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Efficacy of plant extracts against *Alternaria brassicae* under *in- vitro* condition

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Abstract
Experiment was carried out during 2014-15 in the Plant Pathology laboratory, at Narendra Deva University of Agriculture & Technology Kumarganj Faizabad (U.P.). Alternaria blight caused Alternaria brassicae (Berk.) Sacc. is a serious disease of Indian mustard through out the country. Extracts of 54 plants was tested against their efficacy at 5% and 10% concentration *(in-vitro)***.** All the botanicals tested were effective showing different levels of toxicity against Alternaria brassicae in inhibiting the growth of pathogen significantly over control but the degree of inhibition varied from 0.00 *(check)* to 60.61 per cent (Garlic) at 5 per cent and 0.00 (check) to 72.21 (Garlic) at 10 per cent concentration. In this way the maximum inhibition was recorded through Garlic extracts at both the concentration tested fallowed by Eucalyptus. There was no any fungitoxicantic character in found better for the inhibitory effect against Aloevera the pathogens**.** All the 29 botanicals test extracts inhibited the spore germination of the pathogens. Garlic and Eucalyptus was most fungi toxic causing 100% inhibition at 5% and 10% concentration followed by Ashok (90.77% and 100%), Tulsi (87.44% and 100%), Datura (85.09% and 100%), Neem (82.44% and 100%), respectively.

Keywords: *Alternaria brassicae,* Plant extracts, Inhibition, Fungi toxic

Introduction
Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important oil seed crop, grown both in tropical and sub tropical regions of the world. It is a sourced of edible oil, which cannot be replaced. Among the world leader***s*** in rapeseed and mustard growing countries of the world are**,** India, Canada, China, Pakistan, Bangladesh, Germany, France, Sweden and Poland. In India, it had the area of 6.5 mha with production of 7.8 mt and productivity of 1208 kg/ha**,** contributes 28.3% and 19.8% in world acreage and production, respectively and had ranks second after China**.** In India**,** its cultivation is mainly confined to U.P., M.P., Rajasthan, Haryana, Assam, Gujarat, and West Bengal. In Uttar Pradesh it ***is*** grown on 6.39 lakh ha with production of 7.9 lakh metric tonnes and productivity of 1236 Kg/ha and had ranks third in area after M.P. and Rajasthan and second in production after Rajasthan. (Anonymous, 2013).

Among the reasons behind the lower productivity of the crop a number of fungal foliar diseases are most important ones. This crop suffers from devastating diseases like Alternaria blight, caused by *Alternaria brassicae* (Berk) Sacc**,** *Alternaria brassicicola* (Schwein) Wiltshire, white rust caused by *Albugo candida* (Pers. ex Lev.) Kuntze, downy mildew caused by *Peronospora brassicae* (Pers. ex Fr.) Fr. and powdery mildew caused by *Erysiphe cruciferarum* Opiz ex. Junell. These diseases individually or collectively cause greater loss in productivity. The damage due to Alternaria blight disease in the begining may not be prominent not significant but at a later stage of plant growth, the incidence of the disease becomes very high resulting in considerable losses. In severe cases plant may die. According to Saharan (1992), the yield losses to raya crop can be more where infection has taken place at an early stage of crop growth, heavy infection reduces plant height, number of primary and secondary branches, number of pods and number of seeds per pod. The seed remain shrivelled and oil content is reduced to the extent of 3 to 4 per cent.

Material Methods-
Isolation of pathogens from diseased plant tissues
The leaves of mustard having typical symptoms of Alternaria blight were collected and washed with sterilized water to clean and remove the surface contaminants. Small pieces from affected parts were cut and pre treated with 0.1% mercuric chloride solution followed by serial washing with sterilized water and placed the re... on sterilized blotters. These pieces were placed asepticaly on PDA plates and inocubated at 25± 2^0c for 7 days. The hyphal tips from advancing mycelium were picked and placed on slaunts having same media and again

incubated. This process was repetedly done to get the purest form through single spore culture technique.

