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RESPONSE OF DIFFERENT MUNG BEAN VARIETIES AGAINST MACROPHOMINA PHASEOLINA (TASSI) GOID AND IN-VITRO STUDIES OF PLANT EXTRACTS AGAINST PATHOGEN

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A B S T R A C T

Twenty seven varieties/lines of mung bean were tested through artificial inoculation against charcoal rot disease in the field area of Department of Plant Pathology, University of Agriculture, Faisalabad. No variety/line was found immune to charcoal rot disease. Azri 2006, NM 2006 and AUM 9 were found resistant in first disease screening nursery and second disease screening nursery. Azri 2006 was highly resistant in third disease screening nursery. The varieties/lines 8010, AUM 38 and 7009 were found moderately susceptible to susceptible in all the disease screening nurseries. The rest of the varieties show varied results in all the nurseries. Ten plants, namely sufeda (*Eucalyptus camaldulensis*), neem (*Azadirachta indica*), onion (*Allium cepa*), garlic (*Allium sativum L.*), shesham (*Dalbergia sissoo*), akk (*Calotropis gigantea*), chillies (*Capsicum annum*), gardinia (*Gardenia florida*), bakayn (*Melia azedarach*), ginger (*Zingiber officinale*) were selected and their extracts against *Macrophomina phaseolina*. Three concentrations of all the plant extracts were made viz. S, S/2, S/3. Inhibition zone technique was used to study their effect.

Keywords: Mungbean varieties, *Macrophomina phaseolina*, plant extracts, in-vitro.

INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek) belongs to the family *Fabaceae*. It is an ancient, cheap and conventional pulse crop in the world. The crop is cultivated in Asia including Pakistan, India, Burma, Thailand and Philippine (Duke, 1981). The yield of mungbean is affected by several biotic and a-biotic factors. Among the biotic factors, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid, is of prime importance in reducing crop yield. It is one of the most important diseases of field crops in arid regions of the world (Hoes, 1985). This fungal pathogen causes seedling blight, stem rot and pod rot and has more than 500 plant species as a host range (Sinclair, 1982), whereas 67 host species of this pathogen have been reported from Pakistan (Mirza and Qureshi,1982; Shehzad *et al.,* 1988). Varietal screening against this disease in sunflower (Mirza *et al.,* 1982; Hafeez and Ahmad, 2001), in urdbean (Iqbal *et al.,* 2003) and sesame (Mirza *et al.,* 1986) in Pakistan has been reported but such information is lacking in case of mungbean (Iqbal *et al.,* 2003).Green plants represent an unexplored potential reservoir of effective chemo-therapeutants can provide valuable sources of natural pesticides (Balandrin *et al.,* 1985; Mahajan and Das, 2003). There is an urgent need to find the alternative approaches for the management of plant pathogenic organisms considering the adverse effect of synthetic pesticides (Hostmann and Wolfender, 1997). An effective substitute for chemicals is the bio-pesticides (Verma and Dubey, 1999; Kapoor, 2001). Some plant by products have been found that have antimicrobial effect on several pathogenic organisms (Dorman and Deans, 2000; Parameswari and Latha, 2001; Rath *et al.,* 2001; Britto and Senthilkumar, 2001; Byalka et al., 2004; Shimpi and Bendre, 2005; Kilani, 2006). Neem leaf extract, marigold leaf extract and garlic bulb extract at

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5 % as seed treatments significantly reduced the charcoal rot incidence and increased yield (Sinha and Sinha, 2004).

MATERIALS AND METHODS

The pathogen was isolated from the diseased plants of mungbean and identified under light microscope and grown on fresh agar medium in Petri plates. The inoculum was mass cultured on sorghum seeds as the sorghum seeds proved to be the best for the growth of the pathogen (Sahi, 1989; Haq. 1993). Polypropylene bags of 30 x 24 cm size, 2 cm diameter pieces of plastic pipes, cotton plugs and sorghum seeds were used in this process.

The seeds of sorghum were soaked overnight in tap water and then boiled for 2-3 min, boiled seeds and spread over the piece of the paper towel to absorb the excessive moisture of the seeds. About 300 g of the seeds were filled in each polypropylene bag and the open end was passed through the piece of the plastic pipe. A cotton plug was put into the mouth of the bag passing through the ring. The bags were autoclaved at 15-20 psi pressure for 20 min. The autoclaved bags were inoculated with the 6mm diameter disc of the PDA containing the inoculum of the fungus. Streptomycin 20 mg was added in each bag to prevent the growth of the bacterial contaminants. After plugging with cotton plugs the bags were incubated at a temperature 30 ^{o}C for 15 days for the complete development of the sclerotia of the fungus.Twenty seven mungbean cultivars/lines were taken from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad and Pulses Research Institute, Ayub Agriculture Research Institute, Faisalabad and sown in field area of Department of Plant Pathology, UAF. The pathogen was placed into the soil ten days before the sowing by removing 1-2 inches upper soil layer and again covered after the placement of pathogen, at the time of sowing and after the germination of the seedlings have been achieved. Each entry was replicated three times. The disease screening nurseries were developed for the comparison of the three different inoculation time viz. inoculation Ten days before sowing, inoculation at the time of sowing and inoculation after seedling emergence. The experiment was terminated after 90 days. Incidence of the disease was calculated by using the formula

Disease Incidence (%)

$$= \frac{\text{Number infected Plants}}{\text{Total Number of the Plants}} \times 100$$

Saha, (2001).

