# Production Of Cellulases By *Trichoderma* Species

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ABSTRACT: Twelve *Trichoderma* strains (*Trichoderma reesei, Trichoderma harzarium, Trichoderma viride, Trichoderma longibrachiatum, Trichoderma asperellum, Trichoderma arundinaceum, Trichoderma konnigii, Trichoderma pseudokonnigii, Trichoderma ciroviride, Trichoderma fertile, Trichoderma polysporum* and *Trichoderma crassum*) obtained from our prevoious work was used during this study. All the isolates were screened for their ability to produce cellulases on solid agar using carboxymethyl cellulose (CMC) and congo red as an indicator. Six isolates were selected as the best cellulases producer. The cellulases: Filterpase production ranged from 2.2172 - 4.3254 x$10^{-7}$units/ml in which *Trichoderma reesei* had the highest production, Endoglucanase production ranged from 1.5707 - 3.3064 x$10^{-7}$units/ml in which *Trichoderma asperellum* had the highest production and β-glucosidase production ranged from 1.1071 - 3.4668 x$10^{-7}$units/ml. On submerged fermentation, agitation condition had a profound effect on cellulase production. The highest yield of cellulases was recorded at 30$^{o}$C, pH 6, 6days incubation time and Tween80 at 0.15ml concentration. Among the carbon and nitrogen sources tested glucose and urea induced Filterpase and β-glucosidase production (1.1826 - 5.5522 x $10^{-7}$units/ml) by *Trichoderma reseei*, manitol and casein supported Endoglucanase production (0.3513 - 1.5984 x$10^{-7}$units/ml) by *Trichoderma viride*.
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## 1. INTRODUCTION

Fungal species belonging to the genus *Trichoderma* are worldwide in occurrence and are easily isolated from soil, decaying wood, poultry farms, and other forms of plant organic matter. They are mostly classified as imperfect fungi because they have no known sexual stage. There are rapid growth rate in culture and the production of numerous spores (conidia) that varies in shades of green characterize fungi in this genus. The reverse side of colonies is often uncolored, buff, yellow, amber, or yellow-green, and many species produce prodigious quantities of thick-walled chlamydospores in submerged mycelium (Gams and Bisset, 1998). Trichoderma produces a wide array of enzymes, being a saprophyte which adapted to thrive in diverse situations. Industrial quantities of enzyme can be produced by selecting strains that produce a particular kind of enzyme, and culturing them in suspension. T. reesei is used for cellulase and hemicellulase, T. longibratum is used for xylanase, and T. harzianum is used for chitinase (Liming and Xueliang, 2004).

Cellulase is an enzyme which breaks down cellulose to beta-glucose. At least two steps in cellulose degradation by microorganisms begin with the preparatory prehydrolytic first step involving an enzyme which swells and/or hydrates anhydroglucose chains. The second step uses hydrolytic enzymes and beta glucosidase (cellobiase). *Trichoderma reesei* has an extensively studied cellulase enzyme complex. This complex converts crystalline, amorphous, and chemically derived celluloses quantitatively to glucose (Kumar, 1998). Cellulases have a wide range of enormous potential applications in biotechnology; they are used in textile industries, detergent, pulp and paper industry, improving digestibility of animal feeds, in food industry and enzymes account for a significant share of the world (Bhat and Bhat, 1997).

## 2. MATERIALS AND METHODS

### 2.1. Collection of Samples

Soil samples were collected aseptically at a depth of 2cm from three different locations in Ibadan: Cocoa Research institute of Nigeria, University of Ibadan Botanical Garden and a decayed wood sample in Microbiology Department, University of Ibadan. The samples were conveyed to the laboratory in sterile polythene bags for further analysis.

### 2.2. Isolation and identification of *Trichoderma* species from collected samples.

Serial dilution of the collected samples was carried out (Olutiola *et al.*2000) and 1ml of the diluents was pour plated on Potato Dextrose agar (PDA) supplemented with streptomycin. The plates were incubated at 28$^{o}$C for 3 days. Morphological appearances of the inoculated plates (at room temperature) were observed and distinct colonies were sub-cultured to obtain pure isolates which were then maintained on PDA slants and stored at 4□C for further study. Microscopic observations were made for the pattern of conidiation and hyphal branching of the

pure fungi isolates after which identification was done with reference to Barnett's Compendium of Soil Fungi (1980), Rifai (1969) and other relevant electronic documentations on the genus *Trichoderma*.

**2.4. Plate Screening for Cellulase Production**

The isolates were screened using Caboxymethylcellulose – Agar (CMC - Agar) medium was used. This medium consist of: 1.00% (w/v) CMC 0.65% (w/v) $NaNO_3$, 0.65% (w/v) $k_2HPO_4$, 0.03% (w/v) yeast extract, 0.65% (w/v) KCl, 0.3% (w/v) $MgSO_4$, 0.65% (w/v) glucose, 1.7% (w/v) agar and 0.1 % (w/v) triton X-100. Also, conidia from one week old PDA plates were suspended in sterile water. A small well created in the middle of the screening plates and same number of conidia of each isolate was inoculated into the wells. Plates were incubated at $28^0$C for three days followed by 18h at $50^0$C. For cellulolytic activity observations, plates were stained with 1% Congo red dye for 0.5-1hr following by staining with 1M NaCl solution for 15-20min.

