Original Research Article

Isolation of *Cercospora canescens* and Management of Cercospora Leaf Spot (*Cercospora canescens*) of Mothbean Through Botanicals

ABSTRACT

The pathogen was isolated from diseased leaves of mothbean on potato dextrose agar by standard tissue isolation method and pathogenicity was proved following Koch's postulates. On the basis of morphological studies like mycelium was whitish to brownish, hyaline and acircular, straight to variously curved, multiseptate (2-16) many celled, thread like conidia and cultural studies the pathogen was identified as *Cercospora canescens* Ellis and Martin. Among eight botanicals tested at three concentrations (5, 10 and 15 %) under *in vitro* condition against *C. canescens*, neem seed kernel extract was found most effective and inhibiting mycelial growth (58.00 %) at 15% concentration followed by tulsi leaf extract (52.96%) and neem leaf extract (44.68%) also found effective. Least mycelial growth inhibition of *C. canescens* was obtained in turmeric extract (27.31%) and giloy (25.31%) extract at 15% concentration.

Keywords:- Isolation, *Cercospora canescens* , Botanicals, Mothbean, Cercospora leaf spot

INTRODUCTION

Moth bean [*Vigna aconitifolia* (Jacq.) Maréchal], a native crop of the Indian subcontinent is capable of tolerate long dry spells and high temperatures upto 45°C. However, low rains and diseases affect its growth and production drastically often restricting the productivity much below its potential (>200 Kg/ha). Rajasthan, has the highest area (98.25%) and production (97.04%) of moth bean in the country (Patel *et al.,* 2019).

Cercospora leaf spot is an important foliar disease of Mothbean caused by *Cercospora* canescens. The disease was reported first time in Delhi, India (Munjal *et al*., 1962) and can be occurred in all parts of the humid tropical areas of Asia and many other countries. Maximum loss of 61 percentage was observed in Pakistan in case of grain yield (Iqbal *et al*., 1995). In the case of Cercospora leaf spot, infected leaves (especially those more mature) look for brown or rust-colored lesions that vary from circular to angular, are 2-10 mm. Lesions may have a greyish or whitish center with a slightly reddish border. Conidia develop at the center on short conidiophores. Severely affected leaves become chlorotic. Lesions may dry and portions may fall out, giving the leaf a shot-hole appearance. Lesions and blemishes may occur on branches, stems and pods. *Cercospora spp.* they are not easily grown in synthetic media and few species have been successfully isolated so far.

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Sporulation is very scanty and hence, it is necessary to study the sporulation of Cercospora canescens.

Continuous use of fungicides has led to various environmental problems and harmful effect on human health and soil. To sort out these problems plant extracts were observed in laboratory against *C. canescens*. Botanicals are environmentally safe and cheaper means of integrated disease management. Phytoextracts induce resistance in plants against many pathogenic fungi, which is necessary for economically viable and safe alternative means of disease control.

MATERIALS AND METHODS

Isolation and purification

Collection of Cercospora leaf spot infected samples and isolation of *Cercospora canescens* from infested mothbean plants were carried out from mothbean field. The leaf spot causing pathogen was isolated by following standard tissue isolation method. The infected leaves were cut into small leaf bits and surface sterilized in 0.1% sodium hypochlorite solution in Petri dishes for 1-2 minutes followed by repeated washing in sterilized distilled water to remove the excess of mercuric chloride. Then the infected leaf bits were transferred on petri dishes. 1-2 leaf bits were transferred aseptically on potato dextrose agar (PDA) medium in Petri dishes with the help of a sterile forceps and incubated at 27±1°C for 16 days kept in BOD incubator.

Observed periodically for fungal growth and sporulation. Colonies, which developed from the bits, were identified by microscopic observation by taking mycelial and spore character as means for identifying the pathogen. After identification they were transferred to new PDA slants and incubated at 27±1°C for further use.

Pathogenicity

Pathogenicity of the purified cultures of *C. canescens* was tested by growing mothbean plants in pots were sprayed first with distilled water. Twenty five days old plants were sprayed first with distilled water. They were then covered with polythene bags for 24 hours. The mycelial bits of the fungus from 15 day old culture were smeared on leaf surface using cotton plug. Suitable control plants sprayed with sterile distilled water were maintained. The seedlings were kept under greenhouse conditions at 25°C 95% of RH for 120 hours by covering with polythene bags, to ensure successful entry for pathogen into the host. The polythene bags were removed after 120 hours. Regular observations were made for the development of symptoms. The symptoms appeared within 10 days and the fungus was re-isolated from the infected leaves and the culture obtained was compared with the original to confirm the identity.