The verities were evaluated on the basis of percent plant infection basis and arranged according to the following disease rating scale.

Disease Incidence	Level of Resistance/Susceptibility
0	Immune (I)
1-10	Highly Resistant (HR)
11-30	Resistant (R)
31-40	Moderately Resistant (MR)
41-50	Moderately Susceptible (MS)
51-70	Susceptible (S)
71-100	Highly Susceptible (HS)

Aziz-ur-Reman (1992)

Fresh plant parts (Leaves/Branches/bulbs/rhizomes) were washed and placed on paper for drying. Sterilized water (25 ml) was added to the 75 g of the fresh plant parts and macerated with the help of blender. The macerate was passed through the four layers of the muslin cloth and filtered through the Whattman's filter paper No. 4. These extracts obtained were served as standard (S) arbitrary (Ilyas *et al.,* 1997) and then stored at -20 ^{o}C for cold sterilization for the future uses. Wells of 1 cm diameter were made at the two corners of each Petri plate by using sterilized cork borer. Plant extract (1 ml) of different concentrations i.e Standard S, S/2, S/3 was added into the each of plate on one side and pathogen on the other side. The Petri dish with sterilized water was served as control. Plates were placed in the refrigerator at 4 ^{o}C before incubation at 30 ^{o}C. Inhibition zone were measured after 24, 48 and 72 h and data taken was subjected to the analysis of variance and LSD-test to compare the treatments.

RESULTS AND DISCUSSION

All the varieties/lines showed varied response to the disease development. No variety was found immune in all the three disease screening nurseries. However, in first disease screening nursery, three varieties/lines viz. Azri 2006, NM 2006 and AUM 9 were found to be resistant. Twelve varieties were moderately resistant and eleven varieties/lines were found to be moderately susceptible and variety 8010 was susceptible. In second disease screening nursery i.e inoculation at the time of

sowing, seven varieties/lines were found to be moderately resistant. Single variety 8010 which was susceptible in first nursery becomes moderately susceptible in this nursery. All other varieties were found to be resistant. In third nursery i.e inoculation after seedling emergence, results indicate that only one variety i.e Azri 2006 is found to be highly resistant. Moreover AUM 19 found to be moderately susceptible and five varieties/lines were moderately resistant. Remaining all other varieties showed resistant behaviour. Charcoal rot incidence was greatly influenced by the cultivation of the susceptible lines and a coincidence of the environmental conditions to the disease development. Maximum soil temperature, minimum soil temperature and relative humidity of soil have significant interaction with the disease development.Fourteen cultivars of mungbean were found resistant against *M. phaseolina* (Zote *et al*., 1983). Various varieties of mungbean were tested against charcoal rot at Coimbatore, India but no resistant cultivar was resistant (Grover and Sakhuja 1981). However three varieties or cultivars viz. LM 28, MS 93 and MS 85 have been found resistant against charcoal rot disease in mungbean by Grewal (1978). Twenty-nine germplasm accessions were screened by using paper towel technique to identify sources of genetic resistance in mungbean by S. H. Khan and M. Shoaib (1997). They observed 2 genotypes highly resistant, 5 resistant and 6 moderately resistant. Three genotypes were found to be tolerant whereas rest of the accessions was susceptible or highly susceptible. They found the paper towel technique to be efficient for identification of resistance in mungbean for charcoal rot disease.

Table 1: Response of various verities against *Macrophomina phaseolina*

Sr. No.	Variety	Level of resistance/ susceptibility		
		Nursery 1	Nursery 2	Nursery 3
1	AUM 2004	MR	R	R
2	AUM 38	MR	MR	R
3	AUM 19	MR	R	MS
4	MG 1	MS	R	MR
5	AUM 56	MS	R	R
6	8010	S	MS	MR
7	56-2	MS	R	R
8	AUM 31	MS	R	MR
9	93013	MR	MR	R
10	NM 2006	R	R	R
11	7009	MR	R	R
12	7007	MS	MR	R
13	8011	MS	R	R
14	98001	MS	R	R
15	NM 98	MS	R	MR
16	256-1	MS	R	R
17	AUM 18	MR	MR	R
18	6601	MS	R	R
19	AUM 6173	MR	R	R
20	AUM 9	R	R	R
21	AUM 6375-9	MR	MR	R
22	AUM 28	MS	R	R
23	Azri 2006	R	R	HR
24	98002	MR	MR	R
25	97002	MR	R	R
26	97006	MR	R	R
27	8002	MR	MR	R

