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Efficacy of different phytoextracts against *Macrophomina phaseolina*

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Abstract
Mungbean [*Vigna radiate* (L). Wilczek] is an important *Kharif* pulse crop, which is adversely affected by various fungal diseases. Leaf blight/ charcoal rot (*Macrophomina phaseolina*) is one of the most serious diseases, under rainfed conditions. Among phytoextracts tested, garlic clove extracts and neem (15 and 20) per cent was recorded maximum mycelial growth inhibition (92.46 and 100) per cent respectively.

Keywords: mungbean, *M. phaseolina*, phytoextract, inhibition

Introduction
Leaf blight caused by *Macrophomina phaseolina* (Tassi.) Goid. in green gram was found in serious proportion threatening it successful cultivation in Marathwada region of Maharashtra. Considering the seriousness of the problem, investigation was carried out to find out the suitable control measures for the disease. For this purpose eleven phytoextracts and bioagents were tested *in-vitro* to know their inhibitory effect on the growth of *Macrophomina phaseolina* (Tassi) Goid). Phytoextracts posses the great potentialities being used as botanical fungicide without any adverse effect on the environment for the management of plant disease. Earlier workers have reported the effect of plant extracts of various plant species to inhibit the growth of *M. phaseolina in-vitro*. (Upadhyay and Gupta 1990; Dubey and Dwivedi 1991) [8, 1]. Hence, phytoextracts are considered as good alternative for the management of such disease.

Material and methods
The leaves and roots of infested mungbean plants were collected from different farmer's field and Agriculture Research Station, Badnapur and their isolation, purification were done. For the testing the efficacy of following different treatments.

The efficacy of phytoextracts
The efficacy of plant extracts against *Macrophomina phaseolina in-vitro*: Fresh healthy plant parts (leaves/cloves) collected from fields were washed with distilled water and air-dried and 100gm crushed in 100 ml of distilled water (w/v). The extract was filtered through double layered, muslin cloth and further filtrated through WhatsmanNo.1 filter paper using funnel and volumetric flasks (100 ml cap.). The extract obtained formed 100 per cent concentration of 10.0, 15.0, and 20.0 per cent. An appropriate quantity of each plant extract (100%) was separately mixed thoroughly with autoclaved and cooled (40^0C) PDA medium in conical flasks (250 ml cap.) to obtained desired concentrations (10, 15, and 20 per cent). The PDA medium amended separately with plant extracts 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 concentration were replicate thrice. Upon solidification of PDA, all the treatment and control plates were aseptically inoculated by placing in the center 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 ± 2^0C temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus. All these plant extract were evaluated @ 10, 15, and 20 % observation on radial mycelial growth of the test pathogen was recorded at 24 hrs. interval and continued till growth of test pathogen in untreated control plate is fully covered. Per cent inhibition of test pathogen was also calculated by applying the formula given by (Vincent 1927) [10].

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

Where,
C= growth of the test fungus in (mm) untreated control plates
T=growth of the test pathogen in (mm) treated plates

Results and discussion

In-vitro* evaluation of plant extracts against *M. phaseolina

Leaf and clove extract of eleven botanicals were evaluated *in-vitro* (each @10, 15 and 20 %) against *M. phaseolina* and the results obtained on its mycelial growth and inhibitions are presented in the (Table.1).

Results (Table.1) revealed that all the eleven botanicals extracts evaluated were found fungistatic against *M. phaseolina* and which significantly reduced mycelial growth and increased mycelial inhibition of the test pathogen over untreated control. The mycelial growth was found to be decreased and its inhibition was increased with increase in concentrations of the botanicals tested.

Radial mycelial growth

At 10 per cent concentration, radial mycelial growth of the test pathogen was recorded from 20.27 mm (garlic) to 88.86 mm (euphorbia). However, significantly least mycelial growth was recorded with garlic (20.27 mm) and neem (33.87 mm). This was followed by the botanicals *viz*., eucalyptus (37.70 mm) and lantana (39.46 mm) and both were at par, this was followed by ginger (69.48 mm), tulsi (74.79 mm) and bougainvillea (76.10 mm) and both were at par. While botanicals viz., nerium (83.17 mm), mentha (85.66 mm) and par with each other. The plant extract of mulberry exhibited (86.58 mm) and euphorbia (88.86 mm) showed highest radial mycelial growth as against 90.00 mm in untreated control.

