

Original Research Article

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***In vitro* Evaluation of Fungicides Plant Extracts and Bio-agents against *Alternaria helianthi* causing Leaf Blight of Sunflower**

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Alternaria leaf blight of sunflower caused by *Alternaria helianthi* is an important disease of sunflower. The study conducted on *in vitro* evaluation of new molecules of fungicides and plant extracts against *Alternaria helianthi*, causal agent of *Alternaria* blight of sunflower. *In vitro* evaluation of fungicides revealed that, among four systemic fungicides hexaconazole and propiconazole were highly effective in inhibiting the mycelial growth with 100% inhibition among non systemic fungicides the maximum inhibition of mycelial growth was found in captan (86.30%) at 600 ppm concentration followed by copper oxy chloride (84.07%) whereas, among the botanicals neem seed kernel extracts at 10 per cent(43.74%) showed positive effect on inhibition of pathogen mycelium growth followed by neem leaf extracts(25.26%). *Trichoderma harzianum* shows the best bio agents against *Alternaria helianthi* with highest per cent inhibition (72.48%).

Introduction

Sunflower (*Helianthis annuus* L.) belongs to the family Asteraceae and it is one of the most important edible oilseed crops in India. It is a hardy and an herbaceous annual, which grows up to a height of 5 feet. The word *Helianthus* is derived from Greek word ‘Helios’ (sun) and ‘anthos’ (flower). Presently in India Sunflower crop is cultivated in an area of 0.487 million hectares with a production of 296 MT and a productivity of 608 kg/ha

(Anon, 2017). The *Alternaria* leaf blight is a major disease of sunflower which causes an average yield and oil losses in India ranged from 28 to 80 per cent and 31 to 34 per cent, respectively. There is very negligible resistant variety/hybrid available against this disease on sunflower. Thus it has become inevitable to go for fungicidal spray for the management of the disease. In the present study some of the new chemicals were tested in the laboratory to find out their efficacy against *A. helianthi*.

Materials and Methods

A study has been conducted at Department of Plant Pathology, College of Agriculture Navile, Shimoga to know the efficacy of systemic fungicides, non-systemic fungicides and leaf extracts (four each) against *A. helianthi* was assessed by following poison food technique. Both Systemic fungicides and non-systemic fungicides were evaluated at 100, 200, 400 and 600 ppm concentrations and plant extracts were taken at 2.5, 5 and 10 percent each were evaluated. These were assessed by Poison food technique and the efficacy of the fungicides was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the formula.

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

Where,

I = per cent inhibition

C = pathogen growth in control plate

T = pathogen growth in treatment plate

The plant extracts were prepared by grinding fresh leaves in a pestle and mortar by using sterile distilled water. The extract was filtered through double layered muslin cloth and made to the required concentration by adding distilled water.

The details of fungicides / plant extracts tested are given below.

Non systemic fungicides		
Sl. No.	Fungicides	Trade Name
1	Captan 50 WP	Captaf
2	Chlorothalonil 75 WP	Kavach
3	Copper oxy chloride 50 WP	Blitox
4	Mancozeb 75 WP	Indofil M-45

Systemic fungicides		
Sl. No.	Common Name	Trade Name
1	Carbendazim 50 WP	Bavistin
2	Hexaconazole 5 EC	Nectar
3	Propiconazole 25 EC	Tilt
4	Thiophanate methyl 75WP	Roko

Preparation of cold aqueous extract

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 distilled water (1:1 w/v). The extracts were filtered through two layers of muslin cloth and then through Whatman No. 1 filter paper. Finally filtrate thus obtained was used as stock solution. Nimbicidin as commercial formulation available in the market was used.

To study the compatibility as well the antifungal mechanism of plant extracts the poisoned food technique was used (Nene and Thapliyal, 1982). The efficacy of plant products or botanicals was expressed as per cent of radial growth/cfu over the control which was calculated using the formula (Vincent, 1947).

