Research Paper :

Exploration of plant extracts and fungal antagonists against *Macrophomina phaseolina* (TASSI.) Goid causing leaf spot in green gram

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SUMMARY

Green gram [*Vigna radiata* (L.) Wilczek] is nutritionally the most important legume crop and excellent source of high quality protein (25%). The leaf blight of green gram incited by *Macrophomina phaseolina* (Tassi.) Goid. was observed at Agronomy farm, College of Agriculture, Dapoli. *In vitro* evaluation of plant extracts revealed that the bulb extracts of garlic (*Allium sativum*) was most effective in inhibiting the growth of the test fungus followed by ginger and onion. *Trichoderma harzianum* was the most promising antagonist against *Macrophomina phaseolina* among the different fungal antagonists tested, followed by *Trichoderma viride* and *Gliocladium virens*.

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Green gram [*Vigna radiata* (L.) Wilczek] is nutritionally the most important legume among pulse crops grown in India. It is supposed to be easily digestible and hence is preferred by patients. When green gram is allowed to sprout, ascorbic acid (vitamin C) is synthesized. The amount of riboflavin and thiamine are also increased. It is also used as a green manuring crop. In Konkan region of Maharashtra it is grown as a sole crop during late *Kharif, Rabi* and summer seasons. Among the various diseases of green gram, the leaf blight caused by *Macrophomina phaseolina* was noticed at the farm of Agronomy, College of Agriculture, Dapoli during the *Kharif* season in the year, 2008. The disease incidence was observed to be more than 45 per cent. So far, no studies have been undertaken on leaf blight affecting green gram in Konkan region of Maharashtra. Therefore, it was decided to conduct the present investigation.

MATERIALS AND METHODS

For studying antifungal effect of plant extracts against the test fungus, these were selected on the basis of their antifungal activity and below procedure was followed.

Crude extraction:

The fresh, thoroughly washed 100 g plant material was blended in 100 ml sterile water in a mixure. The crude material was then passed through double layered muslin cloth and centrifuged at 40000 rpm for 5 min. After centrifuging, the supernatant was collected and filtered through Whatman No. 1 filter paper. This extract was then passed through Sintered glass filter (to avoid the bacterial contamination) and preserved as stock (100%) solution aseptically in conical flask for further use.

All the plant extracts were tried at 10 per cent concentration against the test fungus using 'Poisoned food technique' on Potato dextrose agar as a basal medium. To obtain 10 per cent plant extract, 90 ml PDA was poured in 100 ml sterilized conical flask and 10 ml of plant extract was poured in each flask with the help of sterilized pipette and mixed thoroughly before solidification. 20ml of such medium was then poured in each sterilized Petri plate. Mycelial discs of 5 mm diameter were cut from seven day old culture of test fungus with the help of a sterilized cork borer and transferred aseptically to the centre of Petri

plate already poured with plant extract medium. Medium without plant extract served as control. Petri plates were incubated at room temperature (27 ± 1^{0}C) for 7 days. Three replications per treatment were maintained. The observations on colony diameter of the fungus and sclerotial formation were recorded after 7 days.

The per cent inhibition of growth was calculated by the following formula (Horsfall, 1956).

$$X = \frac{Y - Z}{Y} \times 100$$

where,
X = Per cent inhibition
Y = Growth of fungus in control (cm)
Z = Growth of fungus in treatment (cm)

Effect of fungal antagonists against *Macrophomina phaseolina:*

Effectiveness of *Trichoderma viride, T. harzianum, T. koningii* and *Gliocladium virens* was assayed against the pathogen. Seven days old culture of the bio-control agents and pathogen were used in this experiment. The inoculation of the test fungus and antagonist was done in two different ways. In first method the culture disc of pathogen (5 mm) was placed aseptically in the centre of plates and three similar discs of the antagonists were placed 4 cm away radially in the same plate and in second, the culture discs of the pathogen and antagonist measuring 5 mm in size were cut and placed aseptically in plate containing 20 ml PDA, keeping three culture discs of pathogen 4 cm away radially and one disc of an antagonist at the centre of the plate. Three replications for each treatment were maintained. The plates containing only the pathogen at the centre, served as control. The plates were incubated at room temperature (27 ± 1^{0}C) and radial growth of the test organism and the pathogen was measured after 5 days.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Effect of plant extracts on growth and sclerotial formation of *Macrophomina phaseolina*:

Data obtained on per cent inhibition of mycelial growth of *Macrophomina phaseolina* by plant extracts (Table 1) revealed that the bulb extract of garlic (*Allium sativum*) recorded the maximum inhibition (88.15%) of the test fungus and was superior to rest of the treatments. Ginger and onion differing significantly recorded 80.37 and 78.15 per cent inhibition of the test fungus, respectively. Sadaphuli (71.48%) was the next effective treatment in order of merit. Tulsi and Neem not being at par and recorded 40.00 and 37.04 per cent inhibition of the test fungus, respectively. Glyricidia recorded the least inhibition (24.81%) of the test fungus.

