

Original Research Article

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Evaluation the Efficacy of Plant Extracts and Bio-agents against *Macrophomina phaseolina* in vitro

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ABSTRACT

The present study was carried to evaluate the efficacy of various plant extracts and bio-agents against *Macrophomina phaseolina*. It is a devastating disease in green gram growing area of Rajasthan. *In vitro* evaluation of bio-agents and seven plants extract (*Datura stramonium*, *Allium sativum*, *Zingiber officinalis*, *Leptidium sparticum*, *Azadirachta indica*, NSKE and *Allium cepa*), revealed that maximum growth inhibition of *Macrophomina phaseolina* was recorded in *Allium sativum* followed by *Zingiber officinalis* at 5%, 10% and 15% concentrations. Among the concentrations 15 % was most effective. *Trichoderma harzianum* was the most promising antagonist against *Macrophomina phaseolina* among the different fungal and bacterial antagonists, followed by *Trichoderma viride* and *Pseudomonas fluorescens*. The Present study was planned to evaluate the efficacy of bio-agents and plant extracts against *Macrophomina phaseolina* causing dry root rot of green gram.

Keywords

Mungbean, Plant
extract, Bio-agent,
*Macrophomina
phaseolina*,
Inhibition, Mycelial

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Introduction

Mungbean/green gram [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crop. It is grown in almost all parts of the country. In India, It is third most important pulse crop after chickpea and pigeonpea and covers an area about 29.36 lakh ha with production of 13.90 lakh tonnes (Anonymous 2014-15), In India, Mungbean is mainly

grown in the states of Rajasthan, Maharastra, Madhya Pradesh, Orissa, Andhra Pradesh, Tamil nadu, and Uttar Pradesh. In Rajasthan, the total area under Cultivation of mungbean is 13.68 lakh ha with the annual production of 6.02 lakh tonnes with the productivity of 441kg/ha (Anonymous, 2015-16). In Rajasthan Mungbean is grown in Jaipur. Bhilwara. Bharatpur. Sriganganagar, Bikaner, Jodhpur, Kota, Ajmer and Udaipur district.

It has proved to be an ideal crop for spring and summer/kharif season. Mungbean belongs to family leguminosae and sub family papilionaceae. Mungbean is an excellent source of high quality protein. It is consumed in different ways as dal, halwa, snacks and so many other preparations. Ascorbic acid (vitamin-C) is synthesized in sprouted seeds of mungbean. The amount of riboflavin and thiamine is also increased in sprouted seeds being a leguminous crop, it has the capacity to fix atmospheric nitrogen through symbiotic nitrogen fixation. It is also used as green manure crop.

Mungbean is prove to different fungal diseases, among them dry root rot incited by *Macrophomina phaseolina* is a soil borne pathogen. *Macrophomina phaseolina* survives in/on seed and persisted in the soil in the form of black sclerotia which are produced in large number on infected host tissues and are subsequently dispersed in soil during tillage operations.

The fungicides presently recommended to manage these diseases provide protection for limited period. The continuous use of traditional fungicides may cause bioaccumulation of the toxic residues besides giving rise to resistant strains. Increased public concern about pesticide utilization and the health hazards necessitates the exploitation of alternative methods of disease control. Now a day's research on disease management all over the world is mostly towards biological control or application of combined treatment of bioagents, fungicide and also biofertilizer. In the last three decades, a lot of researches have been carried out on the antagonistic nature of several fungal and bacterial biocontrol agents (Papavizas, 1985). Blakeman and Fokkema (1982) reported that *Trichoderma* species are the well-known antagonists, particularly in the soil and that they are involved in

competition, antibiosis and hyperparasitic interactions, which makes them the most effective biocontrol agents even on foliar surfaces.

Materials and Methods

All the experiments were conducted under *in vitro* condition during 2016-17 at Department of Plant Pathology, College of Agriculture, Bikaner (Rajasthan).

Plant extract

Different plant extracts were tested for their efficacy against *Macrophomina phaseolina* *in vitro* (Table 1).

Preparation of plant extract

Selected plants were collected from the surrounding areas of Bikaner, and washed thoroughly with tap water and air dried. One hundred gram of plant part was grind using pestle and mortar by adding equal amount (100 ml) of sterilized distilled water (1: 1, w/v). The pulverized mass was squeezed through the cheese cloth and the extracts were centrifuged at 10000 rpm for 5-10 minutes and used as stock solution. To study the antifungal property of plant extracts, poisoned food technique (Nene and Thapliyal, 1993) was adopted.

