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# Evaluation and optimization of agro- industrial wastes for conidial production of *Trichoderma* isolates under solid state fermentation

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## ABSTRACT

*Objective*: The study aimed to evaluate, optimize and determine optimal growth conditions for the control of plant pathogens using high conidia production of *Trichoderma* isolates on different agro- industrial wastes (wheat straw, tea wastes, vegetable wastes, coffee husk and barley bran) under solid state fermentation (SSF).

*Methodology and Results*: The optimum temperature, pH, moisture content, incubation period, inoculums size, inoculums load and effect of light were optimized for maximum conidia production of *Trichoderma* isolates. Among seven isolates of *Trichoderma* only isolates AUT1, AUT2, AUT4 and AUT7 which produced better conidia on potato dextrose agar medium (PDA) were taken for further study in this experiment. Among the substrates tested, wheat straw supported high conidia production of *Trichoderma* isolates AUT1, AUT2 and AUT4 (105± $5\times10^8$ conidia/g of substrate, $117\pm3.5\times10^8$ conidia/g of substrate and $144\pm6\times10^8$conidia/g of substrate respectively), followed by tea waste ($92\pm3\times10^8$ conidia/g of substrate, $96.5\pm2.5\times10^8$ conidia/g of substrate and $119\pm12.5\times10^8$ conidia/g of substrate respectively). *Trichoderma* isolate AUT7 produced maximum conidia on vegetable wastes $405\pm19.9\times10^8$conidia/g of substrate. The optimum temperature for conidia production was between $20^0$C and $30^o$C after 10 and 15 days of incubation. The optimum pH for conidia production was between 4.5 and 5.5 for all *Trichoderma* isolates except isolate AUT7 (pH 7.5). The optimum incubation period for high conidia production of *Trichoderma* isolates was 10 and 15 days. The optimum moisture content for conidia production of *Trichoderma* isolates was 35% and 40% after 5 and 10 days of incubation. The conidia production of *Trichoderma* isolates was maximum in the presence of light all isolates was best under solid state fermentation (SSF).

*Conclusions and application of findings*: wheat straw, vegetable wastes and tea waste supported good conidia production of *Trichoderma* isolates under SSF. These wastes are better substrates for conidia production from economic and environmental point of view. The conidia production of *Trichoderma* isolates was maximal when cultivated in the presence of light than dark condition. The optimum inoculums size and load for conidia production of *Trichoderma* isolates was between 1ml and 2ml inoculums size and $1\times10^6$ conidia/ml inoculum load for all isolates except AUT7 ($1\times10^8$ conidia/ml). Therefore, agro-industrial wastes were suitable for mass production of bioformulated products of *Trichoderma* isolates which are used for the control of coffee wilt disease (*Fusarium xylarioides)* in coffee growing areas of Ethiopia.

**Key words:** Agro-industrial wastes, conidia production, solid state fermentation, *Trichoderma* isolates

## INTRODUCTION

The agricultural wastes and industrial residues adversely affect human and animal health and also pollute the environment. About 147.2 million metric tons of fiber sources are found in the world, while the global output of wheat straw residues and rice straws were estimated at 709.2 and 673.3 million metric tons, respectively, in the 1990s (Belewu and Babalola, 2009). The increasing expansion of agro-industrial activity has led to the accumulation of a large quantity of lignocellulosic residues all over the world (Albores *et al.,* 2006). Lignocelluloses residues are the most abundant renewable biomass and useful substrates for the growth of filamentous fungi, which produce cellulolytic, hemicellulolytic and ligninolytic enzymes by solid state fermentation (Sanchez, 2009). Evaluation and optimization of suitable substrates like corn fiber dry mass, sewage sludge compost (Taweil *et al*., 2009), sawdust, paddy straw (Kumer and Palakshappa, 2009), cow dung, rice bran (Rini and Sulochana, 2007), vegetable waste, fruit juice waste and rotten wheat (Chaudhari *et al.,* 2010) have been studied for mass production of *Trichoderma* species. Large scale production, along with shelf life and establishment of bio-agents in targeted niche, determine the success of biological control (Chaudhari *et al*., 2010). Solid-state fermentation is a cost-effective system for sporulation of fungi (Cavalcante *et al*., 2008). The by-products of coffee processing are mainly coffee pulp, processing effluent, parchment husks and coffee husks. These together with tea and vegetable wastes are accumulating and causing serious environmental, human and animals health problems in Ethiopia. A huge volume of coffee-processing byproducts- mainly coffee pulp and husk, is generated annually from wet and dry coffee processing stations in Ethiopia. This results into bad odors of the surrounding atmosphere, breeding of disease vectors and pollution of ground water and surface water bodies through leaching and run-offs, respectively. A huge amount of household solid wastes, such as coffee husks, tea wastes, vegetable peelings and fruit juice wastes are unattractive for recycling and adversely affect human health. Therefore, it is better to look into the methods to utilize these wastes for mass production of conidia of *Trichoderma* species which can be used for the control of plant pathogens under solid state fermentation (SSF). SSF is a process whereby an insoluble substrate is fermented with sufficient moisture but without free water (Chaudhari *et al.,* 2010). SSF technique isa cost-effective system for sporulation of fungi (Pham *et al*., 2010). *Trichoderma* species are very useful filamentous fungi that have been widely used as antagonistic fungal agents against several plant pathogens as well as plant growth enhancers (Lorito *et al*., 2010).

