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In vitro bio-efficacy of botanicals against *Corynespora cassiicola* (Berk. and Curt.) Wei causing target leaf spot of soybean

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Abstract
Soybean target leaf spot [*Corynespora cassiicola* (Berk. and Curt.) Wei] is gaining importance in major soybean growing regions of Karnataka. To reduce the use of synthetic fungicides for management of the disease, an effort was made to evaluate the efficacy of different botanicals for their antifungal activity against the pathogen. The present *in vitro* study was conducted during September 2018 at Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka, India. Among the six different plant extracts tested, maximum mycelium inhibition was observed in garlic clove extract (73.72%) followed by ginger rhizome extract (65.68%). Whereas, least inhibition of mycelial growth was observed in datura leaf extract (12.74%). Among the commercially available botanicals, complete (100%) mycelial inhibition was observed in herbal mixture which is commercially known as Perfekt. Biological control through the use of plant products is a potential non-chemical means of controlling plant disease by reducing inoculum levels of the pathogens.

Keywords Soybean, target leaf spot, plant extract, botanicals and *Corynespora cassiicola*

Introduction
Glycine max (L.) Merrill commonly known as "soybean" is one of the important oilseed and pulse crops of India belongs to the family Leguminaceae. The crop is considered as wonder crop, golden bean and miracle bean of 20th century. In spite of its usage as a staple food by some of the community, the crop is also used in various industries. Major soybean producing countries are USA, Brazil, Argentina, China and India. In India the crop covers an area of 10.80 million hectare with a production of 12.10 million tonnes and a productivity of 1120 kg/ha (Anon., 2018) [1].

Diseases are one of the most important constraints for successful cultivation of soybean. It is known to be infected by more than 100 pathogens at various stages of crop growth (Hartman *et al*., 1986) [4] of which 35 are economically important including the most devastating disease like Asian rust (Gupta, 2004) [3]. Now a days, some of the minor diseases like target leaf spot are becoming major in soybean growing areas of Karnataka. The disease target leaf spot of soybean was first reported during 1945 in USA (Olive *et al*., 1945) [9]. Now it has been found in most of the important soybean growing states. The disease has also been reported from different countries like Cambodia, Canada, China, Japan and Nicaragua (Sinclair, 1982) [11]. In India, it was reported from Palampur during 1999-2000 and from Jabalpur during 2002-03. In Chhattisgarh it has been reported during 2002 from Raipur (Patel, 2005) [10].

The disease affects all the above ground plant parts like leaves, stems and pods. On leaves spots are rounded to irregular and dark brown in colour and size varies from small specks to big mature spots. These spots are surrounded by a dull green or yellowish green halo. At later stages the leaves become yellow and drop prematurely. On stem and petiole the spots are dark brown and spindle shaped. On pods the spots are mostly circular with slightly depressed having light brown centre and dark brown margin.

Use of fungicides for the management of disease in the absence of resistant genotypes is an old practice but, continuous use of chemicals will leads to problems like, pollution of water, air, soil, residual toxicity, development of fungicide resistant strains of the pathogen. Use of botanicals for the management of the disease is an environmental friendly, non-pollutive, non-phytotoxic, indigenously available, readily biodegradable, easily accessible and relatively cost-effective method on contrary to the use of synthetic fungicides. Hence the biological management is an important plant protection strategy.

Evaluation of plant extracts for knowing their antifungal activity against the pathogen is needed to reduce the use of synthetic fungicides and to obtain an integrated disease management strategy.

Material and Methods

Experimental site

The present laboratory experiment was carried out during September 2018 at Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India.

Isolation of test pathogen

The fresh infected leaves of soybean plant samples were cut into small pieces, surface sterilized with 0.1per cent mercuric chloride ($HgCl_2$) solution followed by three washing with sterile distilled water and placing in moist chamber. After 1 to 2 days fungal mycelium growth was seen, small bits of fungal mycelium was kept on the previously poured and solidified potato dextrose agar (PDA) medium in Petri plates. The plates were incubated at 28 ± 1^0C temperature in incubator.

In vitro evaluation of plant extracts and commercial botanicals by poisoned food technique

Different plant based botanicals were evaluated *in vitro* for their antifungal activity against *Corynespora cassiicola*, causing target leaf spot of soybean. The botanicals used in this experimentation are detailed as under.