**2.5. CELLULASE PRODUCTION**

Spores of *Trichoderma* species were suspended in CMC broth containing g $L^{-1}$ of CMC -1, Yeast Extract - 0.1, $(NH_4)_2SO_4$ -0.5, $KH_2PO_4$-10.0, $MgSO_4$ $7H_2O$ -0.1, NaCl -0.2, pH -5.0 and incubated on orbital shaker at 28 ± $2^0$C for 3days and used to inoculate enzyme production media for submerged fermentation. For enzyme production, Erlenmeyer flasks containing 100ml of basal synthetic medium containing $gL^{-1}$ of $(NH_4)_2SO_4$-0.5, $KH_2PO_4$ -10, $K_2HPO_4$ -5, $MgSO_4$ -0.1, NaCl -0.2, Yeast Extract, 0.1g with 1g of CMC were inoculated $10^6$ spores $ml^{-1}$ prepared inoculums were incubated at $28^0$C ± 2 on orbital shaker (150rpm) for 10days. The mycelium free extract was used as crude cellulose preparation (Kocher *et al*., 2008)

**2.6. Dry Cell Weight Determination**

The mycelium from each flask was filtered and then washed. The washed mycelium was dried in British-made Gallenkamp oven at 110□C to a constant mass and the mass was determined using an automatic electronic balance.

**2.7. Statistical analysis**

Experiments were performed in triplicate and the results were analyzed statistically. The treatment effects were compared and the significant difference among replicates has been presented as Duncan's multiple range tests in the form of probability values.

**3. RESULTS AND DISCUSSION**

A total of twelve fungi belonging to the genera *Trichoderma* (*Trichoderma reesei, Trichoderma harzarium, Trichoderma viride, Trichoderma longibrachiatum, Trichoderma asperellum, Trichoderma arundinaceum, Trichoderma konnigii, Trichoderma pseudokonnigii, Trichoderma ciroviride, Trichoderma fertile, Trichoderma polysporum* and *Trichoderma crassum*) were isolated from soil samples collected from University of Ibadan Botanical garden, Cocoa Research Institute of Nigeria and decayed wood in the Department of Microbiology garden. Identification of genus was based on morphological and cultural characteristics compared to fungi compendium (1980), and an illustrated manual on identification of *Trichoderma* species was used.

Table 1 shows the cultural, morphological and microscopic characteristics of the *Trichoderma* species.

The frequency of occurrence of the fungal isolates is shown in Figure 1. *Trichoderma asperellum* had the highest frequency of occurrence (16.8%) followed by *Trichoderma crassum* (12.6%) and *Tricoderma arundinaceum* (8.9%).

Table 2 shows the screening of isolates for cellulase production on solid agar using CMC as the sole carbon source and congo red as an indicator. *Trichoderma crassum, Trichoderma viride, Tricoderma harzarium* had the highest diameter of 1.8mm, 1.6mm, 1.5 mm on the sixth day of incubation followed by *Trichoderma asperellum, Trichoderma longibractum, Trichoderma reesei* with a diameter of 1.45mm, 1.4mm, 1.35 mm. *Trichoderma konnigii* had the least diameter of 0.7 mm on the sixth day of incubation. Based on screening for cellulases on solid agar, six isolates were selected for further studies using submerged fermentation.

Cellulase productions by the selected *Trichoderma* species cultivated in submerged fermentation are shown in Table 3.1. It was observed that there was variation in cellulase production by the selected isolates during the fermentation period. Optimum production of cellulase was recorded when the fermentation medium was agitated. The cellulase production ranges from 2.2172 - 4.3254 $x10^{-7}$ units/ml in which *Trichoderma reesei* had the highest production for Filterpase, Endoglucanase ranged from 0.5707 - 3.3064 $x10^{-7}$units/ml in which *Trichoderma asperellum* had the highest production and β-glucosidase ranged from 1.1071 - 3.4668 $x10^{-7}$units/ml in which *Trichoderma reesei* had the highest production.

The effect of temperature on **c**ellulase production by selected *Trichoderma* species cultivated in submerged fermentation using CMC as the sole carbon source is shown in Table 3.2a, b and c. It was observed that optimum production of Filterpase and Endoglucanase was attained at $30^o$C and the highest β-glucosidase production was attained at $25^o$C for all

the isolates. Filterpase production ranged from 0.3218 - 6.8033 x$10^{-7}$units/ml, Endoglucanase ranged from 0.0549 - 3.3274 x$10^{-7}$units/ml and β-glucosidase ranged from 2.3558 - 5.5578 x$10^{-7}$units/ml and *Trichoderma reesei* had optimum production in the 3 hydrolytic enzymes.

Incubation time had a profound effect on enzyme production as shown in Table 3.3a, b and c respectively. The best incubation time for enzymes (FPA (Filterpase), Endoglucanase and β-glucosidase) production was at six days of fermentation. Filterpase production ranged from 0.0329 - 1.8544 x$10^{-7}$units/ml in which *Trichoderma reesei* had the highest production, Endoglucanase ranged from 0.1296 - 3.7269 x$10^{-7}$units/ml in which *Trichoderma viride* had the highest production and β-glucosidase ranged from 0.0766 - 5.5336 x$10^{-7}$units/ml in which *Trichoderma reesei* had the highest production. Table 3.4a, b and c show the effect of different pH on the three hydrolytic enzyme productions by the selected isolates. pH 6 was found to be the best for enzymes production. For Filterpase production it ranged from 0.3088 - 3.7473 x$10^{-7}$units/ml, for endoglucanase production it ranged from 0.6048 - 2.9204 x$10^{-7}$units/ml and for β-glucosidase production it ranged from 0.0702 - 2.1977 x$10^{-7}$units/ml.

