IN-VITRO EVALUATION OF FUNGICIDES AND PLANT EXTRACTS AGAINST PATHOGENIC FUNGI OF *Tagetes* spp.

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ABSTRACT

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Ten fungicides i.e. Bavistin 50 WP, Capvit 50 WP, Dithane M-45, Greengel 72 WP, Hayvit 80 WP , Indofil M-45, MC Sulphur 80, Ridomil MZ Gold, Salcox 50 WP and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm were evaluated against three pathogenic fungi viz., *Alternaria alternata* (Fr.) Keissler, *Aspergillus fumigatus* Fresenius and *Curvularia lunata* (Wakker) Boedijn isolated from *Tagete serecta* L. and *Tagetes patula* L. showing blight symptom during the period of 2009 to 2014. Tilt 250 EC completely inhibited the radial growth of the test fungi at all concentrations. Antifungal properties of ethanol extract of *Artocarpus heterophyllus* Lam., *Azadirachta indica* A. Juss.,*Cassia sophera* L., *Citrusmedica* L., *Daturametel* L., *Houttuynia cordata* Thunb., *Lantana camara* L., *Mangifera indica* L., *Moringa oleifera* Lam. and *Vitex negundo* L. at 5, 10 and 20% concentrations were evaluated against the three test pathogens. Eexcept *Lantanac amara*, *Mangifera indica* and *Vitex negundo*, all the seven plant extracts completely inhibited radial growth of the test fungi at 20% concentrations.

Keywords: *In-vitro* evaluation, Pathogenic fungi, Fungicides, Plant extracts, *Tagetes* spp

INTRODUCTION

Tagetes is a genus of annual or perennial, mostly herbaceous plants in the sunflower family (Asteraceae or Compositae). It was described as a genus by Linnaeus in 1753. *Tagetes erecta* and *T.patula* are two common species cultivated in Bangladesh. They are native to North and South America, but now become naturalized around the world. The plant has insecticidal effect (Farjana *et al*. 2009). Seeds of *T. erecta* are a natural pesticide. Leaves are used as blood clotting agents in Ayurbedhic treatment. Plants have antifungal properties also. Plant is also used against fever dysenteries, indigestions, ulcers and eczemas (Ahmed *et al*. 2008). It is most effective against the nematode species *Pratylenchus penetrans*(Abid and Maqbool 1990, Olabiyi and Oyedunmade 2000, Politi *et al.* 2012).Rajasekaran *et al.* (2004) reported mosqutocidal potentiality of the plant. Though marigold is presently a profitable cultivated crop to the farmers in Bangladesh but socioeconomic data and information of this flower are very scare. Ninety five percent farmers in Jessore and Jhenaidah district cultivate marigold as commercial basis. The yield of marigold was 2,650,447 flowers per hectare. The gross margin and net return was Tk. 1, 62,186 and 1, 17,812 per hectare respectively. The net return was 80% higher than lentil, 85% higher than mustard and 6% lower than potato cultivation (Hoque *et al.* 2012). From India Mukerji and Bhasin (1986) reported diseases of marigold. Diseases were major constrain for marigold cultivation. In Bangladesh, due to rapid expansion of commercial marigold cultivation many diseases appear on the plants. From Bangladesh powdery mildew of marigold was reported by Hossain *et al*. (2010), Botrytis grey mold was reported by Sultana and Shamsi (2011) and Sclerotinia rot was reported by Rahman *et al.* (2015). Aktar and Shamsi (2014, 2015 and 2016) reported blight of *Tagetes* spp. caused by *Alternaria alternata*, *Asapergillus fumigatus* and *Curvularia lunata*. Present study was undertaken to evaluate some selected fungicides and plant extracts against causal agents of blight of *Tagetes* spp. *in-vitro*.

MATERIALS AND METHODS

Collection of samples

Altogether 180 samples with blight were collected from BARI, Joydebpur, Gazipur; Dhaka, Chittagong, Comilla, Khulna, Pabna, Rajshahi, Rangpur and Sylhet during the period of 2009 to 2014. Severe blight symptom was recorded on leaves, buds, calyx and petals of two species of *Tagetes.*

Isolation and identification of fungi

Fungi associated with infected leaves, buds, calyx and petals of *Tagetes erecta* and *T. patula* were isolated following 'Tissue Planting' method and 'Blotter method' (CAB 1968). Fungi associated with

infected 184 samples were isolated on PDA (Potato Dextrose Agar) medium. Experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka. Identification of the isolates was done following Ellis (1971and 1976*)* and Thom and Raper (1945). All the specimens were preserved in the Herbarium, Mycology and Plant Pathology section, Department of Botany, University of Dhaka, Bangladesh.