Table 1: Common and Scientific name of Plant parts used

Sl. No.	Common name	Scientific name	Plant parts used
1	Garlic	*Allium sativum* L.	Cloves
2	Ginger	*Zingiber officinale* Roscoe	Rhizome
3	Onion	*Allium cepa* A. Juss.	Bulbs
4	Neem	*Azadirachta indica* L.	Leaves
5	Datura	*Datura stramonium* L.	Leaves
6	Turmeric	*Curcuma longa* L.	Rhizome

Table 2: Commercially available botanicals

SI. No.	Product name	Contents
1	Nimbicidine	Azadirachtin 0.03%
2	Neem ashirvad	Herbal extraction of neem seed kernel
3	Multineem	Azadiractin 0.15%
4	Perfekt	Herbal mixture
5	Crude pongamia oil	-

Preparation of cold aqueous extract from different botanicals

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water (1:1 w/v). The extract was filtered through two layers of muslin cloth and then centrifuged at 10,000 rpm. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts, poisoned food technique was used (Nene and Thapliyal, 1973) [8]. Ten, fifteen and twenty ml of stock solution was mixed with 90, 85 and 80 ml of sterilized molten PDA medium respectively, so as to get 10, 15 and 20 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. To avoid bacterial contamination a little amount of streptomycin was added in each flask before plating.

Commercially available botanicals

The efficacy of five locally available commercial botanicals were tested against *Corynespora cassiicola* by using poisoned food technique. Two, four and six ml of commercially available botanicals were mixed with 98, 96 and 94 ml of sterilized molten PDA medium respectively, to get 2, 4 and 6 per cent concentration. The medium was thoroughly shaken for uniform mixing of extract. To avoid bacterial contamination a little amount of streptomycin was added in each flask before plating.

Twenty ml of the poisoned medium was poured into sterile Petri plates, mycelium of five mm size discs from periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed in the center of each Petri plate containing media. Controls were also maintained by growing the pathogen on PDA plates. Each treatment was replicated four times. Then such plates were incubated at 28 ± 1 ^{0}C temperature and radial growth was taken when maximum growth occurred in the control plates.

The efficacy of botanicals was expressed as per cent inhibition of radial growth mycelium over control which was calculated by using the formula given by Vincent (1947) [12].

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

Where

I = Inhibition of mycelial growth (%)

C = Radial growth of mycelium in control (cm).

T = Radial growth of mycelium in treatment (cm).

Statistical analysis

All the data of per cent mycelial inhibition were statistically analysed by the following procedure of factorial completely randomized design (FCRD). The per cent data of mycelial inhibition were transformed to Arc sine value. Calculations were made after applying the test of significance of the means.

Results and Discussions

In vitro evaluation of plant extracts against *Corynespora cassiicola*

Efficacy of six different plant extracts were evaluated against *C. cassiicola* causing target leaf spot of soybean. Among the plant extracts tested, maximum mycelium inhibition was observed in garlic clove extract (73.72%), which was statistically superior over rest of the treatments. However, ginger rhizome extract showed an inhibition of 65.68 per cent which was statistically on par with neem leaf extracts (62.64%), followed by turmeric rhizome extract (44.11%). Whereas. least inhibition of mycelial growth was observed in datura leaf extract (12.74%) (Table 3, Plate 1 and Fig. 1).

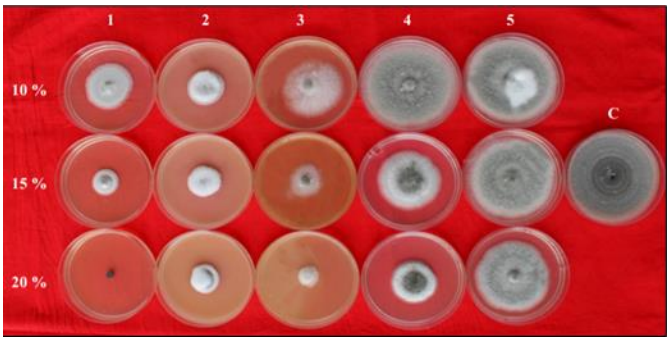
At ten per cent concentration maximum mycelial inhibition was observed in ginger (59.41%), followed by garlic (46.18%) and neem leaf extract (36.47%). Least mycelial inhibition was recorded in datura (4.12%). At fifteen per cent concentration maximum inhibition was observed in garlic (75.00%) followed by neem (70.88%) and ginger (67.06%). Least inhibition was observed in datura (10.29%). At twenty per cent concentration complete inhibition was observed in garlic which was significantly superior to rest of the botanicals used followed by turmeric (85.00%), neem (80.59%) and ginger (70.59%). Least mycelial inhibition was observed in datura (23.82%).