Influence of different concentrations of inducers on enzymes production is shown in Table 3.5a, b and c respectively. Tween80 enhanced the highest production of cellulases at 0.15ml in which *Trichoderma reesei* and *Trichoderma asperellum* had the highest production of Endoglucanase, *Trichoderma reesei* and *Trichoderma crassum* had the optimum production of Filterpase while *Trichoderma viride* has the least production of Filterpase. β-glucosidase production ranged from 3.8086 - 5.7828 x$10^{-7}$units/ml. Effect of different carbon sources on cellulases production is shown in Table 3.6a, b and c respectively. It was observed that Glucose induced the higher level of Filterpase and β-glucosidase production. Filterpase production ranged from 1.1826 - 5.5522 x $10^{-7}$units/ml in which *Trichoderma reesei* had the highest production while β-glucosidase ranged from 0.2052 - 2.0088 x$10^{-7}$units/ml in which *Trichoderma crassum* had the highest production. Carbon sources had effect on Endoglucanase production by the isolates. Manitol supported the production of Endoglucanase and it ranged from 0.3513 - 1.5984 x$10^{-7}$units/ml in which *Trichoderma viride* had the highest production. Table 3.7a, b and c show the effect of different nitrogen sources on cellulase production by the selected isolates. Urea supported the optimal production of Filterpase and β-glucosidase enzyme, Filterpase production ranged from 2.7432 - 7.6862 x$10^{-7}$units/ml, β-glucosidase production ranged from 0.3564 - 1.8608 x$10^{-7}$units/ml in which *Trichoderma crassum* had the highest production. There was a significant difference in Endoglucanase production by the selected isolates in which casein supported the optimum production and *Trichoderma viride* 1.9284a x$10^{-7}$units/ml had the best production.

**Table 1: Cultural and Morphological Characteristic of *Trichoderma* species obtained from soil samples.**

| Isolate code | Appearance on Agar | Growth pattern | Microscopic spore shape | Probable Identity |
|---|---|---|---|---|
| ASB1 | Dark green granular colony | Rapid with coconut odour | Phialides typically crowded arising from broad cells, Conidiophore with branches | *Trichoderma viride* |
| AS2 | Yellow green conidia formed densely over the center and in undulating concentric rings | Fairly rapid | Globose, intercalary hyphae and Terminal phialides | *Trichoderma harzianum* |
| AW3 | Dark green, mottled with white flecks | Fairly rapid | Phialides mainly arising singly, in divergent whorls and typically cylindrical | *Trichoderma longibrachiatum* |
| AW14 | White with a diffusing yellow pigment | Fairly rapid | Phialides in whorls at the tip of fertile branches | *Trichoderma polysporum* |
| AW5 | Wooly green rings | Fairy rapid | Paired lateral branches | *Trichoderma pseudokoningii* |
| AW16 | Yellowish green | Rapid | Phialides held in whorls | *Trichoderma arundinaceum* |
| AS7 | Greenish mycelium | Fairly rapid | Phialides supported by a base cell typically terminating cells of branches in pairs | *Trichoderma citrinoviride* |
| AW8 | Dark green, dense wooly colony | Rapid | Phialides formed on conidiophores within pustules | *Trichoderma asperellum* |
| AS9 | Yellowish brown granules | Rapid | Intercalary within hyphae | *Trichoderma crassum* |
| AW10 | Diffusing yellow pigment | | Phialides held in whorls | *Trichoderma* |

| | | | | |
|---|---|---|---|---|
| | conidiation | Fairly rapid | | *konnigii* |
| NUB5 | Greenish, uniformly dispersed colonies | Fairly rapid | Long straight phialides, typically flask-shaped and enlarged in the middle | *Trichoderma reesei* |
| NUB8 | Conidia formed densely in a central disk and concentric rings of conidial production. No pigment in the agar | Fairly rapid | Basal phialides tending to be held in more or less divergent whorl while terminal phialides slightly hooked | *Trichoderma fertile* |