All the plant extract tested at (@ 15 % exhibited similar trend of mycelial growth as that of 10 %. At 15 per cent concentration, radial mycelial growth of the test pathogen was ranged from 6.77 mm (garlic) to 72.10 mm (euphorbia). However the significantly least mycelial growth was recorded with garlic (6.77 mm) over the rest of the plant extract tested. This was followed by the botanicals *viz*., neem (17.96 mm), lantana (18.55 mm) and eucalyptus (19.47 mm). These three plant extract were found at par with each other followed by ginger (30.05 mm) and tulsi (51.06 mm). This was followed by nerium (57.18 mm), bougainvillea (57.32 mm) and mulberry (57.66 mm) were found at par to each other. The plant extract of mentha (71.04 mm) and euphorbia (72.10 mm) showed the highest mycelial growth as against 90.00 mm in untreated control.

At 20 per cent concentration, all the botanicals exhibited somewhat similar trend of mycelial growth as that of observed at 10 and 15 per cent concentrations of the test botanicals. At 20 per cent concentration, radial mycelial growth of the pathogen was ranged from 0.00 mm (garlic) to 60.04 mm (euphorbia). However, significantly least mycelial growth was recorded with garlic (0.00 mm). This was followed by the botanicals *viz*., neem (11.90 mm), lantana (13.10 mm) and eucalyptus (13.78 mm). These three botanicals were at par and superior over rest of plant extracts tested, followed by botanical ginger (26.96 mm). While other botanicals tulsi (40.74 mm), bougainvillea (42.60 mm) found at par with each other, followed by nerium (45.66 mm) and mulberry (47.46 mm) were at par. Whereas the plants extract mentha (57.37 mm) and euphorbia (60.04 mm) showed the highest mycelial growth as against 90.00 mm in untreated control.

Average radial mycelial growth recorded with all the test botanicals was ranged from 9.01 mm (garlic) to 73.66 mm (euphorbia). However, significantly least mycelial growth recorded with garlic (9.01 mm). This was followed by neem (21.24 mm), lantana (23.70 mm) and eucalyptus (23.65 mm), ginger (42.09 mm), tulsi (55.53 mm) and bougainvillea (58.67 mm), nerium (62.00 mm), (63.90 mm) and mentha (71.35 mm) in order of merit. The maximum mycelial growth was observed with euphorbia (73.66 mm) as against 90.00 mm in untreated control.

Mycelial inhibition

Results obtained on mycelial growth inhibition of the test pathogen with the botanicals tested at various concentrations are presented in the table and depicted in the Table.1.

Results (Table-1) indicated that all the botanicals tested (@ 10, 15 and 20 % each), significantly inhibited mycelial growth of the test pathogen over untreated control (0.00 %). Further, it was found that per cent mycelial growth inhibition of the test pathogen was increased with increased in concentrations of the botanicals tested.

At 10 per cent, mycelial growth inhibition was ranged from 1.26 (euphorbia) to 77.46 (garlic) per cent. However, significantly maximum mycelial growth inhibition was recorded with the botanicals garlic (77.46) was followed by neem (62.36) per cent. This was followed by the botanicals *viz*., eucalyptus (58.10 %) and lantana (56.15 %) were found statistically at par and was followed by ginger (22.79 %), tulsi (16.89 %), bougainvillea (15.43 %), nerium (7.58 %), mentha (4.88 %) and mulberry (3.79 %). The minimum growth inhibition was noticed with euphorbia (1.26 %) as against 0.00 per cent in control.

At 15 per cent, also similar trend in mycelial growth inhibition was observed it ranged from 19.88 (euphorbia) to 92.46 (garlic) per cent. However, significantly highest per cent growth inhibition was recorded with the botanical garlic (92.46) per cent. This was followed by neem (80.04), eucalyptus (78.36) and lantana (79.37) per cent, were found at par to each other. This was followed by ginger (66.60 %), tulsi (43.26 %). While nerium (36.46 %), bougainvillea (36.31 %) and mulberry (35.92 %). were found statistically at par and was followed by mentha (21.06 %) and euphorbia (19.88 %) were at par with each other with least effective in growth inhibition among all the tested plant extracts.

