

In vitro evaluation of botanicals and bioagents against *Phaeoisariopsis griseola* (Sacc) Ferraris causing angular leaf spot of French bean

ADIKSHITA and SANDEEP KANSAL

Department of Plant Pathology

Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan 173230 HP, India

Email for correspondence: sharmaadikshita89@gmail.com

ABSTRACT

Angular leaf spot caused by *Phaeoisariopsis griseola* (Sacc) Ferraris is one of the most important foliar diseases of French bean in mid-hills of Himachal Pradesh. Survey and surveillance studies conducted revealed that the disease occurred in moderate to severe form in different French bean growing localities of Solan, Shimla and Sirmour districts of the state. The in vitro evaluation of botanicals against the mycelial growth *P. griseola* revealed that the extract of garlic bulb at 10 per cent concentration caused significant growth inhibition (84.62%) of the test pathogen followed by neem seed kernel extract. Amongst the different fungal antagonists evaluated in vitro *Trichoderma harzianum* proved most inhibitory to *P. griseola* while testing its antagonistic efficacy through dual culture or through its toxic substances.

Keywords: French bean; angular leaf spot; *Phaeoisariopsis griseola*; eco-friendly management; botanicals; antagonists

INTRODUCTION

French bean (*Phaseolus vulgaris* L) is one of the most important leguminous Kharif pulse and vegetable crops grown throughout the hills of India. In Himachal Pradesh it is a highly profitable crop being grown round the year except winter months over an area of 3749 ha with an annual production of 46372 MT (Anon 2014). Bean production is however constrained by several biotic and abiotic factors. Amongst

the biotic factors, angular leaf spot caused by *Phaeoisariopsis griseola* (Sacc) Ferraris is one of the most widely distributed and damaging diseases of common bean causing yield losses as high as 80 per cent (Ponnappa et al 1976). The disease affects the foliage and pods throughout the growing season and is particularly destructive in areas where warm and moist conditions are accompanied by abundant inoculum from infected plant residues and contaminated

seeds. Though the disease can be controlled to some extent by using different fungicides yet latter are not cost effective. The use of fungicides has also been reported to pollute the eco-environment resulting in soil and water pollution and also posing the residual problem. Besides the pathogen acquires resistance due to continuous use of conventional fungicides (Gangawane and Shaikh 1988). Thus keeping in view the inefficacy of the costly fungicides the present investigation was carried out to evaluate the non-conventional methods including botanicals and bioagents against *P griseola* causing angular leaf spot of French bean under in vitro conditions to obtain useful information for further use under field condition.

MATERIAL and METHODS

In the present in vitro study the efficacy of botanicals viz lantana (*Lantana camara*), soap nut (*Sapindus trifoliatus*), garlic (*Allium sativum*), agave (*Agave americana*), Dharek (*Melia azedarach*), marigold (*Tagetes erecta*) and neem (*Azadiracta indica*) were evaluated against *P griseola* causing angular leaf spot of French bean. Different plant parts (leaves/cloves/fruit rind/seeds) of the botanicals were washed thoroughly with distilled water followed by sterilized distilled water, air-dried, homogenized into paste, extracted through muslin cloth and filtered through sterilized sintered filter. This filtrate was used for antifungal property by poisoned food

technique (Nene and Thapliyal 2011). Required quantity of the extract of the botanicals was added separately into the sterilized molten potato dextrose agar so as to get the desired concentrations of 2.5, 5.0, 7.5 and 10 per cent. The poisoned medium was poured into the sterilized Petri plates (90 mm) and mycelium disc of 5 mm size taken from four days old culture of *P griseola* was placed aseptically at the centre of each agar plate. Control was maintained without adding any botanical extract in the medium. Four replications were maintained for each concentration and plates were incubated at $25 \pm 1^\circ\text{C}$. The radial growth of the test fungus at different concentrations of the botanicals was measured. The efficacy of the plant extracts was expressed as per cent inhibition of mycelial growth over control which was calculated by using the formula of Vincent (1947).

In vitro efficacy of fungal antagonists viz *Trichoderma harzianum*, *T hamatum*, *T viride*, *T polysporum*, *T virens* and *Penicillium pinophilum* was tested for their antagonistic activity against the *P griseola* by dual culture method. In this method the culture disc (3 mm diameter each) of the fungal antagonist and the test pathogen were taken from the margin of vigorously growing cultures and transferred to 90 mm culture plates on two opposite sides. A check having only the disc of test pathogen was also kept for comparison. The inoculated plates were then incubated

at $25 \pm 1^\circ\text{C}$ in darkness. Each treatment was replicated five times. Radial growth of both the fungal antagonist and the test pathogen was recorded after required period of incubation. Per cent inhibition over control was worked out according to formula given by Vincent (1947).