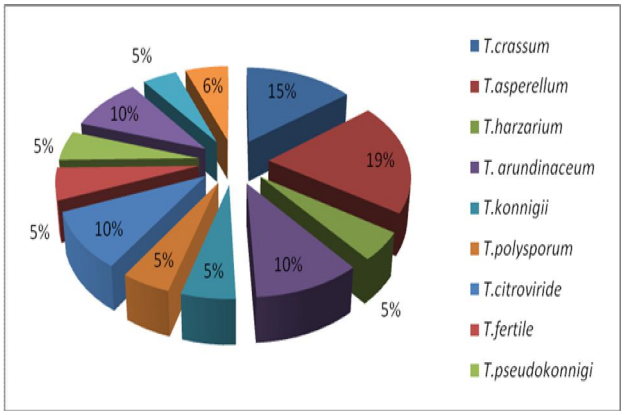


**Figure 1 Percentage frequency of occurrence of microorganisms isolated from the soil samples**

**Table 3.2b: Effect of Temperature on Endoglucanase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | Endoglucanase production (units/ml) x10$^{-7}$ | | | | |
|---|---|---|---|---|---|
| | Temperature ($^{o}$C) | | | | |
| | 25 | 30 | 35 | 40 | 45 |
| *T. reesei* | 1.9224$^{b}$ | 3.3274$^{a}$ | 0.5184$^{d}$ | 0.2916$^{e}$ | 0.6642$^{c}$ |
| *T. viride* | 1.5606$^{b}$ | 1.9656$^{a}$ | 0.594$^{d}$ | 0.7344$^{c}$ | 0.3024e |
| *T. harzarium* | 1.1344$^{b}$ | 1.9224$^{a}$ | 0.4482$^{e}$ | 1.053$^{c}$ | 0.9504$^{d}$ |
| *T. longibrachiatum* | 1.2636$^{b}$ | 2.2194$^{a}$ | 0.6588$^{d}$ | 0.8316$^{c}$ | 0.3672$^{e}$ |
| *T .crassum* | 2.1875$^{b}$ | 3.1752$^{a}$ | 0.7837$^{e}$ | 1.0044$^{c}$ | 0.6318$^{d}$ |
| *T.asperellum* | 3.1374$^{b}$ | 3.3048$^{a}$ | 2.1276$^{c}$ | 0.0972$^{d}$ | 0.0549$^{e}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.2c: Effect of Temperature on β-glucosidase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | β-glucosidase production (units/ml) x10$^{-7}$ | | | | |
|---|---|---|---|---|---|
| | Temperature ($^{o}$C) | | | | |
| | 25 | 30 | 35 | 40 | 45 |
| *T. reesei* | 5.5578$^{a}$ | 4.79682$^{c}$ | 4.6186$^{d}$ | 4.8773$^{b}$ | 3.0904$^{e}$ |
| *T. viride* | 4.5921$^{b}$ | 4.2606$^{c}$ | 4.7422$^{a}$ | 3.9644$^{d}$ | 2.5362$^{e}$ |
| *T. harzarium* | 6.9373$^{a}$ | 5.5566$^{b}$ | 4.3070$^{c}$ | 4.2525$^{d}$ | 3.1696$^{e}$ |
| *T. longibrachiatum* | 4.5191$^{a}$ | 4.32108$^{b}$ | 3.9349$^{d}$ | 4.2154$^{c}$ | 2.3558$^{e}$ |
| *T .crassum* | 5.0970$^{a}$ | 4.97772$^{b}$ | 4.8880$^{c}$ | 4.0829$^{d}$ | 3.1338$^{e}$ |
| *T.asperellum* | 4.7579$^{a}$ | 4.5829$^{b}$ | 4.0905$^{c}$ | 4.1466$^{d}$ | 2.7999$^{e}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.3a: Effect of incubation time on FPA production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | FPA production (units/ml) x10$^{-7}$ | | | |
|---|---|---|---|---|
| | Incubation Time (Days) | | | |
| | 3 | 6 | 9 | 12 |
| *T. reesei* | 0.3672$^{d}$ | 1.8544$^{a}$ | 0.5308$^{b}$ | 0.3974$^{c}$ |
| *T. viride* | 0.0329$^{d}$ | 0.2613a | 0.2527$^{b}$ | 0.1182$^{c}$ |
| *T. harzarium* | 0.1776$^{a}$ | 1.4520$^{b}$ | 0.0725$^{d}$ | 0.5616$^{c}$ |
| *T.longibrachiatum* | 0.2413$^{c}$ | 1.8098$^{a}$ | 0.2100$^{d}$ | 0.3682$^{b}$ |
| *T. crassum* | 0.0453$^{d}$ | 0.1760$^{a}$ | 0.4455$^{b}$ | 0.2062$^{c}$ |
| *T. asperellum* | 0.0469$^{d}$ | 0.5535$^{a}$ | 0.0610$^{c}$ | 0.8289$^{b}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.3b: Effect of incubation time on Endoglucanase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | Endoglucanase production (units/ml) x10$^{-7}$ | | | |
|---|---|---|---|---|
| | Incubation Time (Days) | | | |
| | 3 | 6 | 9 | 12 |
| *T. reesei* | 0.3618$^{c}$ | 1.4644$^{a}$ | 0.2096$^{d}$ | 1.3554$^{b}$ |
| *T. viride* | 0.2106$^{d}$ | 1.3458$^{a}$ | 0.4057$^{b}$ | 0.2758$^{c}$ |
| *T. harzarium* | 0.3024$^{d}$ | 3.7269$^{a}$ | 0.3294$^{c}$ | 0.8802$^{b}$ |
| *T.longibrachiatum* | 0.2376$^{d}$ | 4.9146$^{a}$ | 0.8046$^{b}$ | 0.6642$^{c}$ |
| *T. crassum* | 0.6804$^{d}$ | 1.9512$^{b}$ | 1.0348$^{c}$ | 2.7275$^{a}$ |
| *T. asperellum* | 0.1998$^{c}$ | 0.2594$^{b}$ | 0.6588$^{a}$ | 0.1296$^{d}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.3c: Effect of incubation time on β-glucosidase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | β-glucosidase production (units/ml) x10$^{-7}$ | | | |
