Original Research Article

In- vitro **evaluation of antagonistic activity of native** ***Trichoderma*** **spp. and** ***Pseudomonas fluorescens*** **isolates against** ***Alternaria solani*** **causing early blight of Tomato**

ABSTRACT

The present study was carried out in 2018-2019 at Department of Plant pathology, SASRD, Nagaland University to evaluate the *in vitro* efficacy of 24 native isolates of *Trichoderma* and 18 isolates of *Pseudomonas* against *Alternaria solani* by dual culture technique method. The test pathogen was isolated from disease infected tomato plants collected from an experimental field of Department of Plant pathology, SASRD, Medziphema campus. The results showed that all the isolates had significantly inhibited the mycelial growth of the pathogen. The highest inhibition of mycelial growth of *A. solani* was shown by T-5 (73.34 %) followed by T-11(70.23 %). The lowest inhibition was shown by T-24 (51.55 %). Among the *Pseudomonas* isolates, highest inhibition in mycelial growth of *A. solani* was shown by P-7 (77.73%) followed by P-12(76.00 %) respectively. The lowest inhibition was shown by P-17 (53.78 %). The results indicate that different local isolates of *Trichoderma* spp. and *Pseudomonas fluorescens* were effective against the tested pathogenic fungi which provides their potential in biological management of early blight disease of tomato.

Keywords: *Trichoderma, tomato, Alternaria solani, dual culture*

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.), native to the Andean region of South America is one of the most common horticultural crops cultivated throughout the world. They are important source of vitamins and important cash crop for both small holders and medium scale commercial farmers [1]. The fruit also contains plenty of antioxidant carotenoid lycopene that can prevent cancer, heart disease and muscular degeneration [2]. Among several diseases of tomato, early blight disease caused by *Alternaria solani* has become one of the most destructive diseases all over the world with yield losses up to 80% [3]. *Alternaria solani* (Ellis and Martin) Jones and Grout, is a soil inhabiting air-borne pathogen responsible for leaf blight, collar and fruit rot of tomato. [4]. *A. solani* contains enzymes such as cellulases which degrade the host cell wall and also contain pectin methyl galacturonase which facilitate host colonization [5]. The disease in severe cases can lead to complete defoliation and it is favoured by heavy dew, rainfall, high humidity, and fairly high temperatures. As all

the above ground parts of the plant can be attacked by the pathogen, failure to control this disease can highly reduce the yield [6].

Currently control strategies for managing early blight of tomato include cultural practices, fungicide application, and breeding for resistant varieties [7,8]. Because of issues such as lack of major resistance genes for early blight in tomato cultivars with market desirable traits and the complex nature of inheritable quantitative resistance in tomato cultivars, fungicide application remains the major approach to combat this disease. Protective fungicides such as Mancozeb and systemic fungicides such as azoxystrobin with multisite mode of action are widely used around the world. Several round of fungicide applications are required during tomato growing season to achieve a satisfactory level of disease control [9]. There have been increasing reports on loss of efficacy of commercial fungicides against the pathogen [10,11,12]. Management of plant diseases by chemical pesticides mostly concerned environmental damage since pesticides accumulate in soils as toxic residues, as well as the development of resistance by pathogens resulting from pesticides overuse and single-site fungicides, which enhances the development of specific resistance [13,14,15].

Biological control using microorganisms to suppress plant disease, offers a powerful alternative to the use of synthetic chemicals [16,17]. Biological control of plant pathogens by antagonistic micro organisms is a potential non-chemical means and is known to be a cheap and effective eco-friendly method for the management of crop diseases [15,18,19]. Environmentally, they are also more efficient as they do not release toxic compounds, and it decreases the negative effects of plant pathogens and increases positive responses by the plants [20]. Additionally, they usually have several modes of action, thus reducing the development of resistance [21]. These mutualistic bioagents are considered eco-friendly and have no negative effects on non-targeted organisms, including humans, the useful microflora and host plants. In such tripartite interactions between the host plant, fungal pathogen and mutualistic bioagents, different mechanisms of action were reported and considered responsible for protecting the host plants from pathogens and parasites [22,23,24]. *Trichoderma* spp. is the most widely studied biocontrol agent (BCA) against plant pathogens because of their ability to reduce the population of soil borne plant pathogens. *Pseudomonas fluorescens* is adapted to survival in soil and colonization of plant roots [25].

