39±0.23$^{(a)}$	0.40±0.18$^{(a)}$	0.42±0.17$^{(a)}$
2mm coffee	0.42±0.06$^{(f, e)}$	0.29±0.05$^{(a)}$	0.36±010$^{(a)}$	0.27±0.02$^{(a)}$	0.47±0.05$^{(a)}$	0.36±0.03$^{(a)}$	0.21±0.02$^{(a)}$	0.27±0.06$^{(a)}$
1mm coffee	1.13±0.16$^{(a, c, e)}$	1.86±0.26$^{(b, d)}$	0.98±0.15$^{(a)}$	1.09±0.17$^{(a)}$	1.30±0.12$^{(a, b)}$	0.93±0.10$^{(a)}$	1.29±012$^{(a)}$	1.36±0.25$^{(a)}$
Tea waste	1.90±0.22$^{(a, b, c)}$	2.03±0.24$^{(c, b)}$	2.53±0.50$^{(a, c)}$	1.86±0.37$^{(a, c)}$	1.34±0.45$^{(a, b)}$	1.52±0.18$^{(a, b)}$	2.31±0.64$^{(a)}$	3.62±0.92$^{(b, c)}$
Wheat bran	2.36±0.27$^{(b,)}$	2.03±0.04$^{(d, c)}$	6.34±2.27$^{(b, c, d)}$	5.98±1.59$^{(b)}$	2.00±0.49$^{(a, b)}$	0.91±0.39$^{(a)}$	1.13±0.37$^{(a)}$	0.79±032$^{(a)}$
Vege.waste	1.87±0.27$^{(a, b, c, d)}$	2.96±0.42$^{(c, e)}$	5.19±1.04$^{(a, d)}$	4.96±1.06$^{(b, c)}$	2.21±0.05$^{(b)}$	2.88±0.30$^{(b)}$	5.77±1.53$^{(b)}$	4.24±0.52$^{(c)}$

a) Means in the same column designated by different letters are significantly different, (a–f), at p≤0.05.

There is a gradual increment in conidia count record of AUBI2 as the effect of moisture content increases from 35% to 80% using tea waste as substrate. However, conidia count result of AUBI1 on tea waste is higher at 60% moisture content. The mean conidia yield of AUBI1 on wheat bran was significantly different from AUBI2.

Effect of temperature on conidia production

Incubation temperature markedly affected conidia yield as shown in Table 3. Coffee husk and wheat bran support for high conidia yield of both isolates at 30^0_C. However, vegetable waste gives 6.40±0.6 conidia/gram of AUBI1 when incubated at 27^0_C.

Table 3: Effect of temperature on conidia yield of *Beauveria bassiana* isolates grown at different substrates

Mean±SEM of conidia count (x10^7conidia/gram) for temperature optimization after 14 days of incubation					
Beauveria bassiana					
AUBI1			*AUBI2*		
24^0_C	27^0_C	30^0_C	24^0_C	27^0_C	30^0_C
1.08±0.19$^{(a)}$	0.95±029$^{(a)}$	1.78±0.16$^{(a)}$	1.84±0.08$^{(a)}$	0.63±0.25$^{(b)}$	2.75±0.24$^{(c)}$
3.73±0.39$^{(a, b)}$	1.47±0.04$^{(a)}$	5.77±1.08$^{(b)}$	3.94±0.37$^{(a)}$	1.26±0.94$^{(b)}$	6.25±0.84$^{(c)}$
0.21±0.06$^{(a)}$	0.35±0.04$^{(a, b)}$	0.91±0.22$^{(b)}$	0.70±0.17$^{(a)}$	1.71±0.22$^{(b)}$	1.43±0.14$^{(a, b)}$
3.13±0.74$^{(a)}$	6.40±0.63$^{(b)}$	2.27±0.11$^{(a)}$	3.24±0.33$^{(a, b)}$	1.72±0.71$^{(a)}$	5.28±0.88$^{(b)}$

a) Means in the same row along the substrate within Isolate type designated by different letters are significantly different, (a-c), at p≤0.05.

