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RESEARCH **P**APER

Biological management of pigeonpea stem canker caused by *Macrophomina phaseolina* (Tassi) Goid

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Seven known antagonists were tested *in vitro* for their antagonism to *M. phaseolina* by dual culture method. In this method, *Trichoderma viride, T. harzianum* and *Pseudomonas fluorescens* appeared as strong and potent antagonists against *M. phaseolina* followed by *T. fasciculatum, T. longibrachyatum, T. koningii* and *Bacillus subtilis.*

Key words : Pigeonpea stem canker, *Macrophomina phaseolina*

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INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is known as red gram, tur, arhar, tuvarica, congobean, thogari or gandul, is economically and nutritionally an important legume and is a major source of protein for the poor communities of many tropical and subtropical regions of the world. Globally, it is cultivated on (4.79 M ha) in 22 countries (FAO, 2008) but which only a few major producer in the world. In Asia, India has largest acreage under pigeonpea (3.90 M ha) with a total production and productivity of 2.89 mt and 741 kg/ha, respectively (DAC, 2011). Pigeonpea is attacked by more than 100 pathogens (Nene *et al*., 1989). This includes *viz*., fungi, bacteria, viruses, nematodes and phytoplasmas. Only a few of them cause economic losses and the distribution of the most important diseases is geographically restricted (Kannaiyan *et al*., 1984). Various economic importance diseases are associated with pigeonpea *viz*., Fusarial wilt (*Fusarium udum* B.), Sterility Mosaic (*PPSMV* S.), Phytophthora Blight (*Phytophthora drechesleri* T.), Macrophomina stem canker (*Macrophomina phaseolina*), Alternaria blight (*Alternaria alternate* K.) and pearly cyst nematode (*Globodera rostochiensis* S.) in the Indian sub-continent. In Gujarat, among these diseases, stem canker is one of the major constraints. This is the first report of the occurrence of the disease on pigeonpea from Nepal (Maubl.). The stem canker is caused by *Macrophomina phaseolina* is one of most serious and oldest known disease (Nene *et al*., 1984). Biological control is a potential non chemical means for plant disease control by reducing the harmful effect of a pathogen through the use of living entities.Since the *M. phaseolina* is a soil borne fungus and possess great problem in managing the disease. Soil borne diseases are difficult to control. Seed treatment with fungicides does not protect the crop for long periods. Soil drenching with fungicides are not economical and they may establish imbalances in microbial communityun favourable for activities of beneficial organism (Jeyarajan *et al.,* 1991). In addition, continuous use of the same

fungicides for the same pathogen results in development of resistant strains of pathogen, besides polluting the environment (Muthukrishnan, 1989). It is now widely recognized that biological control of plant pathogens using antagonistic fungi and bacteria is distinct possibility for the future and can be successfully utilized especially within the frame work of integrated disease management system (Muthamilan and Jeyrajan, 1996). Use of antagonistic organism against *Macrophomina phaseolina* has been well documented in several crops.

RESEARCH METHODOLOGY

This work was conducted in Department of Plant Pathology, N.M. College of Agriculture, Navsari agricultural University. Navsari during 2014 to determine the antifungal activity of *Trichoderma viride, T. harzianum* and *Pseudomonas, T. fasciculatum, T. longibrachyatum, T. koningii* and *Bacillus subtilis* against *Macrophomina phasiolina* by dual culture technique (Skidmore and Dickinson, 1976).

Isolation of pathogens:

Pigeonpea plants (GT-1) showing the typical stem canker symptoms were collected from N.A.R.P. Research Station, NAU, Bharuch as well as from the farmers' field and brought to the laboratory and subjected to tissue isolation. After 48 hrs of incubation the isolated fungus initially started to grow as dirty white mycelial growth, then turn to fluffy, blackish mycelial growth on sterilized potato dextrose agar (PDA) medium (potato starch: 20 g, dextrose: 20 g, agar: 20 g and distilled water to make the volume 1 litre. After eight days, minute black sclerotial bodies formed on PDA. The culture was further purified by single hyphal tip method and the purified culture was maintained on PDA slants for further studies. The periodical sub-culturing and multiplication were made on PDA plates to keep the culture fresh and to use throughout the investigations. After purification of the pathogen as described cultural and morphological characters of the fungus on PDA, were studied for identification and compared with those described in the literature. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C), Division of Plant Pathology, I.A.R.I., New Delhi-110 012 and was identified as *Macrophomina phaseolina* (Tassi.) Goid (I.T.C.C. No. 9572.14). The studies on the cultural and morphological characters of isolated *Macrophomina* sp. showed its close identity with *Macrophomina phaseolina* (Tassi.) Goid as described by Nakarni (1991) and Agrawal (1993) were also similar with our present finding. Thus, the *M. phaseolina* causing pigeonpea stem canker.