Therefore, the objective of the present investigation was to assess the efficacy of local isolates of *Trichoderma* and *Pseudomonas* under *in vitro* condition against *A. solani.* The experiment was conducted in Department of Plant Pathology, SASRD, Medziphema campus, Nagaland University.

2. MATERIALS AND METHODS

The dual culture technique described by Dennis and Webster (1971)[26] was followed for the evaluation of antagonistic activities of native *Trichoderma* isolates against *A. solani*. Briefly 20 ml of sterilized PDA medium was poured into each of the sterilized Petri dishes under aseptic condition. After the media gets solidified in the plates, the test fungal pathogen was inoculated at one end of

each Petri plate and the antagonists on the opposite end. A set of control plates inoculated with the test fungal pathogen was maintained. Each set of treatments were replicated three times and incubated at a temperature of 28±1^{0}C for 10 days. The per cent inhibition of growth was calculated following the equation given by Vincent (1947) [27].

Per cent inhibition= PI = $\frac{C-T}{C}$ X 100

Where, C= Radial growth of pathogen in control, T= Radial growth in of pathogen in dual culture plates.

The dual culture technique described by Maurya *et al.* (2014)[28] was followed for the evaluation of antagonistic activities of native bacterial isolates against *A. solani*. *Pseudomonas* isolates were streaked at one side of Petri dish (one cm away from the edge) containing PDA. 10 mm mycelial disc from seven days old PDA culture of the pathogen was placed at the opposite side of Petri dishes perpendicular to the bacterial streak respectively and incubated at 28±1^{0}C for 5-7 days. Petri dishes inoculated with fungal pathogen alone served as control. Three replications were maintained for each isolate. The per cent inhibition of growth will be calculated following the equation given by Vincent (1947)

The experiment was conducted in a Complete Randomised Design (CRD) and three replications were maintained for each treatment. Data were analysed statistically.

3. RESULTS AND DISCUSSION

Altogether 24 native isolates of *Trichoderma* spp. and 18 isolates of *Pseudomonas* spp. were screened for their inhibitory action on the radial growth of *P. infestans* by adopting dual culture technique [29] and the data obtained are presented in Table 1 & 2 and Figure 1 & 2. All isolates screened against *A. solani* were significantly superior over control plate. It was found that the growth of the pathogen in dual culture plates progress until they came in contact with the leading edges of the antagonist. Among the different isolates of *Trichoderma* spp. least radial mycelial growth of the pathogen was recorded in T-5 (2.00 cm) followed by T-11 (2.10 cm), T-20 (2.23 cm) respectively. The per cent inhibition over control showed that T-5 was the most promising isolate against *A. solani* with 73.34 per cent inhibition followed by T-11 (70.23 %) and T-20 (69.34 %). The least antagonistic effect was observed in T-24 (51.55%). Among the different isolates of *Pseudomonas* spp. least radial mycelial growth of the pathogen was recorded in P-7 (1.67 cm) followed by P-12 (1.80 cm), P-14 (1.90 cm) respectively. The per cent inhibition over control showed that P-7 (77.73%) was the most promising isolate against *A. solani* followed by P-12 (76.00%) and P-14 (74.67 %). The least antagonistic effect was observed in P-17 (53.78%). Our findings are in agreement with the findings of earlier workers [30,31].

