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In vitro Evaluation of Bio-efficacy of Botanicals as Water and Ether extracts against *Fusarium oxysporum* f. sp. *radicis-cucumerinum*

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ABSTRACT: Use of natural products like botanical amendments or botanical extracts for the management of fungal diseases in plants is considered as a substitute method to synthetic fungicides, due to their less negative effects on the human and environment health hazard or implications. Botanicals are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. The antifungal properties of plants have been proved in a number of instances as potential means for the control of many diseases. The present study was conducted to evaluate the antifungal efficacy of few botanicals extracted using water and ether against *Fusarium oxysporum* f. sp. *radicis-cucumerinum in vitro.* To study the antifungal mechanism of plant extract, poisoned food technique was used for experiment. Eight plant species *viz., Ipomea carnea, Calotropis gigantean, Allium cepa, Datura stromonium, Catharanthus roseus, Azadirachta indica, Curcuma longa and Piper nigrum* used to test their efficacy against the pathogen at three concentrations (10, 20 and 30 per cent). Among them *A. indica* was highly effective in inhibiting the growth of Pathogen as 58.51, 69.63 and 77.77 per cent growth inhibition in water extract and 62.96, 74.07 and 82.22 per cent growth inhibition in ether extract at 10, 20 and 30 per cent concentrations, respectively.

Keywords: *botanicals, water and ether extract, Fusarium oxysporum* f. sp. *radicis-cucumerinum.*

INTRODUCTION

Cucumber (*Cucumis sativus* L.) belongs to family *Cucurbitaceae* and most important vegetable, which is major source of human edible products and useful fibers. Cucumber popularly known in India as '*khira*' and gherkins are extensively grown in tropics, subtropics and milder temperate zones of India. In India, major cucumber growing states are Karnataka, Andhra Pradesh, Assam, Bihar, Jammu Kashmir, Telangana, Madhya Pradesh, Orissa, Kerala, Jharkhand and almost all states with total production 1.14 million tons in 78 thousand hectare area (Anon., 2017). The productivity of the crop is more affected in the polyhouse as well as in field by insects, pest and diseases. Among them, diseases are one of the major constraints affecting quality and quantity of the crop. Many diseases have been reported on cucumbers from different part of the world, but only few of them cause economic losses. Although an accurate estimate is difficult to obtain, the annual crop loss is probably between 20 and 30% (Anon. 2017). Root and stem rot of cucumber is believed to be caused by a new formae specialis of *F. oxysporum*, presently designated as *F. oxysporum* f. sp. *radicis-cucumerinum* (FORC) (Vakalounakis, 1996). Root and stem rot is the most destructive disease of glasshouse cucumber crops in Canada in 1994, in France in 1998, in China in 1999 and in Spain in 2000, causing significant losses in the yield (Punja & Parker, 2000). When cucumber is infected with the root and stem rot fungus, the primary, secondary and tertiary roots and the basal portion of the stem have brown discolorations. On the stem, this discolouration may extend for 40 to 100 cm above the soil line. *Fusarium* root and stem rot of cucumber has been reported to be favoured at lower soi1 temperatures (17°C) (Vakalounakis, 1996). Botanicals are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many botanicals have been identified to be effective in the control of plant diseases. Presence of antimicrobial substances in plant has attracted attention of many research workers in recent years. A number of plants have been shown to have antimicrobial substances (Spencer *et al.*, 1957; Mercer *et al.*, 1970; Nicalis 1970;

Nene and Kumar 1966; Defoses, 1966). The antifungal properties of plants have been proved in a number of instances as potential means for the control of soil borne disease. Chemical fungicides are used to control Fusarium wilt of cucumber. Unfortunately, these chemical fungicides are not readily biodegradable; tend to persist for years in the environment and few fungi have developed resistance to them. Use of natural products like botanical amendments or botanical extracts for the management of fungal diseases in plants is considered as a substitute method to synthetic fungicides, due to their less negative effects on the human and environment health hazard or implications. In the view of above context *In vitro* experiments were conducted to test the antifungal efficacy of some plant at different concentrations against *Fusarium oxysporum* f. sp. *radicis-cucumerinum.*

