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In vitro evaluation of certain antifungal plant extracts and biocontrol agents against *Alternaria brassicae* (Berk.) Sacc. Causing *Alternaria* leaf spot of cauliflower

HT Valvi, JJ Kadam and VR Bangar

Abstract
Certain plant extracts and bioagents were evaluated under *in vitro* conditions against *Alternaria brassicae* (Berk.) Sacc. All plant extracts at 10 per cent concentration were found significantly effective in inhibiting growth of *A. brassicae* by poisoned food technique. Maximum per cent inhibition of *A. brassicae* over control was achieved due to fruit extract of Soap nut *(Sapindus trifoliatus)* (80.56 %) and it was followed by extracts of Garlic (*Allium sativum*), Nilgiri (*Eucalyptus tereticornis*), Neem (*Azadirachta indica),* Ashoka (*Polyalthia longifolia*), Adulsa (*Justicia adhatoda)* and Nirgudi (*Vitex negundo*) which showed 43.70, 37.04, 25.56, 21.85, 18.52 and 14.07 per cent inhibition of mycelial growth of *A. brassicae*, respectively over control. Three bioagents *viz., Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* were tested by dual culture method. In the present study, *T. viride* recorded maximum per cent inhibition of *A. brassicae* in both conditions when placed at periphery (72.46 %) and at centre (68.48 %) over control. This was followed by *T. harzianum* which showed 67.57 and 62.64 per cent inhibition of the test pathogen. *Pseudomonas fluorescens* showed 61.03 per cent inhibition of *A. brassicae* which when streaked centrally and 55.33 per cent when streaked on two sides of test pathogen.

Keywords: *Alternaria brassicae* (Berk.) Sacc., Plant extracts, Bioagents, poison food technique, dual culture method and Mycelial growth

Introduction
Many fungal and bacterial diseases of cauliflower have been reported from major cauliflower growing regions of the world. The leaf spot disease of Cauliflower (*Brassica oleracea* L. var. *Botrytis*) is caused by *A. brassicae* (Berk.) Sacc. Among the various biotic factors responsible for low production and productivity of cauliflower, *Alternaria* leaf spot is the most common and serious disease.

In present day agriculture, farmers use different strategies involving various inputs, practices and means of managing biotic and abiotic stresses for high yield. However, uses of chemicals dominate all other inputs, thus leads to degradation of the environment, development of fungicidal resistance along with their harmful effect on human beings, beneficial microorganisms and increases cost of cultivation.

Excessive use of chemicals in plant disease management has resulted in number of problems related to fungicide resistance, damage to non-target flora and fauna and other useful organisms along with hazardous effects of residue on environment have become the main concern of scientists at present. So, instead of chemicals ecofriendly ways of control i.e. plant extracts and bio control agents have emerged as a viable alternative under such conditions (Singh, 2006) [19].

The naturally occurring plants with antifungal property have been well recognized and documented, but very few of them have been studied extensively in case of *Alternaria* leaf spot of cauliflower. Some plant extracts have been tested against *A. brassicae* (Patni *et al.*, 2005; Rajendra Prasad and Lallu, 2006; Thaware *et al.*, 2010; Singh *et al*., 2008; and Harde and Suryawanshi, 2014) [14, 21, 20, 5] and also some bioagents have been tested against the pathogen (Patni *et al.*, 2005 and Shrivastav *et al*., 2013) [14, 18].

Considering importance of the crop and disease, present study was planned and conducted with plant extracts and biocontrol agents under *in vitro* conditions to evolve effective and eco-friendly management strategy.

Materials and methods

In vitro evaluation of plant extracts against *A. brassicae*

Ten locally available plants with defined antifungal properties were used in the present study. The plant and their parts such as leaves of Neem, Aloe vera, Nilgiri, Adulsa, Shatavari, Ashoka, Rui, Nirgudi; Bulbs of Garlic and fruits of Soap nut (Ritha) were used individually to prepare extracts.

Preparation of plant extracts

Aqueous plant extracts were obtained as per the method described by Bhatti (1988) [2]. A 100 gram sample of each plant was washed with distilled, sterile water. Then each sample was ground separately by using sterile pestle and mortal in 100 ml distilled sterile water. The extract of each sample thus obtained was filtered separately through a sterilized double layered muslin cloth to remove the bits of plant material is the filtrate. Then this extract was again filtered through a filter paper (Whatman No.1). The filtered extract was centrifuged at 4000 rpm for 5 minutes to get homogenous aqueous solution. After centrifuging, the supernatant of each extract was collected. The extracts thus collected were passed separately through a Sintered glass filter to avoid bacterial contamination. This formed the standard plant extracts solution (100%).

The effect of plant extracts on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1997) [12]. All the glasswares used in the study were sterilized before their use. All the plant extracts were tested at 10 per cent concentration against the test pathogen using PDA as a basal medium. To obtain 10 per cent concentration of plant extracts, 90 ml of lukewarm PDA was mixed with 10 ml of standard plant extracts in 250 ml conical flask, separately and then it was stirred well to obtain homogenized mixture. Fifteen to twenty millilitre of such poisoned medium was then poured in each sterilized Petri plate and allowed to solidify. Mycelial discs of 5 mm diameter were cut from seven day old culture of test pathogen 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. The inoculated Petri plates were incubated at room temperature (27±2 ºC) for further growth of the fungus.

