In vitro cultural characteristics and antagonistic efficacy of major fungal diseases of potato in Manipur

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Abstract

Solanum tuberosum is the most widely cultivated vegetable crop in Manipur during rabi season. The yield loss occurred by two major fungal diseases such as early blight and wilt of potato. To control the damage caused by pathogens many farmers are using hazardous fungicides in order to obtain good yield. The present study was carried out to recognize the antagonistic efficacy of Trichoderma spp. against Alternaria and Fusarium spp. under in-vitro condition by dual culture method. Per cent growth inhibitions were recorded and it was ranged from 61.34 to 75.06% and 64.24 to 70.01% for Alternaria and Fusarium spp. respectively. Trichoderma harzianum revealed best results with 75.06% and 70.01% inhibition of both the pathogen.

Keywords: Dual culture, fungal diseases, per cent inhibition, Trichoderma spp

Introduction

Potato (Solanum tuberosum L.) is one of the most important vegetable and non-grain food crop also known as ‘poor man’s friend’. It is one of the most popular crops in this country for vegetable purposes. Potato is the fourth main food crop in the world. It provides low cost energy to the human diet. It is major source of starch, vitamin C and vitamin B1. In Manipur (Imphal east) potato cultivation area and production are 1.18 ha and 9.92 Mt (Agri Manipur report 2017-18) [15].

Potato is affected by many diseases like fungal, bacterial, viral, and also parasitic nematode. In potato 16% losses in yield due to microbial diseases and out of this 70-80% due to fungal attacks. Major fungal diseases in potato are late blight (Phytophthora infestans), early blight (Alternaria solani), wilt of potato (Fusarium oxysporum), powdery mildew (Erysiphe cichoracearum) and Curvularia disease. In Manipur climatic condition mainly late and early blight, wilt and Curvularia diseases are occured on potato crops. Symptom of Early blight caused by Alternaria solani appeared as small, irregular, dark brown to black spots and the size ranges from a pin point to ½ inch in diameter and also "appearance of concentric rings on older leaves. The lesion gives a characteristic “target-spot” or “bull’s eye” appearance. Early blight is one the major causes of defoliation of potatoes in the North Eastern States the increased disease severity is favored by alternating wet and dry conditions in the plant canopy (Franc, Harrison, and Lahman, 1988) [9]. The disease initially appears on the older leaves causing premature senescence and leaf area reduction (Johnson and Teng, 1990) [17]. Symptoms of Fusarium wilt appeared as yellowing of the leaves, following by wilting, rolling or curling, sometimes affecting leaves on only one side of the plant. The potatoes themselves may be blemished or decayed, often with sunken brown areas, especially at the stem end. Also plant growth stunted and developing of yellow leaves seeing in plant by Garibaldi et al. (2002) [10].

Chemicals are not only costly but also they are creating problems on the environment, human health in all areas of the world (Rahmatzai et al. 2017) [26]. Chemical methods are uneconomical and time-consuming, pollute the atmosphere, and are environmentally harmful, as the chemicals build up in the soil (Nannipieri, 1994) [24].
The repeated use of such chemicals has encouraged the development of resistance among the target organisms (Goldman et al. 1994) [12]. The present study was carried out to find the best bio-control agent like Trichoderma for control the Alternaria and Fusarium spp. causing early blight and wilt of potato respectively.

Materials and Methods
Isolation of fungus cultures
The diseased plants were collected from the potato grown in the experimental field. The early blight and wilt affected leaf and stem portion was cut into small pieces and surface sterilized with 0.1% sodium hypochlorite solution then afterward three times thoroughly washed with water. Potato dextrose agar (PDA) media were prepared and sterilized in an autoclave at 120 °C, 15 lbs. pressure for 20 minutes. The media were poured into Petri plates and allowed to cool for sometimes. Then, the leaf pieces were inoculated into the media and kept for incubation. After seven to eight days the fungal tips were transferred to PDA slants in order to obtain pure cultures. The isolates were confirmed as Alternaria (Van Bruggen 1984) [31] and Fusarium (Majumdar et al. 2008) [20] by microscopic observation of fungal culture.