The botanicals were collected from the surrounding area of NDUA&T Campus, kumarganj, Faizabad. The efficacy of various plant extracts against the *Alternaria brassicae* the 54 plants belonging to different families were tested. Ashoka Leaves *(Polyanthi longifolia),* Madar Leaves *(Calotropis procera* (Act.) R.Er.), Datura Leaves *(Datura stramonium* L.), Adrakh Rhizome *(Zingiber officinalis),* Eucalyptus Leaves *(Eucalyptus occidentalis* L.), Onion Bulb (*Allium cepa* L.*),* Neem Leaves *(Azadirachta indica* L), Tulsi Leaves *(Ocimum sanctum* L.), Parthenium Leaves *(Parthenium hysterophorus* L.), Mehandi Leaves *(Lawsonia inermis* L.), Pudina Leaves *(Mentha arvensis* L.), Garlic Bulb *(Allium sativum* L.*),* Kaner Leaves *(Thevetianeri folia* L.), Krishneel Leaves *(Anagallis arvensis* L.*),* Makoy Leaves *(Solanum nigrum* L.), Bhang Leaves *(Cannabis sativa* L.*),*Peppermint Leaves *(Mentha piperita* L.*)* Sarpgandha Leaves *(Rauwalfia serpentina* L.), Bael Leaves *(Aegle marmelos* L.), Karanj Leaves *(Pongama pinnata* L.), Pepper seed *(Piper nigrum* L.), Tomato Leaves *(Lycopersicon esculentum* Mil.), Rose Leaves *(Rosa chinensis* L), Burmuda grass Leaves *(Cynodon dactylon),* Gurhal Leaves *(Hibiscus rosasinesis* L.), Haldi Rhizome *(Curcuma longa* L.), Motha Leaves *(Cyperus rotundus* L.*),* Arjun Leaves *(Terminallia arjuna),*Aloe Leaves *(Aloe vera),* Karonda Leaves *(Carissa congesta),* Aonla Leaves *(Phyllanthus emblica,)* Peepal Leaves *(Ficusreligiosa* L.), Jamun Leaves *(Syzygium cumini*), Tree jasmine Leaves *(Jasminum arborescens* L.*),* Latjeera Leaves *(Achyranthes aspera),* Cotton Leaves *(Gossypium* sp.), Mustard Leaves(*Brassica campestris*L.), Mahua Leaves *(Madhuka longifolia),* Falsa/Phalsa Leaves (Grewia asiatica), Pigeon pea Leaves *(Cajanus cajan*), Teak Leaves *(Tectona grandis),* Litchi Leaves *(Litchi chinensis* L.), Marigold Leaves (*Tagetes erecta* L.), Motha Bulb *(Cyperus rotundus* L.*),* Ber Leaves *(Zizyhusmauritiana* L), Amaltas Leaves *(Cassia fistula* L), Bakiana Leaves *(Melia azadirachta* L.), Chenopodium Leaves *(Chenopodium album* L.), Satawar Leaves *(Asparagus racemosus* L.), Sissam Leaves *(Dalbergiasissoo* L.), Clerodendron Leaves *(Clerodendrum inerme* L.), Tantani Leaves *(Lantana camara* L.), Chilli Fruit *(Capsicum annum* L.) and Chilli Leaves *(Capsicum annum* L.). Fresh parts of the test plants were collected and washed thoroughly in clean water. Hundred grams of each washed samples were grinded in mortar and pestle by adding equal amount (100 ml) of sterilized distilled water (1:1 W/V) and boiled at 80^0C for 10 minutes in a hot water bath. The grinded material was filtered through muslin cloth followed by filtering through sterilized what man No. 1 filter paper and treated as standard plant extract (100 per cent) and then the mixture was diluted up to 5 per cent & 10 per cent concentration by adding proper volumes of sterilized water. All the plant extracts were tested at 5 per cent and 10 per cent concentration under *in vitro* conditions by using food poison technique to study the inhibitory effect of these botanicals against the mycelia growth of *Alternaria brassicae*.

