Research Paper :

In vitro evaluation of different fungicides, plant extracts and bio-agents against *Alternaria alternata* (Fr.) Keissler causing leaf blight of cowpea

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SUMMARY

The pathogenic fungus was isolated on PDA medium. The colony of *Alternaria alternata* was circular, grayish black with whitish growth on the upper surface on PDA with profuse growth and sporulation. Among different fungicides tested *in vitro*, Mancozeb (0.2 per cent) and Propiconazole (0.05 per cent) completely inhibited the growth of the test fungus. *In vitro* screening of the plant extracts revealed that the bulb extract of garlic was most effective in inhibiting the growth of the test fungus followed by sadaphuli and glyricidia. The antagonistic effect of the fungal bioagents against *Alternaria alternata* revealed that *Trichoderma harzianum* and *Trichoderma viride* significantly inhibited the mycelial growth of the test fungus.

Key words : Leaf blight, *Alternaria alternata*, Fungicides, Plant extracts, Bioagents, Cowpea

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Pulses have been recognized as a major source of vegetable protein with needed minerals and vitamins. India grows a variety of pulse crops on about 223.91 lakh hectares with annual production of 133.81 lakh tonnes. In Maharashtra, pulses are cultivated on 34.32 lakh hectares area with a productivity of about 584 kg^{-ha} (Anonymous, 2007). Among the pulses, cowpea [*Vigna unguiculata* (L.) Walper] is nutritionally the most important legume containing 63.6 per cent carbohydrates, 24.8 per cent proteins, 1.9 per cent fats, 6.3 per cent fibre, 3-3.8 per cent ash and 9-11 per cent moisture. It is a rich source of calcium and iron.

In India, it is cultivated on about 1.5 million hectares with annual production of 0.5 million tonnes (Reddy, 2004). In Maharashtra, it is cultivated on 11800 hectares area with a productivity of about 390 kg ha^{-1} (Apte and Jadhav, 2002). In the Konkan region of Maharashtra, cowpea is grown as a sole crop, mostly during late *Kharif* or *Rabi* or *summer* season after rice on 1200 hectares area with a productivity of 400 kg ha^{-1} (Apte and Jadhav, 2002).

Among various diseases of cowpea, the leaf blight caused by *Alternaria alternata* was noticed in severe form on cowpea crop at the farms of Agric. Botany and Agronomy, College of Agriculture, Dapoli during the summer sown crop in the year 2008. The disease incidence was observed to be more than 40 per cent.

Prevalence of such newly introduced leaf blight disease on cowpea in Konkan region was found very severe. So far no research was undertaken, on this disease in Konkan, hence it was felt necessary to study its control by use of fungicides, plant extracts and bioagents *in vitro*.

MATERIALS AND METHODS

Efficacy of different fungicides against causal organism:

Seven different fungicides belonging to different groups were tested against the causal fungus by using 'poisoned food technique' (Nene and Thapliyal, 1979).The mycelial discs of 5mm diameter were cut from 7 days old culture of test fungus with the help of sterile cork borer and transferred aseptically to the centre of the poisoned PDA poured plates. The PDA plates containing no fungicide but inoculated with fungal culture, served as control. The inoculated plates were incubated at 27 ± 1^0C. Three replications per treatment were maintained. The observations on colony diameter and sporulation were recorded when Petri plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the test fungus was calculated by the following formula (Horsfall, 1956):

$$X = \frac{Y - Z}{Y} x100$$

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

Efficacy of plant extract against the test fungus:

To study the effect of phytoextract, different plants were selected on the basis of their antifungal properties against *Alternaria alternata*

One hundred grams of the fresh plant material was blended in a food processor by adding 100 ml of sterilized distilled water. The macerate was then filtered through double layered muslin cloth and centrifuged at 4000 rpm for 5 min. The supernatant was then filtered through Whatman's filter paper No. 1. The filtered extract was then passed through Sintered Glass Filter to avoid bacterial contamination. Thus, the standard plant extract solution was prepared (100 per cent).

The effect of plant extracts on mycelial growth was studied by 'Poisoned food technique'. All the plant extracts were tested at 10 per cent concentration against *Alternaria alternata* using Potato dextrose agar (PDA) as a basal medium. To obtain 10 per cent plant extract medium, 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. Then 20 ml of medium was poured in each sterilized Petri plate and allowed to solidify. Mycelial discs of 5 mm diameter were cut from 7 days old culture of the fungus with the help of sterilized cork borer and transferred aseptically to the centre of each Petri plate already poured with poisoned medium. Medium devoid of plant extract served as control. Petri plates were incubated at 27 ± 1^{0}C for growth. Three replications per treatment were maintained. The rest of the procedure was the same as described in fungicide evalution.