Pathogenicity of the isolated fungi

The pathogenicity of all the isolated fungi were tested following modified 'detached leaf technique' (Azad and Shamsi 2011) and 'spraying of spore suspension method (Shamsi and Saha 2015).

Culturing of test pathogens

Pure culture of *Alternaria alternata*, *Aspergillus fumigatus* and *Curvularia lunata* from test tube were separately inoculated on PDA plates and incubated for seven days in an incubator at 25° C. Five mm mycelial block from each pathogen was used for *in-vitro* control of test pathogens.

Preparation of fungicide

Ten fungicides i.e. Bavistin 50 WP, Capvit 50 WP, Dithane M-45, Greengel 72 WP, Hayvit 80 WP , Indofil M-45, MC Sulphur 80, Ridomil MZ Gold, Salcox 50 WP and Tilt 250 EC were collected from Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. For each fungicide, a stock solution having the concentration of 10000 ppm was prepared. Then calculated amount of the stock solution of a fungicide was supplemented with sterilized PDA medium to get the final concentration of 100, 200, 300, 400 and 500 ppm. In the control set required amount of sterile water instead of fungicide solution was added to the PDA medium. Five mm mycelial agar disc cut from the margin of actively growing culture of test fungi and then it was placed at the centre of the plate. Three replications were maintained in both the cases. The radial growth of the colonies was measured at the 5th day of incubation (Shamsi and Saha 2015).

Preparation of plant extracts at different concentration

Ethanol extracts of ten plants viz., *Artocarpus heterophyllus* Lam., *Azadirachta indica* A. Juss., *Cassia sophera* L., *Citrusmedica* L., *Daturametel* L., *Houttuynia cordata* Thunb., *Lantana camara* L., *Mangifera indica* L., *Moringa oleifera* Lam. and *Vitex negundo* L at 5, 10 and 20% concentrations were evaluated against the three test pathogen *A. alternata*, *A. fumigatus* and *C. lunata*. The desired parts of each plant was thoroughly washed in tap water, air dried and was prepared by crushing the known weight of fresh materials with ethanol in ratio of 1:1 w/v. The mass of a plant part was squeezed through fine cloth and the supernatants were filtered through Whatman filter paper No. 1 and the filtrate was collected in 250 ml Erlenmeyer conical flasks. The requisite amount of the filtrate of each plant extract was mixed with PDA medium in which plant extracts were in 5, 10 and 20% concentration following Shamsi *et al.* (2014).

The percent of growth inhibition of the test fungi was calculated by the formula described by Shamsi and Saha (2015).The radial growth of the colonies was measured at the 5th day of incubation. The percent growth inhibition of the test fungus was calculated by using the following formula:

$$I = \frac{C - T}{C} x100$$

Where, I = percent growth inhibition, C = growth in control and T = growth in treatment.

The data were collected as inhibition percentage of the radial growth of the pathogen in mm in each replication and evaluated by analysis of variance (ANOVA) by using STAR statistical program and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Blight symptom was recorded on different parts of *Tagetes erecta* and *T. patula* during the tenure of 2009 to 2014 (Plate I). Disease incidence was started from January and gradually increased up to May. The Lowest disease severity (DS 1) was recorded in the month of January and the highest DS was (DS 9) in the month of May. Rainfall and humidity did not show any effect on disease development but temperature shows noticeable effect on disease development. A total of 20 species of fungi were isolated from *Tagetes erecta* and *T. patula.* Among the isolated fungi *Alternaria alternata, Aspergillus fumigatus* and *Curvularia lunata* were found to be pathogenic to *Tagetes erecta* and *T. patula.*

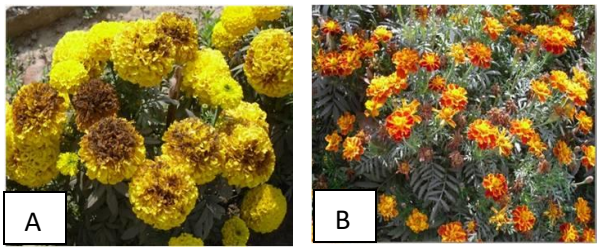

Plate 1. Infected plants showing blight symptom: A. *Tagetes erecta* and B. *T. patula*.

Pathogenicity test on detached leaves and seedlings confirmed *Alternaria alternata* (Fr.) Keissler, *Aspergillus fumigatus* Fresenius and *Curvularia lunata* (Wakker) Boedijn are the potential pathogens of foliage blight of both the species of *Tagetes* (Plate II).