In vitro efficacy of Trichoderma spp. on the growth of fungal pathogens
Antagonistic test of three Trichoderma spp. was done by dual culture technique given by Bell (1982) [2]. A single mycelial disc of 5mm diameter of pathogens was placed aseptically at one end of Petri plate containing culture media. Mycelial disc of 5mm diameter of antagonist isolate was placed at the opposite end of the same plate. Then the plates were incubated at 25 ± 1 °C in B.O.D. incubator. The per cent inhibitions of mycelial growth over control were calculated following the equation given by Edington et al. (1971) [3].

\[ L = \frac{[(C – T)]}{C} \times 100 \]

Where, L = Inhibition of mycelial growth, C = Radial growth measurement of the pathogen in control, T = Radial growth of the pathogen in the presence of Trichoderma isolates.

Bell’s scale with slight modification
Class I: The antagonist completely overgrew the pathogen (100 % overgrowth).
Class II: The antagonist overgrew at least 2/3rd of the pathogen surface (75% over growth)

Table 1: List of different Trichoderma spp. used for this study

| Sl. No | Trichoderma spp.         | Accession number |
|-------|--------------------------|------------------|
| 1.    | Trichoderma asperellum   | KU933477         |
| 2.    | Trichoderma harzianum    | KX0113222        |
| 3.    | Trichoderma viride       | -                |

Table 2a: Effect of pH on growth characteristics of Alternaria spp.

| Different pH | Mycelial growth in diameter(cm) |
|-------------|---------------------------------|
|             | 24 hrs | 48 hrs | 72 hrs | 96 hrs | 120 hrs | 144 hrs | 168 hrs |
| 4.0         | 0.00   | 1.30   | 2.33   | 3.63   | 4.03    | 4.57    | 5.70    |
|             | (0.71) | (1.34) | (1.68) | (2.03) | (2.13)  | (2.25)  | (2.49)  |
| 5.0         | 0.60   | 1.77   | 2.90   | 4.50   | 4.77    | 6.13    | 7.23    |
|             | (1.05) | (1.51) | (1.84) | (2.24) | (2.30)  | (2.56)  | (2.78)  |
| 6.0         | 0.93   | 2.03   | 3.00   | 5.00   | 5.47    | 7.43    | 8.27    |
|             | (1.20) | (1.59) | (1.87) | (2.35) | (2.44)  | (2.82)  | (2.96)  |
| 7.0         | 1.10   | 2.23   | 3.70   | 5.67   | 6.10    | 8.10    | 8.50    |
|             | (1.27) | (1.65) | (2.05) | (2.48) | (2.57)  | (2.93)  | (3.00)  |
| 8.0         | 0.83   | 2.03   | 3.07   | 4.93   | 5.10    | 6.77    | 8.17    |

Effect of solid media on mycelial Growth
Effect of media on mycelial growth of Alternaria and Fusarium spp. were studied in vitro. Potato dextrose agar (PDA), Rose bengal agar (RBA), Sabouraud agar (SDA), Richard synthetic agar (RSA) and Malt extract agar (MEA) media were used for study. After 7 days measurements were recorded.

Effect of different pH on mycelial Growth
Effect of different pH on mycelial growth of Alternaria and Fusarium spp. were studied in vitro. As a basal medium PDA was used. The pH of the medium was adjusted to various levels namely 4, 5, 6, 7, 8 and 9 by adding 0.1 N hydrochloric acid and 0.1 N sodium hydroxide and it was determined by electronic pH meter.

Native cultures of Trichoderma spp. were collected from laboratory of plant pathology, College of Agriculture, CAU, Imphal. Both pathogen cultures and bio-control agents were retained by periodical sub-culturing throughout the study period.