Efficacy of bio-agents against the test fungus:

The laboratory experiment was conducted by dual culture technique with three bioagents *viz*., *Trichoderma harzianum*, *T. viride* and *T. koningii.* The trial was conducted in two possible ways. In the first case, the test fungus was placed at the centre of Petri plates surrounded by bioagent and in second case; the test fungus was placed at the periphery and bioagent at the centre. Bio-agents and test fungus were grown on PDA in Petri plates, separately. The fungal discs of 5 mm diameter were placed in such a way that both the fungi would get equal opportunity for their growth. Each treatment was replicated three times. The plates were then incubated at 27 ± 1^{0}C for seven days. The observations on colony diameter and sporulation of the test fungus were recorded as described in evaluation of bio-agents and phytoextracts.

RESULTS AND DISCUSSION

The data presented in Table 1 reveal that Mancozeb (0.2 %), and Propiconazole (0.05%) completely inhibited the growth of the test fungus followed by Difenconazole (0.05%) and Copper oxychloride (0.2%) with 87.00 per cent and 86.33 per cent inhibition, respectively. Thiophanate methyl (0.1%) and Chlorothalonil (0.1%) recorded 69.22 and 59.22 per cent inhibition, respectively whereas Carbendazim (0.1%) was least effective (27.77 per cent inhibition).

No sporulation was observed in Copper oxychloride (0.2%), Mancozeb (0.1%), Difenconazole (0.05%) and Propiconazole (0.05%). Poor sporulation was recorded in Thiophanate methyl (0.1%) and Chlorothalonil (0.1%) where as moderate sporulation was recorded in carbendazim (0.1%). Excellent sporulation was observed in control treatment. These results are in close conformity with those of Mane (2008) who reported that Mancozeb (0.2%) and Propiconazole (0.05%) completely inhibited the growth of *A. alternata* causing leaf blight of chilli. Similarly, Bagade (2006) also reported that Propiconazole (0.05%) completely inhibited the spore germination of *A. alternata* affecting watermelon.

In the present study, Copper oxychloride (0.2%) recorded 86.33 per cent inhibition of *A. alternata* which seems to be similar to the finding of those of Rao and Rajagopalan (1982) who reported that Blitox-50 (Copper oxychoride) and Captan caused 83.5 per cent and 77.5 per cent inhibition of growth and sporulation of *A. helianthicola*, respectively. Thiophanate methyl (0.1%) and Chlorothalonil (0.1%) caused moderate inhibition of *A. alternata*. These findings seem to be in contrast with those of Viswakarma and Pandey (1995) who recorded complete inhibition of spore germination of *A.alternata* affecting brinjal by Chlorothalonil (0.1%). Carbendazim (0.1%) was found to be least effective fungicide in present investigation with 27.77 per cent inhibition of *A. alternata* over control. Mhasde (2004) also observed that Carbendazim was less effective fungicide against *A. alternata*.

The data of Table 2 reveal that all of the plant extracts tested showed antifungal activity against *A. alternata*. The bulb extract of garlic (*Allium sativum*) recorded maximum inhibition (63.33%) of mycelial growth of test fungus and was significantly superior to rest of the treatments. This was followed by sadaphuli (*Cartharanthus roseus*), glyricidia (*Glyricidia maculata*), neem (*Azadirachta indica*), karanj

Table 1: Efficacy of different fungicides on growth and sporulation of *Alternaria alternata* (Fr.) Keissler

Sr. No.	Common name	Conc. (%)	Mean colony diameter (cm)*	Per cent inhibition	Sporulation
1.	Copper oxychloride	0.2	1.23	86.33	-
2.	Carbendazim	0.1	6.50	27.77	++
3.	Mancozeb	0.2	0.00	100	-
4.	Propiconazole	0.05	0.00	100	-
5.	Thiophanate -methyl	0.1	2.77	69.22	+
6.	Difenconazole	0.05	1.17	87.00	-
7.	Chlorothalonil	0.1	3.67	59.22	+
8.	Control	-	9.00	-	++++
		S.E. ± : 0.09		C.D. (P=0.01) : 0.41	

* Mean of three replications. Sporulation: + + + + Excellent, + + + Good, + + Moderate, + Poor, - Nil