Thakare (2003) evaluated (*in vitro*) the efficacy of some botanicals against *Fusarium oxysporum* and resulted that100 per cent mycelial growth inhibition was obtained with *Allium sativum* (0.1 per cent) followed by 100 percent with *Azadirachta indica* (10 per cent), 37.48 percent in *Ocimum sanctum* (10 per cent) and 47.97 percent was with *Gliricidia maculate* (10 per cent) respectively. Aqueous extract of *A. indica* was most effective in inhibiting mycelial growth (67.8%) of *F. udum* followed by *Datura festilosa* (61.2%), *Tagetes erecta* (52.6%), *Eucalyptus citridora* (52.2%), *Aegle marmelos* (47.9%) and *Mimusops elengi* (45.9%) respectively (Singh *et al.,* 2010). *In vitro* antifungal assay was conducted against *F. oxysporum* f. sp. *lycopersici* (FOL) using plant extracts of fifteen plants. Out of fifteen plants, three plants proved to be potential in inhibiting the growth of the FOL *viz.*, *Solanum indicum* (78.33%), *A. indica* (75.00%), *Oxalis latifolia* (70.33%) at 20% concentration (Anil *et al.,* 2015). Abu-Tahon *et al.* (2014) *in vitro* studied the efficacy of 5 medicinal plant extracts *i.e. E. globules, L. camera, Nerium oleander* and *O. basilicum* against *F. oxysporum* f. sp. *lycopersici* race 3 in Egypt and found that cold distilled water extract of *O. basilicum and E. globulus* were most effective to inhibiting the growth of the pathogen. Singh *et al.,* (2010) reported that aqueous extract of *A. indica* was most effective in inhibiting mycelial growth (67.8%) of *F. udum* followed by *Datura festilosa* (61.2%), *Tagetes erecta* (52.6%), *Eucalyptus citridora* (52.2%), *Aegle marmelos* (47.9%) and *Mimusops elengi* (45.9%) respectively. Devi and Chhetry, (2012) screened antifungal effect of plant extracts against mycelial growth and spore germination of *F. udum* at different concentrations of 5, 10, 15 and 20 per cent using poisoned food technique and cavity slide method. Among them, *A. sativum* at 20% alone recorded 100% inhibition of mycelial growth and spore germination.

MATERIAL AND METHODS

A. Isolation and Pathogenicity test

The infected samples were brought from field of RCA Horticulture farm and RCA Polyhouse during *Kharif* 2017-18. When crop was one month old. For isolation of the pathogen, the diseased roots were thoroughly washed first in the running tap water and finally with sterilized water and aseptically placed on Potato Dextrose Agar (PDA) medium and the plates were incubated at 28 ± 2°C in BOD incubator for incubation and examined daily for emerging any fungal growth. After five days of incubation fungal growth started in the inoculated bits. The white pinkish culture so obtained, was further purified by employing hyphal tip method. The morphological, cultural and formation of macro conidia and micro conidia as chlamydospores were the principle characters to identify the pure cultures, and compared with the standard reference description (Holliday, 1980). The pathogenicity test was conducted on the susceptible variety(Cucumber Long Desi) and pathogenicity was confirmed and the inoculum was prepared using sorghum grains for mass production which was used for further studies (Plate 1 & Plate 2).

B. Preparation of Botanical Extracts

For *In vitro* evaluation of eight plant species for their fungicidal activity against root and stem rot pathogen an experiment was carried out during 2018. The plant species and their parts used in the study are presented in Table 1 & Table 2. These are *Ipomea carnea, Calotropis gigantean, Allium cepa, Datura stromonium, Catharanthus roseus, Azadirachta indica, Curcuma longa* and *Piper nigrum*. They were crushed in a sterilized pestle and mortar by adding a little quantity of ether solvent for the extracts of ether-phytoextract and with distilled water to get water-phytoextract. The extract was collected by filtering through the two layers of muslin cloth. Finally, filtrate thus obtained from the leaves was used as stock solution.

C. Poison food Technique

To study the antifungal mechanism of plant extract, poisoned food technique was followed as suggested by Nene and Thapliyal (1993). 10, 20 and 30 ml of stock solution was taken separately and were mixed with 100 ml sterilized molten potato dextrose agar medium respectively, so as to get 10, 20 and 30 per cent concentrations.About 20 ml medium was poured into each of the 90 ml sterilized petriplates. Three replications were maintained for each treatment. Suitable control plates were maintained. Each plate was placed with 5 mm mycelial bit aseptically taken from the periphery of 7 days old culture and incubated at 28±2ºC in BOD incubator till the growth of the colony touched the periphery in control plate. Mean colony diameter in each case was recorded.

The efficacy of plant extracts was expressed as percent inhibition of mycelial growth over control which was calculated by using the formula as given by Vincent (1947).

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

Where,

I = Percent inhibition

C = Colony diameter in control; T = Colony diameter in treatment

D. Statistical Analysis

The data from various experiments were subjected to analysis for coefficient of deviation. For laboratory and pot trials, completely randomized design was followed. Means of the experiments were used to compare for efficacy of treatments.

