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Management of *Alterneria cucumerina* var. *cyamopsidis* through Plant Extracts, Bio products and Fungicides *in-vitro* and *in-vivo*

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A B S T R A C T

Laboratory studies were conducted to study the effect of six botanicals (neem leaf extract, garlic bulb extract, eucalyptus leaf extract, castor oil, mustard oil, neem oil) and two bio products (vermin wash, cow urine) were evaluated against against *A. cucumerina* var. *cyamopsidis* under *in-vitro* condition. Maximum mycelium inhibition was recorded in Garlic bulb extract followed by Neem leaf extract and Cow urine. Nine fungicides [Difenconazole (25EC), Chlorothalonil (75WP), Carbendazim 12% + Mancozeb 63%WP, Mancozeb (75), kitazin (48%EC), Hexaconazole 50%, Tebuconazole 50% + Trifloxystrobin 25%, Azoxystrobin, Tricyclazole] evaluated against *Alternaria cucumerina* var. *cyamopsidis* under *in-vitro* condition. All the fungicide inhibited the mycelial growth at all concentrations of fungicides. Difenconazole (100%), Tebuconazole 50% + Trifloxystrobin 25% (100%) and Azoxystrobin (100%), completely inhibited the growth at 500ppm concentration. A field experiment conducted to know the efficacy of four botanicals (Neem leaf extract, Garlic bulb extract, Neem oil, Cow urine) and six fungicides (Carbendazim 12% + Mancozeb 63%WP, Difenconazole (25EC), Tebuconazole 50% + Trifloxystrobin 25%, Chlorothalonil (75WP), Azoxystrobin) against Alternaria blight in foiler disease. Among the treatments, minimum disease incidence was recorded in Difenconazole (25EC), followed by Carbendazim 12% + Mancozeb 63%WP, Chlorothalonil (75WP) and Azoxystrobin. Among the botanicals, minimum disease incidence was recorded in Garlic bulb extract.

Introduction

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub] is an important leguminous crop of *kharif*season in arid and semi-arid region of India. It is a very hardy and drought tolerant crop. Alternaria blight caused by *Alternaria cucumerina* var. *cyamopsidis,* which is a major foliar disease of clusterbean in northern India.. In India, this crop is mostly grown in the state of Rajasthan, Haryana, Punjab, U.P. and M.P. About 80% area of the state is in gird zone. In India, clusterbean cultivation is accounted for about 75 percent of global trade and 80 percent to total guar production in the world (Swamy and Naveena, 2015). In M.P., clusterbean is cultivated as pure crop in 75280 hectares and as mixed crop in 54782 hectares

area. Seeds of cluster bean contain 28 to 33 per cent gum and its use in almost all types of industries *viz*., textile, paper, petroleum, pharmaceuticals, food processing, cosmetics, mining, explosives and oil drilling, etc. Besides, it increases fertility of soil by fixing considerable amount of atmospheric nitrogen. India is the leading country in the world, where it occupies 52-55.8 lakh hectares with annual production of 28.32 lakh tonnes (Anonymous, 2017-18). India produces 85 per cent of total guar production in the world followed by 15.6 per cent by Pakistan and 12.1 per cent by USA, Brazil and others. Little attention has been paid on Alternaria blight of cluster bean caused by *A. cucumerina* var. *cyamopsidis* which has become a serious problem in hampering the production in all the clusterbean growing areas especially in rainfed condition. Symptoms of blight appeared on cotyledons, leaves, petioles, stems and pods but mainly. Dark brown more or less circular to irregular lesions up to 10mm diameter were observed on leaves. The lesions turned grayish to dark brown lines inside the lesions. In some lesions distinct darker marginal ridges were seen, also lesions with and without chlorotic halo. At times, several lesions coalesce involving most of the leaf surface and such leaves with petiole infection get defoliated. Similar types of lesions were also seen on cotyledons. On petiole and stem, the lesions were sunken and enlarged up to 10-15 mm. lesions on pod were small, brown to black with concentric zonation. Orellana and Simmons (1965) observed two types lesion occur on guar leaves (A) brown, more or less circular lesions up to 10 mm diameter with distinct, darker marginal ridges and (B) medium brown, spreading lesions without definite marginal ridges and with or without chlorotic halos. Stem lesions appear to develop more rapidly after defoliation. Brown to black lesions is superficial to sunken, up to 5 mm in diameter, with or without distinct ridges. Early pod attack by *Alternaria* appears to interfere with seed development. Symptoms on leaves were small, circular, necrotic spots that develop quickly forming typical concentric rings. Later, these spots coalesce and cause blighting of leaves. The spots were initially light brown which later turned dark brown. On stems, spots were sunken, with concentric rings. In severe infection, defoliation and drying of infected leaves, branches and flower buds was observed.Application of biological agents and extract is eco-friendly and a sustainable approach apart from being a promising alternative to fungicide application. In the absence of resistant cultivars, chemical fungicides provide the most reliable means of disease control. The present study was aimed at determining a cost effective management of Alternaria leaf spot.