Therefore, using suitable agro-industrial wastes for the production of potential indigenous *Trichoderma* isolates, which can be utilized to control coffee wilt disease (*Fusarium xylarioides*) in the coffee growing areas of Ethiopia. Therefore, the present study was carried out to evaluate and optimize different agro-industrial wastes for conidia production of *Trichoderma* isolates and to investigate optimum conditions for conidia production under solid state fermentation technology for sustainable environmental and agricultural development using formulated *Trichoderma* species to control coffee wilt disease.

## MATERIALS AND METHODS

**Sources of Trichoderma isolates cultures:** The standard cultures of *Trichoderma* isolates were obtained from the Mycology Laboratory, Microbial, Cellular and Molecular Biology Program Unit, College of Natural Sciences, Addis Ababa University (AAU). The experiment was conducted from September 30, 2010 to November 30, 2011 in the Mycology Laboratory, AAU. All *Trichoderma* isolates used in this study were previously isolated from soil samples collected from coffee growing areas of Jimma zone. Isolates were designated as AUT1, AUT2, AUT3, AUT4 AUT5, AUT6 and AUT7 which designated for Addis Ababa University *Trichoderma* isolates. Among these isolates only isolates AUT1, AUT2, AUT4 and AUT7

which produced better conidia on potato dextrose agar (PDA) medium were taken and kept at 4°C for further study.

**Preparation of conidia suspension of Trichoderma isolates:** The isolates of *Trichoderma* were grown on malt extract agar (MEA) for 7 days, at 25°C. An aliquot of 10 ml of distilled sterile water (DSW) was added to each plate and the mycelium was scraped with a spatula until the culture surface was free from mycelia and the suspension was collected in a 100 ml conical flask. The conidial suspension *of Trichoderma* isolates was passed through three layers of cheese cloth and conidial concentration was adjusted by using a Haemocytometer (Niranjana *et al.,* 2009).

**Assessment of Trichoderma isolates conidiation on substrates:** One gram of conidiated substrates were mixed with 9 ml of distilled and sterilized water. Ten flasks which each contained one hundred milliliter of the mixture of conidiated substrates were agitated in a rotary shaker at 121rpm for 1hr. The mixture of conidiated substrates were filtered through three layers of cheese cloth. The number of conidia was determined by a Haemocytometer (Pham *et al*., 2010) and calculated as conidia/g of substrate.

**Evaluation of different agro-industrial wastes for conidia production of Trichoderma isolates:** Different agro industrial wastes (wheat straw, barley bran, coffee husk, vegetable wastes and tea waste) were evaluated for maximum conidia production of *Trichoderma* isolates under solid state fermentation. Wheat straw, barley bran and coffee husk were collected from local agricultural product processing units and local farms. Tea and vegetable wastes were obtained from Addis Ababa University student cafeteria. The agricultural waste materials were cleaned to remove any unwanted debris such as stones and foreign plant matters. Vegetable wastes and wheat straw were cut into small pieces after being dried under shade. Humidification of wheat straw, vegetable wastes, barley bran and tea wastes was done by adding 63 milliliters fixed amounts of water because of its instantaneous water absorption. The humidification of coffee husk was carried out by immersing 50g of substrate into 1000 milliliters of distilled water for different times (30min, 1hr, 2hr and 3hr). The moisture level of each substrate was adjusted to 60%, using oven dry method by adding known quantity of water considering the initial moisture content for wheat straw, vegetable wastes, barley bran and tea waste. For coffee husk the moisture content was adjusted by immersing the substrates into 1000 milliliter distilled water for different times (30min, 1hr, 2hr and 3hr). Then the moisten substrates were put into autoclavable plastic bags and autoclaved, at 121°C for 15min. The autoclaved substrates were transferred to plastic bags (30×20cm) and inoculated with 1ml of conidia suspension ($1x10^6$conidia/ml) of *Trichoderma* isolates using a sterile syringe (Cavalcante *et al*., 2008). Small pores were made on the plastic bags to allow aeration. The Inoculated bags were incubated, at 30°C for 10 days and conidia count was done using Haemocytometer.

**Optimization of temperature for conidia production of *Trichoderma* isolates:**In order to determine the optimum temperature for conidia production of *Trichoderma* isolates, fermentation was done, at 20°C, 25°C, 30°C, 40°C and 50°C. Samples were taken after 7, 10 and 15 days of incubation to obtain optimum temperature for different incubation periods (Pham *et al*., 2010).

