

## Evaluation of Some Plant Extracts and Fungal Antagonists for the Biological control of pre-emergence damping off of Brinjal (*Solanum melongena*)

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### ABSTRACT

Eight fungal antagonists and eight leaf extracts were evaluated *in vitro* against *Pythium aphanidermatum* (Edson) Fitzpatrick and the effective treatments selected for pot culture evaluation. Pot culture studies with brinjal seeds showed that *Eucalyptus* leaf extract as seed soak treatment for 30 minutes prior to sowing was effective against *P. aphanidermatum*. Seed treatment with *Trichoderma harzianum* at  $5 \times 10^9$  conidia per ml, *Lactisaria arvalis* at 0.66 mg sclerotia per ml and Captan at 0.25 per cent also effectively controlled *P. aphanidermatum*.

**KEY WORDS:** Plant extracts, fungal antagonists, biological control, damping off brinjal

Pre-emergence damping off caused by *Pythium* sp. poses a serious problem in the establishment of brinjal plants in the nurseries. Plant extracts applied as seed treatment and soil drench are reported to control damping off caused by different species of *Pythium* in crop plants (Gupta and Bilgrami, 1970; Alice, 1984). Fungal antagonists like *Trichoderma harzianum*, *T. koningii* (Lifshitz *et al.*, 1986) *T. hamatum* (Harman *et al.*, 1980), *T. viride* (Boston, 1980; Dumitras and Sesan, 1979) and *Laetisaria arvalis* (Krishnamoorthy, 1987) were applied as seed treatment in different crops. The present study was taken up to evaluate the efficacy of seed treatment with plant extracts and antagonists for the control of pre-emergence damping off of brinjal.

### MATERIALS AND METHODS

Brinjal plants showing damping off were collected from the orchard of Tamil Nadu Agricultural University, Coimbatore and the pathogen isolated and purified by single spore isolation from seeded clear agar plate, single oospore was picked up with a sterile needle and developed as pure culture. The pathogenicity of the isolate was confirmed by mixing the inoculum in soil and sowing brinjal seeds which showed pre-emergence damping off.

#### *In vitro* studies

Cold water extracts of the leaves of different plants (Table 1) were prepared by washing the leaves, chopping and grinding them in a mortar and pestle with the addition of cold distilled water at the ratio of 1 : 2 (1 part leaf : 2 parts water). The extracts were squeezed through cotton wool, and used immediately (Alice, 1984). *In vitro* assay of the leaf extracts was done by filter paper disc bioassay (Sharville and Pelletier, 1956). Filter paper discs soaked in Captan 0.25% and sterile distilled water served as controls. Potato dextrose agar

(PDA) medium was used throughout the lab studies. Each treatment was replicated thrice and radial growth of the pathogen measured after 24 and 48 h. The different fungal antagonists (Table 2) were screened by the dual culture technique (Dennis and Webster, 1971) the fungal disc of pathogen was placed on the PDA medium 24 h after placing the fungal disc of the antagonists. Growth of the pathogen was measured after 24 and 48 h.

#### Pot culture studies

The selected plant extracts were applied to brinjal seeds ( $Co_2$ ) by soaking the seeds in the extract for 30 minutes. The seeds were then dried in shade for 2 h and sown. The antagonistic fungi were applied by the seed treatment method described by Krishnamoorthy (1987). A spore concentration of  $5 \times 10^9$  conidia per ml was used for isolates of *T. viride* and *T. harzianum*. For *L. arvalis*, 0.66 mg sclerotia per ml was used. Three ml of the suspension was used to coat 10 g of brinjal seeds, which were shade-dried for 2 h and sown. Seed dressing with Captan at 0.25 per cent was done 24 h before sowing.