Results and Discussion
Effect of different pH on growth
The mycelial growth of Alternaria spp. were studied at six different pH levels, ranged from 4.0 to 9.0 on PDA medium and the results are presented in Table – 2a. The maximum radial growth of 8.5 cm was recorded at pH-7.0 at 7 days after inoculation. The minimum radial growth of 6.1 cm was recorded at pH-4.0 at 7 days after inoculation. The Alternaria spp. produced dark black colony with cottony puffy growth with regular margin in 7 pH level. Very fast growth observed in pH 7. These findings are supported by the research done by Gawai and Manganiyal, (2018) [11] and Chohan et al. (2015) [8] and Hubballi et al. (2010) [14] who also found relatively similar types of result on average mycelial growth rate.
The maximum radial growths of 8.5 cm were recorded at pH 6.0 at 7 days after inoculation, and the minimum radial growths of 5.7 cm were recorded at pH 4.0 at 7 days after inoculation and the results are presented in Table – 2b. The *Fusarium* spp. produced whitish puffy colony at initial stage later turns into straw colour mycelia with regular margin in 6 pH level. Very fast growth observed in pH 6. These findings are supported by the research done by Duarte *et al*. (2003) [6] and Tyagi, S. and Paudel, R., (2014) [23] and Jaruhar and Prasad (2011) [16].

### Table 2b: Effect of pH on growth characteristics of *Fusarium* spp.

| Different pH | Mycelial growth in diameter(cm) |
|-------------|---------------------------------|
| 4.0         | 0.67 (1.08) 1.17 (1.29) 1.53 (1.43) 3.17 (1.92) 4.23 (2.18) 5.20 (2.39) 6.10 (2.57) |
| 5.0         | 1.27 (1.33) 2.80 (1.82) 4.03 (2.13) 5.07 (2.36) 6.60 (2.67) 7.37 (2.81) 7.83 (2.89) |
| 6.0         | 1.47 (1.40) 3.10 (1.90) 4.43 (2.22) 5.23 (2.39) 7.47 (2.82) 8.13 (2.94) 8.50 (3.00) |
| 7.0         | 1.37 (1.37) 2.90 (1.84) 4.17 (2.16) 4.80 (2.30) 6.77 (2.70) 7.93 (2.91) 8.23 (2.96) |
| 8.0         | 1.00 (1.22) 2.50 (1.73) 3.43 (1.98) 4.50 (2.24) 6.33 (2.61) 7.63 (2.85) 8.03 (2.92) |
| 9.0         | 0.93 (1.20) 2.43 (1.71) 3.30 (1.94) 4.07 (2.14) 5.73 (2.50) 6.77 (2.70) 7.10 (2.76) |
| SE (d)      | 0.0881 (0.1427 0.2494 0.1699 0.1539 0.1621 0.1885 |
| CD at 5%    | 0.1921 (0.3109 0.5434 0.3703 0.3354 0.3533 0.4108 |

*Mean of three replications  **Figures in the parenthesis are square root transformed values

**Fig 1:** Effect of different pH on growth of *Alternaria* and *Fusarium* spp.

**Effect of media on growth**

The mycelial growth of *Alternaria* spp. was studied on five different media and the results are presented in Table – 3a. The maximum radial growth of 8.5 cm was recorded in potato dextrose agar at 7 days after inoculation. The minimum radial growth of 4.90 cm was recorded in rose bengal agar at 7 days after inoculation. *Alternaria* spp. showed dark black colony with cottony puffy growth white pigment with regular margin in potato dextrose agar the cultural media used. Slow to very fast growth were observed (Table – 3a, Plate – 3a). These findings are supported by Shabana *et al*. (2015) [27] and Koley and Mahapatra (2015) [18] and Mishra and Versha (2012) [22] and Somappa *et al*. (2013) [28] who also performed test on growth performance, and other cultural characteristics of *Alternaria* spp. by using different nutrient media.
Table 3a: Effect of different media on growth characteristics of *Alternaria* spp.