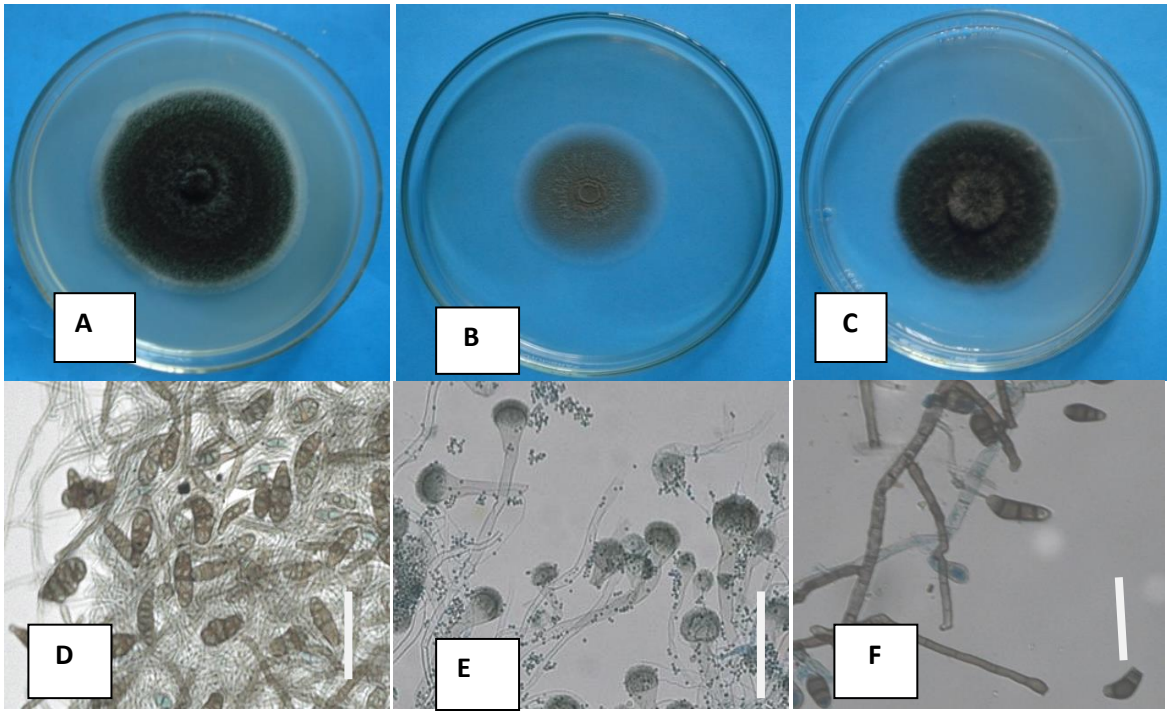

Plate II. A. and D. Colony., Conidiophore and conidia of *Alternaria alternata*, B and E. Colony, Conidiophore and conidia of *Aspergillus fumigatus*, C *and* F. . Colony, Conidiophore and conidia of *Curvularia lunata* (Ba =50.µm)

Amongst the ten fungicides used in the present investigation, Bavistin, Dithane M-45 and Indofil were systemic while Sulphur, Tilt and Salcox were protective fungicides. All the fungicides inhibited the radial growth of the pathogens but complete inhibition of the test pathogens were observed with Tilt 250 EC at all the concentrations used (Fig. 1-3).

On the radial growth of *A. alternata* Salcox 50 WP and Tilt 250 EC were responsible for complete inhibition at all the concentration tested. Bavistin 50 WP and Capvit 50 WP also inhibited the growth completely at 300, 400 and 500 ppm. Bavistin showed 28.70 and 54.63 % inhibition at 100 and 200 ppm respectively. Capvit showed 53.77 and 79.88 % inhibition at 100 and 200 ppm respectively. Dithane M-45 and MC Sulphur 80 also completely inhibited growth of the fungus at 400 and 500 ppm. Dithane showed 48.74, 54.09 and 55.97 % inhibition of test fungus at 100, 200 and 300 ppm respectively. Sulphur showed 18.24, 31.45 and 40.25% inhibition of the fungus at 100, 200 and 300 ppm respectively. Indofil M-45 also completelyinhibited growth of the fungus at 500 ppm. Indofil showed 42.81, 48.93, 52.90 and 83.79 % inhibition of the fungus at 100, 200, 300 and 400 ppm respectively. Greengel 72 WP showed 18.72, 28.77, 35.62, 42.47 and 48.41% inhibition at 100, 200, 300, 400 and 500 ppm respectively. Hayvit 80 WP showed 2.75, 18.35, 22.93, 26.91 and 41.90 % inhibition of radial growth of *A. alternata* at 100, 200, 300, 400 and 500 ppm respectively. Ridomil MZ Gold showed 14.46, 24.22, 32.70, 39.62 and 52.52 % inhibition at 100, 200, 300, 400 and 500 ppm respectively.The toxicity of these fungicides against *A. alternata* at 100 ppm concentration in descending order was Tilt / Salcox > Capvit > Dithane > Indofil > Bavistin > Greengel > MC Sulphur >Ridomil > Hayvit(Fig. 1).