Fifty treated seeds were sown in 10 cm pots each containing sterile soil mixed with 50 g of inoculum of pathogen multiplied on sand maize (19:1) medium. Controls were maintained with Captan seed treatment (0.25 per cent) and untreated seeds. Untreated seeds sown in uninoculated soil served as another control. The pots were watered regularly and the seedling emergence recorded.

### RESULTS AND DISCUSSION

The fungus isolated from the brinjal plants were identified as *Pythium aphanidermatum* (Edson) Fitzpatrick. This isolate was proved to cause pre-

emergence damping off of brinjal as it could be reisolated from infected seedlings in the pathogenicity test. The results of *in vitro* assay of the leaf extracts for fungicidal / fungistatic property against *P. aphanidermatum* indicated that all the leaf extracts were less effective than the fungicide treatment. Extracts of *Azadirachta*, *Eucalyptus*, *Emelia* and *Vinca* were more effective than the other plant extracts.

In the dual culture test, *T. harzianum* (IARI) showed superiority over other antagonists (Table 2). *T. viride* (IARI) was on par with *T. harzianum* (IARI) and also with *T. viride* (Local) and *L. arvalis*. Other fungal isolates were inferior to these isolates although all of them showed antagonistic effect. In the pot culture study, seed coating with *T. harzianum* (IARI) was found to be the best treatment which was on par with uninoculated control and the fungicide treatment. Seed pelleting with *L. arvalis* also was on par with seed coating with *T. harzianum*. Seed coating with *T. viride* (IARI) was inferior to *T. harzianum* but on par with *L. arvalis*. Among the leaf extract tried, *Eucalyptus* extract showed distinct superiority over the others and was on par with *T. harzianum*, *L. arvalis* and *T. viride* (IARI). *Azadirachta* leaf extract though inferior to *Eucalyptus* leaf extract, was more effective than other leaf extracts tried (Table 3). Extract of *Emelia* and *Vinca* did not afford protection *in vivo* indicating the influence of soil conditions which may inactivate or degrade their active principles. Similarly, *T. harzianum* (Local) also failed to give good protection *in vivo*. Besides the influence of soil conditions on growth and activity of the antagonists, their efficiency also depends on the strain of the antagonist used (Hardar *et al.*, 1984).

The effectiveness of *T. harzianum* as seed treatment for the control of pre-emergence damping off caused by *Pythium* was reported by several workers (Ruppel *et al.*, 1983; Sivan *et al.*, 1984; Hardar *et al.*, 1984). *T. viride* was reported to control pre-emergence damping off of pea (Lifshitz *et al.*, 1986) and tomato (Krishnamoorthy, 1987). *L. arvalis* was reported as an effective antagonist of *Pythium* (Martin *et al.*, 1986; Krishnamoorthy, 1987). The present study confirms these findings and points out their effectiveness in reducing pre-emergence damping off of brinjal also.

The effectiveness of *Eucalyptus* leaf extract observed in this study agrees with the report of Sivasithambaram *et al.* (1981) who found that the bark

extracts of *Eucalyptus* was effective in the control of *Phytophthora cinnamomi*. However, *Bougainvillea* leaf extract which was reported to be effective against *Pythium monosporum* (Alice, 1984) was found ineffective against the isolate of *P. aphanidermatum*, used in the present study. The use of plant extracts and fungal antagonists identified in this study offer a cheaper and environmentally safer alternative to the use of fungicides for seed treatment.

Table 1. *In vitro* bioassay of plant extracts for the control of *Pythium aphanidermatum*