Effect of pH levels on conidia production

Initially the natural pH values of the substrates were 6.6, 6.3 and 5.4 for wheat bran, vegetable and tea waste respectively. When the pH value adjusted to 3.5 on coffee husk it significantly affects conidia yield of AUBI1 (11.63±2.84 conidia/gram of substrate) as shown in Table 4. This is the highest result obtained in the study under the tested substrate types and it flipped the productivity of coffee husk from the list supporting substrate to high productive substrate if treated under appropriate pH value. The conidia yield result of AUBI1 on Table 4 shows coffee husk was significantly different from conidia yield on tea waste, wheat bran and vegetable wastes treated at 4.5 pH concentrations. All the substrates except coffee husk gave the lowest conidia yield record than their natural pH values in all tested pH ranges.

Table 4: Effect of pH on conidia yield of *Beauveria bassiana* isolates under different substrates

Substrates	Mean±SEM of conidia count at ($x10^7$ conidia /gram) for PH optimization after 14 days of incubation		
	Beauveria bassiana Isolates		
	PH	AUBI1	AUBI2
Coffee husk	3.5	$11.63\pm2.84^{(a)}$	$3.17\pm1.08^{(a)}$
	4.5	$3.46\pm0.33^{(b)}$	$3.53\pm0.08^{(b)}$
	5.5	$4.25\pm1.32^{(f)}$	$2.88\pm2.28^{(d)}$
Tea waste	3.5	$0.25\pm0.03^{(a)}$	$0.87\pm0.10^{(a)}$
	4.5	$0.63\pm0.12^{(d)}$	$1.37\pm0.03^{(c)}$
	5.5	$0.59\pm0.16^{(g, h)}$	$0.52\pm0.11^{(d)}$
Wheat bran	3.5	$1.73\pm0.37^{(a)}$	$2.29\pm0.15^{(a)}$
	4.5	$1.59\pm0.29^{(c, d)}$	$1.55\pm0.22^{(b, c)}$
	5.5	$2.23\pm0.30^{(f, g, h)}$	$1.73\pm0.48^{(d)}$
Vegetab waste	3.5	$0.53\pm0.19^{(a)}$	$0.78\pm0.33^{(a)}$
	4.5	$0.47\pm0.002^{(c, d, e)}$	$2.02\pm0.85^{(b, c)}$
	5.5	$0.36\pm0.06^{(h)}$	$0.45\pm0.07^{(d)}$

a) Means in the same column within the same PH values designated by different letters are significantly different, (a-h), at $p\leq0.05$.

Effect of incubation period on conidia production

The profile of conidia produced at different incubation periods was evaluated. The maximum conidial harvest of all isolates was recorded on vegetable waste with its optimum incubation period of 21 days. AUBI1 and AUBI2 were favored to produce high conidia (4.31±1.18 and 5.07±0.76 conidia/ gram respectively) yield on vegetable wastes with respect to other substrates used in the study as shown on Table 5. The optimum incubation period for both the isolates tested on coffee husk and wheat bran was 28 days of incubation.

Table 5: effect of incubation period on conidia yield of *Beauveria bassiana* Isolates

Substrates	Incubat period	Mean±SEM of conidia count at (x10^{7}conidia/gram) for incubation period optimization	
		Beauveria bassiana Isolate	
		AUBI1	AUBI2
Coffee husk	14 days	1.19±0.01	1.23±0.17
	21 days	1.06±0.19	0.99±0.13
	28 days	1.61±0.14	1.32±0.13
Tea waste	14 days	1.17±0.20	1.37±0.48
	21 days	0.99±0.06	1.71±0.06
	28 days	1.13±0.28	2.07±0.37
Wheat bran	14 days	1.47±0.04	1.26±0.09
	21 days	1.57±0.23	1.41±0.23
	28 days	3.84±0.65	2.84±0.20
Vegetable	14 days	0.50±0.13	0.53±0.17
	21 days	4.31±1.18	5.07±0.76
	28 days	0.90±0.30	0.50±0.05

