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EVALUATION OF FUNGICIDAL POTENTIAL OF TWENTY MEDICINAL PLANTS AGAINST *MACROPHOMINA PHASEOLINA* CAUSING CHARCOAL ROT OF GREEN GRAM

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ABSTRACT

The possible use of plants with antifungal activities for the control of *Macrophomina phaseolina* is an area that has not been fully exploited. The objective of the present study was, therefore, to evaluate twenty antagonistic plants found in the country for their antifungal activity against *M. phaseolina*, as no information is available on the antifungal activities of these plants against the fungus. All the test plants when used as seed treatment, significantly enhanced seedling emergence (P > 0.001). Of all the test plants, *Azadirachta indica* showed the maximum increase in emergence of black gram (58.33%) over control followed by *Nigella sativa* (57.50%) and *Carum copticum* (51.67%). On the other hand, *Nicotiana tabacum*, *Foeniculum vulgare* and *Lawsonia inermis* appeared to be the least effective in reducing the damage of the pathogen showing 30, 30.83 and 32.5% increases in emergence. The maximum individual increase in seedling emergence of black gram (73.33%) was attained with 10% concentration of *A. indica*. The minimum of 20% increase in plant emergence was obtained with *F. vulgare* at 1% concentration. Other plants showed intermediate increases in seedling emergences. Significant effects (P > 0.001) of concentrations were also observed on seedling emergence. Maximum seedling emergence was recorded at 10% concentration of decoctions of test antagonistic plants. As the concentration of medicinal plants decreased, the effects on seedling emergence also decreased. The effect of concentrations was found to be directly proportional to seedling emergence.

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INTRODUCTION

Black gram (*Vigna mungo* L.) Hepper is one of the commonly grown pulse crops in many South Asian countries of the world. It is known with different names in different regions in different languages as mash bean, urad bean, mash kalai, uzhunnu parippu, ulundu paruppu, minapa pappu, Uddu (in Kannada) or black matpe. Its genus was changed to *Vigna* from *Phaseolus* similar to its relative, the green gram. The commodity sold as black lentil is usually the whole urad bean, whereas the split bean (the interior being white) is called white lentil. It should not be confused with the much smaller true black lentil (*Lens culinaris*).

Black gram has originated in South Asia, where it has been

cultivated since ancient times. It is one of the most highly prized pulses of South Asian countries where it is widely used in different cuisines. In this region, it is known as mash daal and is a popular side dish that goes with curry and rice as a platter. Black gram has also been introduced to other tropical areas such as the Caribbean, Fiji, Mauritius, Myanmar and Africa. In Pakistan, black gram is one of the important pulses grown mostly in Kharif season.

Black gram is very nutritious as it contains high levels of protein (25 g/100 g), potassium (983 mg/100 g), calcium (138 mg/100 g), iron (7.57 mg/100 g), niacin (1.447 mg/100 g), Thiamine (0.273 mg/100 g), and riboflavin (0.254 mg/100 g). Black gram complements the essential amino acids provided in most cereals and plays an important role in the diets of the people of South Asian countries (Anonymous, 2006). Black gram is also very high in folate (628 µg/100 g raw, 216 µg/100 g cooked) (Brink and Belay, 2006). Its seed contains about 24% protein, 60% carbohydrates and 1.3% fats. Dry fodder (pod husk) is nutritive for milch animals. It is also referred to as cover crop. Being leguminous crop, it fixes nitrogen from the atmosphere to an extent of 70-90 kg/ha and thus ameliorates soil fertility. In Pakistan, ehe crop is grown under a wide range of agro-ecological zones in the country. The average yield in Pakistan is very low as compared to its potential yield obtained in many other countries.

The lucrative production of black gram in Pakistan is threatened by numerous biotic and abiotic limitations. Among biotic factors, diseases negatively impact yield of black gram. The diseases incur yield losses to pulse crops to the tune of 44 percent, depending upon the crop variety (Bashir and Malik, 1988). Black and green grams are attacked by about 26 diseases in the world (Charles, 1978). Among several diseases of economic importance, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid, is of primary significance in reducing crop yield especially in arid regions of the world (Hoes, 1985).