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The fungus was sub-cultured on Potato dextrose agar slants and allowed to grow at 25°C for 16 days and such slants were preserved in a refrigerator at 4°C and sub-cultured once in 30 days.

In vitro evaluation of plant extracts

Plant based pesticides which are relatively economical, safe and non-hazardous can be used successfully against the plant pathogenic fungi. In this experiment, different plant extracts were tested for their efficacy at the concentration of 5, 10 and 15 percent against *Cercospora canescens* under *in vitro* condition.

Chart 1 : List of plant extract and their use

Plant extract	**Botanical Name**	**Plant part used**
$T_{1;}$ Neem leaf extract	*Azadirachta indica*	Leaf
$T_{2;}$ Turmeric extract	*Curcuma longa*	Rhizome
$T_{3;}$ Tulsi leaf extract	*Ocimum tenuiflorum*	Leaf
$T_{4;}$ Ginger extract	*Zingiber officinale*	Rhizome
$T_{5;}$ Onion bulb extract	*Allium cepa*	Bulb
$T_{6;}$ NSKE	*Azadirachta indica*	Seed
$T_{7;}$ Tumba extract	*Citrullus colocynthis*	Fruit
$T_{8;}$ Giloy extract	*Tinospora cordifolia*	Stem
$T_{9;}$ Control	-	-

Preparation of plant extract

Selected plants were collected from the surrounding areas of Bikaner, and washed thoroughly with tap water and air dried. One hundred gram of plant part was grind using pestle and mortar by adding equal amount (100 ml) of sterilized distilled water (1:1, w/v). The pulverized mass was squeezed through the cheese cloth and the extracts were centrifuged at 10000 rpm for 5 - 10 minutes and used as stock solution. To study the antifungal property of plant extracts, the poisoned food technique was adopted. The stock solutions of different botanicals (5, 10 and 15%) were mixed with 95, 90, and 85 ml of PDA media, respectively so as to get 5, 10 and 15 per cent concentrations and sterilized. Twenty ml of such medium was poured under aseptic conditions into sterile Petri plates allowed to solidify. Mycelial discs (5mm) were cut out using sterile cork borer from periphery of actively growing culture of *Cercospora canescens* and one such disc was placed on the centre of each Petri plate. The treatments were replicated thrice. Control was maintained by growing the pathogen on PDA plates without plant extracts. Plates were incubated at room temperature (28±2°C) for 7 days and radial growth was taken at the time when maximum growth occurred in the control plates. The efficacy of plant products or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the Vincent (1947) formula.

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Formula;

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

I = Per cent inhibition

C = Radial growth of *C. canescens* in control (mm)

T = Radial growth of *C. canescens* in presence of plant extracts (mm)

RESULTS AND DISCUSSION

Collection of disease sample

The Cercospora leaf spot diseased leaf samples were collected from mothbean field of Experimental Farm, College of Agriculture, Bikaner and subjected to isolate the causal organism. The causal organism isolated from diseased leaves was used in further investigations.

Isolation

Isolation of the causal organism from diseased mothbean leaves showing typical symptoms of Cercospora leaf spot. Isolation was done in Petri dishes using Potato Dextrose Agar (PDA) medium. The surface sterilized of diseased leaf bits yielded the fungus after 16 days at 25°C and sub-cultured once in 30 days.

Purification of the fungus

The isolated pathogen was purified by repeated isolation from the culture plates (Plate 1). The fungus was identified on the basis of mycelial characters and spore morphology. The colonies generally appeared to be creamy whitish colour. The isolate pathogen grow very slow on potato dextrose agar medium. Based on morphological and pathological characters the pathogen was identified as *Cercospora canescens* Ell. & Mart.