The stock solutions of different botanicals (5, 10 and 15%) were mixed with 95, 90, and 85 ml of PDA media, respectively so as to get 5, 10 and 15 per cent concentrations and sterilized. Twenty ml of such medium was poured under aseptic conditions into sterile Petri plates allowed to solidify. Mycelial discs (5 mm) were cut out using sterile cork borer from periphery of actively growing culture of *M. phaseolina* and one such disc was placed on the centre of each Petri plate. The treatments were replicated thrice. Control was

maintained by growing the pathogen on PDA plates without plant extracts. Plates were incubated at room temperature (28±2°C) for 7 days and radial growth was taken at the time when maximum growth occurred in the control plates.

Formula

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth of *M. phaseolina* in control (mm)

T= Mycelial growth of *M. phaseolina* in presence of plant extracts (mm)

In vitro evaluation of antagonistic potential of test bio-agents

The selected antagonists were purified and their antagonistic activities were tested against *M. phaseolina in vitro* as described below.

Evaluation of antagonistic potential of fungal antagonists

Dual culture technique (Dennis and Webster, 1971) was followed in order to ascertain the antagonistic capacity of *Trichoderma* spp. and other fungal antagonists. One mycelial disc (5 mm diameter) of each of the pathogen and antagonist was kept on the surface of potato dextrose agar medium in Petri dishes at 5 cm apart.

The inoculated Petri dishes were incubated at 28±2°C for 7 days. Three replications were kept for each fungal antagonist. In case of control, the Petri dishes were inoculated with mycelial disc of the test pathogen only.

The mycelial growth of test pathogen was measured after 4 days of inoculation. Per cent growth inhibition was calculated by following formula as: (Vincent., 1927)

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth of *M. phaseolina* in control (mm)

T= Mycelial growth of *M. phaseolina* in presence of antagonist (mm).

Evaluation of antagonistic potential of bacterial antagonists

Paper disc inoculation method was followed in order to test the antagonistic capacity of bacterial antagonists. Stock cultures of *P. fluorescens* and *B. subtilis* were streaked on Pseudomonas agar *fluorescens* and nutrient agar media slants respectively and incubated at 28±1°C for 8 hours.

Ten ml sterilized distilled water was added to each slant containing the fresh colony of respective bacterial antagonists and suspension was prepared by scrapping the bacterial growth with the help of sterilized inoculating needle.

The suspension was transferred to sterilized Petri dishes. Sterilized filter paper discs (5 mm diameter) were dipped in respective bacterial suspension. Four such inoculated discs were placed in opposite directions on the surface of nutrient-agar media in Petri dishes.

Mycelial discs (5 mm diameter) taken from periphery of actively growing culture of *M. phaseolina* raised on nutrient-agar media was placed at the center of Petri dishes containing the inoculated paper discs In case of control, the Petri dishes were inoculated with mycelial discs of the test pathogen only.

Three replications were kept for each bacterial antagonist. The inoculated Petri dishes were incubated at 28±1°C in BOD incubator. Mycelial growth of *M. phaseolina*

was recorded after 7 days of incubation. The inhibition of mycelium growth by the respective bacterial antagonists was calculated by using the following formula (Vincent, 1927)

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth of *M. phaseolina* in control (mm)

T= Mycelial growth of *M. phaseolina* in presence of antagonist (mm)

Results and Discussion

Effect of plant extracts on growth of *Macrophomina phaseolina* under *in-vitro*

The results revealed that all the plant extracts at 5%, 10% and 15% concentrations were significantly inhibited the growth of the fungus as compared to the control. Among the above tested concentrations, 15% concentration is most effective in inhibiting the growth of fungus as compared to 10% and 5%. At 15% concentration of plant extract, the *Allium sativum* extract proved significantly superior (82.00%) in checking the fungal growth over the rest. The next best in order of merit was *Zingiber officinalis* (61.11%) (Table 3).

The extracts of *Allium cepa* (55.50%), *Datura stramonium* (50.00%), NSKE (44.40) and *Azadirachta indica* leaf extract (38.80%) were effective against the pathogen while *Leptidium sparticum* (27.00%) was comparatively less effective. The results indicated that the *Allium cepa* extract gave maximum inhibition followed by extract of *Zingiber officinalis*.