The efficacy of plant extracts against *C. cassiicola* revealed that, all the plant extracts showed differential inhibitory action based on the concentration used and were less effective at lower concentration (10 and 15%) as compared to their higher concentration (20%).

Table 3: *In vitro* evaluation of plant extracts against *Corynespora cassiicola*

Sl. No.	Common name	Scientific name	Plant parts used	Inhibition of mycelium growth (%)			
				Concentration (%)			Mean
				10	15	20	
11	Garlic	*Allium sativum* L.	Cloves	46.18 (42.81)*	75.00 (60.01)	100.00 (90.00)	73.72 (64.27)
22	Ginger	*Zingiber officinale* Roscoe	Rhizome	59.41 (50.43)	67.06 (54.98)	70.59 (57.16)	65.68 (54.18)
33	Neem	*Azadirachta indica* Juss.	Leaves	36.47 (37.14)	70.88 (57.35)	80.59 (63.87)	62.64 (52.78)
44	Turmeric	*Curcuma longa* L.	Rhizome	18.82 (25.70)	28.53 (32.28)	85.00 (67.22)	44.11 (41.73)
55	Onion	*Allium cepa* L.	Bulbs	5.29 (13.28)	32.65 (34.79)	59.12 (50.26)	32.35 (32.77)
66	Datura	*Datura stramonium* L.	Leaves	4.12 (11.55)	10.29 (18.68)	23.82 (29.21)	12.74 (19.81)
Mean				28.38 (30.15)	47.40 (43.01)	69.85 (59.61)	48.54 (44.25)
Sources						S.Em. ±	CD @ 1%
Botanical (B)						0.38	1.43
Concentration (C)						0.27	1.01
Botanical × Concentration (B×C)						0.66	2.49

* Angular transformed values



1) Garlic 2) Ginger 3) Neem 4) Onion 5) Datura C) Control

Plate 1: *In vitro* evaluation of plant extracts against *Corynespora cassiicola*

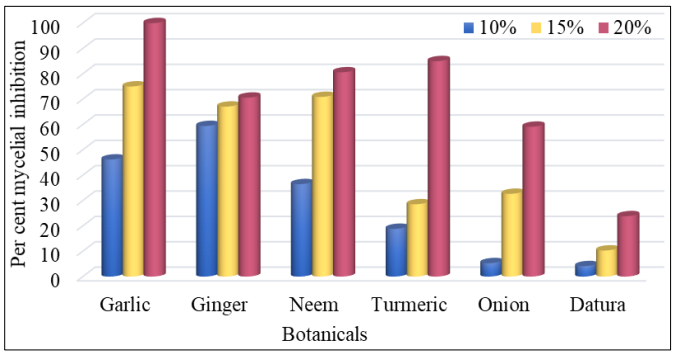


Fig 1: *In vitro* evaluation of plant extracts against *Corynespora cassiicola*

Commercially available botanicals

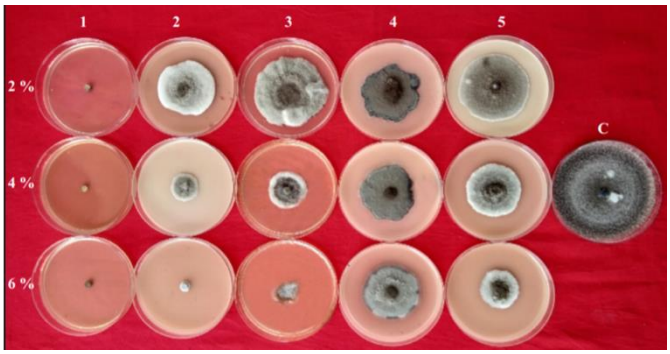
Efficacy of five commercially available botanicals were evaluated for inhibition of *C. cassiicola* at three concentrations (2, 4 and 6%). The results revealed that, complete (100%) mycelial inhibition was observed in herbal mixture which is commercially known as Perfekt at all the three concentrations tested, which was significantly superior to all other treatments, followed by Multineemore (66.37%) and Crude pongamia oil (59.80%). Whereas, least inhibition was observed in Neem ashirvad (47.74%). The results obtained are presented in Table 4, Plate 2 and Fig. 2.