RESULTS AND DISCUSSION

The symptoms of root and stem rot of cucumber showed that rotting of roots, lower stems, crowns and rotting of seeds and seedlings (damping-off) (Agrios, 2005). This proved the pathogen was *F. oxysporum* f. sp. *radicis-cucumerinum* and pathogenicity was confirmed (Plate 1 & Plate 2).

Infected field view

Typical symptoms on stem

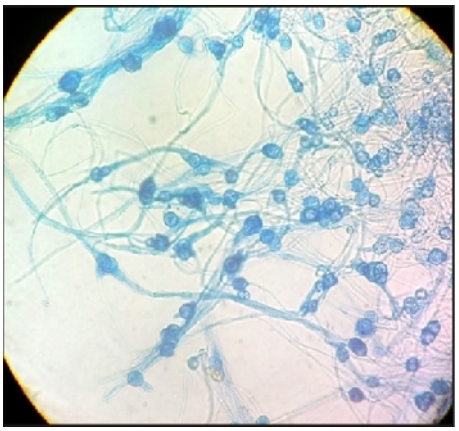
Infected root

Plate 1. Infected root and stem rot caused by *F. oxysporum* f. sp. *radicis-cucumerinum*

Gaint culture and pure culture of *F. oxysporum* f. sp. *radicis-cucumerinum*

Pathogenicity of *F. oxysporum* f. sp. *radicis-cucumerinum* by soil inculation method in pot.

Symptoms developed in seedlings after proving pathogenicity



Chlamydospore of *F. oxysporum* f. sp. *radicis-cucumerinum*

Plate 2.

A. Efficacy of Botanical Extracts

Eight botanicals as water and ether extract such as *Ipomea carnea*, *Calotropis gigantean*, *Allium cepa*, *Datura stromonium*, *Catharanthus roseus*, *Azadirachta indica*, *Curcuma longa* and *Piper nigrum* were evaluated for their efficacy against *F. oxysporum* f. sp. *radicis-cucumerinum* under *in vitro* conditions by Poison Food Technique at three different concentrations (10, 20 and 30 %).

The results (Table 1 and Plate 3) revealed that none of the plant extracts with water could completely inhibit the growth of *F. oxysporum* f. sp. *radicis-cucumerinum* even at 30 per cent concentration but were significant over the control. In these, *A. indica* extract was significantly superior and highly effective in inhibiting the growth of Pathogen as 58.51, 69.63 and 77.77 per cent growth inhibition with 37.33, 27.33, 20.00 per cent mycelial growth at 10, 20 and 30 percent concentrations, respectively. *C. longa*, *P. nigrum*, *D. stromonium*, *C. gigantean*, *A. cepa*, *I. carnea* and *C. roseus* caused 53.33, 50.37, 45.92, 41.85, 38.88, 36.29 and 34.07 per cent growth inhibition with 42.00, 44.67, 48.67, 52.33, 55.00, 57.33 and 59.33 per cent mycelial growth of *F. oxy*sporum f. sp. *radicis-cucumerinum* at 10 percent concentration, respectively.

Table 1. Effect of different Botanicals (Water extract) on mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum in vitro.*

Sr. No.	Treatments/ Botanicals	Common name	Part used	Radial growth of Pathogen (mm)*at different conc. (%)			Inhibition per cent(%)		
				10	20	30	10	20	30
1.	*Ipomea carnea*	Morning glory	Leaves	57.33	47.00	39.33	36.29 (37.03)	47.77 (43.70)	56.29 (48.59)
2.	*Calotropis gigantea*	Calotropis	Leaves	52.33	42.33	34.00	41.85 (40.29)	52.96 (46.68)	62.22 (52.05)
3.	*Allium cepa*	Onion	Bulb	55.00	45.00	36.67	38.88 (38.56)	50.00 (44.98)	59.25 (50.31)
4.	*Datura stromonium*	Datura	Leaves	48.67	38.67	30.00	45.92 (42.64)	57.03 (49.02)	66.66 (54.71)
5.	*Catharanthus roseus*	Periwinkle	Leaves	59.33	49.33	43.00	34.07 (35.69)	45.18 (42.21)	52.22 (46.25)
6.	*Azadirachtaindica*	Neem	Leaves	37.33	27.33	20.00	58.51 (49.88)	69.63 (56.53)	77.77 (61.85)
7.	*Curcuma longa*	Turmeric	Root	42.00	32.00	24.00	53.33 (46.89)	64.44 (53.37)	73.32 (58.88)
8.	*Piper nigrum*	Black pepper	Seed	44.67	34.67	28.00	50.37 (45.19)	61.48 (51.62)	68.88 (56.07)
9.	Control			90.00	90.00	90.00	0.00	0.00	0.00
SEm ±				**0.43**	**0.42**	**0.50**	**0.28**	**0.27**	**0.34**
CD at 5%				**1.29**	**1.25**	**1.49**	**0.84**	**0.81**	**1.02**