Materials and Methods

The experiment was laid out in a complete randomized design (CRD) with nine treatments including untreated control and replicated thrice with three concentrations. Eight bio product *viz.*, Neem leaf extract, Garlic bulb extract, Eucalyptis leaf extract, Castor oil, Mustard oil, Neem oil, Cow urine and Vermi wash were evaluate against A. cucumerina var. cyamopsidis. The present study was undertaken in the laboratory conditions to find out their relative efficacy to inhibit the radial growth of the pathogen on PDA medium by poisoned food technique (Nene and Thapliyal 1979). The calculated quantity of bio-product and bio extracts were added to potato dextrose agar (PDA), mixed thoroughly and poured into sterilized Petriplates and allowed to solidify. After solidification, each plate was inoculated with a 5 mm diameter disc obtained from an actively growing margin of *A. cucumerina* colony on PDA. The Petri dishes were incubated at 25+1ºC in BOD incubator and

allow to growth. The data of efficacy of bio product against *A. cucumerina* var. *Cyamopsidis* was recorded after 7 days after inoculation (DAI) for growth of pathogen at 25± 1ºC. Per cent growth inhibition over control was calculated by the following formula suggested by Vincent (1947) and confirmed by Hegde *et al.*, (2014).

Per cent growth inhibition =

$$\frac{C-T}{C} \text{ x100}$$

Where as,
C= Mycelial growth of *A. cucumerina* var. *cyamopsidis* in control (mm)
T= Mycelial growth of *A. cucumerina* var. *cyamopsidis* in treatment (mm)

Field experiments was conducted with clusterbean germplasm were laid out during *kharif* 2018-19 at experimental Field, College of Agriculture, Gwalior. The crop was sown on 21th July, 2018 and keeping plot size 3 x 5 m^2 with 30 x 15 cm row to row and plant to plant spacing following Randomized Block Design with three replications.

The fungicide, bio product and plant extract which performed better under *in-vitro* condition against *Alternaria cucumerina* var. *cyamopsidis* were again tested under *in-vivo* conditions to test their efficacy to manage the disease. The effective fungicide (Carbendazim 12% + Mancozeb 63%WP, Difenconazole (25EC), Tebuconazole 50% + Trifloxystrobin 25%, Chlorothalonil (75WP) and Azoxystrobin) bio-products (Neem leaf extract, Garlic bulb extract and Neem oil) and plant extracts (Cow urine) were sprayed at just appearance of disease. Two subsequent sprays of chemical and plant extracts were given at 10 days interval. The spraying was done using manually operated high volume (Knapsack) sprayer.

Disease intensity was recorded after 7-10 days of second spray. Per cent disease control was calculated by following formula:

$$\text{Per cent disease control} = \frac{C - T}{C} \text{ x } 100$$

Where,
C= Disease intensity in control
T= Disease intensity in treatment

Results and Discussion

Evaluation of botanicals and bio-products against *Alterneria cucumerina* var. *cyamopsidis in-vitro*

To explore the possibility of substituting fungicide with other eco-friendly products, eight plant extracts/bio products (neem leaf extract, garlic bulb extract, eucalyptus leaf extract, castor oil, mustard oil, neem oil, cow urine, vermin wash) of different concentrations *viz.*, (2, 5 and 10%) were tested *in-vitro* against the mycelium growth of *Alterneria cucumerina* var. *cyamopsidis* .