Mycelial growth

All the plant extracts were tested at 5% and 10 per cent concentration under *in vitro* conditions by using food poison technique to study the inhibitory effect of these botanicals against the mycelial growth of *Alternaria brassicae.* PDA**,** medium (100ml) was autoclaved in 250 ml conical flask. The concentration of plant extracts were separately amended and thoroughly mixed after sterilization when it cooled (40^0C) under aseptic condition. Twenty ml medium was poured aseptically into each of the four Petri-dishes used for per treatment. Control treatment was maintained by pouring PDA medium without plant extracts. Three mm circular disc from 3 days old culture of *Alternaria brassicae* were cut with sterilized cork borer and placed in the centre of plant extracts amended Petri-dishes. The Petri-dish having PDA alone were inoculated in the same manner. These Petri-dishes were incubated at 25 ± 1^0C. The observations were recorded on radial growth after 10 days of incubation in each treatment.

Per cent growth inhibition was calculated by using formula (Vincent, 1947) given below

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

Where,

I = Per cent inhibition of fungal growth
C = Radial growth of control
T= Radial growth in treated Petri-dish

Spore germination

Cleaned and sterilized Petri dishes were taken and filter paper was placed in each Petri dish. Filter paper of each sterilized Petri-dishes were soaked with sterilized distilled water and grooved a slides was placed in horizontal position. After preparation of moist chamber two drops of spore suspension and plant product were placed in each groove of slides. They were applied by means of one to two ml pipette. Each drop was approximately 0.05ml liquid. After this, the treated Petri-plates were placed in incubator at 28 ± 2^0C temperature. After 24 hours germinated and non-germinated spores were counted and their percentage were calculated.

Result*s* and discussion

Effect of plant extracts against *Alternaria brassicae In vitro*

The 54 plants extracts namely Garlic (bulb), Eucalyptus, Parthenium, Onion (bulb), *Tulsi, Datura,* Marigold, *Mehandi, Ashok, Neem, Karanj, Madar, Peppermint,* Haldi (Rhizome), Motha bulb, *Sarpgandha,* Adhrakh (Rhizome), *Arjun, Jamun, etc.* belonging to the 35 families were tested for their efficacy at 5 per cent and 10 per cent concentration against the *Alternaria brassicae.*

The experiment was conducted in *in vitro* conditions to assay their effect on mycelial growth and spore germination of pathogen.

Effect on the mycelial growth

The effect of botanicals on the mycelial 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 significantly in comparison to control but the degree of inhibition varied from 0.00 to 60.61 per cent at 5 per cent and 0.00 to 72.21*%* at 10 per cent concentration. Extract of Garlic bulb (60.61%), Eucalyptus (58.83%), Ashok (55.27%) exhibited maximum inhibition to the mycelial growth of *Alternaria brassicae* at 5% concentration, respectively. These were followed by statistically significant with the each other *Datura* leaf (48.88), Neem leaf (48.05) and Clerodendron (48.32), which were at par. Rest of the plants showed either moderate or poor inhibition and accordingly they were categorised.

During screening, two plant extracts ***(***Garlic and Eucalyptus***)*** exhibited between 72.21 to 69.16%, 10 plants (Ashok, Madar, Tulsi, Datura, Neem, Mehndi, Pepermint Haldi, Motha Bulb,

and Marigold) exhibited between 69.16 to 61.05 per cent, 22 plants (Adharakh, Onion, Parthinium, Pudina, Krishneel, Bhang, Sarpgandha, Bael, Karanj, Peeper, Burmuda grass, Aonla, Peepal, Jamun, Litchi, Bakiana, Chenopodium, Satawar, Sissoo, Clerodendron, Motha leaves, and Tantani) exhibited between 60.20 to 45.02 per cent, 13 plants (Cotton leaf, Makoy leaf, Arjun leaf, Ber leaf, Pigeon Pea leaves, Tomato leaves, Chilli leaves, Chilli fruits, Mustard leaves, Karonda, Tree jasmine, Latjeera, and Kaner) exhibited between 42.83 to 32.62 per cent and 6 plants (Mahua Leaves, Teak, Falsa, Amaltas, and Gurahal and Rose) exhibition between 31.38 to 8.08 per cent inhibition of mycelia growth of the test pathogen at 10% concentration. Zero per cent inhibition was recorded in Aloevera extracts at both (5% and 10%) concentrations.