| Treatment                            | Radial growth @ (mm) * |                   |
|--------------------------------------|------------------------|-------------------|
|                                      | 24 h                   | 48 h              |
| <i>Azadirachta indica</i> Juss.      | 15.0 <sup>a</sup>      | 20.3 <sup>c</sup> |
| <i>Eucalyptus teraticornis</i> Sm.   | 4.0 <sup>b</sup>       | 15.0 <sup>b</sup> |
| <i>Emelia sonchifolia</i> (L) D.C.   | 5.0 <sup>b</sup>       | 15.6 <sup>b</sup> |
| <i>Sida acuta</i> Burm.              | 41.0 <sup>h</sup>      | 45.0 <sup>e</sup> |
| <i>Bougainvillea glabra</i> Chois.   | 29.6 <sup>c</sup>      | 44.3 <sup>e</sup> |
| <i>Ocimum sanctum</i> Land. Mont.    | 39.0 <sup>g</sup>      | 44.6 <sup>e</sup> |
| <i>Leuciana leucocephala</i> L.      | 36.0 <sup>f</sup>      | 45.0 <sup>c</sup> |
| <i>Vinca rosea</i> L.                |                        |                   |
| ( <i>Cathranthus roseus</i> G. Don.) | 17.6 <sup>d</sup>      | 31.0 <sup>d</sup> |
| Captan 0.25%                         | 0.0 <sup>a</sup>       | 0.0 <sup>a</sup>  |
| Sterile distilled water              | 41.0 <sup>h</sup>      | 45.0 <sup>e</sup> |

\* Mean of 3 replications

@ In a column, means followed by similar letters are not different statistically (P=0.05) by D.M.R.T.

Table 2. *In vitro* screening of antagonistic fungi against *Pythium aphanidermatum*

| Treatment                    | Source      | Growth of pathogen * (mm) |                     |
|------------------------------|-------------|---------------------------|---------------------|
|                              |             | 24 h                      | 48 h                |
| <i>Trichoderma harzianum</i> | Pantnagar   | 34.66 <sup>c</sup>        | 43.00 <sup>d</sup>  |
| "                            | IARI        | 11.66 <sup>a</sup>        | 13.00 <sup>a</sup>  |
| <i>Trichoderma viride</i>    | IARI        | 13.33 <sup>a</sup>        | 16.33 <sup>ab</sup> |
| "                            | CMI         | 17.66 <sup>b</sup>        | 27.33 <sup>c</sup>  |
| "                            | Local       | 11.33 <sup>a</sup>        | 20.00 <sup>b</sup>  |
| "                            | Netherlands | 39.66 <sup>d</sup>        | 53.33 <sup>e</sup>  |
| <i>Laetisaria arvalis</i>    | Pantnagar   | 11.33 <sup>a</sup>        | 20.33 <sup>b</sup>  |
| Control                      | --          | 44.00 <sup>e</sup>        | 72.33 <sup>f</sup>  |

\* Mean of 3 replications

In a column, means followed by similar letters are not different statistically (P=0.05) by D.M.R.T.

Table 3. Effect of seed treatment with plant extracts and fungal antagonists on the control of pre-emergence damping off of brinjal

| Treatment                            | emergence @ *        |
|--------------------------------------|----------------------|
|                                      | %                    |
| <i>Azadirachta indica</i>            | 39.33 <sup>d</sup>   |
| <i>Eucalyptus teraticornis</i>       | 56.66 <sup>bc</sup>  |
| <i>Emelia sonchifolia</i>            | 20.00 <sup>e</sup>   |
| <i>Vinca rosea</i>                   | 12.00 <sup>e</sup>   |
| <i>Trichoderma viride</i> (IARI)     | 45.33 <sup>cd</sup>  |
| <i>Trichoderma harzianum</i> (IARI)  | 66.66 <sup>a</sup>   |
| <i>Laetisaria arvalis</i> (Pannagar) | 54.66 <sup>c</sup>   |
| <i>Trichoderma viride</i> (Local)    | 21.33 <sup>e</sup>   |
| Captan 0.25%                         | 63.33 <sup>abc</sup> |
| Control (Inoculated)                 | 12.66 <sup>e</sup>   |
| Control (Uninoculated)               | 68.00 <sup>a</sup>   |

\* Mean of 3 replications

@ In a column, means followed by similar letters are not different statistically (P=0.05) by D.M.R.T.

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