*Mean of three replications; Figures in parentheses are arcsine √ per cent angular transformed values

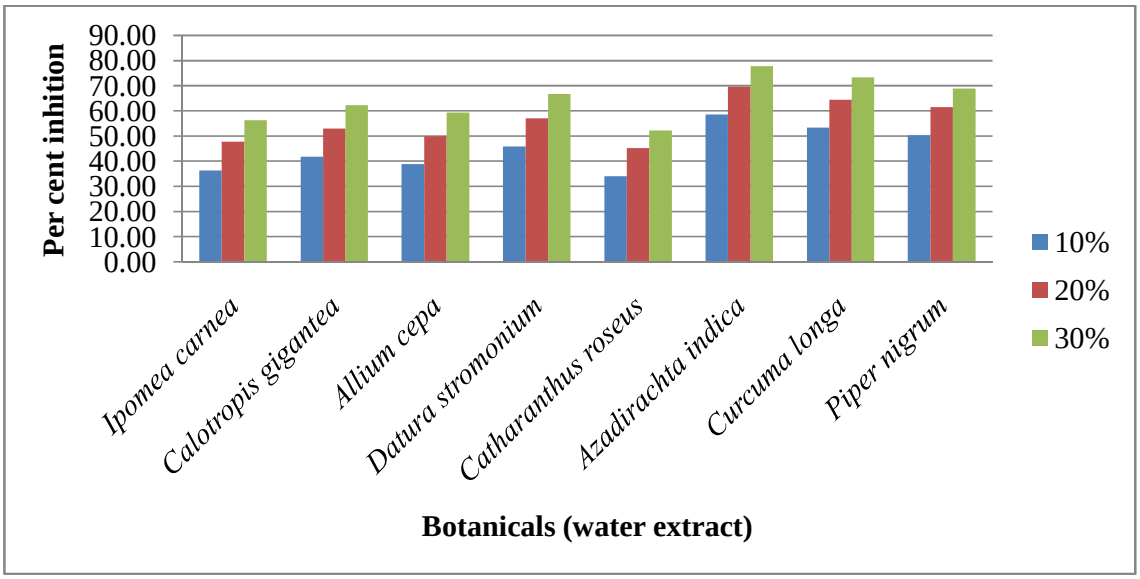


Fig. 1. *In vitro* efficacy of different Botanicals (water extract) against *F. oxysporum* f. sp. *radicis-cucumerinum.*