Trichoderma spp. are capable of producing extracellular lytic enzymes that are responsible for their antagonistic activity [32]. Mechanism used by *Trichoderma* spp. for control of plant pathogen includes competition, mycoparasitism, antibiosis and induced resistance of the plant host [33]. Fluorescent pseudomonas also produced anti fungal compounds such as pseudobactin, HCN, salicylic acid and 2- hydroxy phenazine to suppress plant pathogenic fungi [34]. The antifungal metabolites produced by *P. fluorescens* might be attributed as the reason for the reduction in the

growth of the pathogen and *P. fluorescens* were known to produce an array of low-molecular weight metabolites some of which were potential antifungal agents [35]. Moreover, the difference in their potential may probably be correlated with the differences in levels of hydrolytic enzymes produced by each species or isolates when they attack the mycelium of the pathogens.[36,37]. They are soil borne fungi and show significant activity against a wide range of plant pathogenic fungi [33].

Table 1: Antagonistic activity of native *Trichoderma* isolates against *A. solani*

Treatment	**Inhibition of *Alternaria solani* growth**		
	Radial growth (cm)	**Radial growth inhibited (cm)**	**Inhibition %**
T_0 *(* Control)	07.50	00.00	00.00 (4.05)
T_1 *(A.solani* + T-1)	02.86	04.63	61.78 (51.81)
T_2 (*A.solani* + T-2)	02.36	05.14	65.78 (54.21)
T_3 (*A.solani* + T-3)	03.10	04.40	60.89 (51.30)
T_4 (*A.solani* + T-4)	03.03	04.46	59.55 (50.50)
T_5 (*A.solani* + T-5)	02.00	05.50	73.34 (58.91)
T_6 (*A.solani* + T-6)	02.80	04.76	62.67 (52.34)
T_7(*A.solani* + T-7)	02.83	04.67	62.23 (52.08)
T_8 (*A.solani* + T-8)	03.26	04.24	56.89 (48.95)
T_9 (*A.solani* + T-9)	02.86	04.64	61.78 (51.82)
T_{10} (*A.solani* + T-10)	02.73	04.77	63.56 (52.86)
T_{11} (*A.solani* + T-11)	02.10	05.40	70.23(56.93)
T_{12} (*A.solani* + T-12)	02.36	05.14	65.78 (54.21)
T_{13} (*A.solani* + T-13)	03.23	04.27	56.89 (48.95)
T_{14} (*A.solani* + T-14)	03.40	04.10	54.67 (47.67)
T_{15} (*A.solani* + T-15)	02.30	05.20	68.00 (55.57)
T_{16} *(A.solani* + T-16)	03.56	03.94	52.45 (46.40)
T_{17} *A.solani* + T-17)	03.00	04.50	60.00 (50.76)
T_{18} (*A.solani* + T-18)	03.44	03.86	63.11 (52.60)
T_{19} (*A.solani* + T-19)	03.26	04.24	56.89 (48.95)
T_{20} (*A.solani* + T-20)	02.23	05.27	69.34(56.41)
T_{21} (*A.solani* + T-21)	02.50	05.00	65.34 (53.93)
T_{22} (*A.solani* + T-22)	03.40	04.10	54.66 (47.67)
T_{23} (*A.solani* + T-23)	03.03	04.47	59.55 (50.50)
T_{24} (*A.solani* + T-24)	03.63	03.87	51.55 (45.89)

Sem +-	0.01	0.02	0.39
C.V. (%)	2.53	1.72	3.27
CD (p=0.01)	0.16	0.17	4.23
CD (p=0.05)	0.12	0.13	3.17

