

MANAGEMENT OF CHOANEPHORA LEAF BLIGHT (*CHOANEPHORA INFUNDIBULIFERA*) IN SOYBEAN (IN-VITRO CONDITION)

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Abstract : Out of fourteen medicinal plant leaf extracts, Citrus and Eucalyptus inhibited significantly the radial growth of *C.infundibulifera*. *Trichoderma viride* was not effective in inhibiting the mycelial growth of *C.infundibulifera*. Complete inhibition of radial growth of *C.infundibulifera* was observed with M2 and Sixer *in-vitro* evaluation of fungicides and out of 66 varieties of soybean screened, 7 varieties showed Absolutely resistance 43 were Highly resistance and 16 were found Moderately resistance.

Keywords: *Choanephora infundibulifera*, medicinal leaf extract, fungicides, bioagent, screening

INTRODUCTION

Choanephora leaf blight is the most important disease of soybean causing heavy losses of crop Throughout the world. Hence the important of these fungus and very less management practice has been done . So because of these required to study of different method of management the pathogen with low cost. All the work done in lab condition undertaken at the Indira Gandhi Krishi Vishwavidyalay Raipur, during 2009-2010.

MATERIAL AND METHOD

Medicinal plant leaf extract

Antifungal activity of different medicinal plant leaf extracts were studied under *in-vitro* taking plant leaf extract dextrose agar medium. The fourteen medicinal plant species viz. Lemon grass (*Cymbopogon flexuosus*), garlic (*Allium sativum*), citrus (*Citrus aurantifolia*), sinduri (*Bixa orellana*), neem (*Azadiracta indica*), tulsi (*Occimum spp.*), bel (*Aegle marmelos*), Japanese pudina, madar (*Hibiscus spp.*),

Aloe vera, eucalyptus (*Eucalyptus globules*), safed oak (*Calotropis procera*), blue oak (*Calotropis spp.*), Datura (*Datura stramonium*) were used, for antifungal activity. Twenty gm. leaf of each medicinal plant was taken in 100 ml of water and boiled till the softening of the leaf and then extract was filtered. 2 g. of dextrose and 2 g. agar-agar were mixed in filtrated leaf extract, the volume was make-up to 100 ml and then sterilization was done by autoclaving at 15 lbs for 20 minutes. To avoid bacterial contamination, a little amount of streptomycin sulphate was added at the time of pouring of media. In each Petri plate 20 ml medium was poured in sterilized Petri plates and allowed to solidify. PDA without extract served as control. A 7 mm disc from seven days old culture of the test pathogen by the help of sterilized cork borer and was placed at the center of medium, three replications were kept in each treatment along with a control. The inoculated Petri plates were then incubated at $25 \pm 2^{\circ}\text{C}$ and observation was recorded at an 1st, 2nd and 3rd days after inoculation.

Bio-control

The experiment was conducted to study the bio-efficacy of bio-control agent against Choanephora leaf blight pathogen. *Trichoderma viride* was tested by dual culture method. In this method, sterilized medium was poured in Petri plate under aseptic condition. After solidification of the medium a seven mm mycelial disc of the test fungus (7 days old culture) and bio-control agent (*Trichoderma viride* 4 days old culture) were placed at opposite end of the Petri plates at distance of five mm from the periphery. Petri plate without test microorganism served as control. Seven plates replicated for each treatments. Plates were then incubated at $25 \pm 2^{\circ}\text{C}$. Radial growth of the test fungus were recorded after 1st, 2nd and 3rd days of inoculation

Fungicides

Poisoned food technique was employed for the evaluation of fungicides in the laboratory. Ten fungicides viz. Dhanucop-50% (Copper oxychloride), Indofil M-45(Mancozeb), Dithan M-45 (Mancozeb), Dhan-team(Tricyclazole-75%WP), Curzate M-8(Cymoxanil 8%+ mancozeb 64%) Jatayu (Chlorothalonil 75%WP), Kocide(Copper hydroxide 77%WP), Dhanustin (Carbendazim 50%WP), M2(matalaxyl 8%+ mancozeb 64% WP) and Sixer (carbendazim active 12% + mancozeb 64% WP) were evaluated against *Choanephora infundibulifera*. Three concentrations i.e., 100 ppm, 200 ppm, & 300 ppm of each treatment were used. The required quantity of fungicide was mixed with PDA at the time of pouring. Three replication were maintained for each fungicide for each of it's concentration in CRD. The media was shaken well so as to enhance proper mixing of the fungicides. To avoid bacterial contamination a little amount of streptomycin was added in each flask before plating; seven mm disc was cut with the help of sterilized cork borer from seven days old culture of the test fungus and was placed in the center of the medium in the reversed position to maintain continuous contact of the pathogen with poisoned medium. PDA plates without fungicide served as control. The radial growth

of the colony was measured when the growth in control plates reached the rim of the Petri plates; percent growth inhibition under the influence of different fungicides was calculated on the basis of the control.

