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Effect of Fermentation Parameters for the Mass Cultivation of Trichoderma *viride* via Submerged and Solid-State Fermentation Studies On Cellulase Production

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Abstract

The present investigation was carried out to evaluate different fermentation parameters and to optimize the fermentation parameters to cultivate Trichoderma *viride* (T. *viride*) via submerged and solid-state fermentations. The different parameters such as, effect of pH, temperature, inoculum size, media volume, incubation period and different nitrogen sources. The highest growth yield noticed was 14.221g L^{-1} at 15 g L^{-1}, 0.15 g L^{-1}, 40 ^{o}C, 2.0, 7.0 and 200 carbon, nitrogen, temperature, volume of the medium, pH, RPM respectively. Following, 12.187 g L^{-1} yield of T. *viride* was achieved with 15 g L^{-1}, 0.15 g L^{-1}, 40 ^{o}C, 2.0, 7.0, 200 carbon, nitrogen, temperature, volume of the medium, pH, RPM respectively. the effect of different incubation period on the cellulase activity was ranged from 0.76U/mL/min to 3.02 U/mL/min. The effect of different incubation period on the cellulase activity was ranged from 0.63 U/mL/min to 2.88 U/mL/min. The effect of different pH on the cellulase activity was ranged from 0.436 U/mL/min to 2.481 U/mL/min. the highest cellulase activity was noticed at pH 5.5 with 2.481 Nitrogen sources, five (5) namely, NH_4Cl, NH_4, CH_3COO, $(NH_4)_2SO_4$, $NH_4H_2PO_4$, NH_4NO_3 are identified as significant sources for cultivation of T. *viride* and subsequent production and activity of cellulase. In accordance to results obtained, the inoculum size of 4mL found significant with 2.321 U/mL/min Out of Seven (7), three (3) media volumes such as 30mL, 40mL, and 50mL produced highest cellulase production with 2.964 U/mL/min, 2.331 U/mL/min.

Key words: Trichoderma *viride*, pH, temperature, inoculum size, media volume, incubation period and different nitrogen sources

1.0 INTRODUCTION

Trichoderma *viride* (T. *viride*), a mould belonging to Hypocreaceae family, order- Hypocreales, and class- Ascomycetes, is widely recognised for its biological control and as well as for cellulose and chitinase production (Bailey et al., 2004). The environmental damage brought on by the overuse of chemical pesticides in recent years has boosted interest in integrated pest management, which uses biopesticides instead of chemical pesticides to treat plant diseases and pests.

Plant diseases are a big issue nowadays that is reducing agricultural productivity across the globe. The Food and Agriculture Organisation of the United Nations (FAO) estimates that each year, losses in global agriculture brought on by pests and phytopathogenic fungi account for around 25% of the crop's potential yield (Strange and Scott, 2005; Oerke, 2006; Savary et al, 2012; Draganova et al., 2014). Currently, scientists working in the subject of phytopathology devote a lot of time and energy to researching the physiology, morphology, biochemistry, and genetics of phytopathogens in an effort to lessen the severity of their harm and prevalence (Lorito et al., 1993; Mathivanan et al., 1998; Poloni et al., 2009; Martínez et al., 2016; Shivalee etal., 2016; Muhammadiev and Bagaeva, 2018; Van et al., 2019). The most often utilised technique for protecting plants in agricultural practise is the use of chemical fungicides. Although using fungicides has many important advantages, doing so can also have undesirable effects, including a decrease in soil fertility, disruption of the soil-ecological balance, extinction of insect species, deterioration of agricultural products, and a host of other problems (Dudkin and Karseev, 2014; Yu and Vologdin, 2015; Dudkin, 2016; Myazin, 2017; Myazin et al., 2018; Gryznova et al., 2019; Yushkova et al., 2019). It is currently important to develop substitute and efficient plant protection techniques as well as associated medications in order to limit their use.

