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# INTEGRATED DISEASE MANAGEMENT APPROACH FOR SOME RHIZOSPHERIC PATHOGENS OF ARID REGION

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## Abstract

The present study was undertaken to prepare a package of integrated management of soil borne pathogens *i.e.*, *Ganoderma lucidum*, *Rhizoctonia solani*, *Fusasrium oxysporum* and *Macrophomina phaseolina* isolated from the rhizosphere of tree species of arid region *viz*., *Tecomella undulata* and *Prosopis cineraria*. Biological control agents, chemicals and plant extracts were tested against four soil borne pathogens. The results showed that Bavistin was found to inhibit all the pathogens tested, but cannot be used in IDM programe alone. For *M. phaseolina* either *Trichoderma viride* or Bavistin can be used. *R. solani* can be managed by using combination of *T. viride*, Blitox and plant extract while *T. viride* and plant extracts can be used in combination for *G. lucidum* and *F. solani.*

***Key words :*** Arid region, IDM, biocontrol agents, plant extract.

## Introduction

Diseases and insect pests constitute major biological determinants of forest productivity, particularly in nurseries and plantations. Large-scale mortality in the nursery due to disease problems could seriously affect the plantation programme resulting in the reduction of biomass production or loss of valuable germplasm collections. Thus, the economic loss resulting from nursery diseases and insect-pests are considerable. Therefore, raising disease free, healthy tree seedlings is not only important for maintaining a good nursery stock but also essential in establishing healthy stand in the field for better productivity (Mohan, 2000).

The root rot fungi which pose serious threats to forest nurseries include the species of *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Macrophomina*, and *Cylindrocladium* (Huang and Kuhlman, 1990; Asiegbu *et al*., 1999; Waffa and Haggag, 2002). These pathogens often invade terminal unsuberized roots of young seedlings and cause late damping off or root rot, wilt thereby killing the host. Damping-off disease in forest nurseries is one of the economically important diseases causing heavy losses in different parts of the world. Besides inflicting significant economical losses, the disease might disturb the whole forthcoming planting program.

Many rhizospheric microorganisms are known to be equipped with antagonistic potential against soil borne pathogens (Cook and Baker, 1983; Elad *et al*., 1986). Biological control has attracted attention from researchers for over last three decades, because of the interest in developing more eco-friendly means of disease management in the absence of pesticides. Besides, there are many plant species, which have antifungal properties and can be used for management of diseases integrating it with bioagents and chemicals. Integration of biological control agent with chemical fungicides reduces the amount of fungicides to be applied minimizing the associated residual problems. This also helps to overcome biocontrol limitations and to improve its efficacy providing a reliable disease control that cannot be provided by the biocontrol agent alone (Elad, 2003; Omar *et al*., 2006). Hence, it is compelling to look for alternative disease management practices, which include the integrated use of bicontrol agents, chemicals and botanicals.

Thus, in the present study chemicals along with biocontrol agents and plant extracts having antifungal properties were used to develop a suitable package to reduce the loss due to soil borne plant pathogens by reducing the inoculum build up in nurseries.

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## Materials and Methods

The present study was undertaken to prepare a package of integrated management of soil borne pathogens i.e., *Ganoderma lucidum*, *Rhizoctonia solani*, *Fusasrium oxysporum* and *Macrophomina phaseolina* isolated from the rhizosphere of tree species of arid region viz., *Tecomella undulata* (Rohida) and *Prosopis cineraria* (Khejri). These pathogens were screened with two chemicals, one bioagent and plant extracts having antifungal properties.

**Collection of soil samples**

Soil samples from the rhizosphere of Khejri and Rohida were collected from different localities of Jodhpur to isolate plant pathogens as well as bioagents. 50 g soil samples from 2-5 cm depth were collected from various places of the field. The five samples were mixed to make a composite sample. *G. lucidum* was isolated from the infected bark of Khejri tree. One gm soil sample was plated through dilution plate technique. Out of five different cultures of *T. viride* one actively growing and sporulating isolate was selected for screening.