Table 2 : Effectiveness of different plant extracts on growth and sporulation of *A. alternata* (Fr.) Keissler

Sr. No.	Common name	Conc. (%)	Mean colony diameter (cm)*	Per cent inhibition	Sporulation
1.	Garlic	10	3.33	63.33	+
2.	Neem	10	6.03	33.00	+
3.	Karanj	10	6.33	29.66	+++
4.	Glyricidia	10	5.73	36.33	++
5.	Sadaphuli	10	5.67	37.00	++
6.	Tulsi	10	6.50	27.77	+++
7.	Ashok	10	6.70	25.55	+++
8.	Control	-	9.00	-	+ + + +
		S.E. ± : 0.08		C.D. at 1% : 0.33	

* Mean of three replications.
Sporulation: + + + + Excellent, + + + Good, + + Moderate, + Poor, - Nil.

(*Pongamia pinnata*), tulsi (*Ocimum sanctum*), and ashok (*Polyalthia longifolia*). These results are almost similar to those of Kadam (1997) who observed that, garlic caused 89.96 per cent inhibition of *A. alternata* causing leaf spot of gerbera. Karade and Sawant (1999) also observed the effectiveness of *Allium sativum* against *A. alternata*. Similarly, Jadhav (2003) and Washimkar *et al*. (2005) also reported that bulb extract of *A. sativum* was most effective against *A. alternata*. Garlic was not only effective in inhibiting the mycelial growth but also suppressed the sporulation.Tulsi leaf extract also showed antifungal property against *A. alternata* with 27.77 per cent inhibition. Mamata and Yashoda (2006) also demonstrated effectiveness of tulsi extract against *A. alternata* causing leaf blight of turmeric.

The leaf extracts of ashok (*Polyalthia longifolia*) was found to be least effective (25.55 PI) against *A. alternata.* However, contrary to the present findings, Patni *et al*. (2005) observed that leaf extract of ashok caused 100 per cent inhibition of *A. brassicae*. This might be due to inherent physiological differences in different *Alternaria* species. The fungitoxicity of plant extracts in the present study might be due to antifungal metabolites present in different plant species.

It was revealed from the data presented in Table 3 that the bioagents *T. harzianum*, *T. viride* and *T. koningii* significantly inhibited the mycelial growth of the test fungus. Maximum inhibition (85.88% and 80.00%) was observed in *T. harzianum* when it was placed at peripheri and at the centre, respectively. *T. viride* and *T. koningii* were also effective in inhibiting the growth of the test fungus (77.44 – 81.88 and 73.33 – 78.11 per cent inhibition, respectively).

Trichoderma harzianum was most effective against *A. alternata* and caused 85.88 per cent inhibition, followed by *T. viride, and T. koningii* causing 81.88 per cent and 78.11 per cent inhibition over control, respectively, when the bioagents were placed at the perpheri. These findings are almost similar to those of Mane (2008) who reported the inhibition of *A. alternata* by *T. harzianum, T. viride* and *T. koningii* to the tune of 86.11 per cent, 81.33 per cent and 79.66 per cent, respectively. Similarly, *Trichoderma harzianum* was also reported to cause inhibition of *A. alternata* by Sivapalan (1993), Indra and

Table 3 : Effect of different bioagents on growth and sporulation of *Alternaria alternata* (Fr.) Keissler

Sr. No.	Placement details	Mean colony diameter (cm)*	Per cent inhibition over control	Sporulation
1.	Aa Th Aa Aa Th	1.80	80.00	-
2.	Aa Th Th Th Aa	1.27	85.88	-
3.	Aa Tv Aa Aa Tv	2.03	77.44	+
4.	Aa Tv Tv Tv Aa	1.63	81.88	+
5.	Aa Tk Aa Aa Tk	2.40	73.33	+
6.	Aa Tk Tk Tk Aa	1.97	78.11	+
7.	Control	9.00	-	+ + + +
	S.E. ± : 0.10		C.D. (P=0.01) : 0.304	

* Mean of three replications.
Sporulation: + + + + Excellent, + + + Good, + + Moderate, + Poor, - Nil
where,
Aa = *Alternaria alternata* Th = *Trichoderma harzianum* Tv = *Trichoderma viride* Tk = *Trichoderma koningii*

Thiribhuvanmala (2002) and Kadam (1997) under *in vitro* conition.

Further, the data revealed that there was no sporulation in treatment comprising *T. harzianum*. Poor sporulation was observed in treatments comprising *T. viride* and *T. koningii*. However, excellent sporulation was observed in control treatment.

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