Widespread approval has been given to Trichoderma spp. as efficient BCAs (Biological Control Agents) against number of commercial phytopathogens. Due to their many BCA traits, including antagonism and plant-growth encouragement, these antagonistic fungi are the most prevalent fungal biocontrol agents (Punja and Utkhede, 2003). Trichoderma spp. mass manufacturing would therefore have a lot of potential for commercial application. Despite of BCA function Trichoderma sps exhibit a great ability to produce different types of enzymes such as xylanase, cellulase etc (Deschamps et al., 1985; Hawary and Mostafa, 2001). Cellulases are produced and used extensively in the bioprocessing industry because they generate 30% more hydrolysis than acids (Headon & Wash, 1994). The advantages of enzymatic hydrolysis over chemical processes have been greatly increased by the mode of action of cellulases in a variety of industries, including animal feed, fruit processing, textile wet processing, preparation of dehydrated vegetables, food products, essential oils, flavours, starch processing, botanical extracts, pulp and paper production, jams, juice, production of plant protoplasts for genetic manipulation, wine production, and biomass conversion.

Looking through the literature, we found that submerged fermentation has been used most frequently to produce cellulase from T. *viride* (Shuwu et al., 2010; Sourav et al., 2014). This made us to design the current study to optimise the fermentation parameters for maximise submerged and solid-state fermentation parameters and consequent cellulase output.

2.0 MATERIALS AND METHODS

2.1 Submerged Fermentation (SmF)

2.1.1Fermentation Parameters (Plackett–Burman design) for cultivation of T. *viride*

For the purpose of estimating T. *viride* productivity and consequent cellulase production, fermentation factors including various amounts of nitrogen, glucose. different pH (4.5, 5.5 and 7.0), incubation temperature (30ºC, 35 ºC and 40ºC) and speed rotation (100 RPM, 150 RPM and 200 RPM), Inoculum volume (1ml, 1.5 ml and 2ml), Volume of the liquid medium (250ml), have been optimized. Table 1.0 and 1.1 displays the parameter of optimization.

2.1.2 Fermentation Media

A complex medium made up of (g L^{-1}) ammonium sulphate, (0.5, 0.10, 0.15), magnesium sulphate heptahydrate (4.5), dipotassium phosphate (13.5), calcium chloride (0.25), $NaHPO_4$ (4.0), yeast extract (3.5), trace elements (1.5 mL), and glucose 5, 10, 15 (g L^{-1}) was used to carry out the fermentation in shake flasks. Vegetative inoculums that were 6 days old were used to inoculate flasks with 200 mL of fermentation media. The fermentation was carried out for 7 days.

2.2 Solid State Fermentation (SSF)

2.2.1Fermentation Parameters (Plackett–Burman design) for cultivation of T. *viride*

The fermentation parameters such as different pH (5.0, 5.5 and 6.0), incubation temperature (30ºC, 35 ºC and 40ºC) and inoculum volume (1 mL, 1.5 mL and 2 mL), were also investigated for T. *viride* productivity and consequent cellulase production. The fermentation is carried out for 7 days. Table 1.2 and 1.3 displays the parameter of optimization.

2.2.2 Substrate Feed stock

The sugarcane bagasse was purchased from the local sugarcane juice market, Hanamkonda. The material was bought to laboratory and ground approximately to 1-2 mm size. The ground material was stored at room temperature for further step of pre-treatment.

2.2.3 Bagasse Pre-treatment

The preparation of the lignocellulosic biomass included both physical and mechanical processes. 200 g of the substrate were ground to 1-2 mm particle size in a lab mill. After heat the substrate was boiled around 30 min in 2 L of distilled water and allowed to cool to 25 °C.

2.3 Estimation of dry mass of T. *viride*

Mycelium was filtered via filter paper (Whatman No. 40) to ascertain the fungal biomass produced via SmF and SSF. It was cleaned twice with distilled water. Mycelium that has been cleaned was dried to a constant mass at 105°C. The mass was calculated after it was placed in the desiccators.

2.4 Production of cellulase

2.4.1 T. *viride* starter culture

T. *viride* cultivated via SmF have been selected as starter culture. The selection is directly proportional to its significant yield via SmF (shown in the results).