For isolates tested on wheat bran, it falls between 21 and 28 days of incubation. The incubation period on tea waste varied among the isolates tested. The optimum incubation period for AUBI1 being 14 days and 28 days for AUBI2. Similarly, using two sample t-test statistical analysis the mean conidia yield of AUBI1 on vegetable waste ($M=4.31*10^7$, $SD = 2.05*10^7$, $N = 3$) was not significantly different from mean conidia yield on tea waste ($M = 1.17*10^7$, $SD = 3.50*10^6$, $N=3$) $t(2.12)= 3.14*10^7$, $P =0.05$. The mean conidia yield of AUBI2 on vegetable wastes ($M=5.07*10^7$, $SD=1.32*10^7$, $N=3$) was not either significantly different from mean conidia yield on coffee husk ($M=1.92*10^7$, $SD= 2.22*10^6$, $N =3$) $t (2.11) =3.15*10^7$, $P=$ 0.05.

Effect of inoculum concentration on conidia production

The results shown in Table 6 indicate that the highest level of conidia yield of AUBI1 and AUBI2 with (6.43±1.97 and 6.03±0.02 conidia/gram) on wheat bran was achieved using inoculum concentration of $1x10^5$ and $1x10^3$ conidia/ml respectively. However, Inoculum concentration optimization test of both isolates on vegetable wastes was best favored at $1x10^5$. Tea waste and coffee husk were also having different optimum concentrations for each isolate.

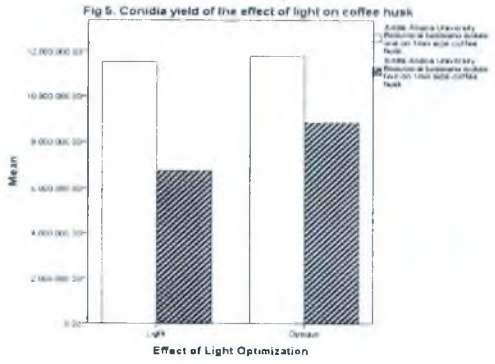
Table 6: Effect of inoculum concentration on conidia yield of *Beauveria bassiana* Isolates

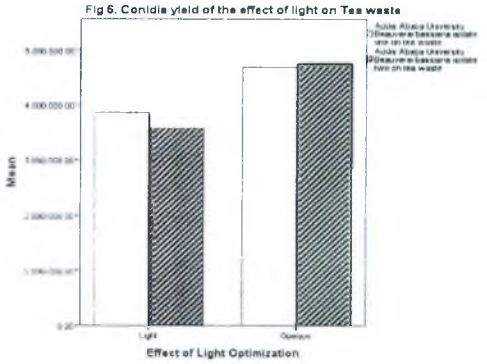
Substrates	Inoculum con	Mean±SEM of conidia count at ($x10^7$ conidia/gram) *Beauveria bassiana* Isolate	
		AUBI1	AUBI2
Coffee husk	$1x10^3$	2.21±0.11	0.70±0.17
	$1x10^4$	1.76±0.30	1.10±0.21
	$1x10^5$	1.59±0.36	1.36±0.33
Tea waste	$1x10^3$	0.45±0.30	0.72±0.21
	$1x10^4$	1.09±0.74	0.29±0.01
	$1x10^5$	0.50±0.02	0.73±0.25
Wheat bran	$1x10^3$	1.46±0.45	6.03±0.02
	$1x10^4$	1.12±0.61	3.43±0.74
	$1x10^5$	6.43±1.97	2.50±0.31
Vegetable	$1x10^3$	0.76±0.02	0.52±0.03
	$1x10^4$	0.71±0.17	0.38±0.11
	$1x10^5$	5.22±1.13	5.68±1.49

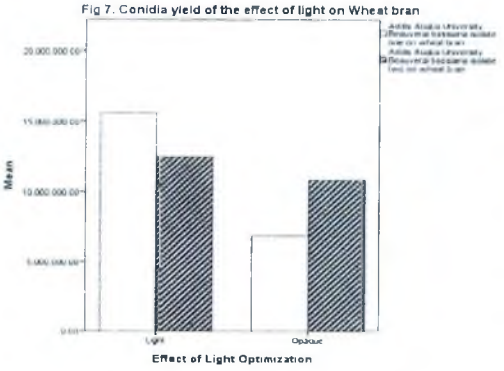
AUBI1 was favored for maximum conidia productivity at $1x10^4$ conidia/ml of inoculum concentration with (1.76±0.30 and 1.36±0.33) whereas AUBI2 was produced a maximum yield (1.09±0.74 and 0.73±0.25) when inoculated with $1x10^5$ conidia/ml.