At 10% concentration the minimum mycelium growth was found in garlic bulb extract (28.00 mm) followed by neem leaf extract (33.33 mm), neem oil (40.33 mm), cow urine (36.67 mm), mustard oil (43.00 mm), eucalyptus leaf extract (48 mm), castor oil (51.67 mm), vermi wash (57.67mm) while the maximum mycelial growth was recorded in control (82.67 mm).

In general all plant extracts showed antifungal activity against the pathogen at all concentrations. The effectivity of the extracts increases with increase in concentration of extracts and the minimum growth of fungus was recorded in garlic bulb extract at all concentrations which was significantly superior over rest of the plant extract in respect of mycelial growth.

Table.1 *In-vitro* evaluation of bio-products and plant extracts against mycelial growth of *A. cucumerina* var. *cyamopsidis* at different concentration

S. no	Plant extract / bio products	Mycelium growth (mm) and per cent inhibition at different concentrations					
		2%		5%		10%	
		Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition
1	Neem leaf extract	43.00	48.38	40.33	51.60	33.33	59.67
2	Garlic bulb extract	37.33	55.17	33.67	59.57	28.00	66.11
3	Eucalypus leaf extract	57.00	31.78	53.33	35.98	48.00	41.92
4	Castor oil	58.67	29.58	54.67	34.39	51.67	37.47
5	Mustard oil	56.33	32.39	50.00	40.00	43.00	47.97
6	Neen oil	48.67	41.57	44.00	47.19	40.33	51.23
7	Cow urine	49.67	40.37	46.33	44.39	36.67	55.67
8	Vermi wash	66.33	20.39	61.33	26.36	57.67	30.21
9	Control	83.33	0.00	83.33	0.00	82.67	0.00
SEm±		**0.59**		**1.13**		**0.56**	
C.D.at 5%		**1.77**		**3.39**		**1.66**	

Table.2 *In-vitro* evaluation of fungicides on mycelial growth of *Alternaria cucumerina* var. *cyamopsidis*

S.no	Fungicide	Mycelium growth (mm) and per cent inhibition at different concentrations									
		100 PPM		200 PPM		300 PPM		400 PPM		500 PPM	
		Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition	Mycelial growth (mm)	Per cent inhibition
1	Difenconazole(25EC)	8.33	90.31	4.79	94.55	1.67	98.14	0.00	100.00	0.00	100.00
2	Chlorothalonil (75WP)	22.67	73.65	18.67	78.79	13.67	84.80	8.33	90..73	4.33	95.18
3	Carbendazim 12% + Mancozeb 63%WP	25.67	70.16	21.67	75.37	16.67	81.47	12.67	85.92	7.67	91.88
4	Mancozeb(75)	33.67	60.84	27.67	68.55	21.67	75.91	14.33	84.07	9.67	89.25
5	kitazin(48%EC)	47.33	45.16	43.33	50.75	40.67	54.80	36.33	59.62	32.66	63.71
6	Hexaconazole 50%	39.33	54.46	35.67	59.46	31.33	65.18	26.67	70.36	22.67	74.80
7	Tebuconazole 50% + Trifloxystrobin 25%	16.67	80.62	13.67	84.47	9.33	89.62	0.00	100.00	0.00	100.00
8	Azoxystrobin	15.67	82.09	11.67	86.74	9.67	89.25	4.33	95.18	0.00	100.00
9	Tricyclazole	53.67	37.59	50.33	42.80	46.33	48.51	41.33	54.07	36.67	59.25
10	Control	86.00	0.00	88.00	0.00	90.00	0.00	90.00	0.00	90.00	0.00
SEm(±)		**0.69**		**0.62**		**0.72**		**0.53**		**0.67**	
C.D at 5 %		**2.05**		**1.87**		**2.15**		**1.58**		**1.99**	

Table.3 *In-vivo* evaluation of bio-products, plant extracts and fungicides against *Alternaria cucumerina* var. *cyamopsidis*