**Optimization of pH for conidia production of *Trichoderma* isolates:** Forty gram of substrates (wheat straw for isolates AUT1, AUT2 and AUT4 and vegetable wastes for isolate AUT7) were extracted with 1liter of distilled water. After addition of 20g of dextrose and agar into the extracted substrates the pH was adjusted to (3.5, 4.5, 5.5, 6.5 and 7.5) using 0.1N HCl (hydrochloric acid) and NaOH (sodium hydroxide) to determine the optimum pH for conidia production of *Trichoderma* isolates (Agosin *et al.,* 1997). The media was autoclaved at 121°C for 15min and poured into Petri dishes. The media were inoculated with 7 days old culture of *Trichoderma* isolates (0.5mm diameter) and incubated, at 25°C (Jayaswal *et al*., 2003). After 10 days of incubation the conidia was harvested by adding 10ml of distilled and sterile water to each plate and scraping the mycelia with spatula. Conidial suspension was passed through three layers of cheese cloth and the suspension was collected in a 100ml conical flask. The conidia yield was determined using Haemocytometer.

**Optimization of moisture content for conidia production of *Trichoderma* isolates:** The optimum moisture levels for conidia production of *Trichoderma* isolates were done by adjusting the moisture content to different levels (35%, 40%, 50% and 60%) using distilled water. The moisture content was determined by oven dry method and known quantities of water considering the initial moisture content added to obtain the desired substrate moisture levels (Cavalcante *et al*., 2008).

**Optimization of the incubation period for conidia production of *Trichoderma* isolates:** The conidia production of *Trichoderma* isolates was conducted, at 30$^{o}$C using wheat straw for isolates and vegetable wastes at a moisture level of 60% in plastic bags with three small aeration filters. The substrates were inoculated with 1×10$^{6}$conidia/ml of conidia suspension and incubated for 5-15 days (at 5 days interval samples were taken) to determine the effect of incubation period on production of conidia (Pham *et al*., 2010).

**Optimization of inoculum size and load for conidia production of *Trichoderma* isolates:** The optimum inoculum load and size for the conidia production of *Trichoderma* isolates was determined by using different inocula sizes and load. To optimize the inocula size the moistened substrates (60% moisture levels) were inoculated with different inoculum sizes (1, 2, 3 and 4ml) of $10^{6}$ conidia/ml and optimum inocula load was determined by inoculating moisten substrates with different inocula load ($10^{4}$, $10^{5}$, $10^{6}$, $10^{7}$ and $10^{8}$ conidia/ml of conidia suspension) of 1ml inocula size (Pham *et al*., 2010). Inoculated bags were incubated, at 30 $^{o}$C for 10 days and conidia count was done using Haemocytometer.

**Effect of light on production of conidia *Trichoderma* isolates:** Fifty gram of substrates were moistened (60% moisture level) and autoclaved, at 121$^{o}$C for 15 min. The substrates were inoculated with 1 ×10$^{6}$ of conidia suspension and incubated, at 30 $^{o}$C for 10 days. To determine the effect of light on conidia production, *Trichoderma* isolates were exposed to light and the control was covered with opaque plastics with small aeration pores (Papavizas, 1985). The conidia yield was determined using a Haemocytometer.

**Statistical Analysis:** All experiments were performed in duplicates, statistically evaluated by excel and SPSS (version 17) and results have been presented as mean ± SEM. (standard error of mean).

## RESULTS

**Evaluation of different agro-industrial wastes for conidia production of Trichoderma isolates:** Among the substrates tested, wheat straw supported high conidia production of isolates AUT1, AUT2 and AUT4 (105±5 ×10$^{8}$ conidia/g of substrate, 117±3.5×10$^{8}$ conidia/g of substrate, and 144±6×10$^{8}$ conidia/g of substrate respectively), followed by tea waste (92±3×10$^{8}$ conidia/g of substrate, 96.5±2.5×10$^{8}$ conidia/g of substrate and 119±12.5×10$^{8}$ conidia/g of substrate respectively) (Table 1). The isolate AUT7 produced high conidia yield on vegetable wastes (405±19.95×10$^{8}$ conidia/g of substrate) followed by tea waste (378±17.5×10$^{8}$ conidia/g of substrate). The lowest conidia count was recorded on coffee husk by all *Trichoderma* isolates except isolate AUT2 which produced minimum conidia yield on vegetable wastes than that of coffee husk. The maximum conidia yield was recorded by isolate AUT7 on all tested substrates followed by isolate AUT4 on wheat straw, tea waste and vegetable wastes. The lowest conidia yield was recorded by isolate AUT1 on wheat straw, tea waste, coffee husk and barley bran followed by isolate AUT2 on the same substrates. The conidia yield of isolate AUT2 was maximal on coffee husk and barley bran next to isolate AUT7 when compared to conidia yield of other isolates on these substrates. The lowest conidia yield was recorded on coffee husk and barley bran by isolate AUT1 followed by isolate AUT4. The conidia yield of all isolates was not significantly different on barley bran when compared to other substrates.