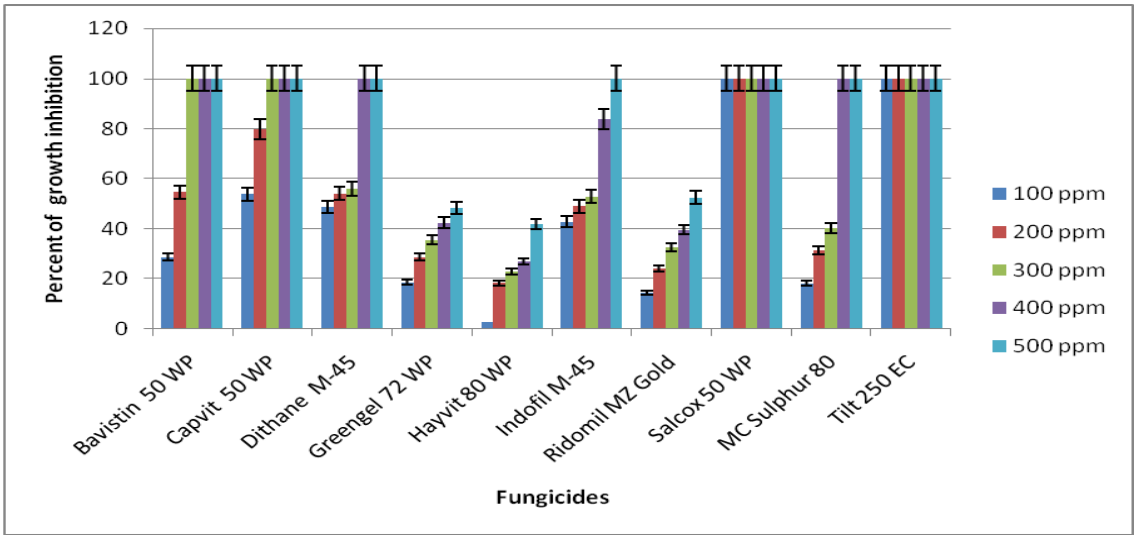

Fig. 1. Percent inhibition of radial growth of *Alternaria alternata* owing to fungicides at different Concentrations.

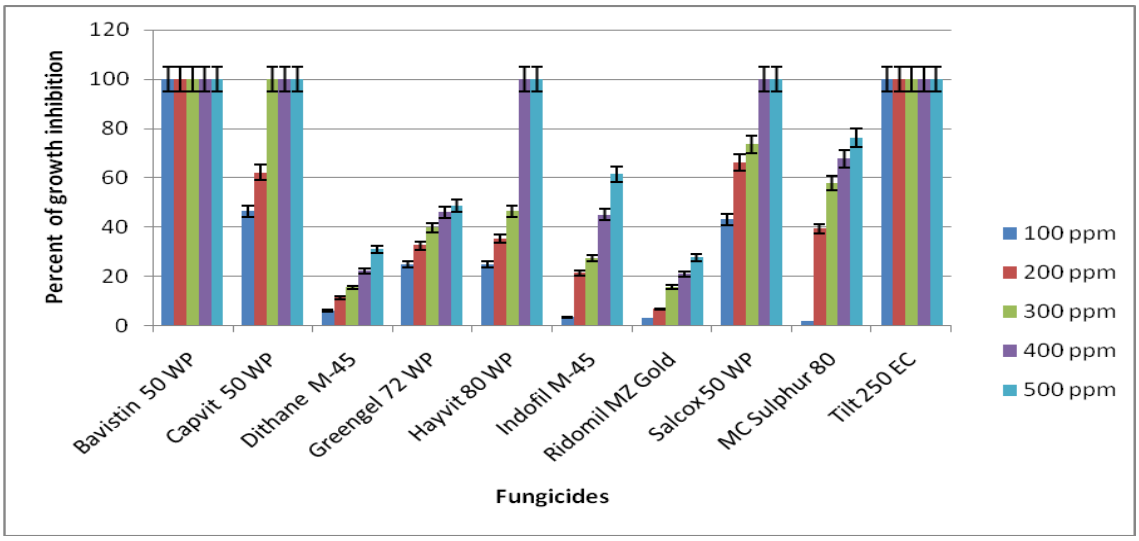

Fig. 2. Percent inhibition of radial growth of *Aspergillus fumigates* owing to fungicides at different Concentrations.