S.NO	FUNGICIDE	45 DAI		60 DAI	
		Disease intensity per cent	Disease control per cent	Disease intensity per cent	Disease control per cent
1	Neem leaf extract	26.46 (30.49)*	53.30	30.50 (33.49)*	51.06
2	Garlic bulb extract	21.36 (27.51)*	62.30	25.83 (30.53)*	58.55
3	Neem oil	29.96 (31.26)*	48.18	33.33 (35.24)*	46.52
4	Cow urine	39.35 (38.81)*	30.58	40.26 (39.35)*	35.40
5	Carbendazim 12% + Mancozeb 63%WP	12.56 (20.74)*	77.83	18.53 (25.44)*	70.27
6	Difenconazole (25EC)	16.10 (23.63)*	71.58	17.83 (24.93)*	71.39
7	Tebuconazole 50% + Trifloxystrobin 25%	21.60 (27.68)*	61.87	24.40 (29.58)*	60.85
8	Chlorothalonil (75WP)	17.20 (24.48)*	69.64	21.26 (27.79)*	65.08
9	Azoxystrobin	18.40 (25.38)*	67.52	22.80 (28.50)*	63.42
10	Control	56.66 (48.23)*	0.00	62.33 (52.12)*	0.00
SEm(±)		0.72		0.94	
C.D. at 5%		2.14		2.81	

*Value parenthesis are angular transformed

Fig.1

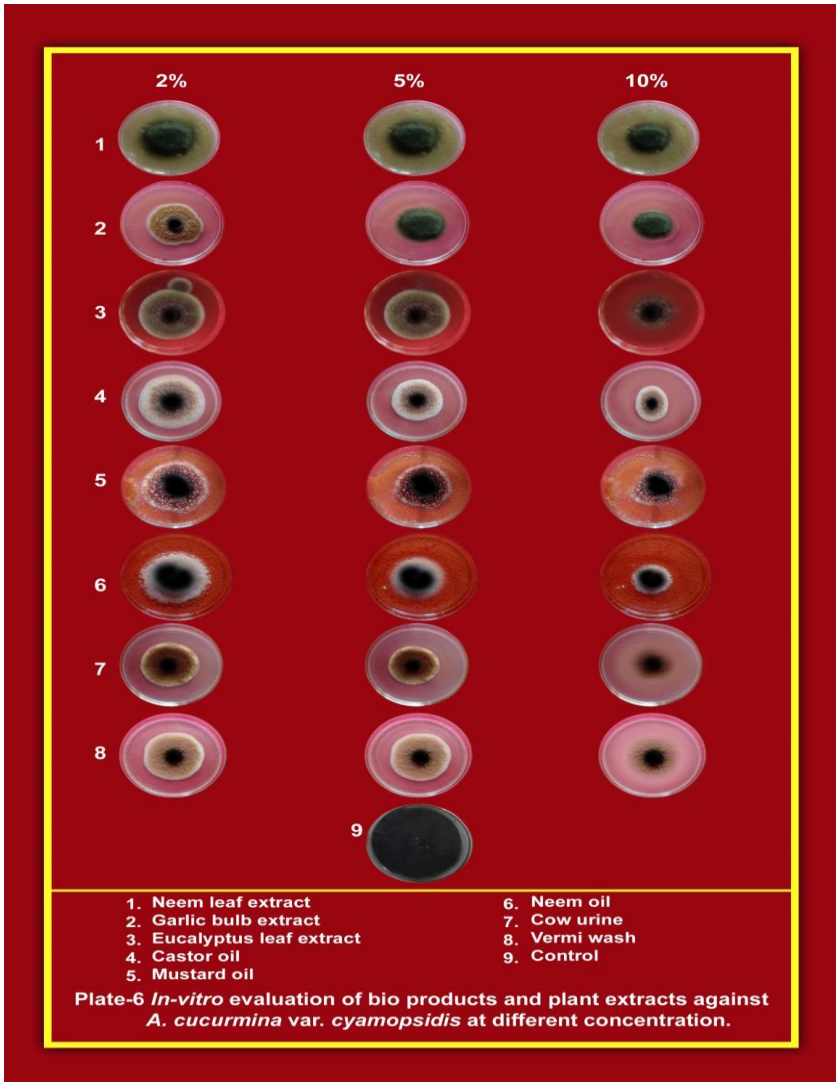
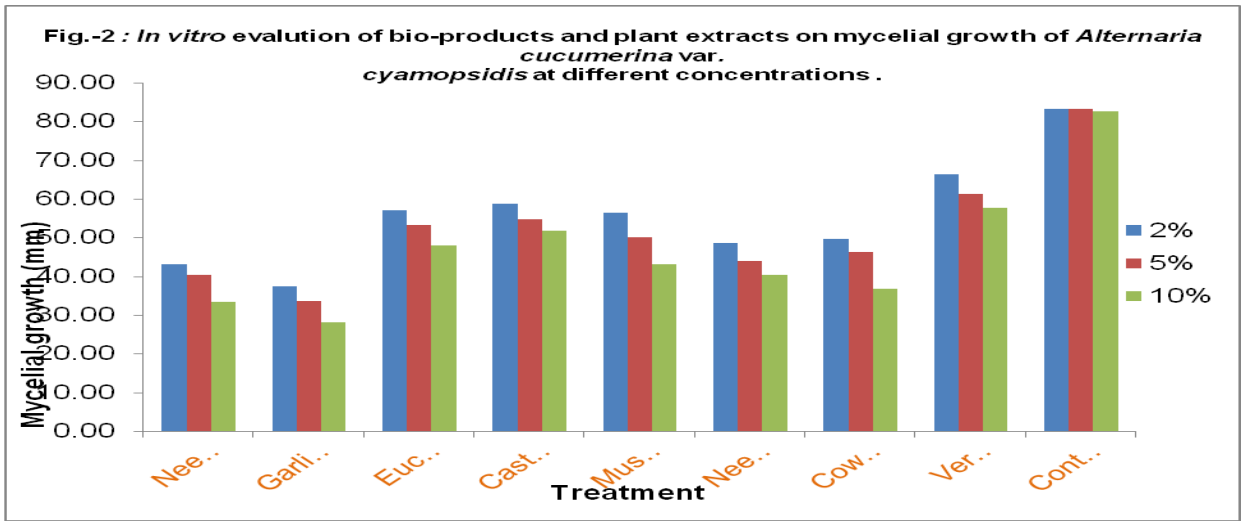