**Table 1:** Conidia yield of *Trichoderma* isolates on different agro industrial wastes

| Substrates | Mean ±SEM of conidia count (×10$^{8}$conidia/ g of substrate) after 10 days of incubation | | | |
|---|---|---|---|---|
| | *Trichoderma* isolates | | | |
| | AUT1 | AUT2 | AUT4 | AUT7 |
| Wheat straw | 105±5 | 117±3.5 | 144±6 | 203±4 |
| Tea wastes | 92±3 | 96.5±2.5 | 119±12.5 | 378±17.5 |
| Coffee husk | 34.5±1.5 | 47±1 | 36.5±3.5 | 83.5±4.5 |
| Vegetable wastes | 52.5±2.5 | 40.5±1.5 | 113±3 | 405±19.95 |
| Barley bran | 65.5±10.5 | 80±10 | 69.5±10.5 | 88±10 |

**Table 2:** The conidia yield of *Trichoderma* isolates cultivated at different temperature after 7, 10 and 15 days of incubation.

| Temperature ($^0$C) | **Mean± SEM of conidia count (×10$^8$conidia/ g of substrate) after 7, 10 and 15 days of incubation** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AUT1** | | | **AUT2** | | | **AUT4** | | | **AUT7** | | |
| | 7 | 10 | 15 | 7 | 10 | 15 | 7 | 10 | 15 | 7 | 10 | 15 |
| 20 | 11.6±0.37 | 14.1±0.12 | 35.9± 0.88 | 21.4±0.37 | 23.8±0.75 | 20± 0.28 | 10.8± 0.19 | 15.9± 0.87 | 15.1± 1.24 | 17.4± 0.62 | 55.4± 0.88 | 50.3±1 |
| 25 | 15.6± 0.62 | 21.3± 0.75 | 37.5± 0.71 | 21.8± 0.18 | 23.8± 0.75 | 25± 0.14 | 28.1± 0.37 | 32.3± 2.25 | 39.1± 0.18 | 29.5± 0.5 | 35.9± 0.37 | 57.1± 0.53 |
| 30 | 16.5± 0.5 | 35.6± 0.62 | 31.5± 0.21 | 23.8± 0.75 | 30.3± 0.75 | 11.9± 0.18 | 29.5± 0.5 | 22.9± 2.12 | 26± 0.35 | 38.3± 0.5 | 30.6± 0.62 | 28.4± 0.53 |
| 40 | 54.1± 3.87 | 33.4± 0.62 | 20.3± 0.35 | 53± 0.25 | 29.6± 0.37 | 10.8± 0.35 | 90.1± 0.12 | 22.4± 0.37 | 20.1± 0.18 | 40.5± 0.5 | 28.5± 1.25 | 20± 0.53 |
| 50 | 28.1± 0.62 | 27.5± 0.75 | 18± 0.11 | 28.1± 0.62 | 29.1± 0.37 | 10.4± 0.16 | 39.4± 1.37 | 20.4± 1.37 | 12.5± 0.35 | 35.6± 0.87 | 24.1± 0.37 | 10.1± 0.18 |

**Effect of temperature on conidia production of Trichoderma isolates:** The result in Table 2 indicated that optimal temperature for conidia production of all isolates was 40°C after 7 days of incubation, whereas 30°C was optimal after 10 days of incubation of isolates AUT1 and AUT2 (35.6± 0.62×$10^8$ conidia/ g of substrate and 30.3± 0.75×$10^8$ conidia/ g of substrate respectively). The optimal temperature for conidia production of all isolates was at 25°C after 15 days of incubation. After 10 days of incubation the conidia yield of isolate AUT7 (55.4± 0.88×$10^8$ conidia/g of substrate) was maximal at 20°C. The optimal temperature for conidia production of all isolates after 7 days of incubation was 40 °C followed, by 50°C (Table 2). All *Trichoderma* isolates were produced maximum conidia at 40°C after 7 days of incubation except isolate AUT7 which produced maximum conidia at 25°C after 15 days of incubation.

**Effect of pH on conidia production of Trichoderma isolates:** The result in Table 3 showed that pH markedly affect conidia yield of *Trichoderma* isolates. The optimal pH for conidia production of most isolates was between 4.5 and 5.5 (Table. 3). Isolates AUT4 and AUT2 produced maximum conidia (35.6±0.37×$10^8$ conidia/ml and 42.1±4.5 ×$10^8$conidia/ml respectively) at pH 5.5 but, isolate AUT1 produced high conidia at 4.5pH (45±1×$10^8$conidia/ml). The conidia yield of all isolates drop at pH 7.5 except AUT7 (37.5±1.75×$10^8$conidia/ml) which conidiated well at this pH value. But, the pH 3.5 and 7.5 were not suitable for conidiation of most isolates (Table 3).

