Original Research Article

Efficacy of Bio-agents and Media against the Early Blight of Potato

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ABSTRACT

Early blight of potato caused by Alternaria alternata is one of the major diseases causing considerable yield loss. Efficacy of bio-agents was studied and revealed the all four Trichoderma species significantly reduced the mycelial growth of the pathogen over the control by dual culture method. Significantly maximum inhibition of mycelial growth was obtained with T. viride (57.08%) followed by T. azospirillum (50.00%), T. harzianum (43.26%) and T. virens (37.08%) of the pathogen after 48 hours of incubation. In present study, T. viride was observed more inhibitory against A. alternata. In vitro efficacy of leaf extract media from host as well as synthetic and semi synthetic media were used for the optimum mycelial growth and sporulation of the Alternaria alternata. Among the media tested, the potato leaf agar was significantly superior and most effective medium for growth as well as dry mycelial weight followed by PDA. However potato leaf agar medium was also superior for sporulation of A. alternata. Whereas, the potato stem agar, V-8 juice agar, pea agar, oat meal agar and czapek’s agar medium were least effective in mycelia growth.

Keywords
Early blight, Potato, Potato leaf agar, Trichoderma and Alternaria

Introduction

Worldwide success of potato as a crop is in part due to the tubers being highly nutritious and providing a good source of fiber, minerals, proteins and vitamins C and B6. Important in the adoption of potato as a human food is its wide adaptability to varying environmental conditions. In India, it was introduced by Portuguese in the seventeenth century (Chakraborty, 2012). The intensive and extensive cultivation under the most favourable environmental conditions for potato crop production in the state failed to provide significant strides in potato yields, because of a number of production constraints, of which frequent occurrence of may be due to fungi, bacteria or viruses (Khurana, 2004). Early blight is one of the most important foliar diseases of potato (Vander-walls et al., 2001). It is caused by two species of genus Alternaria, i.e. Alternaria solani and Alternaria alternata worldwide on potato crops (Gudmestad and Pasche, 2007). Alternaria solani and Alternaria alternata both are more risk-important pathogens on potato crops and significant impact on the tuber yield (Kapsa,
Due to this disease, heavy losses on crop productivity and tuber quality recorded. 5 to 40% of yield losses have been reported in Israel (Rotem and Feldman, 1965) and 20 to 30% in the USA (Shitienberg et al., 1990). The average annual yield losses due to early blight vary enormously from 5-78% but it depends on weather conditions and type of variety grown (Waals et al., 2004). In view of the seriousness of the disease, the studies were planned to undertake on bio-agent and media against pathogen in vitro condition.

**Materials and Methods**

**Dual culture antagonism test**

Various known bio-agent *Trichoderma* species viz., *Trichoderma virens*, *Trichoderma azospirillum*, *Trichoderma harzianum*, *Trichoderma viride* were collected from Department of Plant Pathology and screened for their effectiveness against *Alternaria alternata* by using CRD for their effectiveness using dual culture technique. Twenty ml of potato dextrose agar was poured into 90 mm diameter Petri dishes and permit to solidify. Mycelial disc of (6 mm) from seven day old actively growing culture of the bio-agents and the test pathogen were cut separately with the help of sterilized cork borer and placed on solidified PDA approximately, 4 cm away from each other. Each treatment was replicated five times and incubated at 25 ± 1°C.

The activity of antagonistic organisms were recorded by measuring the colony diameter of *A. alternata* in each treatment and compared with control. The percent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

\[
I = \frac{C - T}{C} \times 100
\]

Where; \( I \) = Per cent inhibition of mycelium, \( C \) = Growth of mycelium in control, \( T \) = Growth of mycelium in treatment

**Media study**

A modified method suggested by Koley and Mahapatra (2015) was followed to find favorable medium. Various leaf decoction media from plant species as well as synthetic and semi synthetic media as listed below was prepared and evaluated. Mycelial growth and dry mycelial weight was recorded. Various eight media viz., V-8 juice agar, Czapek’s agar, Potato Dextrose agar, Oat meal agar, Potato stem agar, Potato leaf agar, Potato carrot agar and Pea agar were taken during study. Sterilize by autoclaving at 15 lbs pressure 121°C for 15 minutes. The broth media of above mentioned media prepared and dry mycelium weight was calculated (mg). The sporulation of the spore suspension was estimated as per (Krishna et al., 2018).

| Number of spore per microscopic field | Designation   |
|--------------------------------------|---------------|
| 0                                    | - (nil)       |
| 1-10                                 | + (poor)      |
| 11-20                                | ++ (moderate) |
| 21-30                                | +++ (good)    |
| 31-40                                | ++++ (excellent) |

**Results and Discussion**

**Efficacy of bio-agents**

The result revealed that all the bio-control agents were significantly superior in inhibiting the mycelial growth of test fungus over the control. The mycelial growth of *Alternaria alternata* was least in presence of *T. viride* (19.25 mm) followed by *T. azospirillum* (22.25 mm), *T. harzianum* (25.25 mm) and *T. virens* (28.00 mm).

In present study, fungal antagonist *T. viride* exhibited 57.08 per cent inhibition of mycelial growth, while *T. azospirillum* (50.00%), *T.
harzianum (43.26%) and T. virens (37.08%) showed poor antagonism. The result is in conformity with the results of Kumar et al., (2018) they reported that maximum percent inhibition (78.97%) of the Alternaria solani by Trichoderma viride followed by Trichoderma harzianum inhibition percent (75.33%). Murmu et al (2015) also founded T. viride was highly effective with 52.39 percent diseases reduction over control.