The pathogen causes seedling blight; stem rot and pod rot on more than 500 plant hosts (Sinclair, 1982). The pathogen has been reported to invade over 67 hosts from Pakistan (Hyder et al., 2018; Mirza and Qureshi, 1982; Shahzad and Ghaffar, 1986; Shahzad et al., 1988). Tropical crop plants are seriously affected by this pathogen (Malaguti, 1990). *M. phaseolina* is classified as a Deuteromycete which shows two asexual sub-phases, a mycelial phase named as *Rhizoctonia bataticola* (Taub) Butler (1925) and the other a pycnidial phase called *M. phaseolina* (Dhingra and Sinclair, 1978). The fungus is classified in the Botryosphaeriaceae according to recent phylogenetic data (Crous et al., 2006).

The fungus produces dark brown lesions on the epicotyls and hypocotyls of seedlings ensuing seedling death because of obstruction of xylem vessels and wilting. The pathogen produces red to brown lesions and then dark mycelia and black microsclerotia on roots and stems of adult plants. The stems manifest longitudinal dark lesions resulting in defoliation and wilting of plants (Abawi and Pastor-Corrales, 1990). The asexual structures produced by the fungus are pycnidia and microsclerotia. The black, 0.1–1 mm sized microsclerotia are formed in soil, infected seeds or host tissues and constitute the primary inoculum source of the pathogen (Abawi and Pastor-Corrales, 1990; Bouhot, 1968; Dhingra and Sinclair, 1978). They can survive up to 15 years depending on environmental conditions, and whether or not the sclerotia are associated with host residues (Cook et al., 1973; Iqbal and Mukhtar, 2014a; Papavizas, 1977; Short et al., 1980). Secondary dispersal is by Pycnidiospores produced on infected stem and leaf tissues (Ali and Dennis, 1992).

M. phaseolina is a heat tolerant pathogen since sclerotia could withstand a temperature range of 60–65°C (Bega and Smith, 1962; Mihail and Alcorn, 1984). The evidence suggests that it is primarily a root inhabiting fungus and produces tuber or cushion shaped 1-8 mm diameter black sclerotia. These sclerotia serve as a primary means of survival (Kaiser and Horner, 1980; Smith, 1969). Charcoal rot causes 60% losses to the black and green grams (Deshkar et al., 1974). The pathogen is managed by different methods which have specific limitations (Iqbal and Mukhtar, 2020a, 2020b; Iqbal et al., 2010; Iqbal et al., 2014b; Shahjahan et al., 2018).

Generally, the control of fungal diseases including charcoal rot mainly relies on synthetic fungicides (Wang et al., 2014a; Wang et al., 2014b) which not only increase agricultural costs but also are associated with potential hazards to the environment and human health. Use of antagonistic plants can be a possible substitute to deleterious chemicals (De Corato et al., 2014; Koch et al., 2013; Paredes et al., 2013). Numerous medicinal and antagonistic plants have been reported to possess antimicrobial, nematicidal and antifungal activities against a multitude of plant pathogens (Bashir et al., 2020; Liu et al., 2013a, 2013b; Švecová et al., 2013; Trigui et al., 2013a; Trigui et al., 2013b; Vogt et al., 2013). The possible use of

plants with antifungal activities for the control of *M. phaseolina* is an area that has not been fully exploited. The objective of the present study was, therefore, to evaluate twenty antagonistic plants found in the country for their antifungal activities against *M. phaseolina*, as no information is available on the antifungal activities of these plants against the fungus.

MATERIALS AND METHODS

The pathogen *M. Phaseolina*

The fungus used in the study was isolated from stem bark tissues of black gram bearing fungal sclerotia and characteristic charcoal rot symptoms on Chloroneb Mercury Rose Bengal Agar (CMRA) medium (Meyer, 1973) and identified on the basis of standard key (Barnett and Hunter, 1972). For pot assay, the fungus was multiplied on sorghum seeds. For this purpose, sorghum seeds were water soaked overnight, air dried under room temperature and placed in conical flasks. The mouth of each flask was plugged with cotton wool, wrapped in aluminum foil and autoclaved at 15 psi (121°C) for 20 minutes. After cooling, the seeds in flasks were inoculated with 4 mm mycelial plugs from a 7-day old culture of *M. phaseolina* and incubated at 25±1°C for 15 days. The flasks were shaken at alternate days for uniform colonization of the grains. The inoculum thus produced was used in pot assay.