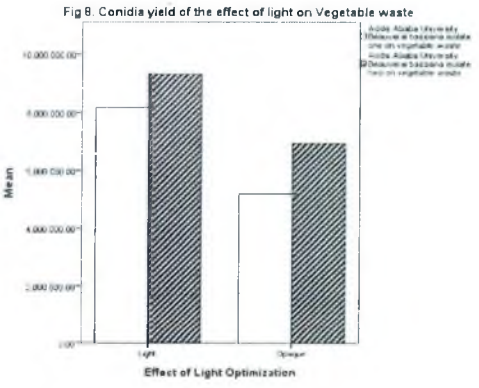
Effect of light on conidia production

The conidia yield of AUBI1 and AUBI2 in the absence of light was larger than in the presence of light on coffee husk (Fig. 5). Wheat bran as substrate afforded comparatively higher conidia growth for both isolates when treated under sufficient light exposure. Excellent growths of the fungal isolates were afforded by the substrate tea waste at opaque condition. 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 affects the conidia productivity of AUBI1 on wheat bran (Fig. 7). Growth of AUBI2 did not show as much 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 as the results shown on (Fig 8). The conidia production of AUBI1 and AUBI2 was highly light dependent.









Discussion

Biological pest management using entomopathogenic fungi as microbial agents primarily requires mass production on cheap cultivation media (*Mohammed 2006; Masoud. et al 2013*). Different evaluated agricultural wastes were potential producers of high conidial number. AUBI2 was 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 p. and Shrivastava p., 2011*) where it records $31.2x10^8$ CFU of *Trichoderma viride*.

Initial moisture content and water activity are the key factors in solid state fermentation reaction (*Rajan and Nair, 2011*). The availability of water strongly affects microbial growth. Therefore, the moisture content of the substrate should be within the suitable range. In this study, the best result was obtained at 60% on vegetable waste for all isolates.

Overall coffee husks in the present study at any moisture content were less efficient for conidia production of *B.bassiana* isolates when compared to wheat bran, vegetable and tea wastes. Similarly, (*Zuriash Mamo and Tesfaye Alemu, 2012*) have observed that the lowest conidia count of *Trichoderma* isolates was recorded on coffee husk under SSF

technique. This might be due the strong lignocellulosic material is too hard to be assimilated by the secreted enzymes from the fungi.

Wheat bran exhibited the highest conidia count population of AUBI1 (6.34±2.27 conidia/gram of substrate) at 60% moisture content. These finding agrees with (*Rosane, et al. 2008*) who has observed that wheat bran under SSF medium gives higher conidia yield 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 *B.bassiana* on wheat bran decreases when moisture content increased from 66 to 80%. Reduction in conidia count when moisture content increased above the optimum may be related to the fact that excess water occupies the space between particles of the substrate and restricts mass oxygen flow across (*Pandey, 2003*).

As the result shown in Table (4) pH optimization test completely flipped conidial productivity potential of each substrate. The high conidia productive vegetable waste at natural pH values of wheat bran, vegetable and tea waste handovers to coffee husk. AUBI1 was produced the maximum conidia yield ($11.6x10^7$ conidia/gram of substrate) at pH 3.5 and AUBI2 at pH 4.5 yielding 3.53±0.08 conidia/gram. Similarly, (*Zuriash Mamo and Tesfaye Alemu, 2012*) have reported that the optimum pH for conidia production of *Trichoderma* isolates was 4.5-5.5. Except for coffee husk the overall conidia count record of the substrates on their natural pH was significantly higher than the initial pH value used for optimization. It is also important to mention that the high productivity of coffee husk was perhaps due to release of its organic matter after highly degraded by 1N HCl used to adjust the pH.

Acknowledgement

We are grateful to acknowledge the Department of Microbial, Cellular and Molecular Biology (DMCMB) at Addis Ababa University School of Graduate Studies, for their financial and technical support during this study.

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