Bavistin 50 WP and Tilt 250 EC were responsible for complete inhibition on the radial growth of *A. fumigatus*, at all the concentration tested. Capvit 50 WP, Hayvit 80 WP, and Salcox 50 WP also completely inhibited the growth of the fungus at 400 and 500 ppm. Capvit also inhibited the growth completely at 300 ppm. Capvit showed 46.41 and 62.09 % inhibition at 100 and 200 ppm respectively. Hayvit showed 24.84, 35.29 and 46.41 % inhibition at 100, 200 and 300 ppm respectively. Salcox showed 42.94, 66.10 and 73.45 % inhibition at 100, 200 and 300 ppm respectively. Dithane M-45 showed 6.04, 11.35, 15.46, 21.98 and 30.92% inhibition of radial growth of *A. fumigatus* at 100, 200, 300, 400 and 500 ppm respectively. Greengel 72 WP showed 24.64, 32.46, 39.71, 45.80 and 48.70% inhibition at 100, 200, 300, 400 and 500 ppm respectively. Indofil showed 3.37, 21.35, 27.34, 44.94 and 61.62 % inhibition at 100, 200, 300, 400 and 500 ppm respectively. Ridomil MZ Gold showed 3.08, 6.67, 15.64, 21.03 and 27.44% inhibition at 100, 200, 300, 400 and 500 ppm respectively. Sulphur showed 1.49, 39.30, 57.71, 67.66, 76.12 % inhibition at 100, 200, 300, 400 and 500 ppm respectively. The toxicity of these fungicides against *A. alternata* at 100 ppm concentration in descending order was Bavistin/ Tilt > Capvit >Salcox >Hayvit> Greengel>Dithane > Indofil > Ridomil > MC Sulphur (Fig. 2).

On the radial growth of *Curvularia lunata* Tilt 250 EC was responsible for complete inhibition at all the concentration tested. Bavistin, Dithane and Sulphur also completely inhibited growth of the fungus at 400

and 500 ppm. Bavistin and Dithane also inhibited the growth completely at 300 ppm. Bavistin showed 63.74 and 77.65% inhibition at 100 and 200 ppm respectively. Dithane showed 47.52 and 65.96% inhibition at 100 and 200 ppm respectively. Sulphur showed 39.0551.91 and 71.90% inhibition at 100, 200 and 300 ppm respectively. Capvit showed 35.80, 46.91, 64, 82, 75 and 80.25 % inhibition of radial growth of *C. lunata* at 100, 200, 300, 400 and 500 ppm respectively. Greengel 72 WP showed 17.65, 31.04, 53.27, 64.71 and 72.55 % inhibition at 100, 200, 300, 400 and 500 ppm respectively. Hayvit showed 5.40, 14.41, 21.62, 27.03 and 30.63 % inhibition at 100, 200, 300, 400 and 500 ppm respectively. Indofil showed 18.07, 56.22, 63.45, and 69.08 % inhibition at 100, 200, 300 and 400 ppm respectively. Indofil also inhibited the growth completely at 500 ppm. Ridomil showed 25.31, 41.05, 58.33, 66.98 and 73.45% inhibition at 100, 200, 300, 400 and 500 ppm. Salcox showed 5.41, 9.91, 16.22, 20.72 and 26.13 % inhibition at 100, 200, 300, 400 and 500 ppm respectively. Among the ten fungicides, Tilt showed best result. The toxicity of these fungicides against *A. alternata* at 100 ppm concentration in descending order was Tilt >Bavistin>Dithane>Ridomil>MC Sulphur> Capvit>Ridomil>Indofil>Greengel Hayvit> Salcox (Fig. 3).