Plant materials

Leaves or seeds or flowers of twenty antagonistic plants belonging to 13 families used in the studies were collected from different locations of Pakistan. Leaves or seeds or flowers of test plants were surface sterilized for 2 minutes in 70% ethanol. Samples were then rinsed twice in sterilized distilled water, dried under room temperature for 21 days and ground separately and used for evaluation against *M. phaseolina*. For preparation of decoctions, 10, 25, 50 and 100 g dried material of each test plant was boiled in 1 liter of water for 5 minutes to get concentrations of 1, 2.5, 5 and 10 percent. The decoctions were squeezed through double cheese cloth sheets and filtered through Whatman No. 1 filter paper. The decoctions were further passed through Millipore filter of 0.2 µm pore size to avoid the bacterial contamination and stored at 4°C until use.

Pot assay for assessment of antifungal activities of antagonistic plants

Seeds of black gram cv. Mash-98 were surface sterilized for 10 minutes in 5% commercial sodium hypochlorite solution, washed in sterilized distilled water and air dried. The seeds were then soaked in different concentrations of decoctions of test plants for 2 hours and air dried for 3 hours in a laminar flow chamber. Seeds treated with sterilized distilled water served as control. The treated seeds were planted in soils amended with sorghum seeds colonized with *M. phaseolina* @ 2g/kg of soil. Ten seeds were planted in each pot. Each treatment was replicated five times. The pots were kept under field conditions in a completely randomized design in an iron cage. Data on seedling emergence was recorded after 20 days and percent increase over control was calculated.

Statistical analysis

Percent increases in seedling emergence were calculated over controls prior to statistical analysis. All the data were subjected to Analysis of Variance (ANOVA) using GenStat package 2009, (12th edition) version 12.1.0.3278 (www.vsni.co.uk). The differences among means were compared by Fisher's protected least significant difference test at ($P \leq 0.05$).

RESULTS AND DISCUSSION

All the test plants when used as seed treatment, significantly enhanced seedling emergence ($P > 0.001$). Of all the test plants, *Azadirachta indica* showed the maximum increase in emergence of black gram (58.33%) over control followed by *Nigella sativa* (57.50%) and *Carum copticum* (51.67%). On the other hand, *Nicotiana tabacum*, *Foeniculum vulgare* and *Lawsonia inermis* appeared to be the least effective in reducing the damage of the pathogen showing 30, 30.83 and 32.5% increases in emergences (Figure 1). The maximum individual increase in seedling emergence of black gram (73.33%) was attained with 10% concentration of *A. indica*. The minimum of 20% increase in plant emergence was obtained with *F. vulgare* at 1% concentration. Other plants showed intermediate increases in seedling emergences. The individual percent increases of seedling emergence at four concentrations of the test plants are given in Table 1. Significant effects ($P > 0.001$) of concentrations were also observed on seedling emergence. Maximum seedling emergence was recorded at 10% concentration of decoctions of test antagonistic plants. As the concentration of medicinal plants decreased, the effects on seedling emergence also decreased. The effect of concentrations was found to be directly proportional to seedling emergence and these relationships have been shown by regression equations (Table 2).

Table 1: Effect of antagonistic plants on plant survival of black gram.