Findings of our experimental are very much similar to findings of S.V. Magar *et al.*, (2011), they evaluated that the efficacy of plant extracts against leaf blight of green gram incited by *Macrophomina phaseolina*

(Tassi) Goid and revealed that the bulb extracts of *Allium sativum* was most effective in inhibiting the growth of the test fungus followed by *Zingiber officinalis* and *Allium cepa*.

Dhingani *et al.*, (2013) observed that among the plant extracts, *Allium sativum* extract was found most effective in reducing root rot incidence followed by *Azadirachta indica* leaf extract. Meena *et al.*, (2014) also observed that garlic extract found most effective in reducing the growth of *Macrophomina phaseolina in vitro*.

Lalita Lakhran and R.R. Ahir (2018) observed in six plant extracts, garlic extract was found most effective in reducing root rot incidence followed by neem leaf extract.

Savaliya *et al.*, (2015) reported the efficacy of various botanicals against *Macrophomina phaseolina* (Tassi.) Goid causing root rot of sesame. The phyto extracts of nine plant species were evaluated *in vitro* by poisoned food technique against *M. phaseolina*. The extract of garlic cloves (*Allium sativum* L.) was proved excellent with maximum inhibiting (77.65 %) of mycelial growth and scanty sclerotial formation followed by onion bulb extract (*Allium cepa* L.) (63.98%), while least growth inhibition (32.34 %) was recorded in ginger rhizome extract. These findings corroborated with our findings that plant extracts is effective in inhibiting the growth of *Macrophomina phaseolina in vitro*.

Efficacy of bio-agents against *Macrophomina phaseolina* under *in vitro*

The antagonists isolated from rhizosphere of healthy mungbean fields were tested for their antagonistic reactions against *Macrophomina phaseolina*. All the isolated antagonists were significantly superior in inhibiting the growth of test fungus over the control. Microorganisms which developed well

marked zones of inhibition on agar plates comprised two fungi viz., *Trichoderma harzianum* and *T. viride*. Maximum growth inhibition was recorded in *T. harzianum* (73.33%) followed by *T. viride* (64.44%), out of two bacteria, maximum growth inhibition was recorded in *Pseudomonas fluorescens* (46.66 %), and this was at par with *Bacillus subtilis* (38.88%). The fungal antagonists *T. harzianum* and *T. viride* and two bacterial antagonists i.e. *Pseudomonas fluorescens* and *B. subtilis* significantly inhibited the mycelial growth of *M. phaseolina* *in vitro* (Table 2 and 4).

These findings very much similar to findings of Priyanka Meena *et al.*, (2017), they observed that *Trichoderma* and other species isolated from infested soil were tested for their antagonism to *Macrophomina phaseolina* on Czapek's dox agar medium in Petri dishes. Four fungi *Aspergillus niger*, *Trichoderma atroviride*, *T. harzianum*, *T. viride* gave distinct antagonistic reactions, showing stunting of *Macrophomina phaseolina* colony and a clear cut inhibition

zone between colonies of antagonist and the pathogen. *Trichoderma harzianum* was most effective among all antagonists in reducing charcoal rot of fenugreek followed by *Trichoderma viride*, *Trichoderma atroviride* and *Aspergillus niger*.

Bimla and Gaur (2016) observed that three fungi and one bacterium viz., *T. harzianum*, *T. viride*, *T. atroviride* and *Bacillus* sp. isolated from soil of crop fields were tested *in vitro* for their antagonistic activity against eight isolates of *Macrophomina phaseolina* on Czapek's dox agar medium. Inhibition of the mycelium growth (%) of pathogen by *T. harzianum*, *T. viride*, *T. atroviride* and *Bacillus* sp. varied from 56.53 to 70.87, 53.56 to 64.88, 50.05 to 62.06 and 45.95 to 55.86. *T. harzianum* inhibited maximum mycelial growth of all the isolates followed by *T. viride*, *T. atroviride* and *Bacillus* sp. *in vitro* condition. These findings very much similar to our findings that bio-agents are very much effective in inhibiting the growth of *Macrophomina phaseolina*.