The effect of non-volatile substances produced by fungal antagonists was evaluated by making use of agar-plate culture method suggested by Dennis and Webster (1971a). For this purpose a single sterilized sheet of cellophane (50 μm thickness) was placed aseptically over each PDA Petri plates (90 mm diameter) and the plates were left overnight to allow excess moisture to evaporate. Culture discs (4 mm diameter) cut from the margins of vigorously growing culture of each fungal antagonist were placed in the centre of each prepared Petri plate. A control having PDA disc instead of antagonist culture disc was also maintained for comparison. The plates were incubated at $25 \pm 1^\circ\text{C}$. After 2 days of incubation the cellophane and adhering fungus (antagonist) were removed gently and a disc (3 mm diameter) of test pathogen was placed immediately on each prepared Petri plate at the central position previously occupied by the antagonist. The prepared Petri plates were further incubated at temperature similar to that used for cellophane culturing. Each treatment including the control was replicated five times. Observations on the radial growth of test pathogen were recorded and per cent growth inhibition of the test pathogen

was worked out according to Vincent (1947).

The effect of non-volatile substances produced by fungal antagonists on the growth of test pathogen was also evaluated by well method. In this method the liquid cultures of fungal antagonists were raised separately in 150 ml flasks having 20 ml of Czapek's Dox medium each and were incubated at $25 \pm 1^\circ\text{C}$. The culture filtrates obtained after filtration through Whatman # 1 filter paper were further filtered through sintered glass filter (G-5) and tested for their efficacy against the growth of test pathogen by well method. For this purpose well (4 mm diameter) was made in the centre of each PDA Petri plate (90 mm diameter) with the cork borer. The thickness of medium in Petri plates was kept about 5 mm. Culture filtrate (0.5 ml) of each fungal antagonist was poured separately in each well by using a sterilized pipette. A check having only the sterilized liquid medium in the well was also maintained. Finally a culture disc (6 mm diameter) of the test pathogen taken from the margin of vigorously growing culture was placed over the well of each prepared plate to cover the well. The effect of culture filtrate was evaluated by incubating the prepared Petri plate at $25 \pm 1^\circ\text{C}$. Each treatment including the check was replicated five times. Observations on the radial growth of the test pathogen was recorded and per cent growth inhibition of the test pathogen was calculated according to the Vincent (1947).

The effect of volatile compounds produced by fungal antagonists was tested by using the method suggested by Dennis and Webster (1971b). For this purpose PDA plates (75 mm diameter) were inoculated separately in the centre with disc cut from the margin of vigorously growing culture of each antagonist and kept for incubation at $25 \pm 1^\circ\text{C}$. At an interval of 0, 5, 10 and 15 days of incubation the lid of each prepared plate was replaced by bottom containing PDA (15 ml) inoculated centrally with the growing culture of test pathogen. The plates were taped together with adhesive tape. The lids of the control plates which had not been inoculated with the antagonists were also replaced in the same way. Test plates and control plates were replicated five times. Observations on the dial growth of test pathogen were recorded in each treatment and per cent growth inhibition of the test pathogen was calculated according to the formula given by Vincent (1947).

RESULTS and DISCUSSION

The results of study (Table 1) revealed that garlic clove extract proved most effective in inhibiting the mycelial growth (75.50%) of *P. griseola* closely followed neem kernel extract. Marigold, lantana and agave leaf extracts were also potent and caused marked inhibition of the mycelial growth of the test pathogen. Extract of soap nut fruit rind also reflected considerable inhibition of the mycelial

growth of *P. griseola*. Moderate inhibition (61.83%) was caused by Dharek extract. Irrespective of botanicals used a significant increase in inhibitory response was recorded with the increase in concentration levels of different botanicals used. The antifungal activity of *A. sativum* may be due to sulphur containing compounds S-methyl-L-cysteine sulfoxide (Whitemore and Naidu 2000). *T. erecta* and *L. camara* have also been reported inhibitory to *Colletotrichum lindemuthianum* causing anthracnose of French bean (Gupta et al 2005).

The evaluation of fungal antagonists against the growth of test pathogen by dual culture technique (Table 2) revealed that *T. harzianum* causing 69.7 per cent mycelial growth inhibition proved most inhibitory closely followed by that of *T. viride* (66.7%) and *T. hamatum* (46.3%). *T. polysporum* (31.7%) and *Penicillium pinophilum* (30.0%) were recorded least inhibitory and were statistically similar in their efficacies.

Amongst the different antagonists evaluated under well method (Table 2) the non-volatile substances of *T. viride* proved most inhibitory (43.0%) closely followed by *T. harzianum*, *T. virens* and *T. hamatum*. *P. pinophilum* caused considerably less inhibition while *T. polysporum* was found least inhibitory. The study further revealed that the non-volatile toxic substances of fungal antagonists

Table 1. In vitro efficacy of different botanicals at different concentrations against mycelial growth of *Phaeoisariopsis griseola*