| Different media | Mycelial growth in diameter (cm) | Mycelial characteristics |
|-----------------|---------------------------------|--------------------------|
|                 | 24 hrs | 48 hrs | 72 hrs | 96 hrs | 120 hrs | 144 hrs | 168 hrs |                     |
| RBA             | 0.15* (0.81)** | 0.83 (1.15) | 1.33 (1.53) | 2.43 (1.71) | 3.33 (1.96) | 4.00 (2.12) | 4.90 (2.32) | Thin, sparse mycelial growth |
| ME              | 1.05 (1.25) | 2.28 (1.67) | 2.85 (1.83) | 4.03 (2.13) | 4.90 (2.32) | 6.15 (2.58) | 7.40 (2.81) | Rigid, light brown to black colour, pluffy growth |
| PDA             | 1.58 (1.44) | 2.70 (1.79) | 3.35 (1.96) | 4.58 (2.25) | 5.45 (2.44) | 7.00 (2.74) | 8.50 (3.00) | Dark black colony with cottony pluffy growth |
| SA              | 0.73 (1.11) | 1.93 (1.56) | 2.58 (1.76) | 3.38 (1.97) | 4.13 (2.15) | 5.65 (2.49) | 7.15 (2.77) | Rigid, light brown to black colour, pluffy growth |
| RA              | 0.20 (0.84) | 1.30 (1.34) | 2.38 (1.95) | 3.98 (2.12) | 4.90 (2.32) | 6.03 (2.56) | 7.58 (2.84) | Whitish, thin, sparse mycelial growth |

*Mean of four replications
**Figures in the parenthesis are square root transformed values

The mycelial growth of *Fusarium* spp. was studied on five different media and the results are presented in Table – 3b. The maximum radial growth of 8.5 cm was recorded in potato dextrose agar at 7 days after inoculation. The minimum radial growth of 4.18 cm was recorded in rose bengal agar at 7 days after inoculation. *Fusarium* spp. showed Whitish puffy colony at initial stage later turns into straw colour mycelia Dark black colony with cottony puffy growth white pigment with regular margin in potato dextrose agar the cultural media used. Slow to very fast growth were observed (Table – 3b). These findings are supported by Gupta *et al.* (2010) [13] and Pradeep *et al.* (2013) [25] and Yadav *et al.* (2017) [32] who also performed test on growth performance, and other cultural characteristics of *Fusarium* spp. by using different nutrient media. Among the 5 media used, potato dextrose agar had shown best results.

Table 3b: Effect of different media on growth characteristics of *Fusarium* spp.

| Different media | Mycelial growth in diameter (cm) | Mycelial characteristic |
|-----------------|---------------------------------|--------------------------|
|                 | 24 hrs | 48 hrs | 72 hrs | 96 hrs | 120 hrs | 144 hrs | 168 hrs |                     |
| RA              | 0.63* (1.06)** | 1.23 (1.32) | 2.78 (2.03) | 3.63 (2.03) | 4.53 (2.24) | 4.90 (2.32) | 5.95 (2.47) | Very thin whitish mycelial growth |
| RBA             | 0.18 (0.83) | 1.18 (1.30) | 1.75 (1.50) | 2.40 (1.70) | 3.08 (1.89) | 3.38 (1.97) | 4.18 (2.16) | Very thin whitish mycelial growth |
| PDA             | 2.13 (1.62) | 3.10 (1.90) | 4.10 (2.15) | 5.15 (2.38) | 6.13 (2.58) | 7.43 (2.82) | 8.50 (3.00) | Whitish pluffy colony at initial stage later turns into straw color mycelia |
| SA              | 1.05 (1.25) | 1.80 (1.52) | 2.85 (1.83) | 3.70 (2.05) | 4.98 (2.34) | 5.78 (2.51) | 7.03 (2.74) | Whitish growth at peripheral region , light brownish yellow at center |
| ME              | 1.30 (1.34) | 2.20 (1.64) | 3.03 (1.88) | 4.13 (2.15) | 5.23 (2.39) | 5.88 (2.53) | 7.58 (2.84) | Cottony White growth |
| SE (d)          | 0.097 | 0.1839 (0.221) | 0.217 (0.250) | 0.099 | 0.1449 (0.1172) | 0.3088 (0.2499) |                     |
| CD at 5%        | 0.2104 | 0.3920 (0.4720) | 0.5342 (0.2122) | 0.2088 |                     |                     |                     |