Pathogenicity

The pure culture of the *Cercospora canescens* was used for prove the pathogenicity. The fungus was spray and artificial inoculation of the pathogen on leaves as described in Materials and Methods to prove the Koch's postulates and symptom expression. The characteristic disease symptoms appeared ten days after inoculation (Plate 2). Initially, the minute specks appeared on the leaf which developed into irregular, reddish brown spots and later whitish spots with narrow reddish brown margin. The symptoms appeared mainly on mature leaves and then young leaves. The pathogen was re-isolated from infected leaves and confirmed its, identity by comparing with original culture of *Cercospora canescens.*

In vitro evaluation of plant extracts

Eight plant extracts were evaluated at three concentrations in the laboratory for their efficacy against *C. canescens* through poison food technique. The data are presented in Table 1, depicted in Fig 1 and Plate 3. Amongst the eight plant extracts evaluated, neem seed kernel extract at 15, 10 and 5 per cent concentration was found best in inhibiting the mycelial growth of *C. canescens* (58.28%, 49.21% and 41.40%) and found significantly superior over all the other extracts, followed by tulsi leaf extract (52.96%, 40.93% and 34.21%), neem leaf extract (44.68%, 37.18% and 30.00%), ginger extract (40.93%, 31.71% and 25.00%), onion bulb extract (39.68%, 30.46% and 22.50%) and tumba extract (32.03%, 22.81% and 14.37%). No more effective inhibition of mycelial growth of *C. canescens* was recorded in turmeric extract (27.65%, 20.00% and 13.43%) and giloy extract (25.31%, 19.38% and 10.31%) at all concentrations.

The pathogen *C. canescens* was isolated from diseased leaf by following the standard tissue isolation method. The pure culture obtained from sub-cultured on petri dishes and slants containing potato dextrose agar and keep in refrigerator for use in more other studies. The culture on potato dextrose agar produced creamy whitish growth of fungus. The fungus colour was initially whitish, later turn to dark black colour suggesting possible secretion of Cercosporin toxin by the pathogen. Various workers (Khandar *et al.,* 1985 and Chandrasekharan and Rangaswami, 1960) have isolated the fungus pathogen from infected leaf.

The identification of the pathogen on the basis of important morphological characters such as; shape, size and septations of conidiophores and conidia. The morphological characters given by Chupp (1953) were compared with the pathogen under investigations and they were found to be identical, hence the pathogen was identified as *C. canescens*.

The pathogenicity was tested by artificially inoculating *C. canescens* on mothbean plants in pots. In the present research work, mycelial and conidial suspension of *C. canescens* was sprayed on 30-35 days old mothbean plants and the symptoms were observed after 15 days of inoculation. Initially the symptoms appeared as minute brown specks, then changed into irregular grey to dirty grey spots with reddish brown margin on leaf surface. The pathogen was re-isolated from the diseased leaves and identity of the fungus was confirmed by comparing with the pure culture of *C. canescens*.

Munjal *et al.,* (1962) observed that, the symptoms showed first on mature leaves and later progressed upward on to younger leaves. Similar symptoms noticed by many workers (Chandrasekhara and Rangaswami, 1960; Vakili, 1977; Jamadar, 1988).

Botanicals are environmentally safe and cheaper means of integrated disease management. In the present investigation, eight plant extracts were evaluated under *in vitro* condition against *C. canescens* to know the efficacy on pathogen. In present

investigation, complete inhibition of the pathogen was not observed in any of the plant extracts, but considerable amount of mycelial inhibition was observed in some of them. Among plant extracts tested against *C. canescens*, neem seed kernel extract was effective and maximum inhibiting mycelial growth (58.00%, 49.21% and 41.40%) at 15%, 10% and 5% concentrations. Further, tulsi leaf extract (52.96%, 40.93% and 34.21%) and neem leaf extract (44.68%, 37.18% and 30.00%) inhibiting mycelial growth at 15%, 10% and 5% concentrations also effective. Minimum inhibition of mycelial growth was recorded at all three concentrations of in turmeric extract (27.65%, 20.00% and 13.43%) and giloy extract (25.31%, 19.38% and 10.31%). Similar results were obtained by Reddy *et al.,* (2009) observed that neem oil inhibited the mycelial growth of *Cercospora moricola.* The findings of this trial are in agreement with the results of Khalil and Javeed (2019) observed that *Azadirachta indica* 74.40% and *Allium cepa* 33.80% growth inhibition of mycelium at 25% concentration. Tripathy *et al.,* (2018) reported that minimum growth of the pathogen used clove extracts of Garlic, finger extract of Ginger followed by neem and onion extract against leaf blight disease of mung bean caused by *Macrophomina phaseolina.*