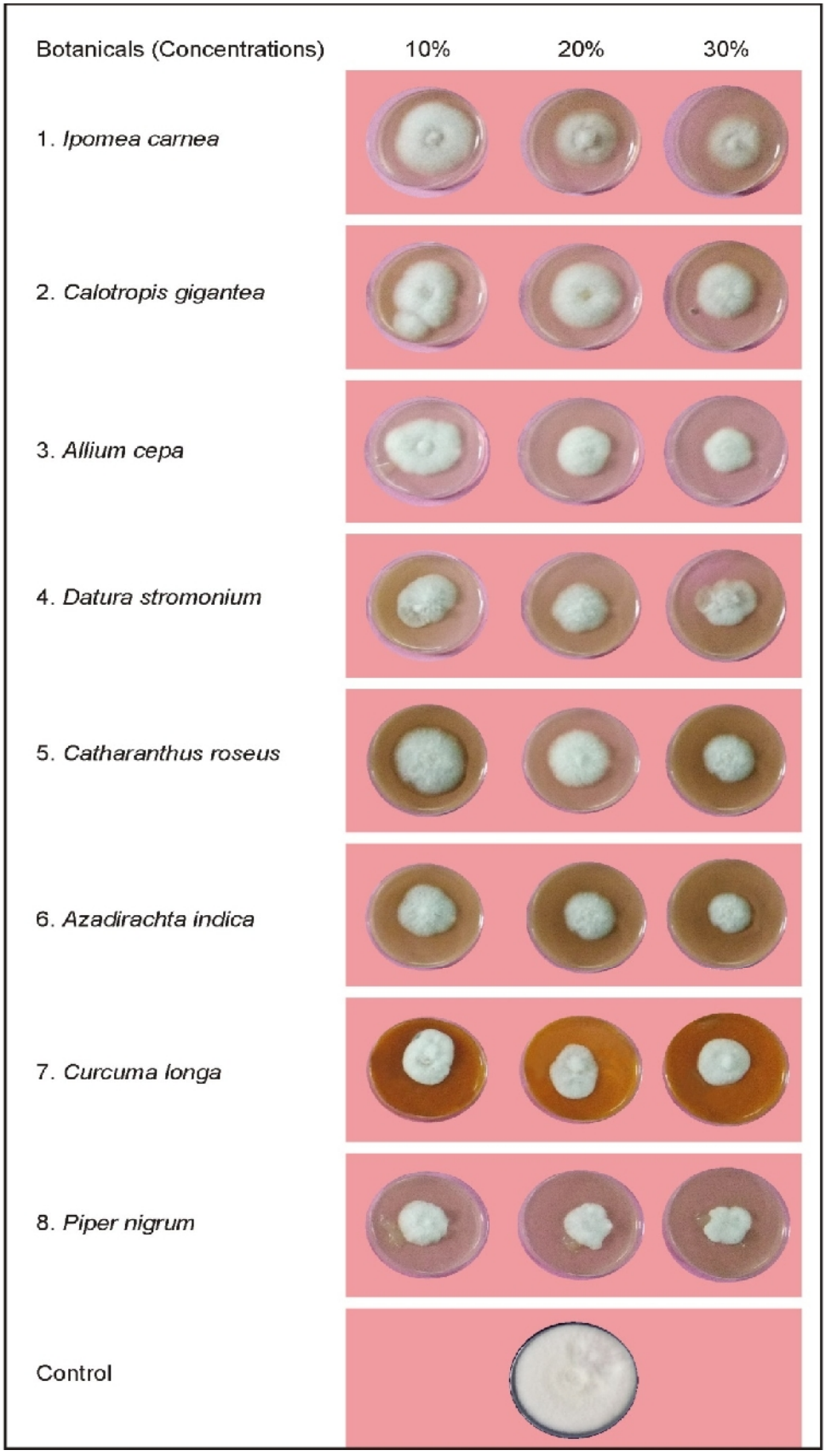


Plate 3. *In vitro* evaluation of different botanicals as water against *F. oxysporum* f. sp. *radicis-cucumerinum.*

At 20 per cent concentration, *C. longa, P. nigrum, D. stromonium, C. gigantean, A. cepa, I. carnea* and *C. roseus* caused 64.44, 61.48, 57.03, 52.96, 50.00, 47.77 and 45.18 per cent inhibition of growth with 32.00, 34.67, 38.67, 42.33, 45.00, 47.00 and 49.33 per cent mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum*, respectively. At 30 per cent concentration, *C. longa, P. nigrum, D. stromonium, C. gigantean, A. cepa, I. carnea* and *C. roseus* caused 73.32, 68.88, 66.66, 62.22, 59.25, 56.29 and 52.22 percent inhibition of mycelial growth with 24.00, 28.00, 30.00, 34.00, 36.67 and 43.00 per cent mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum*, respectively.

Similar results were seen even with the ether extracts, the results (Table 2 and Plate 4) revealed that none of the plant extracts could completely inhibit the growth of *F. oxy*sporum f. sp. *radicis-cucumerinum* even at 30 per cent concentration and were significant over the control. Among these, *A. indica* extract was significantly superior and highly effective in inhibiting the growth of Pathogen as 62.96, 74.07 and 82.22 percent with 33.33, 23.33 and 16.00 per cent mycelial growth at 10, 20 and 30 percent concentrations, respectively.

C. longa, P. nigrum, D. stromonium, C. gigantean, A. cepa, I. carnea and *C. roseus* caused 57.77, 54.81, 50.37, 46.29, 43.33, 40.74 and 38.51 inhibition of mycelial growth with 38.00, 40.67, 44.67, 48.33, 51.00, 53.33 and 55.33 per cent mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum* at 10 percent concentration, respectively. At 20 per cent concentration, *C. longa, P. nigrum, D. stromonium, C. gigantean, A. cepa, I. carnea* and *C. roseus* caused 68.88, 65.92, 61.48, 57.40, 54.44, 52.22 and 49.63 percent inhibition of mycelial growth with 28.00, 30.67, 34.67, 38.33, 41.00, 43.00 and 45.33 per cent mycelial growth of *F. oxysporum* f.sp. *radicis-cucumerinum*, respectively. At 30 per cent concentration, *C. longa, P. nigrum, D. stromonium, C. gigantean, A. cepa, I. carnea* and *C. roseus* caused 77.77, 73.33, 71.11, 66.66, 63.70, 60.74 and 56.66 percent inhibition of mycelial growth with 20.00, 24.00, 26.00, 30.00, 32.67, 35.33 and 39.00 per cent mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum*, respectively.