Plant Extract	% increase over control				
	1%	2.5%	5%	10%	Average
Azadirachta indica	46.67±0.33	50.00±0.00	63.33±0.33	73.33±0.33	58.33
Cannabis sativa	33.33±0.33	36.67±0.33	43.33±0.33	50.00±0.58	40.83
Olea europaea	40.00±0.58	43.33±0.33	50.00±0.58	56.67±0.33	47.50
Mentha Piperita	23.33±0.33	26.67±0.33	36.67±0.33	46.67±0.33	33.33
Melia azadirachta	26.67±0.33	33.33±0.33	40.00±0.58	43.33±0.33	35.83
Nerium indicum	23.33±0.33	33.33±0.33	43.33±0.33	46.67±0.33	36.67
Carum Lopticum	36.67±0.33	40.00±0.58	60.00±0.58	70.00±0.58	51.67
Calotropis procera	23.33±0.33	26.67±0.33	36.67±0.33	46.67±0.33	33.33
Ocimum americanum	33.33±0.33	36.67±0.33	53.33±0.33	56.67±0.33	45.00
Foeniculum vulgare	20.00±0.00	26.67±0.33	36.67±0.33	40.00±0.58	30.83
Dalbergia sissoo	26.67±0.33	33.33±0.33	36.67±0.33	43.33±0.33	35.00
Trigonella foenumgraecum	23.33±0.33	26.67±0.33	43.33±0.33	50.00±0.58	35.83
Cassia fistula	26.67±0.33	30.00±0.58	40.00±0.58	46.67±0.33	35.83
Anethum graveolens	23.33±0.33	30.00±0.00	40.00±0.58	46.67±0.33	35.00
Nigella sativa	43.33±0.33	50.00±0.00	60.00±0.58	76.67±0.33	57.50
Matricharria chammomilla	26.67±0.33	23.33±0.33	33.33±0.33	50.00±0.00	33.33
Cassia angustifolia	36.67±0.33	40.00±0.58	50.00±0.58	60.00±0.58	46.67
Parthenium hysterophorus	30.00±0.58	33.33±0.33	43.33±0.33	50.00±0.58	39.17
Lawsonia inermis	23.33±0.33	23.33±0.33	40.00±0.00	43.33±0.33	32.50
Nicotiana tabacum	23.33±0.33	23.33±0.33	30.00±0.58	43.33±0.33	30.00

LSD Value= 5.96

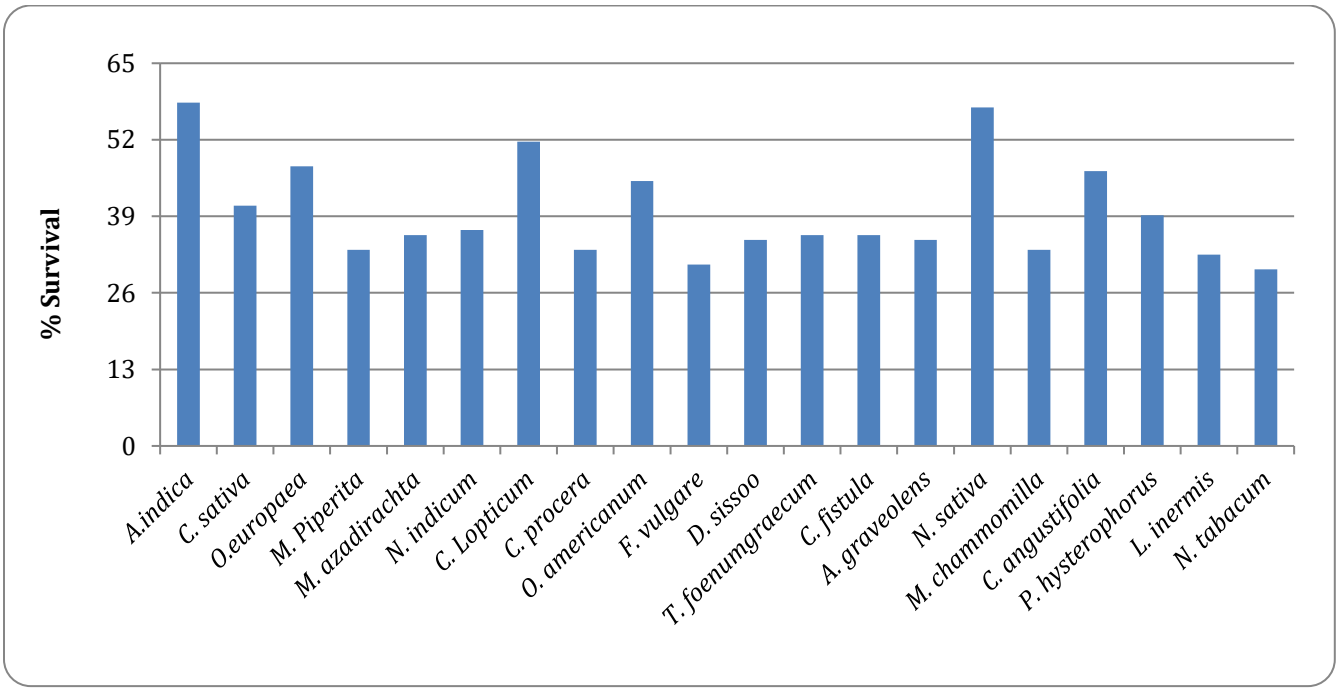

Figure 1: Effect of medicinal plants on average survival percentage of black gram.