Results of *in vitro* evaluation of commercially available botanicals against *C. cassiicola* revealed that a herbal mixture which is commercially known as Perfekt showed complete (100%) mycelial inhibition at all the three concentrations tested and it was significantly superior over rest of the botanicals used. Remaining botanicals were concentration dependent, which showed less inhibition at lower concentration as compared to their higher concentration.

Table 4: *In vitro* evaluation of commercially available botanicals against *Corynespora cassiicola*

Sl. No.	Commercially available botanicals	Active ingredient	Inhibition of mycelial growth (%)			
			Concentration (%)			Mean
			2	4	6	
1	Perfekt	Herbal mixture	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
2	Multineemore	Azadiractin 0.15%	38.82 (38.54)	72.06 (58.11)	88.24 (69.94)	66.37 (55.53)
3	Crude pongamia oil	-	42.94 (40.94)	64.41 (53.37)	72.06 (58.09)	59.80 (50.80)
4	Nimbicidine	Azadiractin 0.03%	38.53 (38.33)	49.41 (44.66)	66.18 (54.45)	51.37 (45.81)
5	Neem ashirvad	Herbal extraction of neem seed kernel	43.53 (41.28)	47.35 (43.48)	52.35 (46.34)	47.74 (43.70)
Mean			52.76 (49.82)	66.64 (57.92)	75.76 (63.76)	65.05 (57.16)
Sources					S.Em. ±	C.D @ 1%
Botanical (B)					0.33	1.26
Concentration (C)					0.25	0.98
Botanical × Concentration (B×C)					0.57	2.19

* Angular transformed values



1) Perfekt 2) Multineemore 3) Pongamia oil 4) Neem ashirvad 5) Nimbicidine C) Control

Plate 2: *In vitro* evaluation of commercially available botanicals against *Corynespora cassiicola*

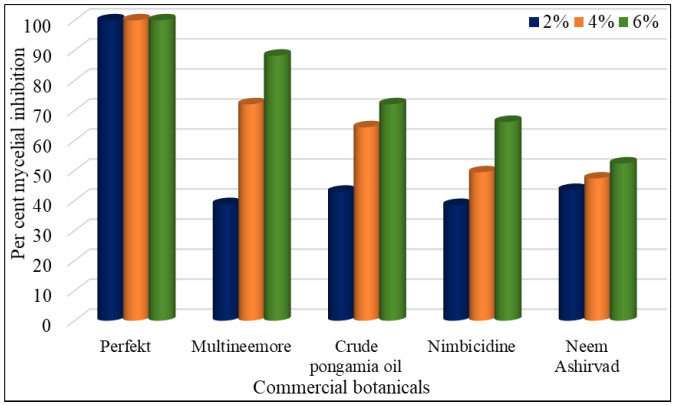


Fig 2: *In vitro* evaluation of commercially available botanicals against *Corynespora cassiicola*

The results are in confirmative with Manju *et al.* (2014) [7] who studied the efficacy of different plant extract against *C. cassiicola* and reported that garlic clove extract (60.50%) found to be best for inhibiting the mycelial growth of the pathogen. Similar results were also obtained from Kumar *et al.* (1979) [6]. Lakshmanan (1990) evaluated ten plant extracts to know their antifungal property against *C. cassiicola* causing boll rot of cotton. Among the plant extracts tested, maximum inhibition of 95.80 per cent of mycelial growth and 78.60 per cent sporulation was observed from garlic clove extract, this might be due to the presence of antifungal compound in *Allium sativum* known as Allicin which contain sulphur and has strong toxic properties against *C. cassiicola*. Biological control through the use of plant products and antagonistic microorganisms is a potential non-chemical means of controlling plant disease by reducing inoculum levels of the pathogens. These are the cheaper and safe means of disease management which reduce not only toxicity hazards but also render eco-friendly disease management approach (Kumar and Gupta, 1999) [6].

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