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Table: 1 Efficacy of plant extracts against *Cercospora canescens* under *in vitro* conditions

Treatment	Mycelial growth (mm) at different conc.			Per cent Growth inhibition at different conc.		
	5%	10%	15%	5%	10%	15%
$T_{1;}$ *Azadirachta indica* (Neem leaf extract)	44.80 (41.99)*	40.20 (39.33)	35.40 (36.50)	30.00	37.18	44.68
$T_{2;}$ *Curcuma longa* (Turmeric extract)	55.40 (48.08)	51.20 (45.67)	46.30 (42.86)	13.43	20.00	27.65
$T_{3;}$ *Ocimum tenuiflorum* (Tulsi leaf extract)	42.10 (40.44)	37.80 (37.92)	30.10 (33.26)	34.21	40.93	52.96
$T_{4;}$ *Zingiber officinale* (Ginger extract)	48.00 (43.84)	43.70 (41.36)	37.80 (37.92)	25.00	31.71	40.93
$T_{5;}$ *Allium cepa* (Onion bulb extract)	49.6 (44.75)	44.50 (41.82)	38.60 (38.39)	22.50	30.46	39.68
$T_{6;}$ *Azadirachta indica* (NSKE)	37.50 (37.75)	32.50 (34.74)	26.70 (31.10)	41.40	49.21	58.28
$T_{7;}$ *Citrullus colocynthis* (Tumba extract)	54.80 (47.73)	49.40 (44.64)	43.50 (41.25)	14.37	22.81	32.03
$T_{8;}$ *Tinospora cordifolia* (Giloy extract)	57.40 (49.24)	51.40 (45.78)	47.80 (43.72)	10.31	19.68	25.31
$T_{9;}$ Control	64.00 (53.11)	64.00 (53.11)	64.00 (53.11)	-	-	-
S.Em ±	0.49	0.46	0.39			
C.D (P=0.05)	1.47	1.41	1.18			

*Figures in parenthesis are angular transformed value

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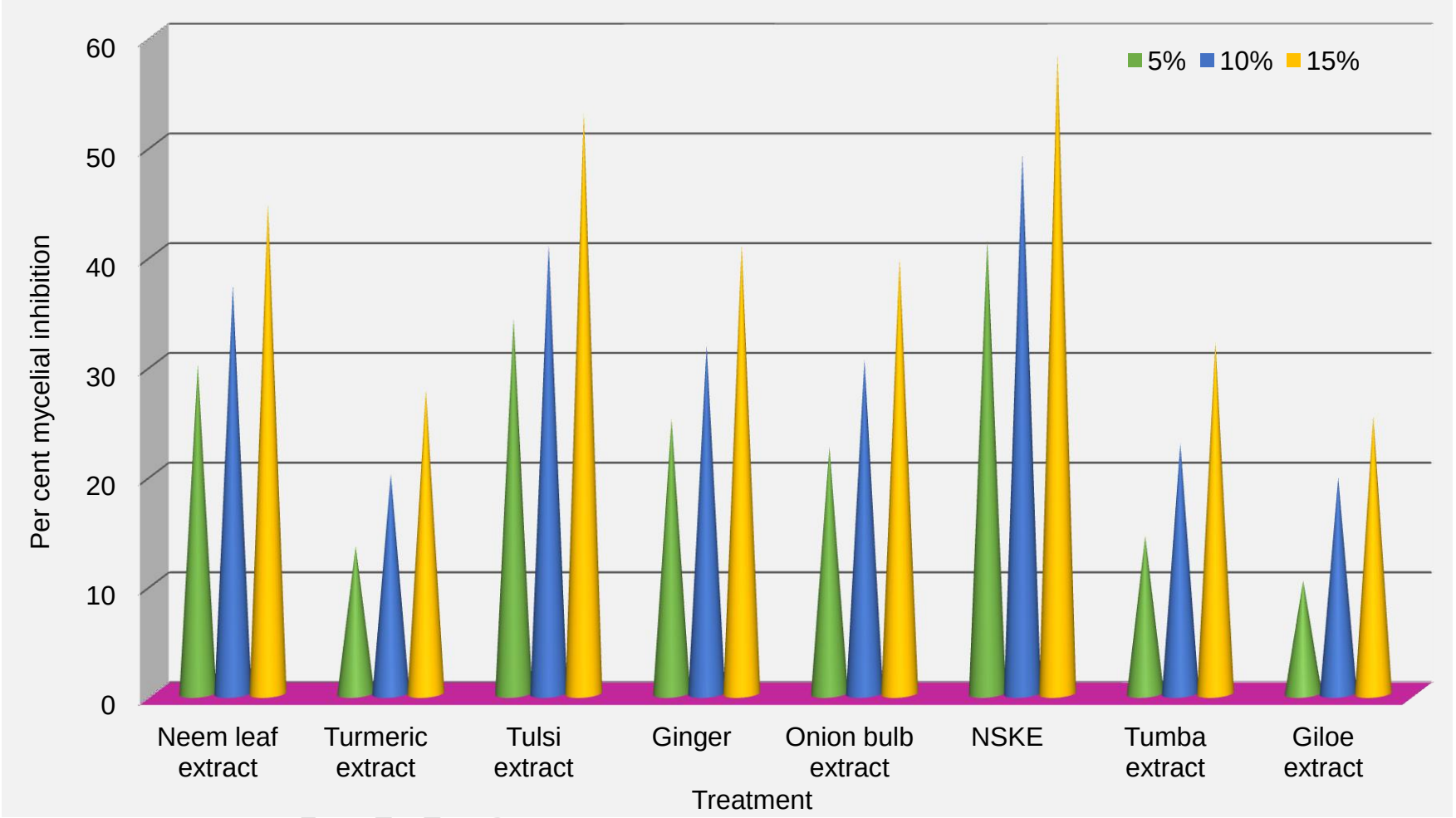
Figure: 1 Efficacy of plant extracts against *Cercospora canescens* under *in vitro* conditions