At 20 per cent, similar trend of mycelial inhibition as that of 10 and 15 per cent recorded from 33.28 (euphorbia) to 100.00 (garlic) per cent. However significantly highest mycelial growth inhibition was recorded with the botanicals garlic (100.00 %). This was followed by the botanicals *viz*., neem (86.77 %). Whereas botanical lantana (85.44 %) and eucalyptus (84.35 %) were found statistically at par. Followed by ginger (70.26 %), tulsi (54.72 %), and bougainvillea (52.65 %), nerium (49.25 %) and mulberry (47.26 %) were found at par with each other. The least effective botanicals noticed in inhibition of where mentha (36.24 %) and euphorbia (33.28 %) among all botanicals tested.

Mean per cent mycelial inhibition recorded with all the test botanicals was ranged from 18.14 (euphorbia) to 89.97 (garlic) per cent. However, significantly highest mycelial growth inhibition was recorded with the botanicals *viz*., garlic (89.97 %). This was followed by neem (76.39 %), eucalyptus (74.83 %) and lantana (73.65 %), garlic (53.21 %), tulsi (38.29 %), bougainvillea (34.79 %) and nerium (31.09 %)

were found at par and was followed by mulberry (28.99 %), mentha (20.72 %) and euphorbia (18.14 %) in order of merit. The least average inhibition was recorded with botanical euphorbia (18.14 %) over untreated control (0.00%).

The results of present investigation resembling the findings of earlier workers, Tandel *et al*. (2010) [6], Murugapriya *et al*. (2011) [7] reported that the *Allium spp*. (20%) completely inhibited the growth of fungus in agar well diffusion method. Savaliya *et al*. (2015) [5] reported that the phytoextracts 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 %) mycelial growth and scanty sclerotial formation.

Table 1: *In-vitro* bio-efficacy of plant extracts against *Macrophomina phaseolina*.

Treatment	Colony dia. (mm) at conc.*			Av.	% Inhibition at conc.*			Av. Inhibition
	10%	15%	20%	(mm)	10%	15%	20%	(%)
Ginger (*Z. officinale*)	69.48	30.05	26.96	42.09	22.79(29.49)	66.60(54.68)	70.26(56.93)	53.21(46.72)
Eucalyptus (*E. globulus*)	37.70	19.47	13.78	23.65	58.10(49.34)	78.36(65.29)	84.35(66.68)	73.60(60.43)
Nerium (*N. olender*)	83.17	57.18	45.66	62.00	07.58(14.97)	36.46(37.12)	49.25(44.55)	31.09(32.56)
Garlic(*A. sativum*)	20.27	06.77	00.00	9.01	77.46(60.96)	92.46(74.09)	100(90.00)	89.97(75.08)
Euphorbia (*E. antiquorum*)	88.86	72.10	60.04	73.66	01.26(3.88)	19.88(26.46)	33.28(35.21)	18.14(21.85)
Neem (*A. indica*)	33.87	17.96	11.90	21.24	62.36(48.51)	80.04(63.45)	86.77(68.69)	76.39(60.21)
Mulberry (*Morus spp*.)	86.58	57.66	47.46	63.9	03.79(11.72)	35.92(36.79)	47.26(43.41)	28.99(30.49)
Lantana camera (*L. aculeate*)	39.46	18.55	13.10	23.70	56.15(52.14)	79.37(62.97)	85.44(67.57)	73.65(60.89)
Tulsi (*O. tenuiflorum*)	74.79	51.06	40.74	55.53	16.89(22.95)	43.26(41.11)	54.72(47.69)	38.29(37.70)
Bougainvillea (*B. spectabilis*)	76.10	57.32	42.60	58.67	15.43(24.23)	36.31(37.03)	52.65(46.50)	34.79(35.56)
Mentha (*M. longifolia*)	85.66	72.10	57.37	71.71	04.88(12.67)	19.88(27.30)	36.24(36.92)	20.33(25.41)
Control	90.00	90.00	90.00	90.00	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
S.E. (m) ±	01.01	00.91	00.82	-	00.92	01.21	00.57	-
C.D. @ 1 %	02.98	02.68	02.41	-	02.70	03.56	01.69	-

*Mean of three replication, Dia. Diameter, Av.- Average, Conc- Concentration.
Figures in parenthesis are arc sine transformed value.

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