At standard concentration (S), the Leaf Extracts of sufeda (*eucalyptus camaldulensis)* was most effective in controlling the pathogen followed by neem (*Azadirachta indica*) and chillies (*Capsicum annuum*). The minimum inhibition zone has been observed in case of onion (*Allium cepa)* followed by sheesham (*Dalbergia sissoo*) and ginger (*Zingiber officinale*). Similar results have been found in case of other two concentrations viz. at S/2 the most effective results have been shown by sufeda (*Eucalyptus camaldulensis*) followed by neem (*Azadirachta indica*) and then chillies (*Capsicum annum*).

The graph shows that the minimum inhibition was observed in case of S/2 are shown by onion (*Allium cepa*) then sheesham (*Dalbergia sissoo*) and ginger (*Zingiber officinale*) as compared to control. Similar response was observed for maximum control at S/3 concentration viz. sufeda (*Eucalyptus camaldulensis*), neem (*Azadirachta indica*) and chillies (*Capsicum annuum*) and onion (*Allium cepa*), ginger (*Zingiber officinale*) and sheesham (*Dalbergia sissoo*) for minimum control.Bajwa and Iftikhar (2005) found that aqueous leaf extracts of *E. camaldulensis* exhibited the strong antifungal activity against *Alternaria alternate*, *Drechslera tetramera* and *D. hawaiiensis*. Jaiswal *et al*., (1984) founded that plant extracts have not been used commonly against different plant pathogens in the field, although some in-vitro studies have given excellent results in terms of effectiveness. Egawa *et al*., (1977) and Kumar *et al*., (1979) reported that leaf extracts of the various species of *Eucalyptus*, *Allium cepa*, *Allium sativum*, *Parenthium histopum* and *Phaseolus atropuporeus* that inhibited the mycelial and conidial germination of *Alternaria* spp. *Fusarium oxysporum*, *Drechlera rostrata* and *Cochliobolus migabeans*.

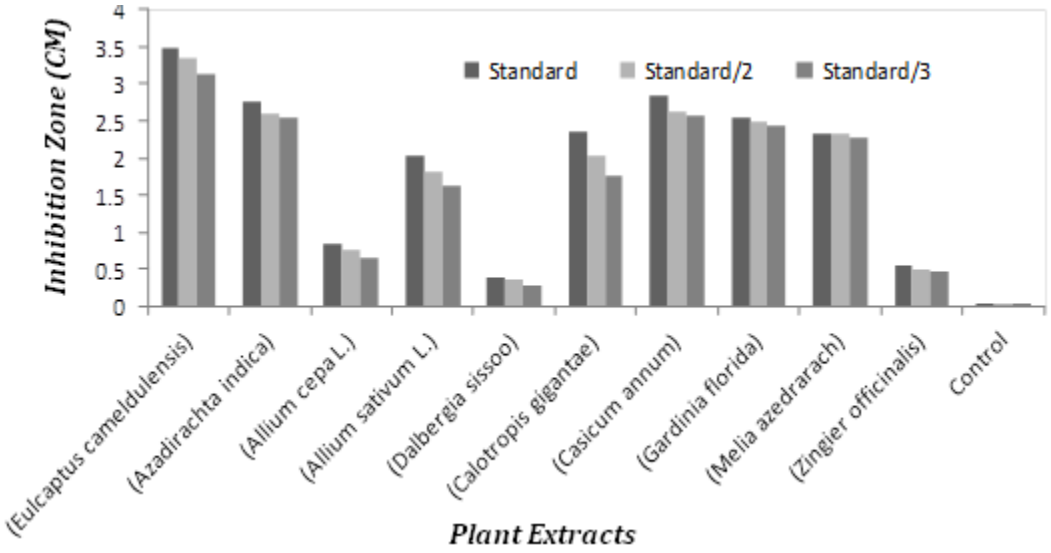

Figure: Effect of plant extracts on *Macrophomina phaseolina*

CONCLUSION

It can be concluded that all the varieties/lines showed the negative effect of the disease. No Variety/line has been found immune however, Azri 2006, NM 2006 and AUM 9 showed the better performance as compared to all other varieties/lines respectively. Azri 2006 proved best among all the tested varieties. sufeda (*Eucalyptus camaldulensis*) gave excellent results in controlling the pathogen by showing maximum inhibition zone followed by chillies (*Capsicum annum*) and then neem (*Azadirachta indica*) while the leaf extract of shesham (*Dalbergia sissoo*), bulb extract of ginger (*Zingiber officinale*) and onion (*Allium cepa*) were found to be very poor in controlling the pathogen as they showed the minimum values of inhibition zone at all the concentrations.

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