2.4.2 Preparation of Starter Culture

In the current investigations, the spore suspension was used as the inoculum. A loopful culture from 5-day old was transferred to 10 mL of sterilised 0.005% Monoxal O.T (Diacetyl ester of Sodium sulphosuccinic acid) and transferred to its corresponding inoculum preparation medium. The flasks were incubated at 27^{o} C for five days. A sterilised wire loop was used to scratch the spores, creating a uniform suspension of spores. Haemocytometer was used to measure the spore count.

2.4.3 Production media

The production medium consisting of (%,w/v); 0.14: KH2PO4, 0.03 Urea; 0.20 (NH4)2SO4; 0.00014 ZnSO4.7H2O; 0.03 MgSO4.7H20; 0.00016 MnSO4; 0.0005 FeSO4.7H2O; 0.0002 CoCl2; 2.0 mL; 0.0002 CaCl2; Tween-80; 0.10 Polypeptone, glucose 6g L^{-1} is transferred to the individual 250 mL cottonwool plugged conical flasks and autoclaved at 15 lb/inch2 for 15 min. After cooling to normal temperature, the flasks were inoculated with 1 ml of this inoculum containing 1.2X106 ml^{-1} of spores, and then incubated at 30°C at 200 rpm in an orbital shaker incubator. The fermented broth was centrifuged at 6000 rpm for 10 minutes after 72 hours, and the supernatant's enzyme activity was measured.

2.4.4 Fermentation parameter

Fermentation parameters such as different incubation periods (20h, 40h, 60h, 80h and 100h), different incubation temperatures (27^{o}C, 28^{o}C, 29^{o}C, 30^{o}C and 31^{o}C), variation in the medium pH (4.5, 5.0, 5.5, 6.0 and 6.5), different nitrogen sources (NH4Cl, KNO3, (NH4)2SO4, (NH4) CH3COO, NH4H2PO4, NaNO3, NH4NO3), effect of inoculum size (2 mL, 4 mL, 6 mL, 8 mL), different volumes of culture medium (10mL, 20mL, 30mL, 40mL, 50mL, 60mL, 70mL) have been studied.

2.5 Enzyme Assay

Cellulase enzyme assay was carried out according to method described by (Mandels & Sternberg, 1976). Briefly, 50 mg rolled Whattman filter paper No. 1, measuring approximately 1x6 cm was suspended in 1.0 mL of diluted enzyme. Following, 1.0 mL of 0.05 M Sodium citrate buffer (pH 4.8) was transferred and incubated at 50^{o}C for about 1 h. Samples are estimated for reducing sugar.

Table 1.0 Submerged State Fermentation parameters for mass cultivation of T. *viride*

Number of Trail Runs	Carbon (g L^{-1})	Nitrogen (g L^{-1})	Temperature (^{o}C)	Volume of the inoculum (ml)	pH	RPM	Biomass (g L^{-1})
1	5	0.5	30	1	5.0	100	5.140
2	10	0.10	35	1.5	5.5	150	8.554
3	15	0.15	40	2.0	6.0	200	7.822
4	5	0.5	30	1	4.5	100	8.991
5	10	0.10	35	1.5	5.5	150	4.893

6	15	0.15	40	2.0	7.0	200	12.187
7	5	0.5	30	1	4.5	100	3.660
8	10	0.10	35	1.5	5.5	150	7.416
9	15	0.15	40	2.0	7.0	200	6.225
10	5	0.5	30	1	4.5	100	8.692
11	10	0.10	35	1.5	5.5	150	11.453
12	15	0.15	40	2.0	7.0	200	14.221
13	5	0.5	30	1	4.5	100	5.730
14	10	0.10	35	1.5	5.5	150	10.615
15	15	0.15	40	2.0	7.0	200	8.449
16	5	0.5	30	1	4.5	100	3.361
17	10	0.10	35	1.5	5.5	150	5.843
18	15	0.15	40	2.0	7.0	200	9.781
19	5	0.5	30	1	4.5	100	7.610
20	10	0.10	35	1.5	5.5	150	5.898
21	15	0.15	40	2.0	7.0	200	7.994
22	5	0.5	30	1	4.5	100	8.127
23	10	0.10	35	1.5	5.5	150	8.364