* Data in the parentheses are angular transformed values

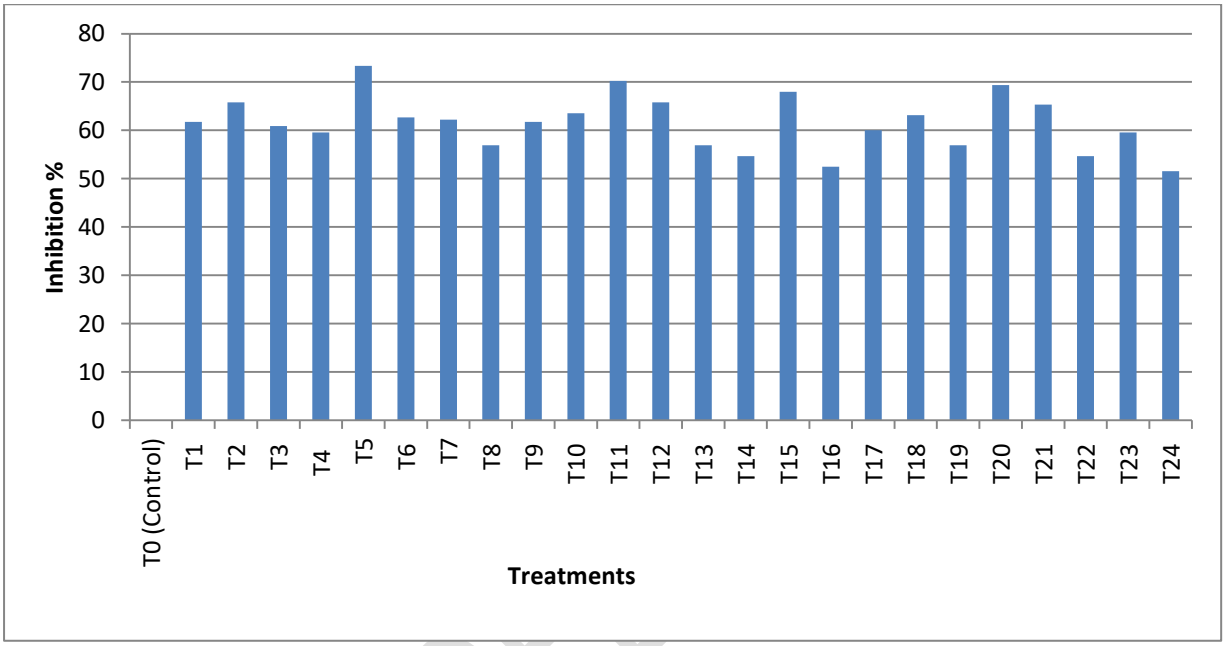

Figure 1: Antagonistic activity of native *Trichoderma* isolates against *A. solani*

Table 2: Antagonistic activity of native *Pseudomonas* isolates against *A.solani*

Treatment	**Inhibition of *Alternaria solani* growth**		
	Radial growth (cm)	**Radial growth inhibited (cm)**	**Inhibition %**
T_0 (Control)	07.50	00.00	00.00 (4.05)
P_1 (A. solani +P-1)	02.93	04.57	60.93 (51.29)
P_2 (*A.solani* +P-2)	02.76	04.74	63.20 (52.62)
P_3 (*A.solani* +P-3)	02.33	05.17	68.94 (56.10)
P_4 (*A.solani* +P-4)	02.63	04.87	64.93 (53.67)
P_5 (*A.solani* +P-5)	03.00	04.50	60.00 (50.78)
P_6 (*A.solani* +P-6)	03.40	04.10	54.67 (47.68)
P_7 (*A.solani* +P-7)	01.67	05.83	77.73 (61.89)
P_8 (*A.solani* +P-8)	03.23	04.27	56.88 (48.95)

P_9 (*A.solani* +P-9)	03.26	04.23	56.44 (48.95)
P_{10} (*A.solani* +P-10)	03.00	04.50	60.00 (50.78)
P_{11} (*A.solani* +P-11)	02.90	04.60	61.34 (51.55)
P_{12} (*A.solani* +P-12)	01.80	05.70	76.00 (60.67)
P_{13} (*A.solani* +P-13)	03.23	04.27	56.88 (48.95)
P_{14} (*A.solani* +P-14)	01.90	05.60	74.67 (59.78)
P_{15} (*A.solani* +P-15)	03.10	04.40	65.34 (51.30)
P_{16} (*A.solani* +P-16)	02.30	05.20	69.34 (56.37)
P_{17} (*A.solani* +P-17)	03.46	04.03	53.78 (47.14)
P_{18}(*A.solani* +P-18)	02.06	05.44	72.54 (58.34)
SEm+-	0.02	0.02	0.47 (0.28)
C.V. (%)	4.71	3.09	3.41 (2.47)
CD (p=0.01)	0.31	0.31	4.57 (2.75)
CD (p=0.05)	0.23	0.23	3.41 (2.06)