Over all, it was concluded that both Garlic as well as Eucalyptus are equally effective against alternariya blight of Indian mustard. Concurrent with present findings Singh and Tiwari (2007) have reported maximum inhibition of mycelial growth with different concentration of garlic bulb extracts, while Patni and Kolte (2006) and Singh *et al.* (2013) also reported maximum inhibition 92.5% and 77.80%, respectively with Eucalyptus leaf extract in mycelial growth of *Alternaria* brassicae. A lot of work has been reported regarding the botanical extracts against *Alternaria* spp. from different places in different years (Shekhawat and Prasad, 1971; Sheik and Agnihotri, 1977; Neetha Sharma and Sharma, 1992; Ram, 1997; Shivpuri *et al.* 1997; Kumar *et al.* 1998; Rao, 2006; Shenoi *et al.* 1998; Karade and Kalekar, 2000; Ferdous *et al.* 2002; Kota, 2003; Meena *et al.* 2004; Patni *et al.* 2005; Patni and Kolte 2006; Singh *et al.* 2007; Sitara *et al.* 2008; Meena and Sharma 2012; Sasode *et al.* 2012; Tawre *et al.* 2014, Singh and Singh, 2016).

Table 1: Effect of mycelial growth of plant extracts against *Alternaria brassicae in vitro* at different concentrations.

S. No.	Treatments	Reduction in mycelial growth			
		Concentrations			
		Mycelial growth (mm)		Per cent inhibition	
		5%	10%	5%	10%
1	Ashoka	40.25	28.62	55.27	68.19
2	Madar	50.25	34.60	44.16	61.55
3	Tulsi	42.50	31.12	52.77	65.41
4	Adrakh	53.00	36.12	41.10	59.85
5	Eucalyptus	37.05	27.50	58.83	69.16
6	Onion	52.75	36.00	41.38	59.99
7	Neem	46.75	32.05	48.05	64.38
8	Datura	46.00	31.52	48.88	64.96
9	Parthenium	62.25	45.12	30.83	49.88
10	Mehandi	49.00	33.75	45.55	62.66
11	Pudina	56.50	36.50	37.21	59.44
12	Garlic	35.44	25.00	60.61	72.21
13	Kaner	79.00	61.75	12.16	31.38
14	Krishneel	59.50	37.45	33.33	58.38
15	Makoy	70.75	54.25	21.38	39.71
16	Bhang	56.50	37.37	37.21	58.46
17	Peppermint	47.75	32.70	46.94	63.64
18	Sarpgandha	51.50	35.82	42.77	60.20
19	Bael	62.00	49.4	31.10	45.10
20	Karanj	56.00	41.2	37.77	54.21
21	Pepper	49.50	36.70	44.99	58.60
22	Tomato	66.75	56.00	25.82	37.77
23	Rose	80.25	65.25	10.99	27.49
24	Burmuda grass	53.25	44.00	40.83	51.11
25	Gurhal	86.00	82.50	4.44	8.08
26	Haldi	49.70	34.07	44.77	62.13
27	Motha leaves	61.50	49.50	30.55	45.02
28	Arjun	70.45	55.2	21.72	38.66
29	Aloe	90	90	-	-
30	Karonda	73.00	59.00	18.88	34.44
31	Aonla	62.75	37.07	30.27	58.24
32	Peepal	63.50	38.50	29.44	57.21
33	Jamun	68.95	37.87	23.38	57.91
34	Tree jasmine	76.95	60.25	14.49	33.65
35	Latjeera	78.45	61.2	12.80	32.62
36	Cotton	63.00	51.45	29.99	42.83
37	Mustard	72.2	58.00	19.77	35.55
38	Mahua	79.95	63.50	11.13	29.44
39	Falsa/Phalsa	82.25	70.75	8.60	21.39
40	Pigeon pea	71.70	55.87	20.33	37.91
41	Teak	81.45	68.25	9.45	24.16
42	Litchi	56.50	44.20	37.21	50.88
43	Marigold	51.15	35.05	43.16	61.05
44	Motha bulb	49.50	34.00	44.99	64.99