Plant extracts		
Sl. No.	Common Name	Scientific name
1	Neem seed kernel extract	<i>Azadirachta indica</i>
2	Neem leaves extract	<i>Azadirachta indica</i>
3	Kari jaali leaves extract	<i>Prosopis juliflora</i>
4	Chromolina leaves extract	<i>Chromolina odonata</i>

Dual culture technique for bioagents

Twenty ml of sterilized and cooled potato dextrose agar medium was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal bio-control agents, mycelial discs of test fungus were inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at one end of the Petri plate to the middle of the Petri plate and the test fungus placed at the other end. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

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

Where,

I = per cent inhibition

C = growth of pathogen in control

T = growth of pathogen in treated plate

List of Bio agents used for study	
Sl no	Bio agents
1	<i>Trichoderma viride</i> (IIHR)
2	<i>T. harzianum</i> (Local)
3	<i>Pseudomonas fluorescens</i> (local strain)
4	<i>P. fluorescens</i> (IIHR)

Results and Discussion

In vitro* evaluation of fungicides against *A. helianthi

Four non-systemic and four systemic fungicides were evaluated for their efficacy

against *A. helianthi* by following poison food technique. The data are presented in Table 1 and 2.

Among different non systemic fungicides tested at 100 ppm the highest inhibition of radial growth was recorded by Captan (50.74%) which differed significantly from other fungicide tested Mancozeb and Chlorothalonil with an inhibition of 35.93 per cent and 29.63 percent respectively. Whereas, Copper oxychloride (17.41%) recorded the least inhibition (Table 1). At 200 ppm Captan recorded the highest inhibition of colony radial growth by 80.86 per cent, followed by Copper oxy chloride (51.48%) and the least inhibition was recorded in Chlorothalonil (35.74%). The highest inhibition of radial growth of 80.86 percent was recorded with Captan at 400 ppm which is superior and significantly differed from other fungicides tested, followed by Copper oxy chloride, Mancozeb with an inhibition of 64.44 per cent and 64.63 percent respectively and was on par with each other. At 600 ppm Captan recorded the highest inhibition of radial growth of 50.74 per cent, which significantly differed from other fungicides tested, followed by Copper oxy chloride (84.07%) and the least inhibition was recorded by Chlorothalonil (47.79%) (Fig. 1).

Among different systemic fungicides tested at 100 ppm the highest inhibition of radial growth was recorded by Hexaconazole (93.52%), followed by Propiconazole (92.59%) and were on par and significantly superior over other fungicides. Followed by Thiophanate methyl (22.41%) and Carbendazim (18.15%) (Table 2 and Fig. 2).

The 100 per cent inhibition of radial growth was recorded by Hexaconazole at 200 ppm whereas; Propiconazole recorded the inhibition of 93.70 per cent, followed by the thiophanate methyl (34.07%) and Carbendazim (24.63%).

Table.1 *In vitro* evaluation of systemic fungicides against *A. helianthi*

SL. No	Fungicides	Per cent inhibition of Radial growth(mm)			
		100 ppm	200 ppm	400 ppm	600 ppm
1	Carbendazim	18.15	24.63	39.07	44.44
2	Thiophanate methyl	22.41	34.07	40.37	46.11
3	Propiconazole	92.59	93.7	100	100
4	Hexaconazole	93.52	100	100	100
Mean		56.67	63.1	69.86	72.63
		S.Em±	CD at 1%	CD at 5%	
Fungicides (F)		0.29	1.11	0.92	
concentrations (C)		0.29	1.12	3.57	
F x C		0.58	2.24	7.16	

Table.2 *In vitro* evaluation of non-systemic fungicides against *A. helianthi*

SL No	Fungicides	Per cent inhibition of Radial growth(mm)			
		100 ppm	200 ppm	400 ppm	600 ppm
1	Mancozeb	35.93	48.89	64.63	73.89
2	Captan	50.74	80.56	85.56	86.3
3	Chlorothalonil	29.63	35.74	39.07	47.59
4	Copper oxy chloride	17.41	51.48	64.44	84.07
Mean		33.42	54.16	63.42	72.96
		S.Em±	CD at 1%	CD at 5%	
Fungicide (F)		0.56	2.20	1.64	
Concentrations (C)		0.56	2.19	6.34	
F x C		1.13	4.39	12.67	

