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Evaluation of some botanical plant extracts for their antifungal activity against *Alternaria brassicae* of cabbage

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Abstract

Black leaf spot disease caused by Alternaria brassicae (Berk.)Sacc. It is a serious disease of Indian crucifers plants throughout the country. Extract of 5 plants Sarpgandha (*Rauvolfia serpentina*), Ashoka (*Saraca asoca*), Amaltash (*Cassia fistula*), Karanj (*Pongomia pinnata*) and Badoh (*Ipomoea carnea*) belonging to 3 families was tested against their efficacy at 25% and 50% concentration in vitro by poisoned food technique. The five extracts found effective in vitro were evaluated on cabbage as pre and post inoculation treatments. All the plant extracts at 50% concentration resulted in significant disease reduction. But maximum reduction was observed with sarpgandha extract followed by amaltash.

***Keywords*:** Cabbage, *Alternaria brassicae*,antifungal activity, medicinal plant extracts ,poisoned food technique

Introduction

Cabbage (Brassica oleracea L. Capitata) is leafy green plant, it is an excellent source of vitamin K, vitamin C, vitamin B6, vitamin B1(thiamine), B2(riboflavin), B3 (niacin),B5 (pantothenic acid), B9 (folate) vitamin-A creates cooling effect in the body, prevents from constipation, helps in digestion and is useful for diabetes. It is also a very good source of mangnesium, calcium, iron, manganese, phosphorus, potassium, sodium, zinc (FAO, 2023). India is the second largest producer of cabbage in the world with a production of 9035 thousand tones from an area of 402 thousand hectares. West Bengal is the leading producer of cabbage in India but the productivity is highest in U.P. (Anonymous, 2023). Cruciferous plant are severely affected by fungi, bacteria, nematodes and viruses. Leaf spot disease caused by *Alternaria brassicae* (Berk) Sacc. And *A. brassicicola* has been reported from all the continents of the world. Losses up to 30% and 47% were caused by Alternaria brassicae in cauliflower (*Brassica oleracea* var. *botrytis*) (Tomayo *et al.* 2001). Black leaf spot disease in cabbage (*Brassica oleracea* L.) is caused by *Alternaria brassicae* (Berk) Sacc. Although this is a common disease of cruciferous vegetables worldwide. The pathogen is easily spread through the contamination of seeds. The leaves and stems of cabbages infected by *A. brassicae* result in the formation of dark-brown, necrotic lesions with a yellow halo; this damage reduces both yield and economic value. Therefore the different botanical were applied to control Alternaria brassicae causing leaf spots in cabbage.

Materials and methods

Experimental site and sample collection

Botanical leaf extract used for this experiment were prepared indigenously. Fresh leaves of Sarpgandha (*Rauvolfia serpentina*), Ashoka (*Saraca asoca*), Amaltash (*Cassia fistula*), Karanj (*Pongomia pinnata*) and Badoh (*Ipomoea carnea*) were collected from the different area of Prayagraj U.P. The experimental site located at 20^0 30' North latitude and 85^0 49' 60" east longitude city and is about 98m above sea level. The city is situated at the confluence of three rivers- Ganga, Yamuna and the invisible Saraswati. The sample of infected leaf were collected from the experimental field for the study.

Fungal and Plant material

Alternaria brassicae isolated from the leaves of cabbage (*B. oleracea* L.) showing symptoms of black leaf spot disease. Which was isolated from diseased cabbage (*A. brassicae*). The cultures were grown on a potato dextrose agar (PDA) (200gm of potato infusion, 20gm of glucose and 20gm of agar in 1L of distilled water).

Isolation and morphological study of the pathogen

The leaf tissues of the plant exhibiting typical symptoms of black leaf spot were selected to retrieved the pure culture of the pathogen. Cut the small piece of the infected leaf and surface sterilized with the sodium hypochlorite solution (NaOCl) of the 4.0% concentration for 3min. and followed by three washing with the sterile distilled water. The water was removed with the help of sterile blotting paper and thereafter the tissues were transferred on the sterile PDA plates and kept on incubation at $25+2^0$C in BOD incubator. The fungal mycelium growth observed after 72h of incubation was transferred on the other PDA plate for pure culture. The morphological and in vitro efficacy of the fungicides was performed from the seven days old culture of the fungus.(Begum *at al.*2010)

Preparation of botanical leaf extract-

Fresh botanical leaf extract was prepared by grinding the required quantity of leaves (100gm) before grinding equal quantity of water was added in the respective plant leaves (1:1 weight/ volume basis). The fresh botanical leaf extract was filtered different leaves was sieved through muslin cloth. The filtered extracts were used @ 25% and 50% concentration by adopting poisoned food technique.(Sasode *et al.*2012)