Plate-6 *In-vitro* evaluation of bio products and plant extracts against *A. cucurmina* var. *cyamopsidis* at different concentration.



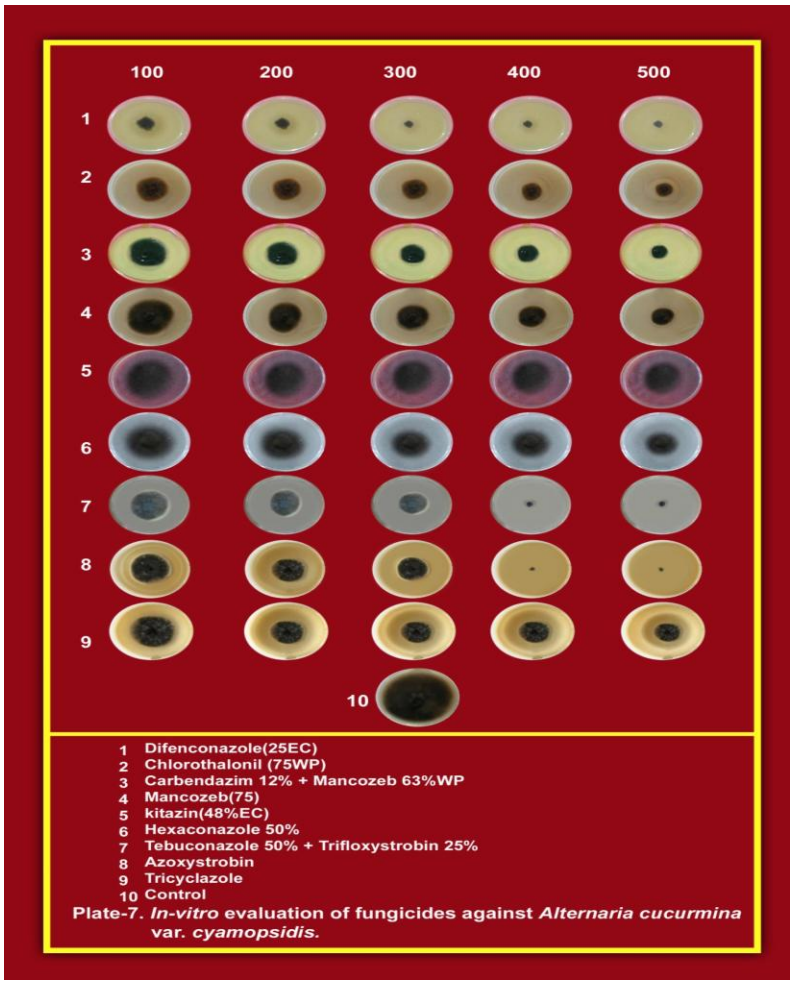
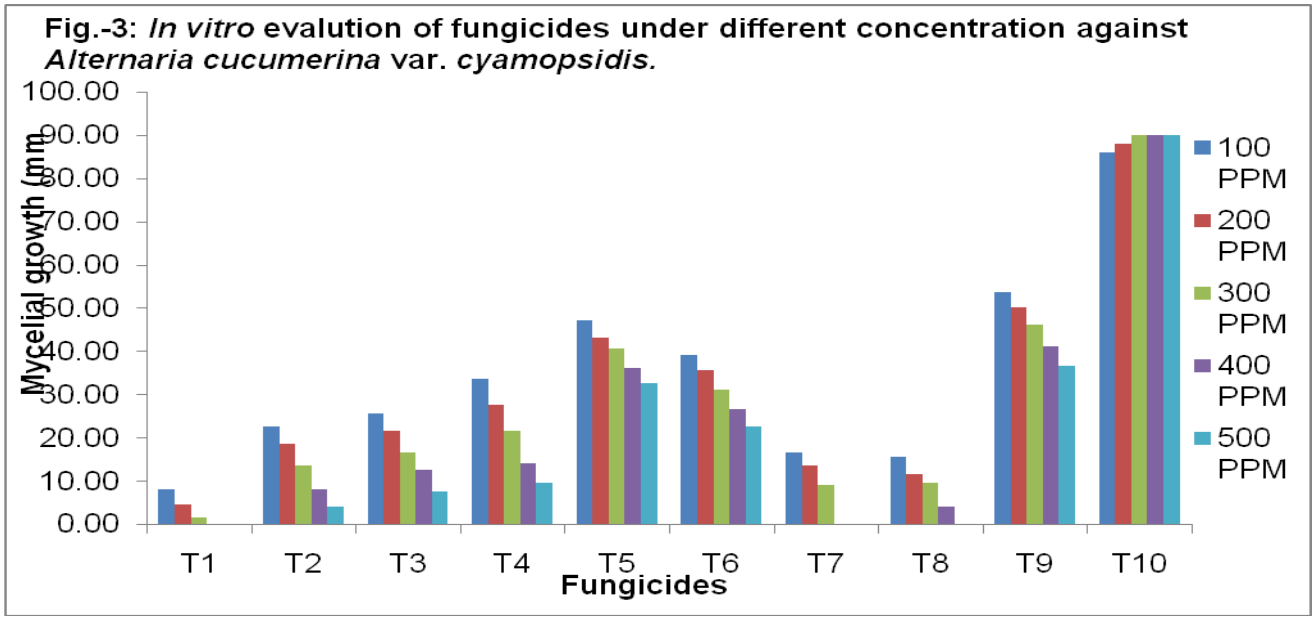


Plate-7. *In-vitro* evaluation of fungicides against *Alternaria cucurmina* var. *cyamopsidis.*