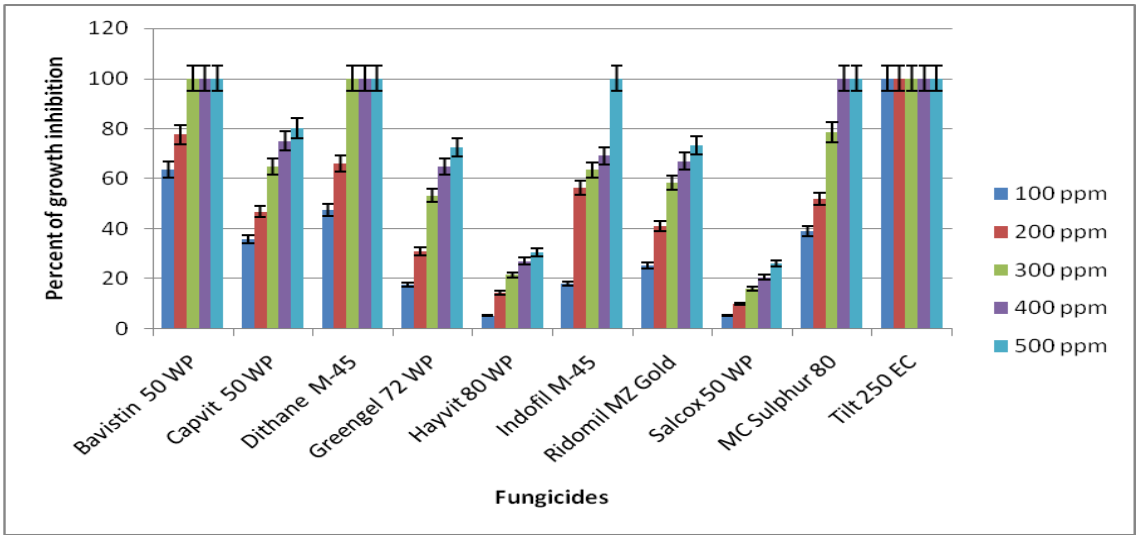

Fig. 3. Percent inhibition of radial growth of *C.lunata* owing to fungicides at different concentrations.

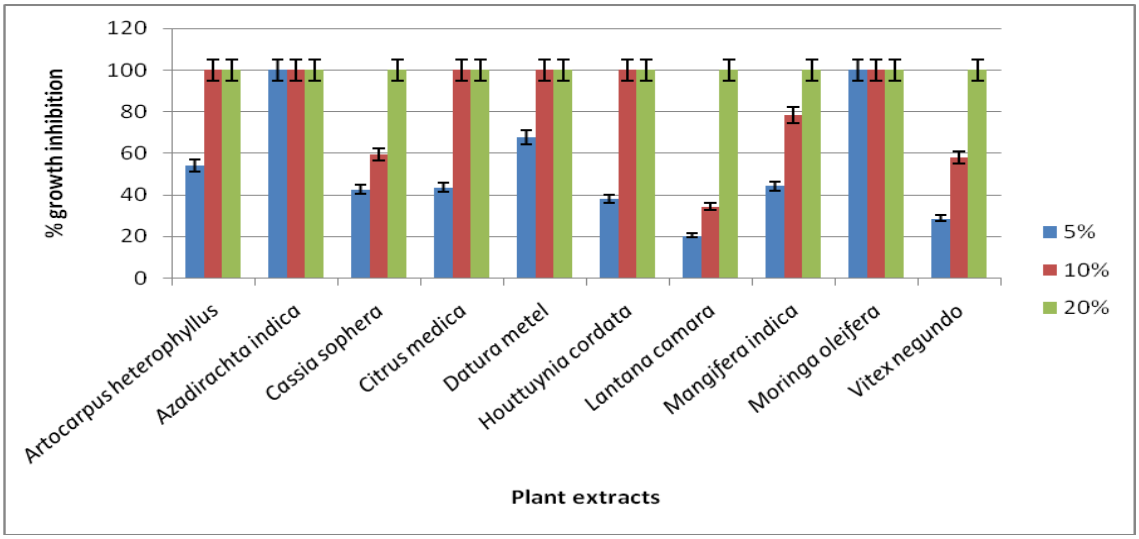

Fig.4. Percent inhibition of radial growth of *Alternaria alternata* owing to plant extracts at different concentrations.

Antifungal properties of ethanol extract of *Artocarpus heterophyllus* Lam., *Azadirachta indica* A. Juss., *Cassia sophera* L,. *Citrus medica* L., *Datura metel* L., *Houttuynia cordata* Thunb., *Lantana camara* L., *Mangifera indica* L., *Moringa oleifera* Lam. and *Vitex negundo* L. at 5, 10 and 20% concentrations were evaluated against the three test pathogens. All the plant extracts completely inhibited the radial growth of the test fungi at 20% concentrations except *Lantana camara*, *Mangifera indica* and *Vitex negundo*(Fig. 5-6).