**Table 3:** The conidia yield of *Trichoderma* isolates cultivated at different pH value after 10 days of incubation.

| | **Mean± SEM of conidia count (×$10^8$conidia/ml) after 10 days of incubation** | | | |
|---|---|---|---|---|
| pH | AUT1 | AUT2 | AUT4 | AUT7 |
| 3.5 | 31.5±0.75 | 19.8±1.25 | 5.75±0.25 | 5.13±0.87 |
| 4.5 | 45±1 | 40.3±2.25 | 11.5±0.5 | 18.5±0.75 |
| 5.5 | 32.5±2.5 | 42.1±4.5 | 35.6±0.37 | 20.8±0.75 |
| 6.5 | 34.3±2.82 | 32.6±4.87 | 31.3±1.25 | 32.1±0.12 |
| 7.5 | 30.5±2.4 | 10.1±0.12 | 11.9±0.12 | 37.5±1.75 |

**Effect of moisture content on conidia production of Trichoderma isolates:** The optimum moisture content for conidia production varied depending on *Trichoderma* isolates and incubation period (Table 4). The results in Table 4 show that 35% moisture level was optimal for conidia production of isolates AUT2 and AUT4 (14.75± 2.25×$10^8$conidia/g of substrate and 11.5± 2×$10^8$conidia/g of substrate respectively) after 7 days of incubation whereas, 40% was optimal for isolates AUT1 and AUT7 (19.56± 0.94×$10^8$conidia/g of substrate and 43.37± 1.62×$10^8$conidia/g of substrate respectively). Optimal moisture level after 10 days of incubation was 40% for all *Trichoderma* isolates except isolate AUT7 (46.5± 2.87×$10^8$conidia/g of substrate) which conidiated well at 50% moisture level. The optimal moisture level for conidia production was 60% for isolates AUT1 and AUT2 (35.25± 5.5×$10^8$conidia/g of substrate and 27.44± 0.06 ×$10^8$conidia/g of substrate respectively) after 15 days of incubation but, for isolate AUT4 (21± 0.12 ×$10^8$conidia/g of substrate) it was 50%. The optimal moisture level for maximum conidia production of isolate AUT7 was 40% after 15 days of incubation. In generally, as the moisture level increased from 35% to 60%, the incubation period extended from 7-15 day for better conidia production was obtained (Table 4).

**Table 4:** The conidia yield of *Trichoderma* isolates cultivated at different moisture levels after 7, 10 and 15 days of incubation.

| Moisture %) | **Mean± SEM of conidia count (×10$^8$ conidia/ g of substrate) of *Trichoderma* isolates after 7, 10 and 15 days of incubation** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **AUT1** | | | **AUT2** | | | **AUT4** | | | **AUT7** | | |
| | 7 | 10 | 15 | 7 | 10 | 15 | 7 | 10 | 15 | 7 | 10 | 15 |
| 35 | 4.94± 1.31 | 15.69± 2.06 | 9.75± 0.62 | 14.75± 2.25 | 12.62±0.01 | 5.05± 0.44 | 11.5± 2 | 5.69± 0.44 | 4.37± 0.62 | 17± 0.75 | 22.12± 1.12 | 9.25± 0.5 |
| 40 | 19.56± 0.94 | 24.37± 2.12 | 8.81± 0.06 | 9.87± 0.37 | 13.87± 0.87 | 8± 0.25 | 8.31± 0.06 | 21.6± 0.62 | 19.25± 2.12 | 43.37± 1.62 | 40± 2.75 | 39± 0.25 |
| 50 | 9.19± 0.81 | 22± 0.42 | 28.37± 0.24 | 8.25± 0.2 | 11± 0.37 | 22.44± 0.06 | 9.5± 0.12 | 12.62± 0.87 | 21± 0.12 | 18.13± 0.87 | 46.5± 2.87 | 21± 0.25 |
| 60 | 8.18± 0.81 | 21.62± 0.25 | 35.25± 5.5 | 9.62± 0.75 | 10.06± 0.06 | 27.44± 0.06 | 10.5± 0.75 | 10.62± 0.87 | 11± 1.0 | 27.75± 0.75 | 34± 0.62 | 24.11±0.2 |

**Effect of incubation period on conidia production of Trichoderma isolates:** The optimum incubation period for conidia production varied depending on *Trichoderma* isolates (Table 5). The optimum incubation period for conidia production was 10 day for isolates AUT2 and AUT7. The maximum conidia yield was recorded after 15 days of incubation for isolate AUT1 and AUT4 (26±0.62×$10^8$conidia/g of substrate and 28.1±1.53×$10^8$conidia/g of substrate respectively).

**Table 5:** Conidia yield of *Trichoderma* isolates after 5, 10 and 15 days of incubation period

| ***Trichoderma* isolates** | **Mean± SEM of conidia count (×$10^8$ conidia/g of substrate)** | | |
|---|---|---|---|
| | **Days after incubation** | | |
| | 5 | 10 | 15 |
| AUT1 | 15.3±0.06 | 21±0.13 | 26±0.62 |
| AUT2 | 20±1.6 | 21.8±1.4 | 18.8±0.53 |
| AUT4 | 16.7±0.77 | 18.5±1.79 | 28.1±1.53 |
| AUT7 | 21.1±0.02 | 25.8±1.28 | 18.7±1.96 |

The conidia yield of isolates AUT1 and AUT4 increased as the incubation period extended from 5 to 15 days (15.3±0.06×$10^8$conidia/g to 26±0.62×$10^8$conidia/g and 16.7±0.77×$10^8$ conidia/g of substrate to 28.1±1.53×$10^8$conidia/g of substrate respectively). But, the conidia yield of isolates AUT2 and AUT7 (20±1.6 to 18.8±0.53×$10^8$ conidia/g of substrate and 21.1±0.02 to 18.7±1.96×$10^8$conidia/g of substrate respectively) was drop after 15 days of incubation period (Table 5).