Table 1.1 Submerged State Fermentation parameters for mass cultivation of T. *viride* continued

Number of Trail Runs	Carbon (g L^{-1})	Nitrogen (g L^{-1})	Temperature (ºC)	Volume of the inoculum (ml)	pH	RPM	Biomass (g L^{-1})
24	15	0.15	40	2.0	7.0	200	7.834
25	5	0.5	30	1	4.5	100	4.153
26	10	0.10	35	1.5	5.5	150	6.221
27	15	0.15	40	2.0	7.0	200	7.891
28	5	0.5	30	1	4.5	100	5.641
29	10	0.10	35	1.5	5.5	150	4.880
30	15	0.15	40	2.0	7.0	200	6.247
31	5	0.5	30	1	4.5	100	9.812
32	10	0.10	35	1.5	5.5	150	7.892
33	15	0.15	40	2.0	7.0	200	8.553
34	5	0.5	30	1	4.5	100	6.221
35	10	0.10	35	1.5	5.5	150	6.925
36	15	0.15	40	2.0	7.0	200	7.012
37	5	0.5	30	1	4.5	100	8.887
38	10	0.10	35	1.5	5.5	150	7.820
39	15	0.15	40	2.0	7.0	200	8.631
40	5	0.5	30	1	4.5	100	3.589
41	10	0.10	35	1.5	5.5	150	5.447
42	15	0.15	40	2.0	7.0	200	8.329

Table 1.2 Solid State Fermentation parameters for mass cultivation of T. *viride*

Number of Trail Runs	Temperature (ºC)	Volume of the inoculum (ml)	pH	Biomass (g L^{-1})
1	30	1	4.5	4.778
2	35	1.5	5.5	8.210
3	40	2.0	7.0	9.333
4	30	1	4.5	6.218
5	35	1.5	5.5	9.880
6	40	2.0	7.0	11.814
7	30	1	4.5	3.601
8	35	1.5	5.5	5.980
9	40	2.0	7.0	6.012
10	30	1	4.5	7.181

11	35	1.5	5.5	8.025
12	40	2.0	7.0	7.996
13	30	1	4.5	5.817
14	35	1.5	5.5	4.992
15	40	2.0	7.0	6.055
16	30	1	4.5	2.813
17	35	1.5	5.5	4.297
18	40	2.0	7.0	6.003

Table 1.3 Solid State Fermentation parameters for mass cultivation of T. *viride* continued

Number of Trail Runs	Temperature (°C)	Volume of the inoculum (ml)	pH	Biomass (g L^{-1})
19	30	1	4.5	8.615
20	35	1.5	5.5	8.981
21	40	2.0	7.0	9.993
22	30	1	4.5	6.230
23	35	1.5	5.5	7.881
24	40	2.0	7.0	8.050
25	30	1	4.5	3.866
26	35	1.5	5.5	4.815
27	40	2.0	7.0	4.622
28	30	1	4.5	6.451
29	35	1.5	5.5	5.867
30	40	2.0	7.0	4.990
31	30	1	4.5	3.215
32	35	1.5	5.5	4.063
33	40	2.0	7.0	4.321
34	30	1	4.5	2.338
35	35	1.5	5.5	3.814
36	40	2.0	7.0	2.944
37	30	1	4.5	5.207
38	35	1.5	5.5	6.334
39	40	2.0	7.0	5.931
40	30	1	4.5	4.447
41	35	1.5	5.5	3.895
42	40	2.0	7.0	3.991