The sporulation of *Choanephora infundibulifera* was recorded separately for each treatment and each concentration. Seven mm diameter disc was placed in 20 ml water in a test tube and was thoroughly shaken. One drop of suspension was placed in a cavity slide and the spores were counted per microscopic field. In one cavity slide, three microscopic fields was focused for counting the spores.

Percent inhibition of radial growth were calculated by the following formula,

$$\text{Inhibition \%} = \frac{C-T}{C} \times 100$$

Whereas C = Diameter of fungus colony (mm) in control plate,

T = Diameter of fungus colony (mm) in treated plate.

Host resistant

The field experiment was conducted at the research farm, IGAU Raipur, in kharif season 2009-10. In screening trail 66 soybean varieties were screened and sown on 7th July 2009. The observation on natural occurrence of *C. infundibulifera* on soybean leaf were recorded after 45 days of sowing. The observation on incidence of disease was recorded. For recording the observation, five plants of each variety were randomly selected and tagged. Total healthy and disease leaf in each plant were counted to observe the disease incidence. While disease severity was calculated by using 0-9 scale

Where –

- 0 – No lesions
- 1 – 1% leaf area covered with lesion
- 3 – 1.1 - 10 % leaf area cover with lesion
- 5 – 10.1 – 25 % of the leaf area covered no defoliation, little damage
- 7 – 25.1 – 50 % leaf area covered, some Leaf drop, death of a few plant , damage conspicuous.
- 9 – More than 50% area cover, lesion very common on all Plants, defoliation common, death of plant common, damage more than 50%.

PDI was calculated by the following formula:

Percent disease index (PDI) =

$$\frac{\text{Sum of individual rating}}{\text{No of leaves examined}} \times \frac{100}{\text{maximum disease rating}}$$

And on the basis of PDI, the varieties were classified as followed-

PDI	Reaction category
0	Absolutely resistant (AR)
0.01-11.11	Highly resistant (HR)
12.22-33.33	Moderately resistant (MR)
34.44-55.55	Moderately susceptible (MS)

56.66-77.77	Susceptible (S)
78.88-100.00	Highly susceptible (HS)

RESULT AND DISCUSSION

Hot water extracts of different plants species were evaluated to observe the inhibitory activity of *C. infundibulifera* under *in-vitro* condition. The data was recorded and presented in Table 1 . It is clear from the table that the radial growth of *C. infundibulifera* differed significantly with respect to different plant extract used. The per cent inhibition in growth of *C. infundibulifera* ranged from (0.29 % to 100%). The maximum inhibition in growth was recorded in the extract of citrus (100%) followed by eucalyptus (73.80%) and garlic (72.52%) at 1 DAI.

The inhibition in growth of *C. infundibulifera* was statistically at par with each other in case of Citrus, Eucalyptus, Garlic, Mint, Bel, Aloe vera and Neem. The minimum growth inhibition was recorded in plant extract of Blue oak (0.29 %). The per cent growth inhibition of 2 DAI by different plant extract ranged between (82.77% to 0.74%). The maximum radial growth inhibition was recorded in plant extract of Eucalyptus (82.77%) followed by Citrus (78.51%) and minimum inhibition was recorded in Lemmon grass (0.12 %).

The isolates of *Trichoderma viride* were used to test antagonistic performance in dual culture with a test pathogen *C. infundibulifera*. This treatment was not effective in inhibiting the mycelia growth. The data on the inhibition of growth of *C. infundibulifera* by *Trichoderma viride* was presented in Table 2. In dual culture technique, *Trichoderma viride* inhibited the growth of test pathogen by 0.5, 22.33 and 21.35 per cent at 1st, 2nd and 3rd day of incubation respectively). *Trichoderma viride* was overlapped by the test pathogen after 3rd days.