Out of eight botanical and plant extract and bio-products against *Alternaria cucumerina var. cyamopsidis* under *in-vitro* condition at (2, 5, 10%) concentration the minimum growth of fungus was recorded in Garlic bulb extract at all concentration. which was significantly superior over rest of the plant extract in respect of mycelium growth. While maximum growth was recorded in vermi wash among the all eight botanical and bio-products. The plant extracts showed antifungal activity and can be used in control of fungus associated with cluster bean. Similar results were reported by Kumar *et al.,* (2005); Govindachari *et al*., (1998). Easier workers have also found garlic clove extract to be effective against growth and or conidial germination of *Alternaria* spp. Mishra and Gupta (2012) found that among plant extracts, clove extracts of *Allium sativum* at 10% resulted in maximum inhibition of growth *of A. porri*and *S. vesicarium*, respectively followed by *Aloe vera* at 10% concentration. Abd El-Ghany*et al.,* (2015) conducted that extracts of *Azadirachta indica* and *Jatropha curca*s were the most effective to inhibit the growth of the tested fungi. Different concentrations of plant extract of A. *indica* and of chemical fungicides were studied on the growth of *Aspergillus flavus* and *Alternaria alternate*. Findings from this study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides. *Azadirachta indica* extract, which was found to be the most efficient extract, might be a promising agent for controlling these fungi. Jhala *et al.,* (2017) concluded that the Neem formulations Azadirachtin was found effective *in vitro* followed by garlic extract and Neem oil proved to be least inhibitor. Barros *et al.,* (1995) reported inhibition of *Alternaria alternate* and *A. longipes* with bulb extracts of Garlic against mycelial growth. Antifungal properties in plant extracts plant extracts have also been observed against growth of *A. alternate, A. brassicae, Colletotrichum capsici, Fusarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclertiarum*. Antifungal activites of extracts of *Azadirachta indica, Ocimum santum, Allium cepa and A. sativum* have also been reported by Shivpuri *et al.,* (1997). Further studies are needed to isolate and characterize the antifungal moieties in these for practical disease control. *Azadirachta indica, Allium capa* and *A. sativum* showed antifungal property against *Alternaria alternate*. The extracts of Eucalyptus sp. And Calotropisprocera inhibited the growth of the fungus Shivpuri *et al.,* (1997) studied the extracts of 10 plant species (*Allium cepa, A.sativum, Azadirachta indica, Calotropis procera, Datura stramonium, Ocimum sanctum, Polyalthia longifolia, Tageteserecta, Vincarosea* and *Klithomia somnifera*). These botanical showed fungitoxic properties against Alternaria and other pathogenic fungi. *Allium sativum* and *Allium cepa* brought about significant reduction in disease caused by alternaria leaf spot on the soyabean crop (Bhosale *et al.,* 2014). Fungicide application can minimize disease and thus increase the genetic potential and ultimately yield. Therefore, it necessities the judicial use of fungicides at proper time.

In the present investigation nine fungicides tested under *in-vitro* condition at 100, 200, 300, 400 and 500ppm all the tested fungicides were capable of inhibiting the growth of pathogen. However difenconazole causes minimum mycelium growth at all concentrations, which was followed by azoxtrobin and carbendazim12%+ manozeb 63% WP. Three fungicides namely Azoxystrobin, Tebuconazole + Trifloxystrobin and Difenconazole inhibited per cent mycelial growth at 500 ppm. Khan *et al.,* (1995) also obtained a complete inhibition of *A. alternata* with propiconazole and tridemorph at 30ppm. Hexaconazole inhibited

cent per cent growth of A. alternata (Dubey *et al*., 2000). tridemorph was shown to be efficient in inhibiting the growth of *A. alternate f.sp. Cucurbitae.* Thiophanate methyl and carbendazim were found average in inhibiting the *A. alternate.* Khan *et al.,* (1995) also reported significantly less mycelial growth of *A. alternate* in carbendazim and thiophanate methyl.