3.0 RESULT AND DISCUSSION

3.1 Mass Production of T. *viride* via SmF and SSF

Understanding a microorganism's fermentation pattern requires knowledge about its growth and biomass production rates. The rate of fungal biomass formation by T. *viride* in SmF and SSF fermentation while incubated at 30°C for different incubation periods is shown in (Table 1 & 2). The influence of carbon, nitrogen concentrations was highly effective in the total biomass productivity. Moreover, the variations in the temperature, pH and RPM also highly influence in the T. *viride* Productivity. The productivity via SmF was ranged from 3.361 g L^{-1} to 14.221g L^{-1}. After incubation period of fermentation, the highest growth yield noticed was 14.221g L^{-1} at 15 g L^{-1}, 0.15 g L^{-1}, 40 °C, 2.0, 7.0 and 200 carbon, nitrogen, temperature, volume of the medium, pH, RPM respectively. Following, 12.187 g L^{-1} yield of T. *viride* was achieved with 15 g L^{-1}, 0.15 g L^{-1}, 40 °C**,** 2.0, 7.0, 200 carbon, nitrogen, temperature, volume of the medium, pH, RPM respectively. On the other hand, the T. *viride* yield via SSF was found less comparing to SmF. The T. *viride* yield was ranged from 2.338 g L^{-1} to 11.814g L^{-1}. The highest growth yield noticed was 11.814g L^{-1} at 40 °C, 2.0, 7.0 temperature, volume of the medium, pH respectively. Following 9.880 g L^{-1} at 35, 1.5, 5.5 temperature, volume of the medium, pH respectively.

3.2 Enzyme Assay

3.2.1 Effect of incubation period

As per the fig 1.0 A, the effect of different incubation period on the cellulase activity was ranged from 0.76U/mL/min to 3.02 U/mL/min. The enzyme activity was found high at 80h with 3.02U/mL/ml. Following, the activity was found significant at 60h with 2.81U/mL/min. At the initial hour (20h), the activity was found very little with 0.76 U/mL/min. The enzyme activity was gradually raised from 20h to 80h and slightly dropped to 2.11 U/mL/min at 100h.

3.2.1 Effect of incubation Temperature

With reference to the fig. 1.0 B the effect of different incubation period on the cellulase activity was ranged from 0.63 U/mL/min to 2.88 U/mL/min. We observed that the cellulase activity at 29^{o}C and 30^{o}C was found significant with 2.36 and 2.88 U/mL/min respectively. The activity was temperature dependent. However, the cellulase activity was dropped at 31^{o}C.

3.2.2 Effect of pH

The effect of different pH on the cellulase activity was ranged from 0.436 U/mL/min to 2.481 U/mL/min. the highest cellulase activity was noticed at pH 5.5 with 2.481 U/mL/min. At pH 6.5 the cellulase activity was inclined with 0.436 U/mL/min. results are shown in Fig 1.1 A.

3.2.3 Effect of Nitrogen source for cellulase production and its activity

This investigation was carried out to determine the effective nitrogen source for the production of cellulase and subsequent its activity. The study was framed out with Seven (7) different nitrogen sources namely, NH_4Cl, KNO_3, (NH_4) CH_3COO, $(NH_4)_2SO_4$, $NH_4H_2PO_4$, $NaNO_3$, NH_4NO_3. Out of Seven (7) nitrogen sources, Five (5) namely, NH_4Cl, NH_4, CH_3COO, $(NH_4)_2SO_4$, $NH_4H_2PO_4$, NH_4NO_3 are identified as significant sources for cultivation of T. *viride* and subsequent production and activity of cellulase (Fig 1.1 B). Within the Five (5), $(NH_4)_2SO_4$ exhibited highest cellulase production and its activity with 2.663 U/mL/min (Fig 1.1 B). Following, NH_4NO_3 and $NH_4H_2PO_4$, showed highest cellulase production and its activity with 2.592 and 2.514 U/mL/min (Fig 1.1 B). Among the tested nitrogen sources, KNO_3 exhibited least cellulase production and activity with 1.993 U/mL/min (Fig 1.1B)

3.2.4 Effect of Inoculum size on cellulase production and its activity

The inoculum size is important fermentation parameters which influence the productivity of any fermentation product. During this study we tested Four (4) different inoculum sizes namely, 2mL, 4mL, 6mL, 8mL (increased number of T. *viride* per mL). In accordance to results obtained, the inoculum size of 4mL found significant with 2.321 U/mL/min (Fig 1.1 C). Following, 6mL size of inoculum also found significant with 2.092 U/mL/min (Fig 1.1 C).