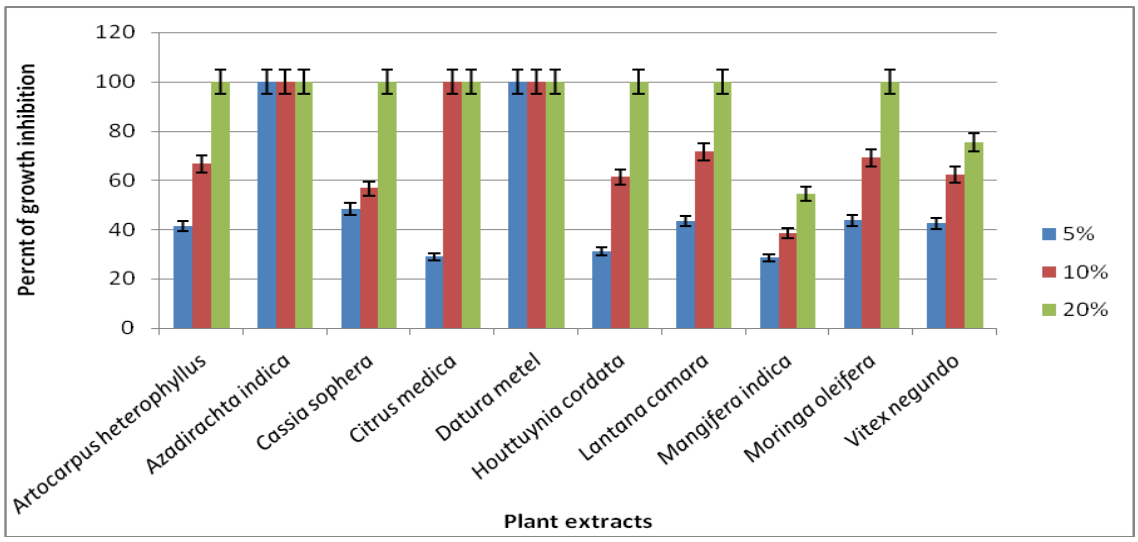

Fig. 5. Percent inhibition of radial growth of *Aspergillus fumigatus* owing to plant extracts at different concentrations

Twenty percent ethanol extract of *Artocarpus heterophyllus*, *A. indica*, *C. sophera*, *C. medica*, *D. metel*, *H. cordata*, *L. amara*, *M.indica*, *M. oleifera* and *V. negundo* were responsible for complete inhibition of radial growth of *Alternaria alternata*. Ten percent ethanol extract of these 6 plants i.e. *A. heterophyllus*, *A. indica*, *C. medica*, *D. metel*, *H. cordata* and *M. oleifera* also showed 100% inhibition of growth of *A. alternata*. At the same concentration *C. sophera*, *L. camara*, *M. indica*, and *V. negundo* showed 59.56%, 34.25%, 78.41% and 58.07% inhibition of the test fungus. Five percent ethanol extract of *A. indica* and *M. oleifera* were responsible for complete inhibition of radial growth of *A. alternata*. At the same concentration *A. heterophyllus*, *C. sophera*, *C. medica*, *D. metel*, *H. cordata*, *L. camara*, *M. indica* and *V. negundo* showed 54.09%, 42.67%, 43.56%, 67.55%, 38.15%, 20.49%, 44.33% and 28.72% growth inhibition against the fungus. The order of effectiveness of plant extracts against *A. alternata* at 5 % concentration was *Moringa oleifera>A. Indica>D. metel>A. heterophyllus>M. indica.>C.medica > C. sophera>H. cordata>L . camara > V. negundo* (Fig. 4).

Twenty percent ethanol extract of *A. heterophyllus*, *A. indica*, *C. sophera*, *C. medica D. metel*, *H. cordata*, *L. camara* and *M. oleifera* were responsible for complete inhibition of radial growth of *Aspergillus fumigatus. Mangifera indica* and *V. negundo* showed 54.59% and 75.36% inhibition of the test fungus. Ten percent ethanol extract of 3 plants i.e. *A. indica*, *C. medica* and *D. metel* also showed 100% inhibition of growth of *A. fumigatus. Artocarpus heterophyllus*, *C. sophera*, *H. cordata*, *L. camara*, *M. indica*, *M. oleifera* and *V. negundo* showed 66.67%,56.77%, 61.48%, 71.50%, 38.65%, 69.05% and 62.32% inhibition of the test fungus. Five percent ethanol extract of *A. indica* and *D. metel* were responsible for complete inhibition of radial growth of *A. fumigatus. Artocarpus heterophyllus*, *C. sophera*, *C. medica*, *H. cordata*, *L. camara*, *M. indica*, *M. oleifera* and *V. negundo* showed 41.27%, 48.44%, 28.99%, 31.11% 43.48% 28.50%, 43.65%, and 42.51% growth inhibition against *Aspergillus fumigatus* at 5% concentration respectively. The order of effectiveness of plant extracts against *A. fumigatus* at 5 % concentration was *Azadirachta Indica* / *D. metel>C. sopheraM. oleifera>L . camara >A. heterophyllus >V. negundo>H. cordata>C. medica > M. indica* (Fig. 5).