Table.3 *In vitro* evaluation of plant extracts against *A. helianthi*

SL No	Plant extracts	Per cent inhibition of radial growth(mm)			Mean
		2.50%	5%	10%	
1	Neem seed kernel extract	22.78	33.89	43.74	33.47
2	Neem leaf extract	11.67	19.44	25.26	18.75
3	Chromolina leaf extract	6.74	19.33	22.93	16.37
4	Prosopis leaf extract	10.56	16.11	23.89	16.85
		S.Em±	CD at 1%		
Plant extracts(P)		0.29	1.13		
concentration (C)		0.29	1.13		
P x C		0.58	2.27		

Table.4 Efficacy of different bio-agents against <i>A. helianthi</i> under <i>in vitro</i>	
Bio agents	Per cent inhibition over control
Trichodermaviride	60.81
<i>T. harzianum</i>	72.48
<i>Pseudomonas fluorescens</i> (local strain)	39.41
<i>P. fluorescens</i> (IIHR)	36.31
S.Em±	1.21
CD at 5%	3.96

Fig. 1: *In vitro* evaluation of systemic fungicides against *A. helianthi*

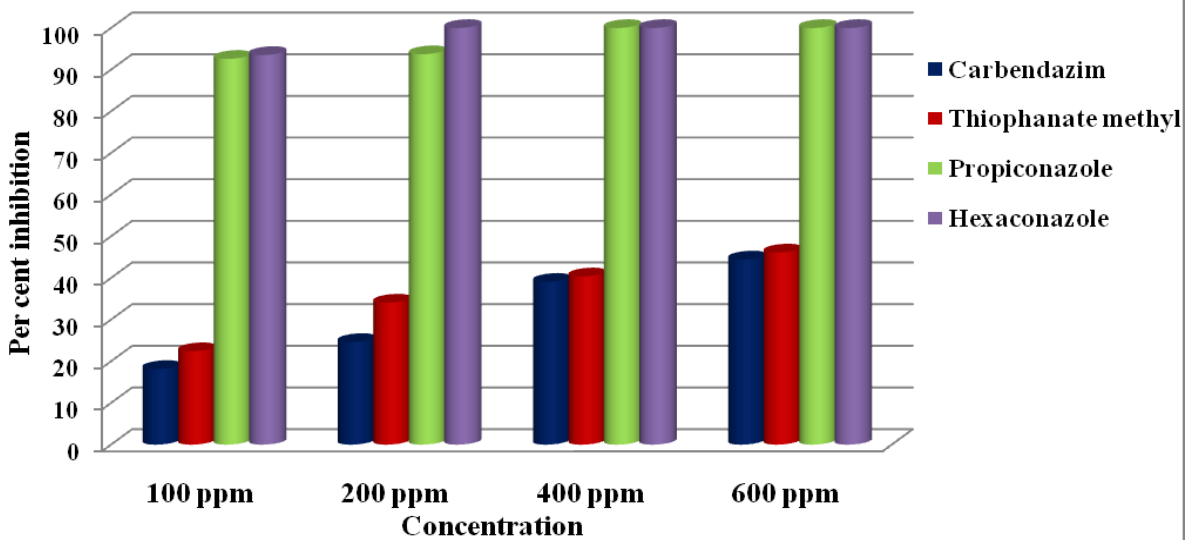


Fig. 2: *In vitro* evaluation of non systemic fungicides against *A. helianthi*

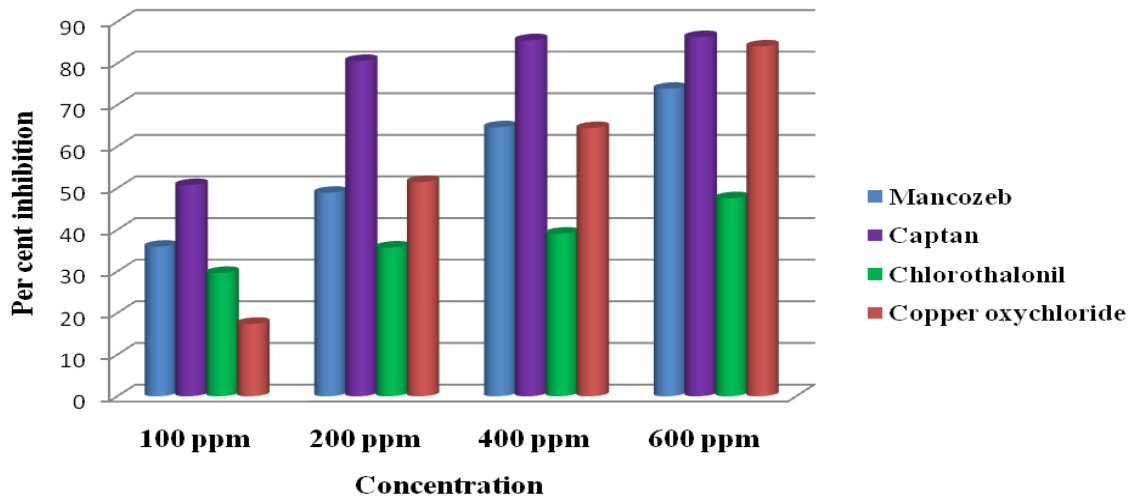


Fig.3: In vitro evaluation of plant extracts against *A. helianthi*

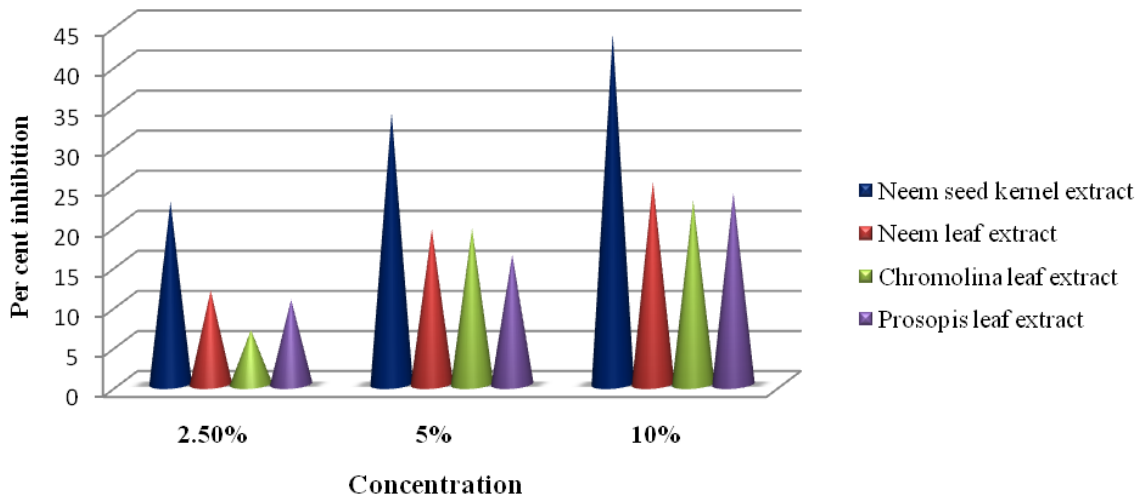
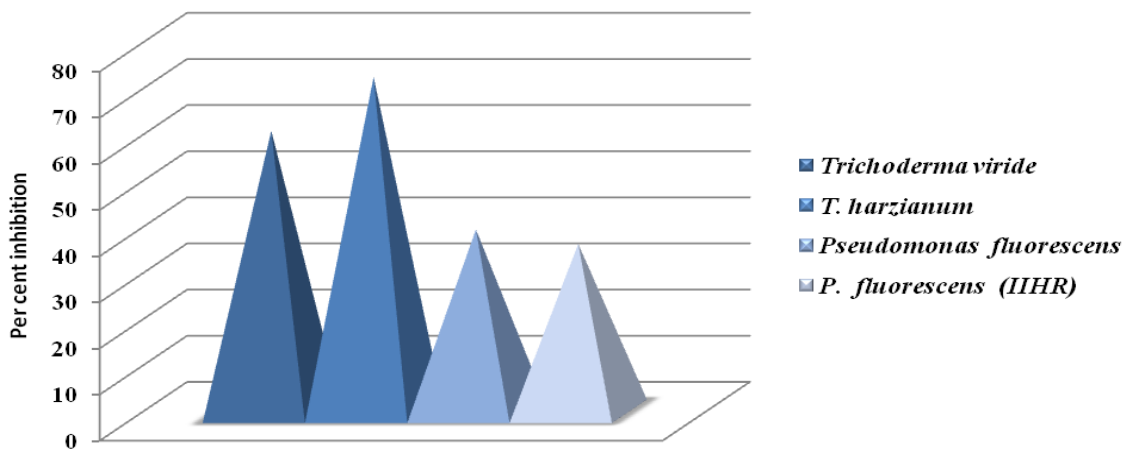