3.2.5 Effect of media volume on cellulase production and its activity

We have tested different media volumes on the cellulase production and its activity. Seven (7) different media volumes such as 10mL, 20mL, 30mL, 40mL, 50mL, 60mL and 70mL. Out of Seven (7), three (3) media volumes such as 30mL, 40mL, and 50mL produced highest cellulase production with 2.964 U/mL/min, 2.331 U/mL/min, 2.012 U/mL/min (Fig 1.1D). the least production and activity was found at 10 mL media volume with 0.761 U/mL/min (Fig 1.1 D).

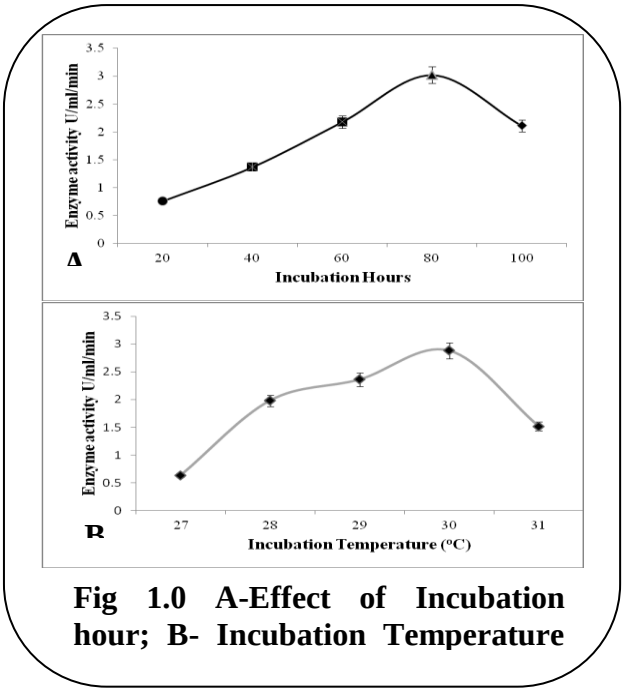

Fig 1.0 A-Effect of Incubation hour; B- Incubation Temperature

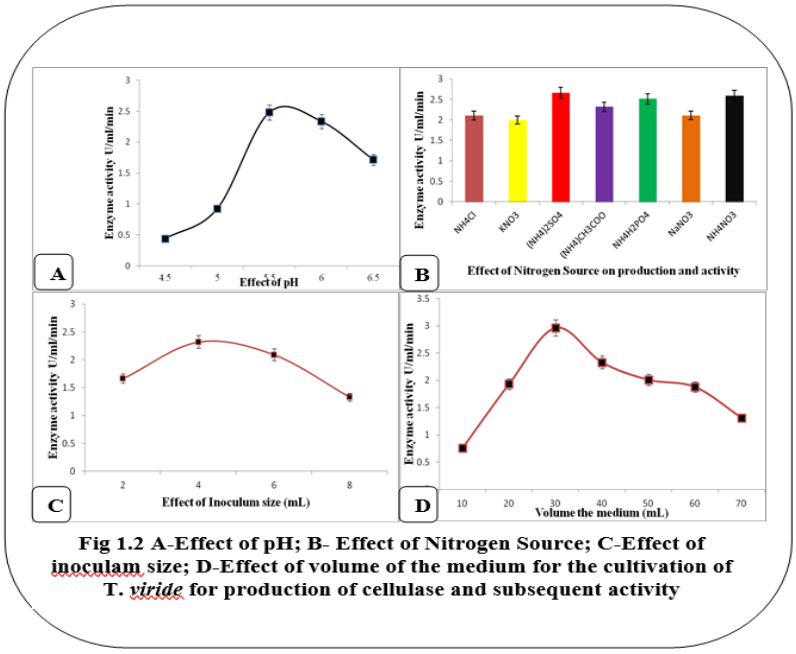

Fig 1.2 A-Effect of pH; B- Effect of Nitrogen Source; C-Effect of inoculam size; D-Effect of volume of the medium for the cultivation of T. *viride* for production of cellulase and subsequent activity

4.0 CONCLUSION

With reference to the results, the authors conclude that, the cultivation of T. viride was found significant with SmF comparing to SSF.

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CONFLICT OF INTEREST

The authors disclose no conflict of financial or nonfinancial interest.