The per cent radial growth inhibition in Citrus, Eucalyptus, Garlic & Mint were statistically at par with each other hence they are superior over other treatments. An average growth inhibition % indicate that *C. infundibulifera* maximum in Citrus (83.29%) followed by Eucalyptus (79.65%) and minimum in Blue oak (0.59%) followed by White oak (4.59%).

The sporulation by pathogen was recorded after complete growth of the fungus in different plant extract media *in-vitro* condition. It's showed lot of variation among different treatments.

Sporulation was varying from 70.78 to 3.11. Maximum sporulation was found in Lemmon grass (70.78) followed by Neem (70.67), Bel (47.33) and Blue oak (43.11). Minimum sporulation was recorded in Eucalyptus (1.5) followed by Aloe vera (3.11), Tulsi (3.77). In citrus sporulation was not found. In present investigation, extracts from 14 different plant were tested to see their influence on the radial growth and sporulation of *C. infundibulifera*. The result indicates that all the plant extract's inhibited the growth of the fungus from 83.29% in Citrus to 0.59%

in Blue oak. The other plant extract showing promising result against *C. infundibulifera* were Eucalyptus, Sindur, Garlic and Mint. The Maximum sporulation found in Lemmon grass & most effective plant extract for inhibition of sporulation was Aloe vera followed by Tulsi, Mint.

Olufolaji (1999) also tested antifungal property of medicinal plant extract under *in-vitro* and *in-vivo* condition and found *Azadiracta indica* (neem) to be effective in inhibition of mycelial growth (13.85%) & sporulation (82.50%).

Ten fungicides at three concentrations (100, 200, 300 ppm) were tested under *in-vitro* condition against *C. infundibulifera* and observation on inhibition of radial growth and sporulation were recorded and

showed in Table 3 & 4. Percent growth inhibition of *C. infundibulifera* in ranged between 84.87% to 19.06% in 100 ppm 1DAI. The maximum inhibition in growth was recorded in the M2 (84.87%) followed by Sixer (79.69%), Dithan M-45 (78.14%) and minimum in Dhanustin (19.06%).

In 200 ppm concentration percent inhibition in growth of *C. infundibulifera* recorded in ranged 81.88 % to 26.22 %, maximum growth inhibition was found in M2 (81.88%) followed by Sixer (81.46%), Curzate (77.91%), Dithan M-45 (77.50%), Indofil M-45 (72.15%) were most effective for radial growth inhibition & minimum radial growth was Dhanustin (26.22).

Table 1: Efficacy of different medicinal plant leaf extracts on inhibition of radial growth & sporulation of *Choanephora infundibulifera*

S.No.	Medicinal Plant	Radial growth (mm)						Average of inhibition (%)	No of spores per microscopic field
		1 st DAI		2 nd DAI		3 rd DAI			
		Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)		
1.	Lemmon grass	36.67	33.33 (35.20)	90	0.12 (0)	90	0 (0)	11.11	70.78
2.	Garlic	14.83	72.52 (58.59)	68.67	23.7 (28.57)	90	0 (0)	32.07	36.89
3.	Citrus	0	100 (18.43)	19.33	78.51 (62.49)	25.83	71.36 (57.65)	83.29	0
4.	Sindur	29.33	46.49 (42.97)	57.83	35.74 (36.70)	69.17	19.56 (26.19)	33.93	11.78
5.	Neem	27	50.50 (45.27)	57.83	23.14 (45.74)	90	0 (0)	24.55	70.67
6.	Tulsi	31.33	42.17 (40.09)	64	28.89 (32.39)	90	0 (0)	23.69	3.77
7.	Bel	22.83	58.99 (50.20)	74	17.59 (24.51)	90	0 (0)	25.53	47.33
8.	Mint	30	62.18 (55.24)	61	32.22 (34.56)	90	0 (0)	31.47	7.22
9.	Madar	31	43.34 (41.11)	80	11.11 (55.13)	90	0 (0)	18.15	19.11
10.	Aloe vera	26.83	50.69 (45.40)	84.33	6.29 (52.84)	90	0 (0)	18.99	3.11
11.	Eucalyptus	14.33	73.80 (59.26)	15.5	82.77 (65.48)	16	81.79 (64.75)	79.45	1.5
12.	White oak	47.33	13.32 (34.17)	89.33	0.74 (2.53)	90	0 (0)	4.69	21.67
13.	Blue oak	55.17	0.29 (29.62)	88.67	1.47 (18.59)	90	0 (0)	0.59	43.11
14.	Datura	42.33	22.72 (27.92)	89.17	0.92 (14.5)	90	0 (0)	7.88	27.11
15.	Control	55.33	-	90	-	90	-	-	58.78
	CD (5%)	5.56	15.16	8.17	19.85	3.75	1.23		
	SEm ±	1.92	5.25	2.83	6.87	1.29	0.43		