*Data in the parentheses are angular transformed values

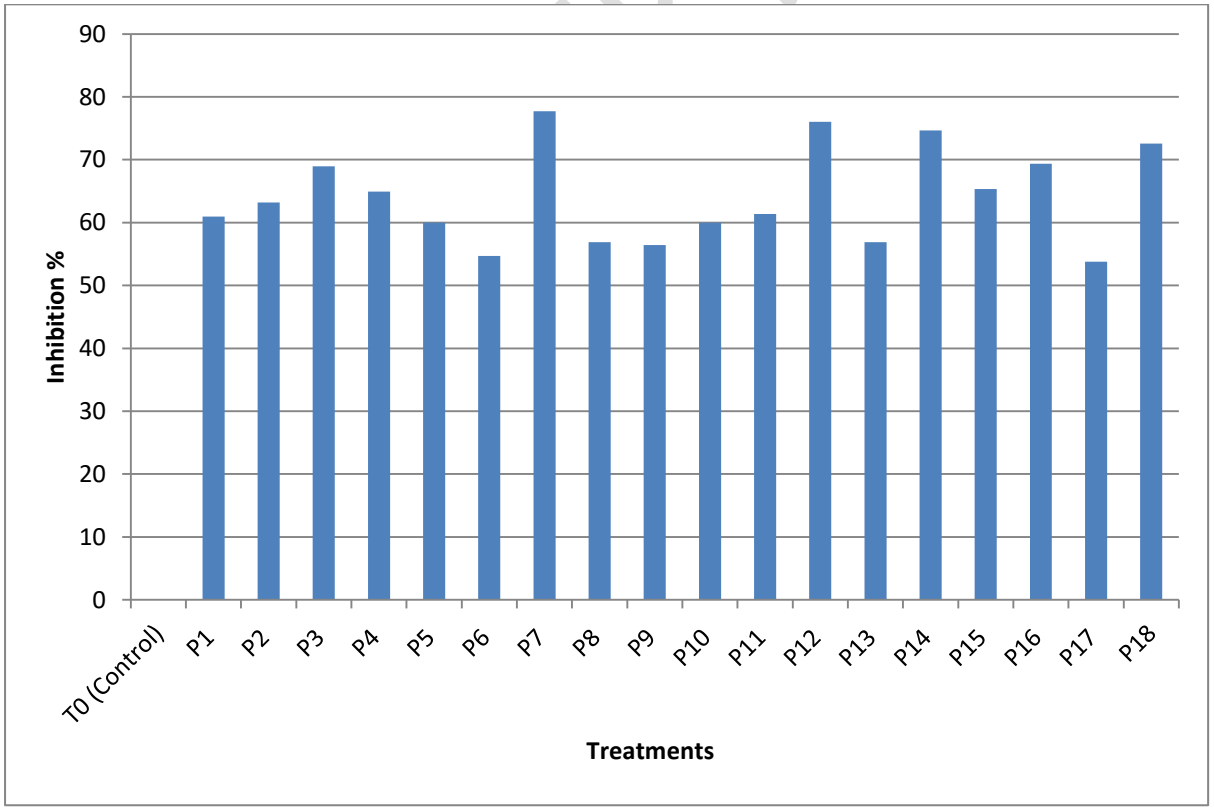

Figure 2: Antagonistic activity of native *Pseudomonas* isolates against *A. solani*

4. CONCLUSION

From the present study, it is observed that all the local isolates of *Trichoderma* and *Pseudomonas* inhibited the growth of the test pathogen, *A. solani* at different ranges. Although, the mechanisms employed by the selected native isolates were not mentioned, our findings indicate that these native isolates can be adopted for biological management of early blight of tomato which ultimately will increase the quality as well as quantity of productivity. Moreover, disease suppression by combination of compatible bioagents could be more effective than the individual ones possibly by the existence of synergism among the metabolites of the strains. However, field trials aimed at understanding the potential use of indigenous isolates of *Trichoderma* and *Pseudomonas* for management of early blight of tomato in agricultural system are suggested for future studies.

