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In vitro bio-efficacy of bioagents and botanicals against *Macrophomina* blight of mungbean caused by *Macrophomina phaseolina* (Tassi) Goid

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Abstract
All the 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 mycelial 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. Also all the 12 botanicals / plant extracts evaluated were found fungistatic / antifungal against *M. phaseolina*. Average mycelial growth inhibition recorded with all the test botanicals was ranged from 6.12 (*L. carnea*) to 95.08 (*A. sativum*) percent. However, significantly highest average mycelial growth inhibition was recorded with the botanicals *A. sativum* (95.08%) and *A. cepa* (93.08%). This was followed by the botanicals *viz.*, *A. indica* (67.43%), *D. metal* (59.24%), and *I. carnia* (6.12%) was found less effective.

Keywords: Bio-efficacy, Blight, Mungbean, *Macrophomina*, Bioagents and Botanicals

Introduction
The Mungbean (*Vigna radiata* (L.) Wilczek, *Phaseolus radiata* L. is one of the thirteen food legumes grown in India and third most important pulse crop of India, after chickpea and pigeonpea. It is commonly called as, mung, moong, mungo, greengram, goldengram, chickasawpea and oreganpea. In India, greengram is more commonly used than mungbean. Mungbean crop has been reported to be affected by about 60 fungal, three bacterial and five viral diseases. The major fungal diseases includes: rust (*Uromyces appendiculatus* Unger), root rot (*Rhizoctoria solani* Kuhn), wilt (*Fusarium* o*xysporum* f. sp. *tricheiphilum*), bean blight (*Aschochyta phaseolrum* Sacc.), leaf spot (*Cercospora canescens* rot (*Phytopthora parasitica* Dast.), anthracnose (*Colletotrichum lindemuthianum* Sacc. And Magn.), leaf and stem gall (*Synchytrium phaseoli*), powdery mildew (*Erysiphe polygnoni* DC) and leaf blight (*Macrophomina phaseolina*), the bacterial diseases are bacterial blight/ canker (*Xanthomonas compestris* pv. *vignicol*a), pod blight (*Xanthomonas phaseoli* var. *faciens*), halo blight (*Pseudomonas phaseolicola*) and viral diseases like common bean mosaic, yellow mosaic, ring spot, leaf curl and leaf crinkle. Among these diseases, *Macrophomina* blight incited by *Macrophomina phaseolina* (Tassi) Goid has been reported as one of the most important disease causing potential yield losses in Mungbean.

In Maharashtra, *Macrophomina* blight and powdery mildew have been reported as most potential and devastating diseases putting farmers into economic loss (Zote *et al.*, 1983) [25]. *Rhizoctonia bataticola* (*Macrophomina phaseolina*) has been reported to infect the Mungbean plant parts *viz*., leaf (leaf blight), stem (stem blight), stalk (stalk rot), root (root rot), collar region (collar rot), blossom and fruit rot (Saksena, 1979) [19]. Considering the importance of the pathogen / disease in the state and the losses incurred by the disease in the farmer's field, the present investigations of biomanagement were undertaken to minimize the development of resistance against fungicides.

Materials and Methods

Bioagents

Seven fungal antagonists *viz.*, *Trichoderma viride, T. harzianum, T. hamatum, T. longibrachiatum, T. koningii*, *Gliocladium virens, Aspergillus niger* and two bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated *in vitro* against *M. phaseolina*, applying Dual Culture Technique (Dennis and Webster, 1971) [4]. Seven days old cultures of the test bioagents and test fungus (*M. phaseolina*) grown on (PDA, NA) were used for the study. Discs (5 mm dia) of PDA along with culture growth of the test fungus and bioagents were cut out with sterilized cork borer. Then two culture discs, one each of the test fungus and bioagent were placed at equidistance and exactly opposite with each other on solidified PDA medium in Petri plates aseptically and plates were incubated at 27 ± 2 ^{0}C. PDA plates inoculated only with culture disc of the test fungus were maintained as untreated control.

Observations on linear mycelial growth of the test fungus and bioagent were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth of the test fungus. Percent inhibition of the test fungus by the bioagents over untreated control was calculated by applying following formula (Arora and Upaddhyay, 1978) [2]. Observations on sclerotia and pycniospores production were recorded at 10 days after incubation using Stereo binocular microscope.

$$\text{Percent Growth Inhibition} = \frac{\text{Colony growth in control plate - Colony growth in Intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

Botanicals/Phytoextracts

Several phytoextracts/botanicals have been found were reported antifungal against *M. phaseolina*. Therefore, locally available plant species *viz.* Mehandi, Ginger, Parthenium, Neem, Garlic, Turmeric, Bougainvillea, Onion, Eucalyptus, Datura, Beshram, Pomogranate were evaluated *in vitro* against *M. phaseolina* applying Poisoned food techniques (Nene and Thapliyal, 1993) [17] and using PDA as basal medium.