|---|---|---|---|---|
| | Incubation Time (Days) | | | |
| | 3 | 6 | 9 | 12 |
| *T. reesei* | 2.2561$^{b}$ | 5.5336$^{a}$ | 0.3029$^{c}$ | 0.1981$^{d}$ |
| *T. viride* | 2.4953$^{b}$ | 4.2584$^{a}$ | 0.1506$^{d}$ | 0.2019$^{c}$ |
| *T. harzarium* | 2.4067$^{b}$ | 5.2666$^{a}$ | 0.1312$^{d}$ | 1.7220$^{c}$ |
| *T.longibrachiatum* | 3.6844$^{b}$ | 4.5851$^{a}$ | 0.0766$^{d}$ | 0.9547$^{c}$ |
| *T. crassum* | 3.9841$^{b}$ | 5.3502$^{a}$ | 1.4315$^{c}$ | 1.0503$^{d}$ |
| *T. asperellum* | 3.2103$^{b}$ | 4.2514$^{a}$ | 0.6598$^{d}$ | 1.2733$^{c}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.4a: Effect of Tween80 on FPA production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | FPA production (units/ml) x10$^{-7}$ | | | | |
|---|---|---|---|---|---|
| | Tween80 concentration (ml/l) | | | | |
| | 0.05 | 0.1 | 0.15 | 0.25 | 0.3 |
| *T. reesei* | 5.5236$^{c}$ | 6.1576$^{b}$ | 7.9632$^{a}$ | 5.3087$^{e}$ | 5.3271$^{d}$ |
| *T. viride* | 4.5311$^{e}$ | 5.5679$^{c}$ | 6.9502$^{a}$ | 6.6047$^{b}$ | 5.2336$^{d}$ |
| *T. harzarium* | 6.1570$^{c}$ | 6.5647$^{b}$ | 6.9595$^{a}$ | 5.6802$^{e}$ | 6.1009$^{d}$ |
| *T.longibrachiatum* | 4.7476$^{e}$ | 6.7483$^{b}$ | 6.8358$^{a}$ | 5.5857$^{c}$ | 4.7968$^{d}$ |
| *T. crassum* | 4.9053$^{e}$ | 6.7640$^{c}$ | 7.7392$^{a}$ | 7.1393$^{b}$ | 6.2823$^{d}$ |
| *T. asperellum* | 5.4556$^{c}$ | 5.2369$^{e}$ | 6.0388$^{a}$ | 5.7148$^{b}$ | 5.4340$^{d}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.4b: Effect of Tween80 on Endoglucanase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | Endoglucanase production (units/ml) x10$^{-7}$ | | | | |
|---|---|---|---|---|---|
| | Tween80 concentration (ml/l) | | | | |
| | 0.05 | 0.1 | 0.15 | 0.25 | 0.3 |
| *T. reesei* | 1.2204$^{d}$ | 2.0736$^{b}$ | 2.1066$^{a}$ | 2.0173$^{c}$ | 0.5076$^{e}$ |
| *T. viride* | 0.2268$^{c}$ | 0.3078$^{b}$ | 0.7028$^{a}$ | 0.2214$^{d}$ | 0.0819$^{e}$ |
| *T. harzarium* | 0.7295$^{d}$ | 1.3608$^{c}$ | 1.6146$^{a}$ | 0.7128$^{e}$ | 1.5126$^{b}$ |
| *T.longibrachiatum* | 0.2754$^{e}$ | 0.5454$^{d}$ | 1.0152$^{a}$ | 0.6485$^{b}$ | 0.6219$^{c}$ |
| *T. crassum* | 0.4914$^{d}$ | 0.9504$^{b}$ | 1.0476$^{a}$ | 0.3024$^{e}$ | 0.8532$^{c}$ |
| *T. asperellum* | 1.6473$^{e}$ | 2.1067b | 2.1546$^{a}$ | 1.7015$^{d}$ | 1.8414$^{c}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.4c: Effect of Tween80 on β-glucosidase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| | β-glucosidase production (units/ml) x10$^{-7}$ | | | | |
|---|---|---|---|---|---|
| | Tween80 concentration (ml/l) | | | | |
| Isolate code | 0.05 | 0.1 | 0.15 | 0.25 | 0.3 |
| *T. reesei* | 4.1596$^{e}$ | 4.2357$^{b}$ | 5.6104$^{a}$ | 4.1817$^{d}$ | 4.2260$^{c}$ |
| *T. viride* | 3.8863$^{e}$ | 3.9884$^{d}$ | 4.1223a | 3.9863$^{c}$ | 4.0638$^{b}$ |
| *T. harzarium* | 4.1720$^{d}$ | 4.5252$^{b}$ | 4.6580$^{a}$ | 4.5241$^{c}$ | 4.0840$^{e}$ |
| *T.longibrachiatum* | 3.9857$^{e}$ | 4.2129$^{c}$ | 4.6018$^{a}$ | 4.0240$^{d}$ | 4.5079$^{b}$ |
| *T. crassum* | 4.4814$^{c}$ | 4.2303$^{d}$ | 4.7806$^{a}$ | 3.8086$^{e}$ | 4.4890$^{b}$ |
| *T. asperellum* | 4.2417$^{e}$ | 4.5096$^{b}$ | 5.7828$^{a}$ | 4.3151$^{c}$ | 4.2897$^{d}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.5a: Effect of pH on FPA production (units/ml) $10^{-7}$ by *Trichoderma* species**