Efficacy of media study

Suitable medium is first step of pathogen for growth as well as sporulation for pathological research work. The results on the above studies indicated that potato leaf agar medium was significantly superior in supporting maximum mycelial growth at 5 days (70.50 mm) and 7 days (80.25 mm) of the fungus followed by Potato dextrose agar medium at 5 days (64.75 mm) and 7 days (78.25 mm). Potato stem agar medium V-8 juice agar medium and pea agar medium were the third group of the media with growth diameter at 5 days and 7 days (59.00 and 67.25 and 57.50 and 64.5, 58.00 and 63.25 mm), respectively, three being statistically at par with each other. The fourth group of culture media included oat meal agar medium and potato carrot agar medium producing growth at 5 days and 7 days (50.00 and 58.25, 46.50 and 53.75 mm), respectively and these both media were statistically at par with the each other. The czapek’s agar medium supported least growth diameter of the fungus at 5 days and 7 days (37.50 and 42.75 mm).

Maximum dry mycelium weight 584.75 mg was recorded on potato leaf agar was significantly superior. Next best dry mycelium weight was recorded on potato dextrose agar 570.25 mg followed by potato stem agar 315.50 mg, V-8 juice agar 285.00 mg, pea agar 268.50 mg, oat meal agar 244.25 mg, potato carrot agar 212.25 mg and czapek’s agar 176.50 mg, while potato leaf agar was good for sporulation.

Table.1 Effect of bio-agents against A. alternata in vitro by dual culture

| T. No | Treatment name         | Mycelial growth At 48 hrs. (mm) | Per cent growth Inhibition |
|-------|------------------------|---------------------------------|----------------------------|
| 1     | Trichoderma virens     | 28.00 (31.94)*                  | 37.08                      |
| 2     | Trichoderma azospirillum| 22.25 (28.14)                   | 50.00                      |
| 3     | Trichoderma harzianum  | 25.25 (30.16)                   | 43.26                      |
| 4     | Trichoderma viride     | 19.02 (26.02)                   | 57.08                      |
| 5     | Control                | 44.50 (41.84)                   | --                         |
|       | S. Em. ±               | (0.47)                          |                            |
|       | CD at 5%               | (1.41)                          |                            |

*Figures in parentheses are arcsine transformed values
Table 2. *In-vitro* evaluation of different media for mycelial growth of *A. alternate*

| T. No. | Treatment name         | Mycelial growth at 5 days (mm) | Mycelial growth at 7 days (mm) | Dry mycelium weight in broth media (mg) | Sporulation |
|--------|------------------------|-------------------------------|-------------------------------|-----------------------------------------|-------------|
| 1      | V-8 juice agar         | 57.50 (49.31)*                | 64.50 (53.44)                 | 285.00                                  | ++          |
| 2      | Czapek's agar          | 37.50 (37.31)                 | 42.75 (40.83)                 | 176.50                                  | +           |
| 3      | Potato Dextrose agar   | 64.75 (53.60)                 | 78.25 (62.21)                 | 570.25                                  | +++         |
| 4      | Oat meal agar          | 50.00 (45.00)                 | 58.25 (49.76)                 | 244.25                                  | +           |
| 5      | Potato stem agar       | 59.00 (50.19)                 | 67.25 (55.10)                 | 315.50                                  | +++         |
| 6      | Potato leaf agar       | 70.50 (57.12)                 | 80.25 (63.63)                 | 584.75                                  | ++++        |
| 7      | Potato carrot agar     | 46.50 (42.99)                 | 53.75 (47.16)                 | 212.25                                  | ++          |
| 8      | Pea agar               | 58.00 (49.62)                 | 63.25 (52.69)                 | 269.50                                  | +           |
| S. Em. ± | (0.86)               | (0.89)                        |                              | 2.73                                    |             |
| CD at 5% | (2.50)               | (2.60)                        |                              | 7.96                                    |             |

*Figures in parentheses are arcsine transformed values*

Fig 1. Effect of *Alternaria alternata* on media
In present study, maximum mycelial growth was observed on potato leaf agar medium followed by potato dextrose agar. Potato leaf agar as host media was found suitable for best mycelial growth of *Alternaria alternata*. These results corroborate the findings of former literature such as Chand and Chandra recorded maximum vegetative growth of *Alternaria brassicicola* on host media. Plant Pathogenic fungi often used to induce sporulation on the source host tissues Photita et al., (2001). Potato dextrose agar was reported as most suitable culture medium for growth and sporulation of *Alternaria* spp, earlier by several workers Kumar et al., (2018), who observed that highest radial growth of *Alternaria solani* on potato dextrose agar (63.00 mm) followed by oat meal agar (42.33 mm) and Czapek’s dox agar (27.73 mm), while, oat meal agar showed good sporulation of *Alternaria* spp. Roopa (2012) reported that maximum dry mycelia weight (249.27mg) of *Alternaria solani* after 9th day of inoculation in potato dextrose broth. However, maximum dry matter was recorded by potato leaf agar medium followed by potato dextrose agar. Other culture media reported suitable for better growth and good sporulation of *Alternaria* spp. in present study Potato stem agar medium, V-8 juice agar medium, pea agar medium oat meal agar medium and potato carrot agar medium, while czapek’s agar medium was observed poor medium against pathogen.

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How to cite this article:

Priyanka Kumari Meena, D. L. Yadav, C. B. Meena, B. K. Patidar and Pratap Singh. 2020. Efficacy of Bio-agents and Media against the Early Blight of Potato. Int.J.Curr.Microbiol.App.Sci. 9(07): 2208-2213, doi: https://doi.org/10.20546/ijcmas.2020.907.258