Preparation of Phytoextracts

Fresh healthy plant parts (leaves/root/bulbs) collected from fields were washed with distilled water and air-dried and100 gm crushed in 100 ml of distilled water (w/v). The extract was filtered through double layered, muslin cloth and further filtrated through Whatsman No. 1 filter paper using funnel and volumetric flasks (100 ml cap.). The extract obtained formed 100 percent concentration, which was further diluted to required concentrations of 10.0, 15.0 and 20.0 percent. An appropriate quantity of each plant extract (100%) was separately mixed thoroughly with autoclaved and cooled (40 ^{0}C) PDA medium in Conical flasks (250 ml cap.) to obtain desired concentrations (10, 15 and 20 percent). The PDA medium amended separately with plant extract was then poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. For each test botanical extract and their respective concentrations, three plates / treatment / replication were maintained. Each plant extract and its respective concentrations were replicated thrice. Upon solidification of PDA, all the treatment and control plates were aseptically inoculated by placing in the centre a 5 mm mycelial disc obtained from a week old actively growing pure culture of *M. phaseolina*. Plates containing plain PDA without any botanical extract and inoculated with mycelial disc of the test fungus served as untreated control. All these plates were then incubated at 27 ± 2 ^{0}C temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus.

Observations on radial mycelial growth of the test fungus were recorded at 24 hrs. interval and continued till growth of the test pathogen in untreated control plate was fully covered. Percent inhibition of the test pathogen was calculated by applying the formula given by Vincent (1927) [24].

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

Where,

C= growth of the test fungus in untreated control plates

T= growth of the test fungus in treated plates

Observations on sclerotia and pycniospores production were recorded at 10 days after incubation.

Result and Discussion

In vitro bioefficacy of bioagents

Results from Table 1 indicated that all the bioagents evaluated exhibited fungistatic activity against *M. phaseolina* and significantly inhibited mycelial growth of the test pathogen over untreated control. Of the seven fungal antagonists tested, *T. harzianum* was found most effective and recorded least linear mycelial growth (20.17 mm) with highest mycelial inhibition (77.59%) of the test pathogen. The second and third best antagonists found were *A. niger* and *T. viride*, which recorded mycelial growth of 28.64 mm and 31.08 mm, respectively and inhibition of 68.17 and 65.46 percent, respectively. This was followed by *T. hamatum* (col. dia.: 33.06 mm and inhibition: 63.26%) *T. longibrachiatum* (col. dia.: 35.91 mm and inhibition: 60.10%) and *T. koningii* (col. dia.: 45.40 mm and inhibition: 49.55%). The fungal antagonist *G. virens* was found least effective which recorded 61.61 mm linear mycelial growth and 31.54 percent mycelial inhibition, respectively. Inhibition of the fungus may be either due to the production of toxins, by the *Trichoderma spp.* or coiling, penetration and lyses of the hyphae of the pathogen by antagonists. The antagonists *P. fluorescens* and *B. subtilis* was also found fungistatic and recorded 35.19 mm and 43.77 mm linear mycelial growth, respectively and 51.37 and 60.90 percent mycelial inhibition, respectively.

Table 1: *In vitro* bioefficacy of bioagents against of *M. phaseolina*

Treatments	Colony dia.* (mm)	% Inhibition
Trichoderma viride	31.08	65.46 (54.01)
T. harzianum	20.17	77.59 (61.76)
T. hamatum	33.06	63.26 (52.70)
T. longibrachiatum	35.91	60.10 (50.83)
Gliocladium virens	61.61	31.54 (34.16)
Aspergillus niger	28.64	68.17 (55.66)
T. koningii	45.40	49.55 (44.74)
Pseudomonas fluorescens	43.77	51.37 (45.78)
Bacillus subtilis	35.19	60.90 (51.30)
Control	90.00	0.00 (0.00)
S.E. ±	1.45	0.96
C.D. (P=0.05)	4.28	3.84

* Mean of three replications

Figures in parenthesis are arc sine transformed value

The bioagents, *T. harzianum, T. viride, A. niger, B. subtilis* were reported as effective antagonists against *Macrophomina phaseolina, Rhizoctonia bataticola and R. solani* by several

workers (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; Lakpale, 2012.) [14, 21, 5, 7, 18, 1, 8, 13, 10, 11]