DAI: Days after inoculation

Figures in parentheses denote arcsine-transformed value

Average of three replication

Table 2: Bio-efficacy of *Trichoderma viride* on the radial growth of *Choanephora infundibulifera*.

S. No.	Treatment	Growth					
		1 st DAI		2 nd DAI		3 rd DAI	
		Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)	Growth (mm)	Inhibition (%)
1.	<i>Choanephora infundibulifera</i>	58	0.51 (39.79)	69.87	22.33 (28.19)	90	21.35 (27.46)
2	<i>Trichoderma viride</i>	18.5	-	43.87	-	78	-
3.	Control	58.37	-	90	-	90	-

DAI: Days after inoculation

Average of seven replication

Table 3 : Evaluation of fungicides at different concentration on inhibition of radial growth of *Choanephora infundibulifera*.

S.No.	Fungicide	100 ppm				200 ppm				300 ppm			
		1 st DAI		2 nd DAI		1 st DAI		2 nd DAI		1 st DAI		2 nd DAI	
		Growth (mm)	Inhibi tion (%)										
1.	Dhanucop	57.5	31.37 (34.04)	90	0 (0)	47.17	43.22 (41.08)	90	0 (0)	14.67	82.48 (65.26)	26.17	70.92 (57.37)
2.	Indofil M-45	28	66.59 (54.68)	85	5.55 (47.83)	23.33	72.15 (58.14)	49.17	45.36 (42.32)	16.5	78.93 (62.75)	30	66.66 (54.73)
3.	Dithan M-45	18.3	78.14 (62.12)	69	23.33 (28.87)	18.83	77.50 (61.74)	66.83	25.73 (30.24)	27	67.81 (55.46)	81.33	9.62 (29.34)
4.	Dhan-team	37.17	55.63 (48.23)	90	0 (0)	33.33	60.21 (50.88)	76	15.55 (23.15)	19.67	76.52 (61.01)	35.67	60.36 (50.98)
5.	Curzate	22.17	73.54 (59.04)	69	23.33 (28.85)	18.5	77.91 (61.98)	52.33	41.85 (40.29)	19.83	76.31 (63.99)	54	39.99 (39.19)
6.	Jatayu	32.5	61.19 (51.46)	90	0 (0)	39.17	53.23 (46.85)	90	0 (0)	34.17	59.10 (49.99)	90	0 (0)
7.	Kocide	58.83	29.81 (33.08)	90	0 (0)	50.33	40.02 (39.20)	90	0 (0)	0	100 (18.43)	15	83.33 (65.95)
8.	Dhanustin	67.83	19.06 (25.87)	83	7.77 (9.62)	61.83	26.22 (30.79)	90	0 (0)	42.67	49.05 (44.44)	90	0 (0)
9.	M2	12.67	84.87 (67.13)	27.5	69.44 (56.44)	15.17	81.88 (64.81)	27	69.81 (56.66)	11.33	86.47 (68.44)	23.5	73.88 (59.26)
10.	Sixer	17	79.69 (63.24)	40.5	54.99 (47.86)	15.5	81.46 (64.52)	26.5	70.55 (57.13)	0	100 (18.43)	18.83	79.06 (62.78)
11.	control	83.83	-	90	-	83.83	-	90	-	83.83	-	90	-
	CD (5%)	2.45	2.02	7.07	15.41	4.35	3.27	5.37	3.87	4.17	2.80	3.95	8.72
	SEm ±	0.84	0.69	2.41	5.25	1.48	1.12	1.83	1.31	1.42	0.95	1.35	2.97

Figures in parentheses denote arcsine-transformed value

Average of three replication

Table 4 : Effect of fungicides at different concentration on sporulation of *Choanephora infundibulifera*