**Isolation and identification of pathogens and biocontrol agents**

Four species of pathogenic fungi were isolated from the rhizosphere soil of Rohida and Khejri. Similarly, *T. viride* was isolated from same plants. Different methods, media and incubation temperatures have been used for isolation of fungi from soil samples. One gram of the soil sample was taken and added to 1 ml of sterilized distilled water to make a dilution of $10^{-1}$. This suspension was then subjected to serial dilution and a dilution of $10^{-5}$ was attained. One milliliter of each dilution viz., $10^{-3}$ to $10^{-5}$ was spread on a potato dextrose agar (PDA) plate which was then incubated at 28°C for 5 days and purified by the spore method. Fungi were isolated from the mixed isolates from each plate and sub cultured on PDA, until a pure isolate was obtained. They were identified on the basis of their morphological characters (Barnett and Hunter, 1972; Bridge *et al*., 2001). The purified and identified cultures were maintained on potato dextrose agar (PDA) medium and used for further study.

**Screening of *Trichoderma* isolates against plant pathogenic fungi**

Antagonistic activities of isolated *T. viride* over pathogenic fungi were tested by employing dual culture technique (Morton and Stroube, 1955). A 5 mm mycelial disc of bioagent and pathogen was cut with the help of cork borer from fresh 7 days old culture and inoculated on the opposite side of the plate at equal distance from the periphery; the cultures were incubated at 25±2$^0$C until the end of the incubation period. In control plates (without *Trichoderma*), the pathogenic disc were placed on a sterile PDA medium. The radial growth of pathogen isolates were measured after every 24 hours. Percent inhibition of average radial growth was calculated in relation to growth of the controls adopting method as given by Hajieghrari *et al*. (2008).

**Screening of pathogen and bioagent with chemicals**

Two chemical fungicides Bavistin 50% WP, Blitox 50% WP were screened for their antifungal activity against the test pathogen using the poisoned food technique (Dey *et al*., 1991). The PDA agar plates having concentration of 0.15% of fungicides were prepared and marked accordingly. A 5 mm disc of each fungus was transferred to the centre of a PDA petri plate then incubated at 25±2$^0$C until the control plate was completely filled. In control plates (without fungicide), the soil borne pathogenic disc were placed on a sterile PDA medium. The radial growth of pathogen isolates were measured after every 24 hours.

**Extraction and preparation of plant extract and screening of pathogens and biocontrol agent**

The plant samples were collected from Jodhpur and its nearby areas. Four plant species viz., *Datura stramonium* roots, *Lawsonia inermis* leaves, *Withania somnifera* leaves and *Datura metel* leaves were used as a plant part to evaluate the antifungal properties. The materials were washed with distilled water and dried in shade. The dried plant materials were ground to fine powder and then extracted with methanol (10% v/v) as done by Green (2004) and Parekh *et al*. (2005). The collected extracts were then stored in a refrigerator at 5°C. The extracts were added to sterilize PDA flasks before solidifying to obtain the proposed concentrations of 50 mg/ml. Poison food technique was adopted for screening of the pathogenic fungi as well as *T. viride* with the extract.

## Results and Discussion

When compatibility of *T. viride* was studied with the chemicals, fungicide and antifungal extract of Datura root, it was observed that *T. viride* was completely inhibited by Bavistin (no compatibility at all) while it had 100 per cent compatibility with the Blitox and Datura root extract (table 1). The results showed that as the bioagent is incompatible with Bavistin, thus, we cannot integrate Bavistin with *T. viride* in IDM programme. While it can be integrated with Blitox and Datura root extracts. According to Haas and Défago (2005), the antagonistic