Bioassay of plant extracts by poisoned food technique-

The experiment was conduct for measurement of mycelium growth inhibition following the poisoned food technique (Sharvelle 1961). The standardized concentration of the fungicides and botanical extracts were mixed in the sterilized PDA and amended PDA poured in the sterilized petri plates. The petri plates of solidified PDA inoculated with a 7mm mycelium disk cut with the sterile cork borer from the 7 days old fungal culture. The inoculated plates were placed at $25+2^0$C in BOD incubator for incubation. Each treatment was replicated four and the growth of fungus colony was measured after 7 days of inoculation. The percent inhibition of the mycelium growth over the control was calculated as;

$$PI = \frac{C - T}{C} \times 100$$

where

PI = Per cent inhibition of fungal growth

C = Mycelium growth of the fungus in absent of treatment

T = Mycelium growth of the fungus with treatment.

Result and discussion

Effect of plant extracts against Alternaria brassicae in vitro

The 5 plants fresh leaf extracts namely sarpgandha (*Rauvolfia serpentina*), Ashoka (*Saraca asoca*), Amaltash (*Cassia fistula*), Karanj (*Pongomia pinnata*) and Badoh (*Ipomoea carnea*) belonging to 3 families were tested for their efficacy at 25% and 50% concentration against the *A. brassicae*. The experiment was conducted in in-vitro conditions to assay their effect on mycelial radial growth of pathogen.

Effect on mycelial radial growth

The effect of botanical on the mycelia growth of *Alternaria brassicae* are presented in the (Table -1). All the botanicals tested were effective showing different levels of toxicity against *Alternaria brassicae* inhibiting the growth of pathogen.

At 25% mycelial radial growth of the test pathogen was ranged from T1 sarpgandha (Rauvolfia serpentina)(21.450mm) to T3 Amaltash (*Cassia fistula*)(40.125mm) however, it was maximum with

T3 Amaltash (40.125mm). This was followed by T2 Ashoka (*Saraca asoca*)(38.575mm), two of which were at par and T4 Karanj (*Pongomia pinnata*) (25.80mm), T5 Badoh (*Ipomoea carnea*) (24.325mm) of which were at par. Comparatively least mycelia growth was recorded with the botanical viz., T1 sarpgandha (*Rauvolfia serpentina*)(21.450mm) and T5 Badoh (*Ipomoea carnea*) (24.325mm)

At 50% radial mycelia growth of the test pathogen was ranged from T1 sarpgandha (*Rauvolfia serpentina*) (12.175mm) to T3 Amaltash (*Cassia fistula*) (20.675mm) however, significantly highest mycelial growth was maximum with T3 Amaltash (*Cassia fistula*) (20.675mm). This was followed by T2

Ashoka (*Saraca asoca*)(19.725) T4 Karanj (*Pongomia pinnata*) (15.950), T5 Badoh (*Ipomoea carnea*) (13.300). Significantly least mycelia growth was recorded with the botanical T1 sarpgandha (*Rauvolfia serpentina*) (12.175mm) as compared to untreated control was 90.00mm.

Mycelial growth inhibition

Results (Table - 1) revealed that all the plant extracts tested (at 25 and 50% each) significantly inhibited mycelial growth of the test pathogen over untreated control (0.00%) further, it was found that percent mycelia growth inhibition of the test pathogen was increased with increase in concentration of the botanicals tested.

At 25% mycelia growth inhibition was ranged from T3 Amaltash (*Cassia fistula*) (45.41) to T1 sarpgandha (*Rauvolfia serpentina*) (66.16) percent. However, significantly highest mycelial growth inhibition was recorded with T1 sarpgandha (*Rauvolfia serpentina*) (66.16). This was followed by T5

T5 Badoh (*Ipomoea carnea*)(62.97), T4 Karanj (*Pongomia pinnata*) (61.33),T2 Ashoka (*Saraca asoca*)(47.13) significantly least mycelia growth inhibition was recorded with T3 Amaltash (*Cassia fistula*) (45.41%) over untreated control (0.00%).