Table 2: Relationships between concentrations of plant extracts and black gram survival.

Plant Extract	Regression equation	R^2
Azadirachta indica	y= 37.324x + 35.005	0.9561
Cannabis sativa	y= 22.668x + 26.665	0.9797
Olea europaea	y= 22.672x + 33.33	0.9796
Mentha Piperita	y= 32.008x + 13.33	0.9601
Melia azadirachta	y= 22.66x + 21.67	0.9796
Nerium indicum	y= 32.008x + 16.66	0.9601
Carum Lopticum	y= 44.008x + 29.995	0.9595
Calotropis procera	y= 32.008x + 13.33	0.9601
Ocimum americanum	y= 34.672x + 23.33	0.9137
Foeniculum vulgare	y= 28.00x + 13.335	0.9692
Dalbergia sissoo	y= 21.328x + 21.67	0.9847
Trigonella foenumgraecum	y= 38.668x + 11.665	0.9398
Cassia fistula	y= 28.00x + 18.335	0.9692
Anethum graveolens	y= 32.008x + 14.995	0.9931
Nigella sativa	y= 47.996x + 21.67	0.9391
Matricharria chammomilla	y= 31.996x + 13.335	0.7577
Cassia angustifolia	y= 31.996x + 26.67	0.96
Parthenium hysterophorus	y= 28.00x + 21.665	0.9692
Lawsonia inermis	y= 30.668x + 13.33	0.8601
Nicotiana tabacum	y= 26.668x + 13.33	0.8334

The enhanced seedling emergence is attributed to the deposition of chemical compounds around seed surface which prevented penetration of the pathogen. These chemicals might have caused lyses of sclerotia and triggered plant growth hormones which resulted in increased emergence and decreased disease incidence.

Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in them may either act on the pathogen directly (Baraka et al., 2006; Liu et al., 2013b; Olabiyi et al., 2020), or induce systemic resistance in host plants resulting in reduction of disease development (Chen et al., 2014; Hussain et al., 2011; Kagale et al., 2004; Kayani et al., 2012; Mukhtar et al., 2013; Narwal et al., 2001; Paul and Sharma, 2002).

The increase in seedling emergence of black gram by antagonistic plants might also be due to the suppression of the fungal growth in the soil. The reduction in fungal growth by antagonistic plants is attributed to the presence of antifungal compounds in plant extracts or decoctions including glycoside, steroid, saponin, medicagenic acid, 3-O-B-D glycopyranoside, (3-GleMA) ajone, tannins, sesquiterpenes, lactones, terpenoids and phobol esters (Johnson and Nunley, 2000; Tiwari and Singh, 2004). Various phenolic acids, namely protocatechuic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, caffeic and syringic acids have also been isolated from a number of plants which have been reported to possess antifungal activities against *M. phaseolina* and other pathogenic fungi (Batish et al., 2007; El-Khatib et al., 2004; Trigui et al., 2013b). Variations in antifungal activities have also been observed among the twenty antagonistic plants. The differences in the toxicity of the plants might be due to the differences in the chemical composition of the plants and concentration of toxic components indicating more antifungal potential at higher concentrations than lower ones.

CONCLUSIONS

The present study demonstrates that of the twenty antagonistic plants *Azadirachta indica, Nigella sativa* and *Carum copticum* possessed potent antifungal activities with potential practical applications in the treatment of charcoal rot disease of black gram. These antifungal plants outstrip synthetic fungicides on account of their

ready availability; cost effectiveness, non-phytotoxicity, biodegradability and being ecofriendly. As these plants and plant materials are commonly found in the country and can therefore, be effectively utilized as a substitute to chemical fungicides particularly by small-scale farmers to protect crops against fungal attack in the organic production of crops.

AUTHOR'S CONTRIBUTION

Both the author designed the study, performed the experiments, collected and analyzed the data, wrote the manuscript and proofread the paper.

CONFLICT OF INTEREST

The author declares no conflict of interest

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