Table 2. Effect of different Botanicals (Ether extract) on mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum in vitro.*

S.No.	Treatments/Botanicals	Common name	Part used	Radial growth of Pathogen (mm)*at different conc. (%)			Inhibition per cent(%)		
				10	20	30	10	20	30
1.	*Ipomea carnea*	Morning glory	Leaves	53.33	43.00	35.33	40.74 (39.65)	52.22 (46.25)	60.74 (51.18)
2.	*Calotropis gigantea*	Calotropis	Leaves	48.33	38.33	30.00	46.29 (42.86)	57.40 (49.24)	66.66 (54.71)
3.	*Allium cepa*	Onion	Bulb	51.00	41.00	32.67	43.33 (41.15)	54.44 (47.53)	63.70 (52.93)
4.	*Datura stromonium*	Datura	Leaves	44.67	34.67	26.00	50.37 (45.19)	61.48 (51.62)	71.11 (57.45)
5.	*Catharanthus roseus*	Periwinkle	Leaves	55.33	45.33	39.00	38.51 (38.34)	49.63 (44.77)	56.66 (48.81)
6.	*Azadirachta indica*	Neem	Leaves	33.33	23.33	16.00	62.96 (52.49)	74.07 (59.37)	82.22 (65.04)
7.	*Curcuma longa*	Turmeric	Root	38.00	28.00	20.00	57.77 (49.45)	68.88 (56.07)	77.77 (61.85)
8.	*Piper nigrum*	Black pepper	Seed	40.67	30.67	24.00	54.81 (47.74)	65.92 (54.26)	73.33 (58.89)
9.	Control			90.00	90.00	90.00	0.00	0.00	0.00
SEm ±				**0.43**	**0.42**	**0.50**	**0.28**	**0.27**	**0.36**
CD at 5%				**1.29**	**1.25**	**1.49**	**0.83**	**0.82**	**1.07**

*Mean of three replications; Figures in parentheses are arcsine √ per cent angular transformed values