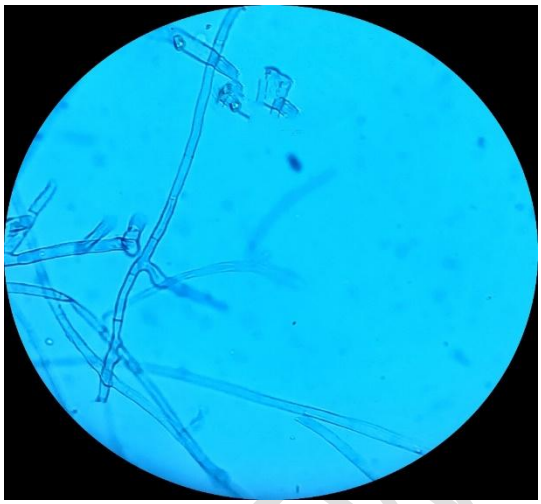


Plate: 1 (A) View of pure culture Plate (B) Microscopic view

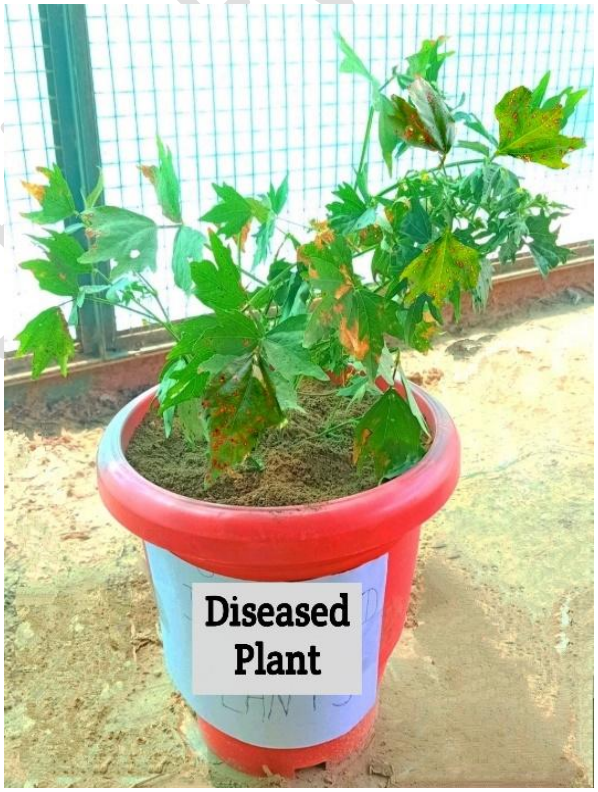