Fig. 4: In vitro evaluation of bio-agents against *A. helianthi*.



At 400 ppm, 100 percent inhibition of radial growth was observed with Hexaconazole and Propiconazole and these differed significantly compared to other fungicide tested.

These were followed by the Thiophanate methyl (40.37%) and Carbendazim (39.07%). At 600 ppm Hexaconazole and Propiconazole was found to be superior over other fungicides tested with 100 per cent inhibition of fungal radial growth followed by COC (84.07%), Thiophanate methyl (40.37%) and

Carbendazim (39.07%). These results in conformity with the results of Mesta *et al.*, (2009) reported that Hexaconazole (63.12 % and 72.87 % respectively) and Propiconazole (61.80 and 76.53 %) were most effective and were found on par.

Thaware *et al.*, (2010) reported that among different fungicides tested *in vitro*, Mancozeb (0.2 per cent) and Propiconazole (0.05 per cent) completely inhibited the growth of the test fungus.

Chethana *et al.*, (2012) recorded that Mancozeb and Propineb (0.3%) caused 100 per cent inhibition of mycelia growth. This was also in confirmation with the work specified Vasudha *et al.*, (2018) reported that Propiconazole (100%) followed by Hexaconazole (99.38%)

In vitro* evaluation plant extracts against *A. helianthi

As natural products isolated from plant appears to be one of the best alternatives in plant disease management as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. Among different plant extracts tested NSKE at 10 per cent showed maximum inhibition of 43.74 per cent, followed by Neem leaf extracts (25.26%), karijaali (23.89%) whereas, Chromolina leaf extract recorded the least (22.93 %) represented in Table 3 and figure 3. At 5 per cent the maximum inhibition of 33.89 per cent was recorded by NSKE which significantly differed compared to others, followed by Neem leaf extracts (19.44%) and karijaali leaf extracts (16.11 %). At 2.5 percent also the maximum inhibition was recorded with NSKE (22.78%) and the least was inhibition was recorded by chromolina (6.74%). The present study agrees with the findings of who observed, among the extract tested Neem seed kernel extract (NSKE) was found superior compared to other plant extracts tested against *A. helianthi* under *in vitro* condition.

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

From the observation it was revealed that, the maximum reduction in colony growth of *A. helianthi* was recorded in *T. harzianum* (72.48%) treated plates which was significantly superior over all other bio-agents tested. However *T. viride* (60.81%), *Pseudomonas fluorescens* (39.41%) of local

strain were also effective in inhibiting the growth of fungus. However the least inhibition of mycelia was noticed in *Pseudomonas fluorescens* (36.31%) IHR strain (Table 4 and Fig. 4).

These findings were in conformity with the observation made by Amaresh (2000), Chethana *et al.*, (2012), Mesta (2006) and Kota (2003) reported that among the bio-control agent *T. viride* and *T. harzianum* were effectively inhibited the growth of *A. helianthi* while the bacterium *P. fluorescens* produced maximum inhibition zone.

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