REFERENCES

1. Ana MV, Abdurrabi S, Benhard L: A Guide to integrated Pest Management in Tomato Production in Eastern and Southern Africa. ICIPE Science Press, Nairobi, Kenya. 2003;144.
2. Wener ZH. 2008. Importance of the tomato. http://agrisupportonlive.com/Article/importance of the tomato html.
3. Chandravanshi SS, Singh BP, Thakur MP. Persistence of different fungicides used against *Alternaria alternata* in tomato. Indian Phytopathology. 1994;47:241-244.
4. Datar VV, Mayee CD. Assessment of loss in tomato yield due to early blight. Indian Phytopathology.1981;34:191-195.
5. Shahbazi H, Aminian H, Sahebani N, Halterman D. Effect of *Alternaria solani* exudates on resistant and susceptible potato cultivars from two different pathogen isolates. Journal of Plant Pathology. 2011;27(1):14-19.
6. Mallik I, Arabiat S, Pasche JS, Bolton MD, Patel JS, Gudmestad NC. Molecular characterization and detection of mutations associated with resistance to succinate dehydrogenase inhibiting fungicides in *Alternaria solani*. Phytopathology. 2014;104:40-49.
7. Adhikari P, Oh Y, Panthee, DR. 2017. Current status of early blight resistance in tomato : An update. International Journal of Molecular science. 2017;18:2019.
8. Foolad MR, Merk HL, Ashrafi H. 2008. Genetics, genomics and breeding of late blight and early blight resistance in tomato. Critical reviews in plant science. 2008;27:75- 107.
9. Abu-El Samen F, Goussous SJ, Jendi AA, Makhadmeh, I. 2015. Evaluation of tomato early blight management using reduced management rates and frequencies of fungicides applications. International Journal of Pest Management. 2015;61:320-328.
10. Abu-El Samen F, Goussous SJ, Al-Shudifat A , Makhadmeh, I. Reduced sensitivity of tomato early blight pathogen (Alternaria solani) isolates to protectant fungicides and implication on disease control. Archive in Phytopathology and Plant protection. 2016;49:120-136.
11. Gudmestad NC, Arabiat S, Miller JS, Pasche JS. 2013. Prevalance and impact of SDHI fungicides resistance in *Alternaria solani*. Plant disease. 2013;97: 952-960.