***In vitro* bioefficacy of botanicals**

The results presented in Table 2 revealed that all the botanicals/plant leaf extracts tested *in vitro* were found significantly effective in reducing the percentage mycelial growth of *M. phaseolina* over untreated control. *A. sativum* and *A. cepa* recorded lowest mean colony diameter (4.42 and 6.22 mm) and highest mean mycelial growth inhibition (95.08 and 93.08%) of the test pathogen over untreated control. This was followed by the botanicals, *A. indica* (Mean Col. Dia. 29.91 mm and mean inhibition 67.43%), *D. metel* (Mean Col. Dia. 36.51 mm and mean inhibition 59.24%), *E. globules* (Mean Col. Dia. 40.74 mm and mean inhibition 54.72%), *P. hysterophorus* (Mean Col. Dia. 42.88 mm and mean inhibition 52.35%), *Z. officinale* (Mean Col. Dia. 49.71 and mean inhibition 44.76%), *L. innermis* (Mean Col. Dia. 65.61 mm and mean inhibition 27.71%) and *P. granatum* (Mean Col. Dia. 71.66 mm), and mean inhibition 20.37%). The least effective found were *C. longa* (Mean Col. Dia. 74.11 mm and mean inhibition 17.64%), *B. spectabilis* (Mean Col. Dia. 76.80 mm and mean inhibition 14.66%) and *I. carnea* (Mean Col. Dia. 84.49 mm and mean inhibition 6.12%). These results support the observations of Magar *et al*. (2011)[13] who reported that bulb extract of *Allium sativum* and *A. cepa*, leaf extract of *A. indica* @ 10% conc. inhibit the growth of *M. phaseolina* causing leaf blight of mungbean. Similar observations have been reported by Tandel *et al*. (2010) [23]. Whereas, Murugapriya *et al*. (2011) [16] reported that only two plant extracts zimmu (*Allium cepa* L. and *Allium sativum* L.) and garlic creeper (*Adenocalymma alliaceum*) were found toxic against the leaf blight pathogen of Mungbean (*M. phaseolina).*

Table 2: *In-vitro* bioefficacy of plant extracts against *M. phaseolina*

Treatments	Colony Dia. (mm)* at Conc.			Ave. (mm)	% Inhibition at Conc.			Ave. inhibition (%)
	10%	15%	20%		10%	15%	20%	
Mehandi (*L. innermis*)	73.50	65.00	58.33	65.61	20.18 (26.65)	27.77 (31.79)	35.18 (36.38)	27.71 (31.60)
Ginger (*Z. officinale*)	52.87	49.87	46.40	49.71	41.26 (39.96)	44.59 (41.89)	48.44 (44.11)	44.76 (41.98)
Parthenium *(P.hysterophorus)*	48.87	43.27	36.50	42.88	45.70 (42.53)	51.92 (46.10)	59.44 (50.44)	52.35 (46.35)
Neem (*A. indica*)	37.80	30.32	19.81	29.31	57.99 (46.49)	66.31 (54.52)	77.99 (62.03)	67.43 (54.34)
Garlic (*A. sativum*)	8.60	4.67	0.00	4.42	90.44 (72.02)	94.81 (76.85)	100.00 (90.00)	95.08 (79.62)
Turmeric (*C. longa*)	76.88	75.00	70.47	74.11	14.58 (22.38)	16.64 (24.07)	21.70 (27.76)	17.64 (24.73)
Bougainveilliea (*B. spectabilis*)	82.13	75.87	72.40	76.8	8.73 (17.12)	15.70 (23.30)	19.55 (26.24)	14.66 (22.22)
Onion (*A. cepa*)	9.20	6.97	2.50	6.22	89.77 (71.39)	92.25 (73.90)	97.22 (80.43)	93.08 (75.24)
Eucalyptus (*E. globules*)	44.97	41.80	35.47	40.74	50.03 (45.02)	53.55 (47.04)	60.59 (51.11)	54.72 (47.72)
Datura (*D. metal*)	41.77	38.33	29.94	36.51	53.59 (47.06)	57.40 (49.26)	66.73 (54.78)	59.24 (50.36)
Beshrum (*I. carnea*)	90.00	85.10	78.37	84.49	0.00 (0.00)	5.44 (13.45)	12.92 (21.06)	6.12 (11.50)
Pomegranate (*P. granatum*)	76.12	73.57	65.30	71.66	15.42 (22.99)	18.25 (25.28)	27.44 (31.59)	20.37 (26.62)
Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E. ±	1.36	0.90	0.59	0.95	1.08	0.72	0.46	0.75
C.D. (P=0.05)	3.95	2.60	1.71	2.75	3.15	2.10	1.33	2.19

* Mean of three replications,
Figures in parenthesis are arc sine transformed value

Botanicals *A. sativum*, *A. cepa*, *A. indica*, *D. metal*, *E. globules*, *P. hysterophorus* and *Z. officinale* were also reported fungistatic against *M. phaseolina*, *R. bataticola* and *R. solani* causing blights and root rot in many crop by several workers (Dubey and Dwivedi, 1991; Sundarraj *et al*., 1996; Kaushal *et al.*, 2003; Lokesha and Benagi, 2004; Dawar *et al*. 2007; Mandhare and Suryawanshi, 2009; Salam *et al.* 2009; Rani *et al*., 2009 and Tandel *et al*. 2010; Lakpale, 2012) [6, 22, 9, 12, 3, 15, 20, 18, 23, 11]. Inhibition of mycelial growth of *M. phaseolina* by plant extracts in the present study may be attributed to the presence of antifungal properties and inhibitory compounds in the extract. In the present investigation, the extracts from garlic and onion inhibited the sclerotial production. These plant extracts can help to reduce the inoculum potential and thereby reduce disease incidence and intensity.

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