**Effect of inoculum size on conidia production of Trichoderma isolates:** The results shown in Table 6 indicated that, the conidia yield of isolates AUT1 and AUT2 was increased with increasing sizes of inoculums from 1ml to 2ml (23.5±3.5×$10^8$conidia/g of substrate to 38.5±0.79×$10^8$conidia/g of substrate and 13.6±0.63×$10^8$conidia/g of substrate to 14.6±1.38×$10^8$conidia/g of substrate respectively). The optimum inoculums size for isolate AUT4 and AUT7 was 1ml (12.9×±0.12×$10^8$conidia/g of substrate and 23.4±0.87×$10^8$conidia/g of substrate respectively). The maximum conidia yield was achieved using 2ml inoculums size for isolates AUT1and AUT2 (38.5±0.79×$10^8$ conidia/g of substrate and 14.6±1.38×$10^8$conidia/g of substrate respectively). The conidia yields of all isolates were dropped with an increasing of inoculums size more than 2ml.

**Table 6:** The conidia yield of *Trichoderma* isolates cultivated using different inoculums size after 10 days of incubation.

| ***Trichoderma* isolates** | **Mean ±SEM of conidia count (×$10^8$conidia/g of substrate) Inoculums size** | | | |
|---|---|---|---|---|
| | 1ml | 2ml | 3ml | 4ml |
| AUT1 | 23.5±3.5 | 38.5±0.79 | 12.1±0.88 | 10.8±0.5 |
| AUT2 | 13.6±0.63 | 14.6±1.38 | 14.6±0.12 | 10±0.5 |
| AUT4 | 12.9×±0.12 | 9.69±0.31 | 5.81±0.69 | 4.75±0.75 |
| AUT7 | 23.4±0.87 | 16.8±0.25 | 8.13±6.6 | 14.1±0.56 |

**Effect of inoculum load on conidia production of Trichoderma isolates:** The results shown in Table 7 indicated that the highest conidia yield for isolate AUT1, AUT2 and AUT4 (22±2×$10^8$conidia/g of substrate, 13.9±0.85×$10^8$conidia/g of substrate and 13.3±0.5×$10^8$conidia/g of substrate respectively) was achieved using an inoculum concentration of 1×$10^6$conidia/ml. Isolate AUT7 produced maximum conidia yield at inoculum concentration of 1×$10^8$conidia/ml (19.6±0.37×$10^8$conidia/g of substrate) (Table 7). Conidia yield of most *Trichoderma* isolates were low at inoculums load of 1× $10^8$ conidia/g. However, the conidia yield of isolate AUT7 increased as the conidia concentration increased from 1×$10^4$ to 1×$10^8$ conidia/g (12.3±1.31×$10^8$conidia/g of substrate to 19.6±0.37×$10^8$conidia/g of substrate).

**Table7:** The conidia yield of *Trichoderma* isolates cultivated using different inoculums load after 10 days of incubation.

| ***Trichoderma* isolates** | **Mean± SEM of conidia count (×10⁸ conidia/g of substrate)** | | | |
|---|---|---|---|---|
| | **Inoculums load** | | | |
| | $1\times10^4$ | $1\times10^6$ | $1\times10^7$ | $1\times10^8$ |
| AUT1 | 6.25±0.25 | 22±2 | 21±2 | 4.55±1.05 |
| AUT2 | 13.2±0.7 | 13.9±0.85 | 5.48±0.6 | 3.63±0.37 |
| AUT4 | 11.6±0.37 | 13.3±0.5 | 12.1±0.31 | 4±0.5 |
| AUT7 | 12.3±1.31 | 19.2±3.68 | 19.4±4.81 | 19.6±0.37 |

**Effect of light on conidia production of Trichoderma isolates:** The result in Fig 1 showed that light affect the conidia yield of *Trichoderma* isolates cultivated on wheat straw (AUT1,AUT2 and AUT4) and vegetable wastes (isolate AUT7) in some extent. The conidia yield of all isolates cultivated in the presence of light was higher than the absence of light. The conidia production of isolate AUT2 (149± $0.62\times10^8$conidia/g of substrate in the presence of light and 51.5± $1.25\times10^8$conidia/g of substrate in the absence of light) was highly light dependent, followed by isolate AUT7 (222± $2\times10^8$conidia/g of substrate in the presence of light and 137± $2.25\times10^8$conidia/g of substrate in the absence of light). The conidia production of isolate AUT1 was less light dependent followed by AUT4 when compared with other isolates (Fig 1).