Table.1 Name of plant extracts tested against *Macrophomina phaseolina* (*in vitro*)

Common Name	Botanical Name	Part used
Datura	<i>Datura stramonium</i>	Leaf
Garlic	<i>Allium sativum</i>	Clove
Ginger	<i>Zingiber officinalis</i>	Rhizome
Kheep	<i>Leptidium sparticum</i>	Stem
Neem leaf	<i>Azadirachta indica</i>	Leaf
NSKE	<i>Azadirachta indica</i>	Seed
Onion	<i>Allium cepa</i>	Bulb

Table.2 Name of Bio-agent tested against *Macrophomina phaseolina* (*in vitro*)

Bio-agent
<i>Trichoderma harzianum</i>
<i>Trichoderma viride</i>
<i>Pseudomonas fluorescens</i>
<i>Bacillus subtilis</i>

Table.3 Efficacy of plant extracts against *M. phaseolina* under *in vitro*

Treatment	Per cent inhibition of mycelial growth					
	5% Conc.		10% Conc.		15% Conc.	
	Mycelial Growth (mm)	Growth Inhibition (%)	Mycelial Growth (mm)	Growth Inhibition (%)	Mycelial Growth (mm)	Growth Inhibition (%)
<i>Datura stramonium</i> (Datura)	60.00	33.30 (35.23)*	54.00	40.00 (39.21)*	45.00	50.00 (44.98)*
<i>Allium sativum</i> (Garlic)	55.00	38.80 (38.51)	40.00	55.00 (47.85)	16.00	82.00 (64.87)
<i>Zingiber officinalis</i> (Ginger)	58.00	35.50 (36.55)	43.00	52.22 (46.25)	35.00	61.11 (51.39)
<i>Leptidium sparticum</i> (Kheep)	88.00	2.22 (8.41)	80.00	11.10 (19.38)	65.00	27.70 (31.74)
<i>Azadirachta indica</i> (Neem leaf)	75.00	16.60 (24.02)	68.00	24.44 (29.61)	55.00	38.80 (38.51)
NSKE	65.00	27.70 (31.74)	57.00	36.66 (37.25)	50.00	44.40 (41.76)
<i>Allium cepa</i> (Onion)	60.00	33.30 (35.23)	52.00	42.22 (40.51)	40.00	55.50 (48.13)
Control	90.00	-	90.00	-	90.00	-
S.Em±		0.79		1.30		1.44
CD (P=0.05)		2.30		3.92		4.25
CV%		4.15		4.90		3.92

*Figure in parentheses are angular transformed values

Table.4 Efficacy of bio-agents against *Macrophomina phaseolina* under *in vitro*

Bio-agent	Per cent inhibition
<i>Trichoderma harzianum</i>	73.33 (59.05)*
<i>Trichoderma viride</i>	64.44 (53.71)
<i>Pseudomonas fluorescens</i>	46.66 (43.34)
<i>Bacillus subtilis</i>	38.88 (38.78)
Control	-
S.Em±	0.51
CD P=0.05	1.56
C.V (%)	2.62

*Figure in parentheses are angular transformed values

Thombre and Kohire (2018) tested seven fungal and two bacterial bioagents / antagonists evaluated *in vitro* were found antifungal / antagonistic against *M. phaseolina*. However, *T. harzianum* was

found most effective and recorded significantly highest mycelial growth inhibition (77.59%) of the test pathogen over untreated control.

The second and third best bioagents / antagonists found were *A. niger* and *T. viride* which recorded mycelia inhibition of 68.17 and 65.46 percent, respectively. The antagonists *P. fluorescens* and *B. subtilis* was also found fungistatic and recorded 51.37 and 60.90 percent mycelial inhibition, respectively. These findings very much similar to our findings that bio-agents are very much effective in inhibiting the growth of *Macrophomina phaseolina*.

Majumdar and Gaur (1996), Sethuraman *et al.*, (1998), Desai and Kulkarni (2002), Gupta *et al.*, (2003), Rani *et al.*, (2009), Anis *et al.*, (2010), Kaur *et al.*, (2010), Magar *et al.*, (2011), Kumari *et al.*, (2012), Kumar *et al.*, (2013), Doley and Jite (2012), Ramezani (2008), Loksha and Benagi (2007), Kaswate *et al.*, (2003) found that *Trichoderma*, *Bacillus* and *Pseudomonas* were antagonistic to *Macrophomina phaseolina* in their studies *in vitro* and confirming the our present findings.

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