These results support the observations of Shinde and Patel (2004) who reported that the growth of *Rhizoctonia solani* was completely inhibited by extracts of garlic bulb at 10 per cent concentration. Similar observations have also been recorded by Sindhan *et al.* (1999) and Kane *et al.* (2002) against *Rhizoctonia solani.* Dubey and Kumar (2003) observed that Azadirachtin at 25 per cent concentration inhibited the radial growth of *Macrophomina phaseolina* by 84.2 per cent. Mandhare and Suryavanshi (2008) reported that extract of *Allium sativum* and *Azadirachta indica* inhibited the growth of *Macrophomina phaseolina* by 88.8 and 58.8 per cent, respectively.

Inhibition of mycelial growth of *Macrophomina phaseolina* by plant extracts in the present study may be attributed to the presence of antifungal properties and

Table 1 : Effect of plant extracts on growth and sclerotial formation of *Macrophomina phaseolina* (Tassi.) Goid

Sr. No.	Common name	Botanical name	Mean colony diameter (cm)*	Per cent inhibition	Sclerotial formation
1.	Garlic	*Allium sativum*	1.07	88.15	-
2.	Sadaphuli	*Catheranthus roseus*	2.57	71.48	-
3.	Tulsi	*Ocimum sanctum*	5.40	40.00	++
4.	Neem	*Azadirachta indica*	5.67	37.04	++
5.	Onion	*Allium cepa*	1.97	78.15	+
6.	Ginger	*Zingiber officinale*	1.77	80.37	+
7.	Glyricidia	*Glyricidia maculata*	6.77	24.81	+++
8.	Control		9.00		++++
		S.E. ± 0.11	C.D. (P=0.01) 0.47		

* Mean of three replications
Sclerotial formation: - No sclerotia, + Poor, ++ Moderate, +++ Good, ++++ Excellent

Table 2 : Effectiveness of different bioagents against *Macrophomina phaseolina* (Tassi.) Goid

Tr. No.	Placement details	Mean colony diameter (cm*)	Per cent inhibition over control	Sclerotial formation
T_1	Tv			
	Mp	1.57	82.59	+
	Tv Tv			
T_2	Mp			
	Tv	1.80	80.00	+
	Mp Mp			
T_3	Th			
	Mp	1.50	83.33	+
	Th Th			
T_4	Mp			
	Th	2.00	77.78	+
	Mp Mp			
T_5	Tk			
	Mp	1.87	79.26	+
	Tk Tk			
T_6	Mp			
	Tk	2.23	75.19	+
	Mp Mp			
T_7	Gv			
	Mp	1.53	82.96	++
	Gv Gv			
T_8	Mp			
	Gv	1.90	78.89	+
	Mp Mp			
T_9	Mp			
	Mp	9.00		++++
	Mp Mp			
	S. E. $\pm$ 0.10	C.D. (P=0.01) 0.35		

Sclerotial formation: - No sclerotia, + Poor, ++ Moderate, +++ Good, ++++ Excellent.
Tv = *Trichoderma viride,* Th = *Trichoderma harzianum,*
Tk =*Trichoderma koningii,* Gv =*Gliocladium virens,*
Mp = *Macrophomina phaseolina,*

inhibitory compounds in the extracts. In the present investigation, the extracts from garlic and sadaphuli completely inhibited the sclerotial production, while poor sclerotial formation was observed in the extracts of onion and ginger. Tulsi and neem supported the moderate sclerotial formation. Good sclerotial formation was observed in glyricidia, while it was excellent in control. These plant extracts can help to reduce the inoculum potential and thereby reduce the disease incidence.

However, it is high time to standardize these plant extracts to enhance their antifungal effects and exploit them at commercial level, so that they can be easily made available to the farming community at feasible rates.

Effectiveness of different bioagents against *Macrophomina phaseolina* (Tassi.) Goid:

The results revealed that all the antagonists inhibited mycelial growth of *Macrophomina phaseolina* (Table 2). Maximum inhibition of mycelial growth of the test fungus was observed in treatment comprising *Trichoderma harzianum* (83.33%) followed by *Gliocladium virens* and *Trichoderma viride* with 82.96, 82.59 per cent inhibition of growth, respectively.

Inhibition of the fungus may be either due to the production of toxin, by the *Trichoderma* spp. or coiling, penetration and lyses of the hyphae of the pathogen by antagonists. Similar types of results were obtained by Lambhate *et al.* (2002) and Jakhar *et al.* (1997) who indicated that *Trichoderma* and *Gliocladium* species

were effective against *Rhizoctonia solani* and *Macrophomina phaseolina in vitro.* The antagonism of *Trichoderma viride* and *T. harzianum* against *Macrophomina phaseolina* has been reported by many workers (Maharshi and Kumavat, 1992; Singh and Majumdar, 1995; Elad *et al.,* 1986 and Majumdar *et al.,* 1996). Ashraf *et al*. (2006) and Mandhare and Suryavanshi (2008) also observed that under *in vitro* condition, the maximum inhibition of *Macrophomina phaseolina* was recorded by *Trichoderma harzianum.* In the present study, *Trichoderma koningii* was found to be less effective with 75.19 per cent inhibition compared to rest of the treatments. The inhibitory effect of *Trichoderma koningii* against various fungal pathogens with varying percentages was reported by Santhosh Priya (2006).

Sclerotial formation was poor in seven treatments (T_1 to T_6 and T_8) and moderate in T_7 treatment. Profuse growth of sclerotial bodies was observed in control (T_9).

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