Plate: 2 (A) Healthy plant (B) Diseased plant

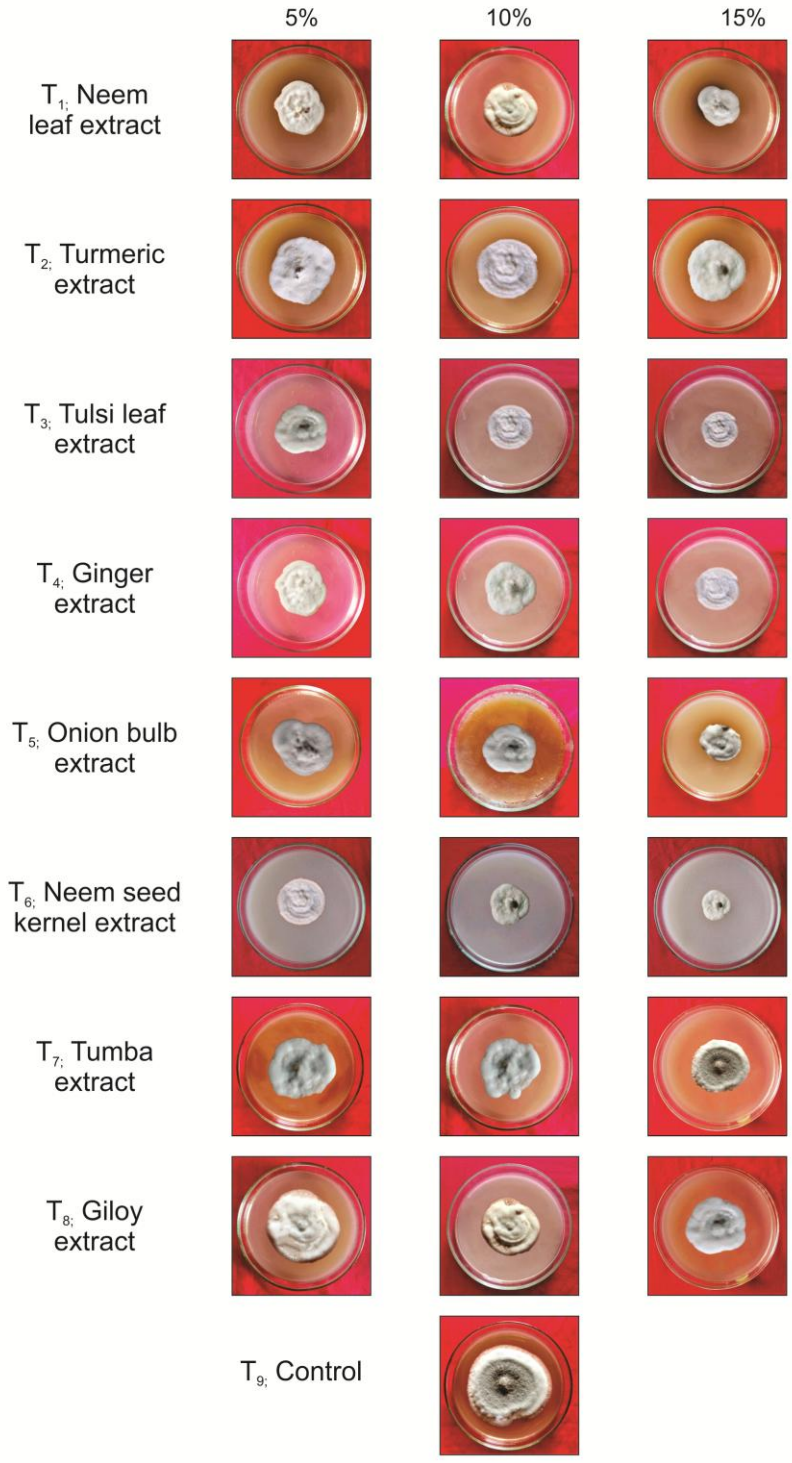


Plate: 3 Efficacy of plant extracts against *Cercospora canescens* under *in vitro* conditions