Three replications of each treatment were maintained. The observation on colony diameter of the fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the test pathogen was calculated.

Per cent inhibition of growth of the test fungus was calculated by following formula (Horsfall, 1956) [6].

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

Where, X = Per cent inhibition; Y = Growth of fungus in control (mm); Z = Growth of fungus in treatment (mm)

Evaluation of bio-agents against *A. brassicae*

In order to study the antagonism of the bio-agents against the test pathogen, two fungal bio-agents (*Trichoderma harzianum* and *T. viride*) and one bacterial bio-agent (*Pseudomonas fluorescens*) were used. The experiment was carried out by dual culture technique (Padder *et al.* 2010) [13]. Fungal bio-agents and the test pathogen were grown on PDA and the bacterial bio-agent was grown on nutrient agar medium. Seven days old culture of each organism was used.

A 5 mm culture disc of the test pathogen was placed in the centre of solidified medium. In the same plate three culture discs of *T. harzianum* were cut in similar way and placed around the pathogen disc in such a way that all the three discs were at equal distance away from the pathogen. Appropriate space was provided for the growth of the pathogen. The procedure was repeated by using the culture of *T. viride.*

In case of the bacterial antagonist, a loopful of bacterial culture was streaked around the culture disc of the pathogen in the centre. The experiment was repeated by placing the culture of bio-agents is the centre and three discs of the pathogen around the bio-agent. Three replications of each treatment were maintained. The observation on colony diameter of the fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the test pathogen was calculated as described above.

Statistical analysis

The data obtained were statistically analysed by the methods suggested by Gomez and Gomez (1986). The standard error and critical difference were worked out and the results obtained were compared statistically.

Results and discussion

In vitro evaluation of plant extracts against *A. brassicae*

The aqueous extracts of ten plant species were tested against *A. brassicae* to exploit their antifungal properties. All the plant extracts were tested at 10 per cent concentration by poisoned food technique. All of the plant extracts under study, showed antifungal activity against *A. brassicae*. The data obtained on the effect of plant extracts on growth and sporulation of the test fungus are presented in Table 1 and Plate-I.

All plant extracts at 10 per cent concentration were found significantly effective in inhibiting growth of *A. brassicae.* Maximum per cent inhibition of *A. brassicae* over control was achieved due to fruit extract of Soapnut *(Sapindus trifoliatus)* (80.56 %) and it was followed by extracts of Garlic (*Allium sativum*), Nilgiri (*Eucalyptus tereticornis*), Neem (*Azadirachta indica)*, Ashoka (Polyalthia longifolia), Adulsa (*Justicia adhatoda)* and Nirgudi (*Vitex negundo*) which showed 43.70, 37.04, 25.56, 21.85, 18.52 and 14.07 per cent inhibition of mycelial growth of *A. brassicae*, respectively over control. Fruit extract of Soap nut was not only effective in inhibiting the mycelial growth but also suppressed the sporulation of the test fungus.

Table 1: Effect of different plant extracts on growth and sporulation of *A. brassicae* (Berk.) Sacc.

T. No.	Name of plant extracts	Conc. (%)	Mean colony Diameter (mm)	Per cent inhibition over control	Sporulation
T_1	Soap nut (*Sapindus trifoliatus)*	10	17.50	80.56	+
T_2	Aloe vera (*Aloe vera)*	10	87.00	3.33	++++
T_3	Nilgiri (*Eucalyptus tereticornis)*	10	56.66	37.04	++
T_4	Garlic (*Allium sativum)*	10	55.06	43.70	++
T_5	Adulsa (*Justicia adhatoda*)	10	73.33	18.52	+++
T_6	Ashoka (*Polyalthia longifolia)*	10	70.33	21.85	+++

T_7	Rui (*Calotropis gigantea)*	10	90.00	0.00	++++
T_8	Neem *(Azadirachta indica)*	10	67.00	25.56	++
T_9	Nirgudi (*Vitex negundo*)	10	77.33	14.07	+++
T_{10}	Shatavari (*Asparagus resimose)*	10	88.33	1.85	++++
T_{11}	Control	-	90.00	-	++++
S.Em.±			0.51		
C.D. at 1%			2.03		

Sporulation

- = No sporulation, +++ = Good,
+ = Poor, ++++ = Excellent.
++ = Fair,

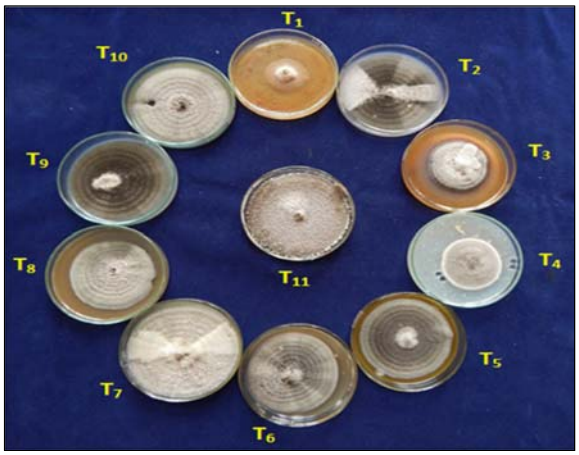

Plate 1: Effect of different plant extracts on growth and sporulation of *Alternaria brassicae* (Berk.) Sacc.