*Mean of four replications
**Figures in the parenthesis are square root transformed values

Fig 2: Effect of different media on growth of *Alternaria* and *Fusarium* spp.
In-vitro efficacy of Trichoderma spp. on the growth

After 7 days of incubation, Trichoderma harzianum showed maximum inhibition of 75.06% on mycelial growth of Alternaria and 70.01% on mycelial of Fusarium spp. However, the Trichoderma asperellum showed the lowest inhibition percentage which is around 61.34% (Alternaria spp.) and 64.24% (Table.4, Plate.1a,1b). In Bell’s Scale Trichoderma harzianum comes under Class II whereas Trichoderma asperellum in Class III for Alternaria spp. For Fusarium, Trichoderma harzianum comes under Class III and Trichoderma asperellum also comes under Class III. T. harzianum can be used as a promising biological control agent against Alternaria and Fusarium spp. by Meena et al. (2017) [21]. T. viride and T. harzianum most strongly suppressed the growth of Alternaria solani by Zafar et al. (2013) [33]. Under greenhouse conditions, the application of Trichoderma harzianum (ANR-1) exhibited the least disease incidence (by 15.33%) reported by Sundaramoorthy and Balabaskar (2013) [29].

Successful reductions of Fusarium wilt in many crops with application of different species of Trichoderma have been found (Bell et al. 1982; Morsy et al. 2009) [2, 23]. Trichoderma has emerged as an alternative mean of management of soil borne diseases (Ansari, et al. 2011) [1]. Lynch et al. (1991a) [19] concluded Trichoderma strains have the potential to consistently increase plant growth. The various antibiotics produced by Trichoderma spp. such as, Trichodermin, Trichodermol, Harzianum A, Harzianolide inhibits the mycelial growth of pathogen (Dennis and Webster, 1971a) [5] and also some cell wall degrading enzymes like chitinases, glucanases that breakdown polysaccharides and other cellular compounds (Elad et al. 2000) [8]. De Mayer et al. (1998) [4] reported that Trichoderma help in induced systemic resistance and competition against the pathogen.

![Fig 3: Percent growth inhibition of Alternaria and Fusarium spp. by Trichoderma spp.](image)

| Sl. no. | Bio-control agents     | Alternaria spp. Per cent growth inhibition over control (%) | Bell's Scale | Fusarium spp. Per cent growth inhibition over control (%) | Bell’s Scale |
|--------|------------------------|------------------------------------------------------------|--------------|----------------------------------------------------------|--------------|
| 1      | Trichoderma asperellum | 61.34* (7.86)**                                           | Class III    | 64.24*(8.05)**                                           | Class III    |
| 2      | Trichoderma harzianum  | 75.06(8.69)                                               | Class II     | 70.01(8.40)                                              | Class III    |
| 3      | Trichoderma viride     | 69.77(8.38)                                               | Class III    | 64.41(8.06)                                              | Class III    |
| 4      | Control                | -                                                         | -            | -                                                        | -            |

SE (d) 1.293
CD at 5% 2.816

*Mean of five replications

**Figures in parenthesis are square root transformed values
control of *Botrytis cinerea*. European Journal of Plant Pathology. 1998; 104:279-286.
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**Plate 1a:** Effect of bio-control agents on *Alternaria* spp.

C: control
T1- *Trichoderma asperellum*
T2- *Trichoderma harzianum*
T3- *Trichoderma viride*

**Plate 1b:** Effect of bio-control agents on *Fusarium* spp.

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