Preparation of culture media:

The Petri plates containing 20 ml PDA medium inoculated aseptically with the pathogen *M. phaseolina* and the test organism (antagonist) by placing 5 mm diameter culture blocks at 70 mm apart from each other. Three repetition of each treatment were kept and the Petri plates with only pathogen at center served as control. All the plates were incubated at 27 ± 2^0 C temperature.

Observations on colony diameter were recorded up to the complete coverage of control plates, which were inoculated with only pathogen. Radial growth of the pathogen was recorded and per cent growth inhibition was calculated by following formula (Vincent, 1947).

$$\text{Growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

where,

C = Growth of pathogen in control after incubation

T = Growth of pathogen in treatment after incubation

RESEARCH FINDINGS AND ANALYSIS

The results presented in Table 1, (Plate 1) and depicted graphically in Fig. 1 revealed that, all the antagonists tested against *M. phaseolina* were effective in checking the growth of the pathogen. Out of seven antagonists tested, least growth of the pathogen was recorded in *Trichoderma viride* (22.00 mm) which was at par with *T. harzianum* (24.00 mm) followed by *Pseudomonas fluorescens* (28.00 mm), *T. longibrachyatum* (31.00 mm) and *T. koningii* (32.00 mm), *T. fasciculatum* (40.00 mm) and *Bacillus subtilis* (46.00 mm).

The result presented in Table 1 revelled that, *Trichoderma viride* (74.42%), *T. harzianum* (72.09%), *Pseudomonas fluorescens* (67.44%), *T. longibrachyatum* (63.95%) and *T. koningii* (62.79%) significantly inhibited the pathogen. Whereas, *T. fasciculatum* (53.49%) and *Bacillus subtilis* (46.51%) were comparatively least effective.

It is evident from these studies that among all the antagonists evaluated by dual culture method, *T. viride, T. harzianum* and *P. fluorescens* consistently showed

Table 1 : Efficacy of various native isolated bio-agents against *Macrophomina phaseolina in vitro*

Sr. No	Test Organism	Colony diameter of pathogen (mm)	Growth inhibition over control (%)
1.	*Trichoderma viride* Navsari isolate	22	74.42
2.	*Trichoderma harzianum* Navsari isolate	24	72.09
3.	*Trichoderma longibrachyatum* Navsari isolate	31	63.95
4.	*Trichoderma koningii*Navsari isolate	32	62.79
5.	*Pseudomonas fluorescens* Navsari isolate	28	67.44
6.	*Trichoderma fasciculatum* Navsari isolate	40	53.49
7.	*Bacillus subtilis* Navsari isolate	46	46.51
8.	Control	86	-
	S.E.±	0.78	
	C.D. (P=0.05)	2.35	
	C.V. %	6.41	

Plate 1 : Antagonistic effects of micro-organisms to *Macrophomina phaseolina*

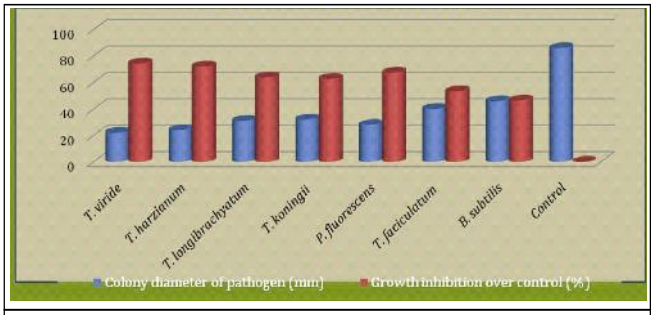

Fig. 1 : Efficacy of various native isolated bio-agents against *Macrophomina phaseolina in vitra*

strong antagonistic property against *M. phaseolina* compared to the other antagonists tested hence considered as potential antagonists. Our results are in harmony with earlier workers Lokesha and Benagi (2007) found that *T. harzianum, T. viride* and *P. fluorescens* significantly inhibited the mycelial growth of *M. phaseolina* (74-76%) by Dual culture technique. Tandel (2004) carried out interaction study of known antagonist by dual culture method and found strong antagonistic effect on *M. phaseolina* with *T. viride, T. harzianum, B. subtilis, T. longibrachyatum, T. koningii* and *Chaetomium globosum in vitro.*

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