Fair sporulation was observed in the treatment of Nilgiri, Garlic and Neem. Good sporulation was observed in Adulsa, Ashoka and Nirgudi. Rui, Shatavari and Aloe vera extracts were less effective in inhibiting the mycelia growth and also showed excellent sporulation of *A. brassicae.*

These results are in line with the findings of Shivpuri *et al.*, (1997) [17] who reported that the leaf extract of *A. indica* was found to be the most fungitoxic against *A. brassicae.* Patni *et al.*, (2005) [14] who reported that *Eucalyptus* showed 80.25 and 89.88 per cent reduction in mycelial growth at 0.5 and 1.0 per cent concentration, respectively. Leaf extract of Ashoka also resulted in 100% reduction in growth at 2 and 5 per cent concentration against *A. brassicae.* Rajendra Prasad and Lallu (2006) also reported that garlic bulb extract and leaf extract of Nilgiri were effective in reducing growth of *A. brassicae*. Also Meena and Sharma (2012) [9] reported highest inhibition of *A. brassicae* by bulb extract of Garlic. Some of the findings of present study are also in close conformity with earlier report of Singh *et al.*, (2008) [20].

In vitro* evaluation of bio-agents against *A. brassicae

The laboratory experiment was conducted by dual culture method with three bioagents *viz., Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens.* 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. The data obtained on the effect of bioagents on growth and sporulation of *A. brassicae* are presented in Table 2 and PLATE-II.

Maximum reduction in colony diameter of the test pathogen was observed when the centrally placed test pathogen was surrounded by bio-agents.

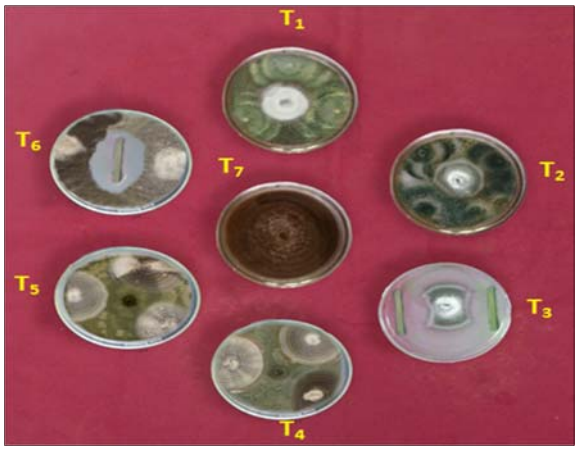

Plate 2: Effect of different bioagents on growth and sporulation of *Alternaria brassicae* (Berk.) Sacc.

In the present investigation *Trichoderma viride* recorded maximum per cent inhibition of *A. brassicae* in both conditions when placed at periphery (72.46 %) and at centre (68.48 %) over control. This was followed by *T. harzianum* which showed 67.57 and 62.64 per cent inhibition of the test pathogen. *Pseudomonas fluorescens* showed 61.03 per cent inhibition of *A. brassicae* which when streaked centrally and 55.33 per cent when streaked on two sides of test pathogen. No sporulation was observed in treatment *T. viride* and *T. harzianum.* Poor sporulation was observed in treatment *P. fluorescens.* These findings are in close conformity with the results of Patni *et al.*, (2005) [14]; Mane (2008) [8]; Abdul kareem (2012) [1]; Ganie *et al.*, (2013) [3]; Rahaman *et al.*, (2015) [16] and Shrivastav *et al.*, (2013) [18].

Table 2: Effect of different bioagents on growth and sporulation of *A. brassicae* (Berk.) Sacc.

T. No.	Placement details	Mean colony diameter (mm)	Per cent inhibition over control	Sporulation
T_1	Th Ab Th Th	33.63	67.57	-
T_2	Tv Ab Tv Tv	28.37	72.46	-
T_3	Pf Ab Pf	40.20	61.03	+

T_4	Ab Th Ab Ab	29.19	62.64	-
T_5	Ab Tv Ab Ab	24.78	68.48	-
T_6	Ab Pf Ab	35.08	55.33	+
T_7	Control	90.00		++++
S.Em.±		0.64		
C.D. at 1%		2.69		

Sporulation

-	=	No sporulation,	+++	=	Good,
+	=	Poor,	++++	=	Excellent.
++	=	Fair,			

Conclusion

On the basis of the results of present study it can be concluded that the herbal extract such as fruit extract of soap nut (10%) is not only effective in inhibiting the mycelial growth but also suppressed the sporulation of pathogen under laboratory conditions In evaluation of bio-agents *viz.*, *Trichoderma viride* and *T. harzianum* are found as the potential antagonists of *A. brassicae*.

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