12. Odilbekov, F, Edin E, Garkava-gustavsson L, Hovmalm HP, Liljeroth E. 2016. Genetic diversity and occurrence of the F129L substitutions among isolates of *Alternaria solani* in south eastern Sweden. Hereditas. 2016;153:10.
13. Wang X, Glawe DA, Kramer E, Weller D, Okubara A. Biological Control of *Botrytis cinerea:* Interactions with Native Vineyard Yeasts from Washington State. Phytopathology. 2018;108:691–701.
14. Kim YC, Hur JY, Park SK. Biocontrol of *Botrytis cinerea* by chitin-based cultures of *Paenibacillus elgii* HOA73. European journal of Plant Pathology. 2019;155:253–263.
15. Jiao X, Takishita Y, Zhou G, Smith DL. Plant Associated Rhizobacteria for Biocontrol and Plant Growth Enhancement. Frontier in Plant Science. 2021;12:17.
16. Howarth FG. Environmental impact of classical biological control. Annual Review of Entomology. 1991;36:485-509.
17. Osburn RM, Milner JL, Oplinger ES, Smith RS, Handelsman J. Effect of *Bacillus cereus* UW 85 on the yield of soybean at two field sites in Wisconsin. Plant Disease. 1995;79:551-556.
18. Harman GE. Seed treatment for biological control of plant diseases, Crop Protection. 1991;10:166.
19. Cook RS, Baker KF. The nature and practice of biological control of plant pathogens. American Phytopathological Society, St Paul, Minn,1983;539.
20. Schalchli H, Tortella GR, Rubilar O, Parra L, Hormazabal E, Quiroz A. Fungal volatiles: An environmentally friendly tool to control pathogenic microorganisms in plants. Critical Review in Biotechnology. 2016;36:144–152.
21. Pertot I, Giovannini O, Benanchi M, Caffi T, Rossi V, Mugnai L. Combining biocontrol agents with different mechanisms of action in a strategy to control *Botrytis cinerea* on grapevine. Crop Protection. 2017;97:85–93.
22. Kiely PD, Haynes JM, Higgins CH, Franks A, Mark GL, Morrisse JP, O'Gara F. Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. Microbial Ecology. 2006;51:257–266.
23. Alabouvette C, Edel V, Lemanceau P, Olivain C, Recorbet G. Diversity and interactions among strains of Fusarium oxysporum: Application and biological control. In: MJ Jegger and NJ Spence (Eds): Biotic interactions in plant-pathogen associations. pp 131-157. CAB Internationals, London, England. 2001
24. Fravel D, Olivain C, Alabouvette C. *Fusarium oxysporum* and its biocontrol. New Phytopathologist. 2003;157:493-502
25. Fuchs JG, Moenne-Loccoz Y, Defago G. Non pathogenic Fusarium oxysporium strain Fo47 induces resistance to *Fusarium* wilt in tomato. Plant disease. 1997;81:492-496.
26. Dennis C, Webster J. Antagonistic properties of species groups of *Trichoderma* II.Production of volatile antibiotics. *Transactions of the British Mycological Society.* 1971(b);57: 41–48.
27. Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947;159:850.
28. Maurya MK, Singh R, Tomer A. *In-vitro* evaluation of antagonistic activity of *Pseudomonas fluorescens* against fungal pathogen. Journal of Biopesticides.2014;7(1):43-46.
29. Magesh M, Sudha R, Kumar R, Devi PA. Impact of rhizosphere *Pseudomonas fluorescens* against *Alternaria solani* in tomato. Journal of Bio innovation.2018;2:242-251.
30. Roy CK, Akter N, Sarkar MKI, Uddin PK, Begum M, Zenat N, Jahan MAA. Control of early blight of tomato caused by *Alternaria solani* and screening of tomato varieties against the pathogen. The Open Microbiology Journal. 2019;13:41-50.
31. Elad Y, Chet I, Henis Y. Degradation of plant pathogenic fungi by Trichoderma harzianum. Canadian journal of Microbiology.1982;28:719-725.
32. Schirmbock M, Lorito M, Wang YL, Hayes CK, Arisan- Atac I, Scala F, Harman GE, Kubicek C. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against

phytopathogenic fungi. Applied Environmental Microbiology. 1994;60:4364-4370.

33. Pandey A, Trivedi K, Palni LS. Characterization of phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (BO) isolated from a Sub-Alpine location in the Indian central Himalaya. Current Microbiology 2006;53:102-107.
34. Reddy BP, Reddy KRN, Subba Rao, BP, Rao KS. Efficacy of antimicrobial metabolites of *Pseudomonas fluorescens* against rice fungal pathogens. Current Trends in Biotechnology and Pharmacy.2008;2:178-182.
35. O'Dowling DN, O'Gara F. Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends Biotechnol. 1994;12:133-141.
36. Sundaramoorthy S, Balabaskar P. Consortial effect of endophytic and plant growth promoting rhizobacteria for the management of early blight of tomato incited by *Alternaria solani.* Journal of Plant Pathology and Microbiology. 2012;3:7.
37. Meera T, Balabaskar P. Isolation and characterization of *Pseudomonas fluorescens* from rice fields. International Journal of Food Agriculture and Veterinary sciences. 2012;2(1):113-120