Botanical	Per cent growth inhibition at different concentrations				Mean
	2.5%	5.0%	7.5%	10.0%	
Lantana	62.78 (52.41)	67.04 (54.97)	71.16 (57.53)	77.41 (61.63)	69.60 (56.63)
Agave	59.00 (50.19)	63.50 (52.83)	69.18 (56.47)	75.00 (60.01)	66.74 (54.88)
Marigold	66.36 (54.55)	69.04 (56.20)	72.24 (58.21)	78.10 (62.18)	71.44 (57.77)
Soapnut	53.50 (47.01)	63.50 (52.84)	67.00 (55.40)	75.00 (60.01)	64.94 (53.81)
Dharek	54.84 (47.80)	59.38 (50.41)	65.24 (53.88)	67.82 (55.44)	61.83 (51.88)
Garlic	67.70 (55.37)	71.98 (58.04)	77.49 (61.68)	84.62 (66.93)	75.45 (60.51)
Neem	71.16 (57.53)	72.24 (58.21)	75.00 (60.0)	77.49 (61.68)	73.95 (59.35)
Mean	62.19 (52.12)	66.66 (54.78)	71.04 (57.59)	76.49 (61.12)	

Figures in the parentheses are arc sine transformed values

CD_{0.05}

Botanicals (F)= 0.48, Concentrations (C)= 0.39, F x C= 0.96

Table 2. In vitro evaluation of different fungal antagonists against mycelial growth of *Phaeoisariopsis griseola* by dual culture method, well method and agar-plate culture method

Fungal antagonist	Per cent mycelial growth inhibition		
	Dual culture method	Well method	Agar-plate culture method
<i>Trichoderma harzianum</i>	69.7(56.60)	43.0 (40.96)	85.0(67.70)
<i>T. viride</i>	66.7 (54.75)	41.1 (39.85)	82.2 (65.24)
<i>T. hamatum</i>	46.3(42.90)	38.1 (38.12)	75.0 (60.05)
<i>T. virens</i>	43.3(41.16)	37.0 (37.44)	51.1 (45.64)
<i>T. polysporum</i>	31.7(34.23)	10.7 (18.99)	21.2 (27.22)
<i>Penicillium pinophilum</i>	30.0(33.19)	20.3 (26.59)	38.2 (38.15)
CD _{0.05}	2.0980	3.4221	5.0522

Figures in the parentheses are arc sine transformed values

reflected significantly higher inhibitory response in agar-plate culture method as compared to their inhibitory effect evaluated through well method.

It is evident from the data (Table 3) that the efficacies of volatile substances

produced by six different fungal antagonists differed significantly in causing growth inhibition of *P. griseola* at all the tests ages tried though to a varying extent. In the 0, 5, 10 and 15 days old culture of fungal antagonist the *T. harzianum* caused marked inhibition followed by *T. viride*, *T.*

Table 3. Effect of volatile substances produced by antagonists on the growth of *Phaeoisariopsis griseola*

Fungal antagonist	Per cent inhibition by fungal antagonists of different ages (days)				Mean
	0	5	10	15	
<i>Trichoderma harzianum</i>	49.6 (44.77)	55.5 (48.19)	62.2 (52.11)	58.7 (50.01)	56.5 (48.77)
<i>T viride</i>	47.9 (43.77)	51.1 (45.64)	59.2 (50.30)	56.9 (48.99)	53.8 (47.18)
<i>T virens</i>	32.4 (34.69)	50.2 (45.13)	56.1 (48.53)	54.7 (47.68)	48.4 (44.01)
<i>T hamatum</i>	34.6 (35.991)	49.3 (44.62)	54.0 (47.33)	52.3 (46.30)	47.6 (43.56)
<i>T polysporum</i>	2.0 (8.03)	3.8 (11.24)	7.9 (16.28)	10.1 (18.23)	5.9 (13.45)
<i>Penicillium pinophilum</i>	0 (0.00)	0 (0.00)	3.1 (10.12)	5.2 (13.17)	2.1 (5.82)
Mean	27.8 (27.87)	35.0 (32.47)	40.4 (37.44)	39.6 (37.40)	

Figures in the parentheses are arc sine transformed values

CD_{0.05}

Fungal antagonist (F)= 1.409, Age (A)= 1.150, F × A= 2.817

virens, *T hamatum*, *T polysporum* and *P pinophilum*.

Irrespective of the age of the antagonists prior to bringing in contact with test pathogen *T harzianum* was adjudged to be the best as it resulted in 56.5 per cent growth inhibition. *T viride* with 53.8 per cent growth inhibition was followed by *T virens*, *T hamatum*, *T polysporum* and *P pinophilum* in order. Irrespective of different fungal antagonists evaluated for their inhibitory effect through their volatile substances, the aging period of 10 days reflected the highest (37.44%) inhibitory effect. Ageing period of 15 days also resulted in marked inhibition and was statistically similar to that of 10 days aging period. Fungal antagonists exhibited increasing inhibitory response with the increase in age up to 10 days.

Dennis and Webster (1971b) also reported that the species group of *Trichoderma* possesses a characteristic smell of coconut. They tentatively identified acetaldehyde as one inhibitory metabolite of *Trichoderma* species. The inhibitory effect of non-volatile and volatile toxic substances produced by *Trichoderma* species against *Colletotrichum capsici* has also been reported by Kaur et al (2006).

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