Among the fungicide plant extract and bio-products under, field condition at 45 and 60 day after sowing the result reveals that all the plant extracts , bio-products and fungicide superior in checking disease. The minimum disease severity was recorded with difencozole at 45 and 60 DAI. Which was followed by Carbendazim12% + Mancozeb63% WP, Chlorothalonil, Azoxystrobin, Tebuconazole 50%+ Trifloxystrobin 25%, Garlic bulb extract, Tricyclazole. Among the botanical and bio products Garlic bulb extracts superior over rest of the botanical and bio-products. Among the fungicides tested under field conditions, the mixture azoxystrobin-difenconazole was the most effective in reducing Alternaria blight intensity (Kandolo *et al.,* 2016). Devananthan and Ramanunjam (1995) observed a best control of *A. solani* by Mancozeb followed by chlorothalonil and copper oxychloride. Meena *et al.,* (2010) observed that the disease intensity was significantly reduced by fungicide spray. The disease severity was low in treatments with Difenconazole and Chlorothalonil compared to that in control. Nimbecidine (*Azadirachtin*) spray treatment provided a good level of disease control (59%). Treatments with aqueous leaf extract of *Calotropis* and *Azadirachta* as well as its seed kernel extract were also effective in controlling blight. The highest and significantly enhanced grain yield was with Difenconazole and the next best was with chlorothalonil. Azadirachtin, Calotropis leaf or Azadirachta seed kernel and leaf extracts also enhanced grain yields.

References

Abd El-Ghany, T., M. Roushdy, M. M., Mohamed, A. and Abboud, Al. (2015).Studies on efficacy of certain plant extracts as safe fungicides against phytopathogenic and mycotoxigenic Fungi. *Agri. Bio. Sci. J.* 1(3): 71-75.

Anonymous, (2017-18). Directorate of Agriculture. Agriculture Statistics. Government of Rajasthan, Jaipur.

Bhosale, Sri Kanth, B., Jadav, D.S., Patil, B.Y. and Chavan Ashok, M. (2014). Bio efficacy of plant extract on Alternaria leaf spot of soybean (*Glycine max (L.)* Merr). *Indian J. Appl. Res.,* 4-11.

Dubey, A. K., Pandya, R. K., Bataria, A. M. and Srivastava, A. K. (2002). Efficacy of plant extracts against *Alternaria tagetics*. *National Seminar on Role of Anti microbial for Sustainable Horticulture*, January 20, 2002 at IGAU, Raipur (India). pp. 278.

Govindachari, T.R., Suresh, G., Geetha, Gopalakrishnan. Balaganesan, Banumathy and Masilamani, S. (1998). Identification of antifungal compounds from the seed oil of *Azadirachta indica*. *Phytoparasitica*. 26(2): 109-116.

Jhala, P., Mal, B. L. and Meena, M. K. (2017).Effective management of purple blotch of onion caused by *Alternaria porri*(Ellis) through host resistance, fungicides and botanicals. *Int.J.Curr.Microbiol.App.Sci. 6*(5): 1737-1745.

Kandolo, S.D., Thompson, A.H., Calitz, F.J., Laurie, S.M., Truter, M., Van Der, J.E., Waals and Aveling, T.A.S. (2016). Field tolerance of selected varieties and fungicide efficacy against Alternaria blight of sweet potato**.***African Crop Sci. J.*, 24(3): 331 – 339.

Kumar Sanjeet; Upadhyay, J. P. and Kumar, Sanjeev. (2005). Evaluation of plant extract for control. *J. Phytopathol*. 7(2):

166-168.

Meena A. K., Godara, S.L. and Gangopadhyay, S., (2010). Efficacy of fungicides and Plant Extracts against Alternaria Blight of Cluster bean. *J. Mycol. Pl. Pathol.* 40(2): 272-275.

Mishra, R. K. and Gupta, R. P. (2012).*In vitro* evaluation of plant extracts, Bio- agents and fungicides against purple blotch and stem phylium blight of onion. *J. Med. Plant Res*. 6(45): 5658-5661.

Nene YL, Thapliyal PN (1979).Fungicides in Plant Disease Control, Oxford and IBH Publishing House, New Delhi. pp. 163.

of Alternaria Leaf spot of *Viciafaba*. *Ann. Pl. Protec. Sci*., 13(1): 213-269.

Orellana, R. G. and Simmons, E.G. (1965).Alternaria blight of guar in the United States. *Pl. Dis. Rep.*, 27: 129-131.

Shivpuri.A. O.P. sharma and S.L. Jhamarina. (1997). Fungi toxic properties of plant extract against pathogenic fungi. J. Mycol. Pl. Pathol. 27: 29-31.

Swamy, Dhananjaya, P.S. and Naveena, K.P. (2015). An analysis of production performance and yield variability of guar (Cluster bean) in India. *J. Prog. Agric.*, 6: 1.

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