| Isolate code | FPA production (units/ml) $10^{-7}$ | | | |
|---|---|---|---|---|
| | pH | | | |
| | 3 | 6 | 10 | 14 |
| *T.reesei* | 3.6666[b] | 3.7473[a] | 0.5626[c] | 0.1544[d] |
| *T.viride* | 0.5194[b] | 1.0319[a] | 0.4973[c] | 0.3088[d] |
| *T.harzarium* | 1.3651[b] | 1.2587[a] | 0.4401[c] | 0.1512[d] |
| *T.longibrachiatum* | 1.0087[c] | 3.8421[a] | 1.7911[b] | 0.3861[d] |
| *T.crassum* | 0.9844[b] | 1.2047[a] | 0.4552[c] | 0.4509[d] |
| *T.asperellum* | 1.9035[b] | 2.3441[a] | 1.2576[c] | 0.9498[d] |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.5b: Effect of pH on Endoglucanase production (units/ml) $10^{-7}$ by *Trichoderma* species**

| Isolate code | Endoglucanase production (units/ml) $10^{-7}$ | | | |
|---|---|---|---|---|
| | pH | | | |
| | 3 | 6 | 10 | 14 |
| *T.reesei* | 2.1384[b] | 2.9204[a] | 1.2332[c] | 1.1286[d] |
| *T.viride* | 0.6048[d] | 1.5444[a] | 1.4526[b] | 1.2312[c] |
| *T.harzarium* | 1.7982[c] | 2.7594[a] | 2.5812[b] | 1.4958[d] |
| *T.longibrachiatum* | 1.0044[d] | 2.0314[a] | 1.0412[b] | 1.0358[c] |
| *T.crassum* | 0.9072[c] | 2.6092[a] | 0.8532[d] | 1.0898[b] |
| *T.asperellum* | 0.8478[d] | 2.9164[a] | 1.9173[c] | 2.0304[b] |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.5c: Effect of pH on β-glucosidase production (units/ml) $10^{-7}$ by *Trichoderma* species**