REFERENCES

1. Bailey, D.J., A. Kleczkowski and C.A. Gilligan, (2004). Epidemiological dynamics and the efficiency of biological control of soil-borne disease during consecutive epidemics in a controlled environment. New Phytol., 161: 569-576.
2. Deschamps, F., C. Giuliano, M. Asther, M.C. Huet and S. Roussos. (1985), Cellulases production by Trichoderma harzianum in static and mixed solid state fermentation reactor under nonasceptic conditions. Biotechnol. Bioengin., 27:28-31.
3. Draganova D, I. Valcheva, Y. Stoykov, Y. Tumbarski, and A. Krastanov. (2014), Isolation and identification of new chitinolytic fungi Petromyces alliaceus H5 Bio prospect Journal 24 : 71-75.
4. Dudkin VI, and A. Y. Karseev. (2014), Measurement Techniques 57: 912-918 (2014)
5. Gryznova E, N. Grebenikova, D. Ivanov, and V. Bykov. (2019), IOP Conference Series: Earth and Environmental Science 390: 012044.
6. Hawary, F.I. and Y.S. Mostafa. (2001), Factors affecting cellulases production by Trichoderma koningii. Acta Alimentaria, 30:3-13.
7. Lorito M, G. E. Harman, C. K. Hayes, R. M. Broadway, A. Tronsmo, S. L. Woo, and A. Di Pietro. (1993), Chitinolytic Enzymes Produced by Trichoderma harzianum Phytopathology 83: 302–307.
8. Martínez VR, T. S. Garza-Romero, V. R. Moreno-Medina, S. HernándezDelgado, and N. Mayek-Perez. (2016), Biochemical basis of tolerance to osmotic stress in phytopathogenic fungus: The case of Macrophomina phaseolina (Tassi) Goid.Revista Argentina de Microbiologia 48: 347-357 (2016)
9. Mathivanan N, V. Kabilan, and K. Murugesan. (1998), Purification, characterization, and antifungal activity of chitinase from *Fusarium chlamydosporum*, a mycoparasiteto groundnut rust, *Puccinia arachidis* Canadian Journal of Microbiology 44: 646-651.
10. Muhammadiev RS, and T. V. Bagaeva. (2018), Chitinase production by Trichoderma viride in submerged state fermentation Indo American Journal of Pharmaceutical Sciences 5: 10388-10395.
11. Myazin N, Y. Neronov, V. Dudkin, and V. Yushkova (2018). MATEC Web of Conference 245, 11013.
12. Myazin NS (2017), Measurement Techniques 60: 491-496.
13. Myazin NS. (2017), Measurement Techniques 60: 183-189.
14. Oerke EC. (2006), Crop losses to pests, Journal of Agricultural Science 144: 31-43.
15. Poloni A, I. S. Pessi, A. P. G. Frazzon, and S. T. Van Der Sand. (2009), Morphology, Physiology, and Virulence of Bipolaris sorokiniana Isolates Current Microbiology 59: 267-273.
16. Punja, Z.K. and R.S. Utkhede. (2003), Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol., 21: 400-407..
17. Savary, MS, A. Ficke, J. N. Aubertot, and C. Hollier. (2012), Food Security 4: 519-537.
18. Shivalee A, M. Divatar, G. Sandhya, S. Ahmed, and K. Lingappa. (2016), ISOLATION AND SCREENING OF SOIL MICROBES FOR EXTRACELLULAR CHITINASE ACTIVITY Journal of Advanced Scientific Research 7: 10-14.
19. Strange RN and Scott PR. (2005), Plant disease: a threat to global food security (2005), Annual Review of Phytopathology 43: 83-116.
20. Van S, A. Cheremisin, A. Chusov, O. Zueva, A. Dolgopolov, E. Nikulina, and F. Switala, (2019). IOP Conference Series: Earth and Environmental Science 390: 0120011.
21. Yu AK and V.A. Vologdin, Journal of Physics: Conference Series 643: 012108 (2015).
22. Yushkova V, G. Kostin, V. Dudkin, and L. Valiullin. (2019). IOP Conference Series: Earth and Environmental Science 390:012016.