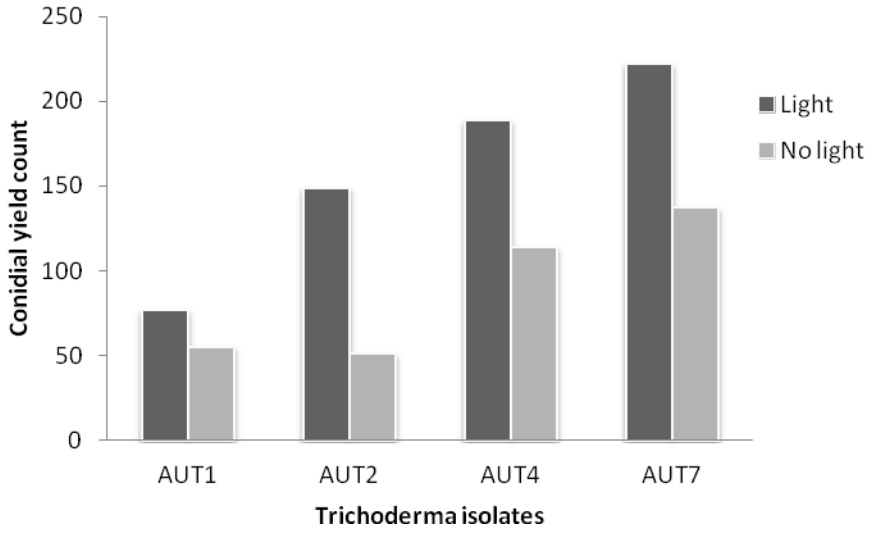


**Figure1:** Effect of light on conidia production of *Trichoderma* isolates

## DISCUSSION

The various agro-industrial wastes substrates evaluated using plastic bags with higher conidia yield was recorded on wheat straw (105±5 to 144±$6\times10^8$ conidia/g of substrate), followed by tea waste (92±3 to 119±$12.5\times10^8$ conidia/g of substrate) for most *Trichoderma* isolates. Similarly, Tewari and Bhanu (2004) reported that wheat straw and paddy straw produced maximum conidial counts of *T. harzianum* ($4.86\times10^8$/g powder and $4.95\times10^8$/g powder, respectively) when compared to saw dust ($2.29\times10^8$/g powder), paper waste ($1.16\times10^8$/g powder) and sugar cane baggesse ($3.73\times10^8$/g powder) after 20 days of incubation of the culture. The present result showed that coffee husk was not suitable for conidia production of all isolates (34.5±1.5 to 83.5±$4.5\times10^8$ conidia/g of substrate). Similarly, Prakash *et al.* (1999) have reported that the population of *T. harzianum* and *T.virens* in coffee husk was minimum ($14\times10^6$cfu/g and $14\times10^5$cfu/g respectively) when compared to tea waste (*490* $\times10^6$cfu/g and *490* $\times10^5$cfu/g respectively). This

may be due to the maximum caffeine content of coffee husk, which was not easily degraded by *Trichoderma* isolates (Roussos *et al*., 1995). In this study the vegetable wastes supported maximum conidia production of isolate AUT7 (405±19.95×$10^8$conidia/g of substrate). Chaudhari *et al. (*2010) also reported that maximum conidia production of *T. viride* was recorded on vegetable wastes (31.2 x $10^8$cfu/g) than fruit juice wastes (24.7 x $10^8$ cfu/g). It has been observed that for isolate AUT1 the conidia yield on wheat straw > Tea wastes > barely bran > vegetable wastes > coffee husk, for isolate AUT2 wheat straw > tea wastes> barely bran > coffee husk > tea wastes, for isolate AUT4 wheat straw > tea wastes> vegetable wastes > barely bran > coffee husk and for isolate AUT7 vegetable wastes> tea wastes> wheat straw > barely bran > coffee husk. All agro-industrial wastes tested in the present study favored the growth and conidia production of *Trichoderma* isolates thereby indicating their high cellulolytic activity. It has been also reported that *Trichoderma* spp are potential cellulose degraders (Schuster & Schmoll, 2010 and Harman *et al*., 2004). In the present study barley bran and vegetable wastes supported higher mycelia growths but lower conidia production, this might be due to suppressive effect of excess nutrients that are found in these wastes. (Belewu and Babalola, 2009) (REFERENCE) The conidia production of isolate AUT7 was maximal in all tested substrates followed by AUT4 (wheat straw, tea waste and vegetable wastes). The present study was conducted using plastic bags as solid medium container for SSF instead of Erlenmeyer flasks. Because of plastic bags can retain temperature and allow the passage of oxygen which stimulate the growth and sporulation of fungal strains during fermentation (Biniyam *et al*., 2010).