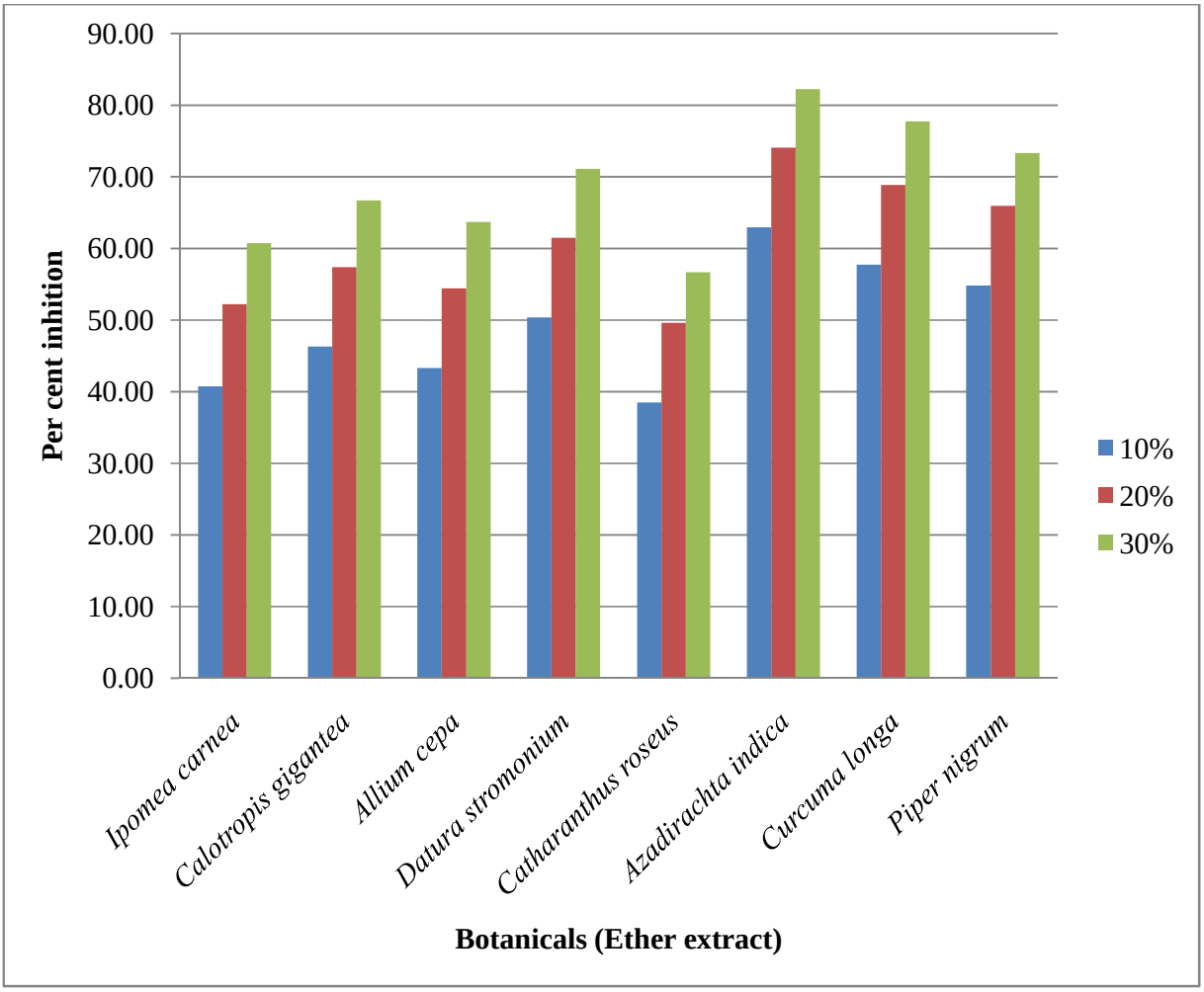


Fig. 2. *In vitro* efficacy of different Botanicals (Ether extract) against *F. oxysporum* f. sp. *radicis-cucumerinum.*

B. Discussion

Both water and ether extracts of the botanicals *A. indica* at 30% concentration showed maximum growth inhibition of the mycelial growth of the pathogen against *F. oxysporum* f. sp. *radicis-cucumerinum In vitro.* The inhibition per cent as water extract was 77.77% and as ether extract was 82.22% with mycelial growth 20.00% and 16.00%, respectively. The growth inhibition of the pathogen was due to the antimicrobial compounds present in the extract which inhibited the growth of the pathogen. The leaf extract of *A. indica* was well supported by ether solution which enhanced the inhibition percentage than that of water. The results discussed above were in tune with the results reported by several workers *viz.*, Thakare (2003) observed 100 per cent growth inhibition of *F. oxysporum* by leaf extract of *A. indica*. The study of Anil *et al.* (2015) resulted in 75.0% growth inhibition of *F. oxysporum* f. sp. *lycopersici* by *A. indica.* Singh *et al.* (2010) reported 67.8% growth inhibition of *F. udum* by *A. indica*. Patil (2003) *in vitro* tested various botanicals against *F. oxysporum* causing wilt of patchouli, per cent inhibition achieved was 76.72 per cent with garlic extract (10%) and inhibition of 69.87 per cent tulsi leaf extract (10%) respectively. Chohan *et al.* (2011) registered that toxic effects of five medicinal plants namely, *Azadirachta indica*, *Ocimum basilicum*, *Datura stramonium*, *Tagetes erecta* and *Allium sativum* were tested at 2, 4, 6 and 8% concentration against *Fusarium oxysporum* f. sp. *gladioli in vitro*. Out of five medicinal plants, extract of *A. indica* showed maximum mycelial growth inhibition both at 8% concentration (83.5) and 2% concentration (34.5%)followed by *T. erecta*, *A. sativum* and *D. stramonium* that suppressed the mycelial growth at 8% concentration *viz.* 58.5, 35 and 28.5% respectively. *O. basilicum* was the least effective in suppressing the mycelial growth of *F. oxysporum.*