| Isolate code | β-glucosidase production (units/ml) $10^{-7}$ | | | |
|---|---|---|---|---|
| | pH | | | |
| | 3 | 6 | 10 | 14 |
| *T.reesei* | 1.1691[b] | 2.1977[a] | 0.2203[c] | 0.2149[d] |
| *T.viride* | 1.0984[b] | 1.7368[a] | 0.4849[c] | 0.4870[d] |
| *T.harzarium* | 0.5718[c] | 1.2652[a] | 1.2128[b] | 0.3407[d] |
| *T.longibrachiatum* | 1.0319[b] | 1.5222[a] | 0.5994[c] | 0.1166[d] |
| *T.crassum* | 0.8148[c] | 1.9286[a] | 0.8693[b] | 0.7360[d] |
| *T.asperellum* | 0.3099[c] | 0.3904[a] | 0.3574[b] | 0.0702[d] |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.6a: Effect of carbon sources on FPA production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | FPA production (units/ml) x10$^{-7}$ | | | | | |
|---|---|---|---|---|---|---|
| | Carbon sources (g/l) | | | | | |
| | Sucrose | Glucose | Fructose | Lactose | Maltose | Manitol |
| *T.reesei* | 1.4504$^{f}$ | 5.5522$^{a}$ | 1.5768$^{e}$ | 2.9862$^{c}$ | 3.0024$^{b}$ | 2.1708$^{d}$ |
| *T.viride* | 3.6187$^{c}$ | 4.3595$^{a}$ | 4.1848$^{b}$ | 2.5164$^{f}$ | 3.5856$^{d}$ | 2.8404$^{e}$ |
| *T.harzarium* | 3.3082$^{c}$ | 4.8178$^{a}$ | 2.7648$^{d}$ | 4.1256$^{b}$ | 1.1826$^{f}$ | 2.7216$^{e}$ |
| *T.longibrachiatum* | 2.6196$^{f}$ | 3.8728$^{a}$ | 3.6724$^{b}$ | 3.2945$^{c}$ | 3.0942$^{e}$ | 3.2562$^{d}$ |
| *T.crassum* | 3.8074$^{e}$ | 5.2542$^{a}$ | 4.2822$^{d}$ | 3.3756$^{f}$ | 4.8924$^{c}$ | 4.9734$^{b}$ |
| *T.asperellum* | 2.1168$^{f}$ | 3.9786$^{a}$ | 3.7908$^{b}$ | 3.2778$^{d}$ | 3.8232$^{c}$ | 2.4138$^{e}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.6b: Effect of carbon sources on Endoglucanase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | Endoglucanase production (units/ml) x10$^{-7}$ | | | | | |
|---|---|---|---|---|---|---|
| | Carbon sources (g/l) | | | | | |
| | Sucrose | Glucose | Fructose | Lactose | Maltose | Manitol |
| *T.reesei* | 0.7294$^{e}$ | 0.5487$^{f}$ | 0.9828$^{d}$ | 1.2966$^{b}$ | 1.1744$^{c}$ | 1.2986$^{a}$ |
| *T .viride* | 1.0856$^{b}$ | 0.6534$^{e}$ | 0.5886$^{f}$ | 0.7293$^{d}$ | 0.8137$^{c}$ | 1.5984$^{a}$ |
| *T. harzarium* | 0.8262$^{e}$ | 0.6858$^{f}$ | 1.2096$^{c}$ | 1.1448$^{d}$ | 1.8576$^{b}$ | 1.3239$^{a}$ |
| *T. longibrachiatum* | 0.7992$^{b}$ | 0.3513$^{f}$ | 0.7074$^{c}$ | 0.5346$^{e}$ | 0.6858$^{d}$ | 0.9774$^{a}$ |
| *T. crassum* | 1.0962$^{b}$ | 0.8802$^{e}$ | 0.9018$^{d}$ | 0.6048$^{f}$ | 0.9726$^{c}$ | 1.2964$^{a}$ |
| *T. asperellum* | 1.1556$^{e}$ | 1.2646$^{cd}$ | 1.0906$^{b}$ | 1.2528$^{d}$ | 0.9180$^{f}$ | 1.0926$^{a}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.6c: Effect of carbon sources on β-glucosidase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | β-glucosidase production (units/ml) x10$^{-7}$ | | | | | |
|---|---|---|---|---|---|---|
| | Carbon sources (g/l) | | | | | |
| | Sucrose | Glucose | Fructose | Lactose | Maltose | Manitol |
| *T.reesei* | 0.5292$^{e}$ | 1.4202$^{a}$ | 0.2052$^{f}$ | 0.6048$^{d}$ | 0.7398$^{c}$ | 1.4634$^{b}$ |
| *T.viride* | 0.1782$^{f}$ | 1.9978$^{a}$ | 1.0701$^{c}$ | 0.8748$^{d}$ | 0.5948$^{e}$ | 1.4904$^{b}$ |
| *T.harzarium* | 0.8100$^{d}$ | 1.7554$^{a}$ | 1.5926$^{b}$ | 0.6966$^{e}$ | 0.4968$^{f}$ | 1.3716$^{c}$ |
| *T.longibrachiatum* | 0.6534$^{d}$ | 1.4472$^{a}$ | 0.5022$^{e}$ | 0.5184$^{d}$ | 0.7776$^{c}$ | 1.4148$^{b}$ |
| *T.crassum* | 0.8154$^{e}$ | 2.0088$^{a}$ | 1.8738$^{b}$ | 0.59446$^{f}$ | 0.9828$^{d}$ | 1.7016$^{c}$ |
| *T.asperellum* | 0.5508$^{d}$ | 1.4824$^{a}$ | 0.3564$^{f}$ | 0.4266$^{e}$ | 0.7668$^{c}$ | 1.358$^{b}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.7a: Effect of nitrogen sources on FPA production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | FPA production (units/ml) x10$^{-7}$ | | | | | |
|---|---|---|---|---|---|---|
| | Nitrogen sources (g/l) | | | | | |
| | Casein | Yeast | $NaNo_3$ | Urea | $NH_4No_3$ | $(NH_4)_2SO_4$ |
| *T. reesei* | 4.0402$^{c}$ | 3.4938$^{d}$ | 4.6548$^{b}$ | 4.7182$^{a}$ | 2.8188$^{f}$ | 3.4837$^{e}$ |
| *T. viride* | 4.7412$^{c}$ | 3.5802$^{e}$ | 3.6993$^{d}$ | 5.7244$^{a}$ | 5.3942$^{b}$ | 2.9268$^{f}$ |
| *T. harzarium* | 3.4678$^{d}$ | 4.9518$^{b}$ | 4.8168$^{c}$ | 4.9842$^{a}$ | 3.0186$^{e}$ | 2.7432$^{f}$ |
| *T.longibrachiatum* | 3.7422$^{e}$ | 4.8978$^{c}$ | 2.6194$^{f}$ | 7.6862$^{a}$ | 5.2468$^{b}$ | 4.2391$^{d}$ |
| *T. crassum* | 5.2542$^{b}$ | 3.6234$^{d}$ | 4.7466$^{c}$ | 6.4854$^{a}$ | 3.4452$^{f}$ | 3.4722$^{e}$ |
| *T. asperellum* | 3.9582$^{c}$ | 3.3966$^{b}$ | 3.4236$^{d}$ | 4.0186$^{a}$ | 3.3514$^{e}$ | 2.9268$^{f}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.7b: Effect of nitrogen sources on Endoglucanase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | Endoglucanase production (units/ml) x10$^{-7}$ | | | | | |
|---|---|---|---|---|---|---|
| | Nitrogen sources (g/l) | | | | | |
| | Casein | Yeast | $NaNo_3$ | Urea | $NH_4No_3$ | $(NH_4)_2SO_4$ |
| *T. reesei* | 1.1082$^{a}$ | 0.1085$^{f}$ | 0.9072$^{c}$ | 1.0962$^{b}$ | 0.8478$^{d}$ | 0.7452$^{e}$ |
| *T. viride* | 1.9284$^{a}$ | 1.1178$^{d}$ | 1.2474$^{c}$ | 0.7884$^{e}$ | 1.3554$^{b}$ | 0.6966$^{f}$ |
| *T. harzarium* | 1.8856$^{a}$ | 1.0886$^{d}$ | 1.0908$^{c}$ | 0.8532$^{e}$ | 0.4536$^{f}$ | 1.1178$^{b}$ |
| *T.longibrachiatum* | 1.1826$^{a}$ | 0.3834$^{f}$ | 0.7992$^{c}$ | 0.5778$^{e}$ | 0.9967$^{b}$ | 0.6534$^{d}$ |
| *T. crassum* | 1.3212$^{a}$ | 0.4482$^{f}$ | 1.0024$^{b}$ | 0.6102$^{c}$ | 0.9072$^{d}$ | 0.7839$^{e}$ |
| *T. asperellum* | 1.6578$^{a}$ | 1.2636$^{b}$ | 0.9504$^{e}$ | 1.1883$^{d}$ | 0.6534$^{f}$ | 1.2312$^{c}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