S.no.	Fungicide	100 ppm	200 ppm	300 ppm
		No of spores per microscopic field	No of spores per microscopic field	No of spores per microscopic field
1.	Dhanucop	58.99	25.89	2.78
2.	Indofil –M-45	96.89	87.11	39
3.	Dithan-M-45	45.78	84.33	56.89
4.	Dhan-team	35.89	29.67	12.33

5.	Curzate	78.11	85.78	38.67
6.	Jatayu	78.89	35.11	36.11
7.	Kocide	45	42.34	5.89
8.	Dhanustin	42	31	73.78
9.	M2	22.89	27.78	16.89
10.	Sixer	33.44	29.56	16
11.	Control	36.44	36.44	36.44
	CD (5%)	69.48	66.41	50.49
	SEm ±	23.69	22.64	17.22

Average of three replication

Table 5 : Evaluation of host resistance in different soybean varieties against *Choanephora infundibulifera*

S. No.	Scale (0 - 9)	Disease Reaction	No of variety	Name of variety
1.	0	AR	7	JS 20-19, SH 2 ,KD 5319, SH 1, JS 93-05, Montana,PS 1475.
2.	(1) 0.01- 11.11	HR	43	MACS -1140, TS-2, RKS-54, DSB-12, JS-20-06, NRC-37, NRC -77, Bragg, JS-335, RKS-18, SL-752, PS-2410, VLS- 75, JS(SH)-2003-2, DSB-16, VLS-74, PS- 1472, DS- 12-13, Himso- 1679, NRC-82, PS -1475, PS -1466,TS- 9, KSL, MACS- 1259, NRC- 84, AMS- 323, PS- 1469, JS -20-21, SL- 794, KHSb -6, KDS- 343, KS -3,Bragg,JS -72-44, JS -75-46, JS-72-280, PK- 262, MACS-58, Bragg, KHSb-2,NRC-7, JS-335.
3.	(3) 12.22- 33.33	MR	16	MACS-1140, TS-2, PS -2410, NS- 084, SL- 799,NRC- 83, MACS- 1281, MAUS- 394, MACS -1254, SL- 795, DSb -15, TS- 7, MACS -1238, NRC-37,JS-71-05, PK- 472.
4.	(5) 34.44-55.55	MS	0	–
5.	(7) 56.66-77.77	S	0	–
6.	(9) 78.88- 100.00	HS	0	–

In 300 ppm concentration per-cent inhibition in growth of *C.infundibulifera* recorded in ranged 100% to 49.05%. The maximum inhibition was recorded in Kocide & Sixer (100%) & minimum in Dhanustin (49.05%). M2, Sixer, Dithan M-45, Curzate, & Kocide were most effective for radial growth inhibition. The radial growth in case of M2, Sixer, Dithan M-45, Curzate, were statistically at par with each other at 100 ppm and M2, Sixer, Curzate, Dithan M-45 & Dhan team were also statistically at par with each other at 200 ppm, and Sixer, Kocide, M2 & Dhanucop, was statistically at par with each other at 300 ppm. Minimum inhibitions in radial growth were recorded in Dhanustin at all

concentration of fungicide. Maximum sporulation was recorded in Indofil M-45 (96.89) at 100 ppm, Indofil M-45 (87.11) at 200 ppm and Dhanustin (73.78) at 300 ppm. Minimum sporulation was recorded in M2 (22.89) at 100 ppm, Dhanucop (25.89) at 200 ppm and Dhanucop (2.78) at 300 ppm.

The similar result obtained by Panja (1999) reported that Captan 50%, Ziram 27% and Copper Oxychloride 50% completely inhibited the mycelial growth and sporulation of *C. spp.*

Gunasekera *et al* (1989) also found Triadimenol, Vinclozolin and bitertanol were most effective among eight systemic fungicides in inhibiting spore

germination and mycelial growth of *C. infundibulifera*.

Out of sixty six varieties of soybean were screened under natural field condition against *Choanephora infundibulifera*. Disease reaction was classified in Absolutely resistant, Highly resistant, Moderate resistant, Moderate susceptible, Susceptible and Highly susceptible. The data presented in table 5 indicated that out of 66 varieties, 07 varieties viz., JS 20-19, SH 2, KD 5319, SH 1, JS 93-05, Montana, PS 1475 exhibited Absolutely resistant reaction, 43 varieties showed Highly resistant reaction while remaining 16 varieties were found Moderately resistant. one of the varieties showed Moderate susceptible, Susceptible and Highly susceptible reaction. Similar work of screening done by Anonymous 2010.

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