Twenty percent ethanol extract of *A. heterophyllus*, *A. indica*, *C. sophera*, *C. medica*, *D.metel*, *H. cordata*, *M. indica*, *M. oleifera* and *V. negundo* were

responsible for complete inhibition of radial growth of *Curvularia lunata*. *Lantana camara* showed 69.77 % inhibition. Ten percent ethanol extract of these 6 plants i.e. *A.heterophyllus*, *A.indica*, *C.medica*, *D.metel*, *H.cordata* and *M. oleifera* also showed 100% inhibition of radial growth of *C. lunata*. *Cassia sophera*, *L.camara*, *M.indica* and *V.negundo* showed 81.13%, 36.18%, 78.75%, and 64.43% inhibition of the test fungus. Five percent ethanol extract of *A. indica* were responsible for complete inhibition of radial growth of *C. lunata*. *Artocarpus heterophyllus*, *C. sophera*, *C. medica*, *D. metel*, *H.cordata*, *L.camara*, *M.indica*, *M. oleifera* and *V. negundo* showed 65.97%, 32.93%, 53.81 %, 78.31%, 25.56%, 30.23%, 53.91%, 54.17% and 30.87% growth inhibition against test fungus respectively. The order of effectiveness of plant extracts against*C. lunata*at 5 % concentration was *Azadirachta Indica*>*D. metel*> *A. heterophyllus* >*M. oleifera*> *M. indica*> *C. medica* > *C. sophera*>*V. negundo*> *L . camara* >>*H. cordata* (Fig. 6).

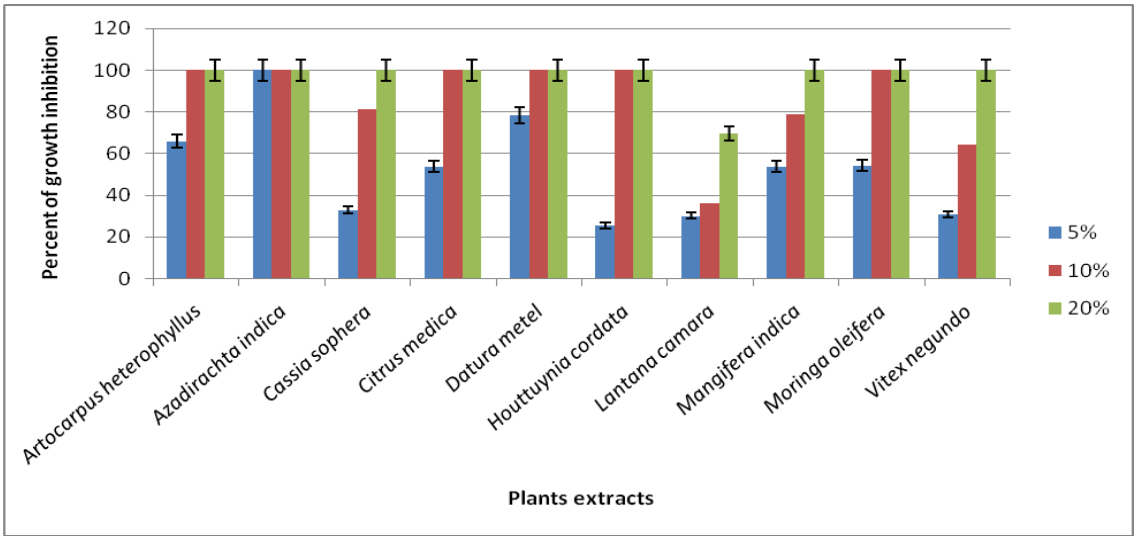

Fig. 6. Percent inhibition of radial growth of *Curvularia lunata* owing to plant extracts at different concentrations

In-vitro experiment showed that Tilt 250 EC completely inhibited radial growth of *Alternaria alternata*, *A. fumigatus* and *C. lunata* at 100 ppm concentration. Ethanol extract of *A. indica* also inhibited the radial growth of the causal agents of blight of *T. erecta* and *T. patula* at 5% concentration.

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