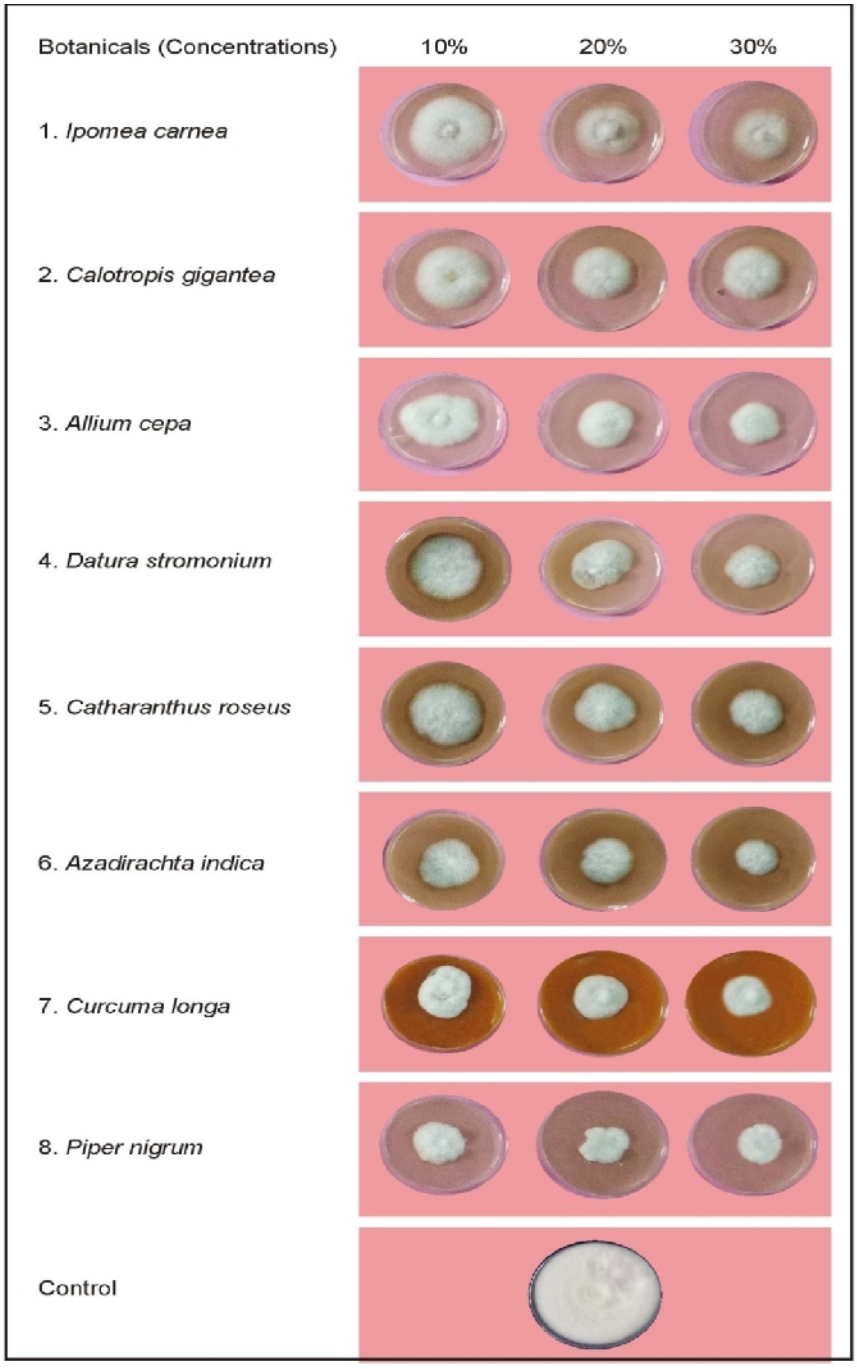


Plate 4. *In vitro* evaluation of different botanicals as ether extract against *F. oxysporum* f. sp. *radicis-cucumerinum.*

SUMMARY, CONCLUSION AND FUTURE SCOPE

To develop effective management strategies, the botanicals should be included in the cultivation practice. Eight Botanicals as water and Ether extract such as *Ipomea carnea, Calotropis gigantea, Allium cepa, Datura stromonium, Catharanthus roseus, Azadirachta indica, Curcuma longa* and *Piper nigrum* were evaluated *in vitro* against mycelial growth of *F. oxysporum* f. sp. *radicis-cucumerinum* at three concentrations *viz*., 10, 20 and 30 per cent by poison food technique. Among them, *A. indica* ether extract was highly effective in inhibiting the growth of Pathogen as 62.96, 74.07 and 82.22 percent at 10, 20 and 30 percent concentrations, respectively. And in water extracts, *A. indica* extract also found effective in inhibiting the growth of pathogen as 58.51, 69.63 and 77.77 percent at 10, 20 and 30 percent concentrations, respectively. The other botanicals were not so effective in inhibiting the growth of the pathogen, the only other botanical that exhibited good per cent inhibition of the pathogen was turmeric at 3 per cent concentration with

73.32 % growth inhibition. *A. indica* was effective in inhibiting the mycelial growth of the pathogen in both water and ether extracts. Due to the antimicrobial properties present in the botanical extracts they are very effective in controlling the disease. Even if they are at higher concentrations such as 30 % they are not harmful for the plants. Hence, the botanicals which were found effective in inhibiting the growth of pathogen and can be incorporated in the management of the disease and can be recommended for use at field level which may help the farmers reducing the cost in use of fungicides and increase in yield which may be attributed by the botanicals applied. In future aspect, Botanicals are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many botanicals have been identified to be effective in the control of plant diseases. Presence of antimicrobial substances in plant has attracted attention of many research workers in recent years. A number of plants have been shown to have antimicrobial substances. The antifungal properties of plants have been proved in a number of instances as potential means for the control of soil borne disease.

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