At 50% concentration growth inhibition was ranged from T3 Amaltash (*Cassia fistula*) (67.02%) to T1 sarpgandha (*Rauvolfia serpentina*) (76.47%) However significantly highest mycelial growth inhibition was recorded with T1 sarpgandha (*Rauvolfia serpentina*) (76.47%). This was followed by T5 Badoh (*Ipomoea carnea*) (75.22%), T4 Karanj (*Pongomia pinnata*) (72.27%), T2 Ashoka (*Saraca asoca*)(68.08%). Significantly least mycelial growth inhibition was recorded with T3 Amaltash (Cassia fistula) (67.02%) over untreated control (0.00%). A lot of work has been reported regarding the botanical extracts against *Alternaria* spp. From different places in different years (Shekhawat and Prasad. 1971; Sheikh and Agnihotri. 1972; Singh *et al*.1993; Tripathi and Shukla. 2002; Bhalona *et al*. 2011; Sitara *et al*. 2008; Sharma *at al*.2007; Kumar *et al*. 2006; Meena *et al*. 2010; Patni and Kolte. 2006; Siva *et al*. 2008; Sundar *et al*. 2010; Yanar *et al*.2011; Singh *et al*. 2013; Singh *et al*.2014; Sasode *et al*. 2012; Zaker , M. 2013; Satish *et al*. 2007; Yadav *et al*. 2019; Meena *et al*. 2020;)

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Table-1 : In-vitro effect of different plant extracts on mycelia growth and % inhibition of *A. brassicae.*

Treatment	Colony diameter (mm) at conc.		% inhibition at conc.	
	25%	50%	25%	50%
T_1 Sarpgandha (*Rauvolfia serpentina*)	**21.450 d**	**12.175 f**	**66.16**	**76.47**
T_2 Ashoka (*Saraca asoca*)	**38.575 b**	**19.725 c**	**47.13**	**68.08**
T_3 Amaltash (*Cassia fistula*)	**40.125 b**	**20.675 b**	**45.41**	**67.02**
T_4 Karanj (*Pongomia pinnata*)	**25.800 c**	**15.950 d**	**61.33**	**72.27**
T_5 Badoh (*Ipomoea carnea*)	**24.325 c**	**13.300 e**	**62.97**	**75.22**
T_0 Control	**90.00 a**	**90.00 a**		
F- test	**S**	**S**		
CD (0.05)	**2.15**	**0.77**		

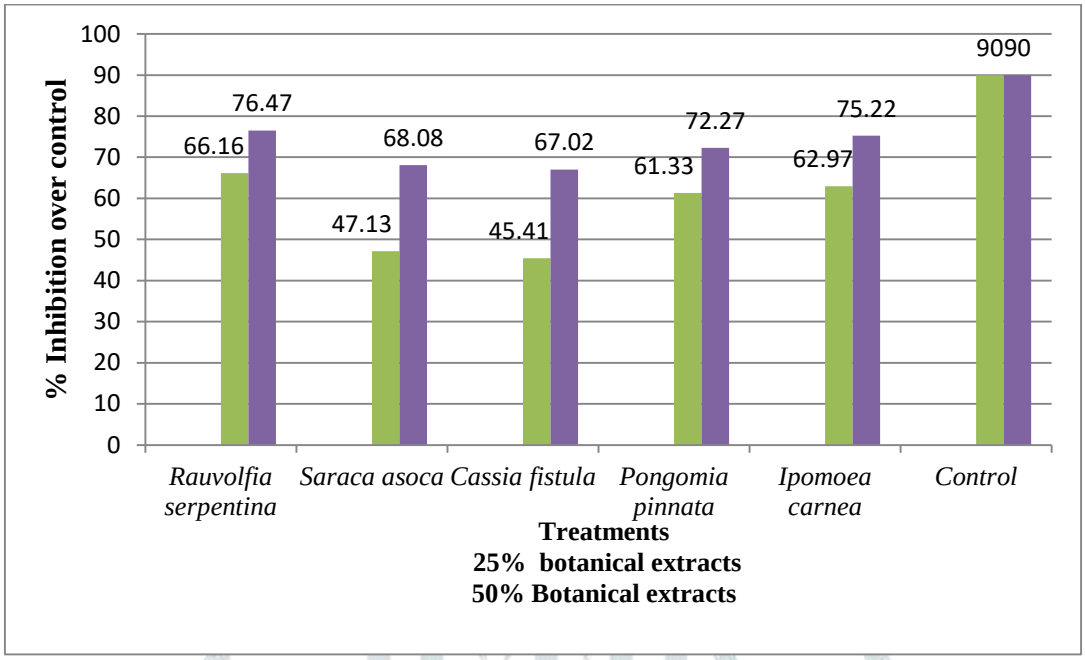

Figure 1: *In- vitro* evaluation of different botanicals against *Alternaria brassicae*

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