45	Ber	63.25	54.75	28.60	39.16
46	Amaltas	82.5	72.00	8.06	19.99
47	Bakiana	61.5	39.70	31.66	55.58
48	Chenopodium	63.5	38.75	29.44	56.93
49	Satawar	71.50	45.75	20.55	49.16
50	Sissaoo	71.75	49.00	20.27	45.55
51	Clerodendron	46.50	44.50	48.32	50.55
52	Tantani	59.50	41.00	33.88	55.42
53	Chilli leaves	67.85	56.5	26.35	37.21
54	Chilli fruit	72.10	57.75	19.22	35.82
55	Control	90.00	90.00	-	-
	SEm±	1.182	1.019	1.386	1.440
	C.D. (0.01)%	3.735	3.217	4.378	4.544

Table 2: Effect of spore germination of plant extracts against *Alternaria brassicae in vitro* at different concentrations.

S. No.	Treatments	Reduction in spore germination			
		Concentrations			
		Average number of spores germinated		Per cent inhibition	
		5%	10%	5%	10%
1	Ashoka	9.12	00.00	90.77	100
2	Madar	25.50	00.00	74.23	100
3	Datura	14.75	00.00	85.09	100
4	Adrakh	41.50	00.00	58.07	100
5	Eucalyptus	00.00	00.00	100	100
6	Onion	39.37	0.00	60.22	100
7	Neem	17.50	00.00	82.44	100
8	Tulsi	12.50	00.00	87.44	100
9	Parthenium	59.50	14.50	39.89	85.34
10	Mehandi	19.25	00.00	80.55	100
11	Pudina	63.50	23.59	35.85	75.26
12	Garlic	00.00	00.00	100	100
13	Kaner	71.12	39.50	28.02	60.09
14	Krishneel	72.12	39.50	27.64	60.09
15	Makoy	63.50	33.12	35.85	66.56
16	Bhang	61.50	19.50	37.87	80.04
17	Peppermint	17.50	00.00	82.44	100
18	Sarpgandha	36.50	00.00	63.12	100
19	Bael	49.50	14.37	49.99	85.59
20	Karanj	43.50	11.50	56.05	88.37
21	Peepal	62.50	22.75	36.86	77.01
22	Arjun	41.75	7.00	57.82	92.92
23	Jamun	42.75	9.25	56.81	90.65
24	Turmeric	23.50	00.00	76.25	100
25	Marigold	25.87	00.00	73.86	100
26	Motha Bulb	20.77	00.00	78.37	100
27	Motha Leaves	78.75	41.50	20.70	58.07
28	Burmuda Grass	79.12	42.25	20.07	57.32
29	Gurahal	66.50	33.50	32.82	66.16
30	Control	99	99	-	-
	SEm±	0.70	0.50	0.71	0.52
	C.D. at (0.01)	2.285	1.639	2.318	1.676

Effect on the spore germination

Per cent inhibition of spore germination was also worked out. both Eucalyptus as well as Garlic Extract did not allowed any spore germination at 5 and 10% concentration levels exhibiting cent- percent inhibition of spore germination (table-2). In this record our findings corroborates the findings reported by Patni *et al.* (2006) Kumar *et al.* **(**2006) have also reported the the inhibitory effect of botanicals against germination of *Alternaria brassicae* spores.

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