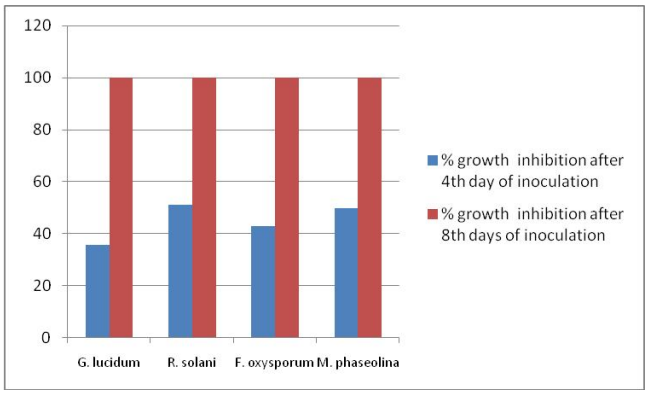


**Fig. 1 :** Antagonism of *T. viride* with soil borne pathogens.

**Table 1 :** Compatibility of *T. viride* with chemicals and antifungal compounds.

| Treatment | Compatibility of *T. viride* with fungicide and plant extract (%) |
|---|---|
| Bavistin | 0 |
| Blitox | 100 |
| *Datura stamonium* roots extract | 100 |
| *Lawsonia inermis* leaves | 100 |
| *Withania somnifera* leaves | 100 |
| *Datura metel* leaves | 100 |

*lucidum* (35.7%) (fig. 1). But after eight days of inoculation *T. viride* was able to inhibit the growth of all the four pathogen completely *i.e.* (100%).

The results showed (table 2) that Bavistin was able to inhibit all the four fungal pathogen upto 100 %. Maximum inhibition of Blitox *i.e.* 83.0 % is of *R. solani* followed by *G. lucidum* (26.2%), but it is unable to inhibit either *F. oxysporum* or *M. phaseolina*. In case of *D. stamonium* root, the microfiltered extract was able to inhibit *F. oxysporum* upto 100.0 per cent followed by *R. solani* (65.8%) and *G. lucidum* (55.8%), where as it was unable to inhibit *M. phaseolina* completely. The leaf extract of *L. inermis* showed maximum growth inhibition 82.35 percent against *F. oxysporum* followed by 78.21, 69.15 and 68.74 against *R. solani*, *M. phaseolina* and *G. lucidum*, respectively (table 2).

## Conclusion

Biological control agents, chemicals and antifungal compound extracted from root of *Datura stramonium* were tested against four soil borne pathogens. The idea was to produce an effective combination/consortia of bioagent, chemical and botanicals that are compatible to each other so that they can be used in development of

**Table 2 :** Per cent growth inhibition of pathogens with chemicals, plant extracts and bio-agent.

| Treatments | Per cent growth inhibition | | | |
|---|---|---|---|---|
| | *G. lucidum* | *R. solani* | *F. oxysporum* | *M. phaseolina* |
| Bavistin | 100 | 100 | 100 | 100 |
| Blitox | 26.2 | 83.0 | 0 | 0 |
| Datura root extract | 55.8 | 65.8 | 100 | 0 |
| *Lawsonia inermis* leaves | 68.74 | 78.21 | 82.35 | 69.15 |
| *Withania somnifera* leaves | 66.82 | 75.12 | 63.54 | 69.76 |
| *Datura metel* leaves | 47.0 | 82.55 | 46.87 | 42.36 |
| *T. viride* | 100 | 100 | 100 | 100 |

microorganisms could compete with pathogens, particularly by producing antibiotic compounds. In soil, these antibiotics could interfere with pathogen development, for example, during spore germination and the onset of root infection. Moreover to effectively manage diseases one spray is not enough as was observed during the study that Blitox initially inhibited but as days passes the fungus overcomes the chemicals and shows significant growth.

When the four pathogen were screened with *T. viride* for its antagonistic activity against the pathogen, it was observed that initially after 4$^{th}$ day of inoculation *R. solani* showed maximum inhibition (51%) followed by *M. phaseolina* (50%), *F. oxysporum* (43.1%) and then *G.*

IDM, which can be further used in nursery stages as well as in plantations. The results show that as the bioagent was not compatible with Bavistin, while it showed compatibility with the root extract and Blitox. Thus Bavistin and *T. viride* should be used separately for managing these soil pathogens. Bavistin was found to inhibit all the pathogens tested but cannot be used in IDM programe alone. For *M. phaseolina* either *T. viride* or Bavistin can be used. *R. Solani* can be managed by using combination of *T. viride*, blitox and Datura extract while *T. viride* and Datura extract can be used in combination for *G. lucidum* and *F. solani*.

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