**Table 3.7c: Effect of nitrogen sources on β-glucosidase production (units/ml) x10$^{-7}$ by *Trichoderma* species**

| Isolate code | β-glucosidase production (units/ml) x10$^{-7}$ | | | | | |
|---|---|---|---|---|---|---|
| | Nitrogen sources (g/l) | | | | | |
| | Casein | Yeast | $NaNo_3$ | Urea | $NH_4No_3$ | $(NH_4)_2SO_4$ |
| *T. reesei* | 1.6146$^{b}$ | 0.7722$^{e}$ | 1.4256$^{c}$ | 1.6958$^{a}$ | 1.2096$^{d}$ | 0.7025$^{f}$ |
| *T. viride* | 1.4742$^{c}$ | 1.5396$^{b}$ | 1.4148$^{d}$ | 1.5742$^{a}$ | 1.3662$^{e}$ | 1.3284$^{f}$ |
| *T. harzarium* | 1.3773$^{e}$ | 1.4526$^{d}$ | 1.4904$^{b}$ | 1.8554$^{a}$ | 1.4688$^{c}$ | 0.8856$^{f}$ |
| *T.longibrachiatum* | 1.3547$^{d}$ | 1.4634$^{b}$ | 1.0746$^{f}$ | 1.7014$^{a}$ | 1.4318$^{c}$ | 1.1826$^{e}$ |
| *T. crassum* | 1.7289$^{b}$ | 1.4958$^{c}$ | 1.2042$^{e}$ | 1.8608$^{a}$ | 1.3237$^{d}$ | 0.4212$^{f}$ |
| *T. asperellum* | 1.5012$^{c}$ | 0.3564$^{f}$ | 1.1349$^{e}$ | 1.7986$^{a}$ | 1.2528$^{d}$ | 1.6578$^{b}$ |

Values are means of three triplicates. Mean values in columns with different superscript letters are significantly different (P≥0.05), NG- NO GROWTH

## 4.0 Discussion

Microbes are an attractive topic of interest for the production of enzyme complexity and extreme habitat variability (Sarkar *et al*., 1995). In the present study, a total of 12 *Trichoderma* species were isolated from soil samples and they were able to grow and produce cellulase at different rates. The enzymes produced enable the organisms to depolymerize crystalline cellulose (Wood and Kellogg, 1988). The cellulase system comprises of endoglucanase which randomly hydrolyze 1, 4-β bonds within cellulose molecules thereby producing reducing and non-reducing ends; Exoglucanase which cleaves cellobiose units from non-reducing ends of cellulose polymer, β-glucosidase which hydrolyze cellobiose and low molecular weight cellodextrins thereby yielding glucose and filter paper activity (Coughlan and Ljundahl, 1998).

Cellulase productions by the selected *Trichoderma* species cultivated in submerged fermentation are shown in Table 3.1 It was observed that there was variation in cellulase production by the selected isolates during the fermentation period. Highest production of cellulase was recorded when the fermentation medium was agitated which is in agreement with the work of Kocher *et al*. (2008). The cellulase production ranges from 2.2172 - 4.3254 $x10^{-7}$units/ml in which *Trichoderma reesei* had the highest production for Filterpase, Endoglucanase production ranged from 0.5707 - 3.3064 $x10^{-7}$units/ml in which *Trichoderma asperellum* had the highest production and β-glucosidase production ranged from 1.1071 - 3.4668 $x10^{-7}$units/ml in which *Trichoderma reesei* had the highest production.

The profile of cellulase at different temperature levels, it was observed that 30$^{o}$C gave the best yield for cellulase enzyme production in submerged state fermentation which is in agreement with the work of Kocher *et al*. (2008). The production of enzyme is very sensitive to the incubation temperature as reported by Smits *et al*. (2003). Among the four pH levels tested, pH 6 supported the optimum production of FPA, Endoglucanase and β-glucosidase after 6days of incubation in which this agrees with the work of Juhasz *et al*. (2004) as they reported a high Endoglucanase, FPA (Filter paper activity) and β-glucosidase production at pH 6 after 6 days of incubation time for *Trichoderma* species.

Cellulase production was increased when Tween80 was added to their fermentation medium but when the concentration was increased to 0.15%, a substantial increase in Filterpase production resulted and is in accordance with the work of El-Halwary and Mostafa, (2001) which reported that supplement like Tween80 enhances enzymes activities by increasing availability of nutrients. The mechanism of enhancement by Tween80 at low concentration increases the permeability of the cell membrane allowing for more rapid secretion of the enzyme which in turn leads to greater enzyme synthesis which is in agreement with the work of Kishen *et al*. (1981).

Glucose, Sucrose, Fructose, Maltose, Manitol and lactose induced the production of cellulases enzymes. Glucose gave the best yield for Filterpase and β-glucosidase enzyme. This could be because glucose can be easily metabolized by the isolate which was in agreement with the work of Ikram *et al* (2006) while manitol gave the highest yield of Endoglucanase production which was not in agreement with the work of Saha (2003).

Urea as a source of nitrogen induced higher Filterpase and β-glucosidase production. This agrees with the studies about the production of extracelluar enzymes used for the bioconversion of rice straw by Khan *et al*. (2007). Casein gave the highest production of Endoglucanase enzyme. Inorganic nitrogen sources gave higher enzyme yield than organic nitrogen as reported by Kudryashova *et al.* (1976) that organic nitrogen sources do not favour cellulase synthesis in *Geotrichum candidum* although some organic sources have been reported to favour the synthesis of cellulolytic and xylanolytic enzymes by Brown *et al.* (1987).

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