In the present study, the *Trichoderma* isolates were grown and conidiated over a broad range of temperature 20-50$^o$C. However, the optimal temperature for high conidia production was depending on the incubation period and fungal isolate. The present study showed that the optimal temperature for conidia production was observed at 25$^o$C after 15 days of incubation of all *Trichoderma* isolates. Similarly, Sanogo *et al*. (2002) have reported that conidia production of *Trichoderma stromaticum* was highest at 20 and 25°C after 14 days of incubation. After 10 days of incubation the conidia yield of all isolates was increased as incubation temperature increased from 20 to 40$^o$C. This result is considerably similar to what was reported by Jayaswal *et al*., (2003) who reported that *T. viride* was grew and sporulated well between temperatures 20 to 37$^o$C. Similarly, Mohd *et al.* (2011) have reported that the most favorable temperature for growth and sporulation of *Trichoderma longibrachiatum* was found in between 25–30$^o$C, followed by 20$^o$C.

The result in Table 2 clearly shows that at lower temperature value (20-25$^o$C) the conidia yield increased with increasing of incubation period from 5-15 days. However, at higher incubation temperature the conidia yield of *Trichoderma* isolates decreased with increasing of incubation period. This may be due to reduction of moisture level as a result of water loss by evaporation at high temperature. All *Trichoderma* isolates produced conidia at all tested temperatures (20-50) but the conidia yield was varied depending on the incubation period and temperature. However, the study that was conducted by Sanogo *et al*. (2002) have reported that *T. stromaticum* produced abundant conidia on PDA at 20 and 25°C, but no conidia at 15, 30, and 35°C after 14 days of incubation.

The optimal pH for conidia production of most isolates was between 4.5 and 5.5. Similarly, Jayaswal *et al*. (2003) have reported that the optimum pH for conidia production of *T. viride* was between 4.5 and 5.5. The pH value of 3.5 and 7.5 were not suitable for conidiation of most *Trichoderma* isolates. But, the optimal pH for conidia production was 7.5 for isolate AUT7. Mohd *et al*. (2011) also reported that the most favorable pH for growth and sporulation of *Trichoderma longibrachiatum* was found in between 6.5 to 7.5. The conidia yield of *T. harzianum* was greater at pH 7.0 than pH 4.0, while faster growth was observed under acidic rather than neutral conditions (Agosin *et al.,* 1997). The optimum pH for the maximum production of conidia varies depending on the *Trichoderma* isolates.

In the present study, 40% moisture level was optimal for conidia production after 10 days incubation of all isolates except isolate AUT7 which produce maximum conidia, at 15$^{th}$ day of incubation. After 7 days of incubation the conidia yield of all isolates was minimal at moisture level of 60%. However, Cavalcante *et al*. (2008) have reported that higher amounts of conidia were obtained at higher moisture contents (≥60%) after 7 days of incubation of *Trichoderma* species. In the present study conidia yield of all isolates was decreased at moisture levels of 35% and 40% as the incubation period increased from 5 to 15 days, but it was increased at moisture levels of 50% and 60% as incubation period increased. The decrease of conidia yield as incubation period increased at lower moisture level is due possibly to decrease of moisture level as a

result of evaporation and metabolic activities of *Trichoderma* isolates.

The optimal incubation period for maximum conidia production varies depending on the *Trichoderma* isolates under SSF. The conidia yield of isolates AUT1 and AUT4 was increased as the incubation period extended from 5 to 15 days. However, the conidia yield of isolates AUT4 and AUT7 was drop after 15 days of incubation period. Similarly, Kumar and Palakshappa (2009) have reported that the population of *T. harzianum* significantly decreased when number of days of incubation increased from 15 to 30 days ($46.67\times 10^6$cfu/g of substrate to $23.67\times 10^6$cfu/g of substrate respectively). Kumar and Palakshappa (2009) also reported that the population of *T. harzianum* cultivated on molasses yeast medium, broken maize grains and sorghum grains was significantly decreased when the number of days of incubation increased from 10 to 30 days. The conidia yield of isolates AUT1 and AUT2 was increased with increasing sizes of inoculums from 1ml to 2ml. The optimum inoculums size for isolate AUT4 was 2ml ($12.9\times\pm0.12\times10^8$ conidia/ml) but, for isolate AUT7 it was 1ml. In the present study the conidia yield of all isolates was decreased with increasing of inoculums sizes more than 2ml, possibly due to increased moisture content of the substrates and it turned to decrease in aeration (Pham *et al*., 2010). The conidia yield of most isolates were low using inoculums load of $10^8$conidia/ml but, the conidia yield of isolate AUT7 was maximal using $10^8$conidia/ml inoculums load.

The present study shows that light induced high conidia production of *Trichoderma* isolates. The conidia yield of all *Trichoderma* isolates cultivated in the presence of light was maximal when compared with those cultivated under dark condition. The conidial production of isolate AUT2 was highly light dependent, followed by isolate AUT7. The conidia production of isolate AUT1 was less light dependent followed by AUT4 when compared with other *Trichoderma* isolates. Papavizas (1985) has observed that the sporulation of *Trichoderma* spp was inducible by pulses of light given to colonies growing in the dark prior to lighting.

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