ASSOCIATION OF FUNGI WITH *TAGETES* SPP. AND THEIR MANAGEMENT

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CERTIFICATE

*This is to certify that the research work embodying the results reported here in this thesis entitled "**Association of fungi with Tagetes spp. and their management**" by **Mahfuza Aktar** has been carried out under my supervision and guidance in the Laboratory of Mycology and Plant Pathology, Department of Botany and field plots of Botanical Garden, Curzon Hall Campus, University of Dhaka.*

It is further certified that the research work presented here is original and suitable for submission for the award of Ph.D. degree.

Supervisor

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DECLARATION

I hereby declare that this dissertation is based on entirely my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of another degree or diploma at any other University. From this research work four papers are published in scientific journals.

Mahfuza Aktar

Date: 02.12.2019

Mahfuza Aktar

ABSTRACT

Tagetes erecta L. and *Tagetes patula* L. are two common ornamental as well as herbal medicinal plants belongs to the family Asteraceae. Blight symptom was recorded on different parts of *T. erecta* and *T. patula* during the tenure of 2009 to 2014. 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. Temperature shows noticeable effect on disease development but rainfall and humidity did not show any effect on disease development.

Twenty species of fungi were associated with blight symptom of different plant parts of *T. erecta* and *T. patula.* The associated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama, *Chaetomium globosum* Kunze, *Cladosporium elatum* (Harz) Nannf., *Corynespora cambrensis* M. B. Ellis, *Curvularia brachyspora* Boedijn, *C. fallax* Boedijn, *C. lunata* (Wakker) Boedijn, *C. stapeliae* (du Plessis) Hughes & du Plessis, *Epicoccum purpurascens* Ehrenb. ex Schlecht., *Fusarium semitectum* Berk. & Rav., *Monochaetia ceratoniae* (Sousa da Camera) Sutton, *Nigrospora panici* Zimm., *Penicillium italicum* Wehmer, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill, *Trichoderma viride* Pers. and *Trichothecium roseum* Link. *Corynespora cambrensis, Monochaetia ceratoniae* and *Nigrospora panici* are new record for Bangladesh.

Among the isolated fungi *Alternaria alternata, Aspergillus fumigatus* and *Curvularia lunata* were found to be pathogenic to both the species of *Tagetes.*
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 WDG, Ridomil Gold MZ 68 WG, Salcox 50 WP and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm were evaluated against the above mentioned pathogenic fungi of *Tagetes.* Bavistin 50 WP and Tilt 250 EC completely inhibited the radial growth of the test fungi at all concentrations. Antifungal properties of ethanol leaf extracts 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. Leaf extracts of *A. indica* and *C. medica* also completely inhibited the radial growth of the test fungi at all concentrations. Except *Lantana camara, Mangifera indica* and *Vitex negundo,* all the seven plant extracts completely inhibited radial growth of the test fungi at 20% concentrations.

Three antagonistic fungi namely as *Aspergillus flavus, A. niger* and *Trichoderma viride* were isolated from the field soil of blight infected *Tagetes* spp. by serial dilution method.

Antagonistic potentiality of aforesaid fungi were evaluated against the pathogenic fungi of *T. erecta* and *T. patula* following 'dual culture colony interaction' and volatile and non-volatile metabolites. In dual culture colony interaction, out of three soil fungi, *T. viride* showed the highest growth inhibition on *A. alternata* (71.03%), *A. fumigatus* (38.49%) and *C. lunata* (60.71%). The maximum inhibition of radial growth of *A. alternata* (74.55%) was observed with the culture filtrates of *T. viride* owing to volatile metabolites. The maximum inhibition of radial growth of *A. fumigatus* (37.43%) was also observed with the culture filtrates of *A. flavus* owing to volatile metabolites. The complete inhibition of radial growth of *C. lunata* was observed with the culture filtrates of *A. niger* owing to volatile metabolites. The complete inhibition of radial growth of *A. alternata* was observed with non-volatile metabolites of *A. niger* and *T. viride* at all concentrations used. The complete inhibition of radial growth of *A. fumigatus* was also observed with non-volatile metabolites of *A. niger* at all concentrations used. The maximum inhibition of radial growth of *C. lunata* was observed with nonvolatile metabolites of *A. niger* at 20% concentrations used. *Aspergillus niger* and *T. viride* may be exploited commercially as a biocontrol agent against blight pathogens of *T. erecta* and *T. patula.*

Field experiment was conducted in Botanical research garden, Department of Botany, University of Dhaka during the tenure of 2015, 2016 and 2017 to evaluate the efficacy of two fungicides and two plant extracts against blight disease of *T. erecta* and *T. patula.* Both the fungicides Bavistin 50 WP and Tilt 250 EC and leaf extracts of *A. indica* and *C. medica* show effective management of the disease over untreated check. However, among the treatments Bavistin 50 WP and Tilt 250 EC at 100 ppm concentration and *A. indica* and *C. medica* at 10% concentration was found significantly superior in controlling PDI (Per cent disease index) and increasing number of healthy flowers. Number of healthy flowers was highest per plant 17.13 in *T. erecta* in the year 2017 and 25.00 in *T. patula* in the year 2016.

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INTRODUCTION

Introduction

Tagetes erecta L. is an erect annual medicinal herb with much segmented leaves and yellow flower heads, planted in gardens as a flower plant. It belongs to the family Asteraceae (Compositae). The herb is Bengali known as "Gendaphul" also known in English as "African Marigolds"/ "American Marigolds". Flowers contain essential oil, coloring matter, pigments, quercetagetin and phenolics, syringic acid, methyl-3, 5-dihydroxy- 4- methoxy benzoate and quercetin. Whole plant yields 0.01% essential oil, thienyl and ethylgallate; dried petals contain queretagitrin and a glucoside of quercetagetin. Juice of leaves and flowers is emmenagogue. Cures bleeding piles and purifies blood. Infusion of plant is used in rheumatism, colds and bronchitis. Leaves are used in kidney troubles, muscular pains, and applied to boils and carbuncles. Extract of root is laxative.

Tagetes patula is annuals and biennials medicinal plant. It is also belongs to the family Asteraceae. The plant is known as French Marigolds. French marigolds are small, bushy plants with flowers up to 2 inches across. Flowers may be single or double, yellow, orange, mahogany-red or bicolored. Plant height ranges from 6 to 18 inches. French marigolds bloom from spring until frost. They hold up better in rainy weather than the larger African marigolds. Actually, all marigolds are native to subtropical America, and have been cultivated in Mexico for over 2,000 years.

Marigold is often intercropped with other plants. Rotation with marigolds reduces diseases of other crops (Ijani and Mmbaga 1988, Abid and Maqbool 1990), and reduces nematode populations (Prasad and Haque 1982, Baghel and Gupta 1986 and Alam *et al.* 1988).

Ninety five per cent 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 were 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.

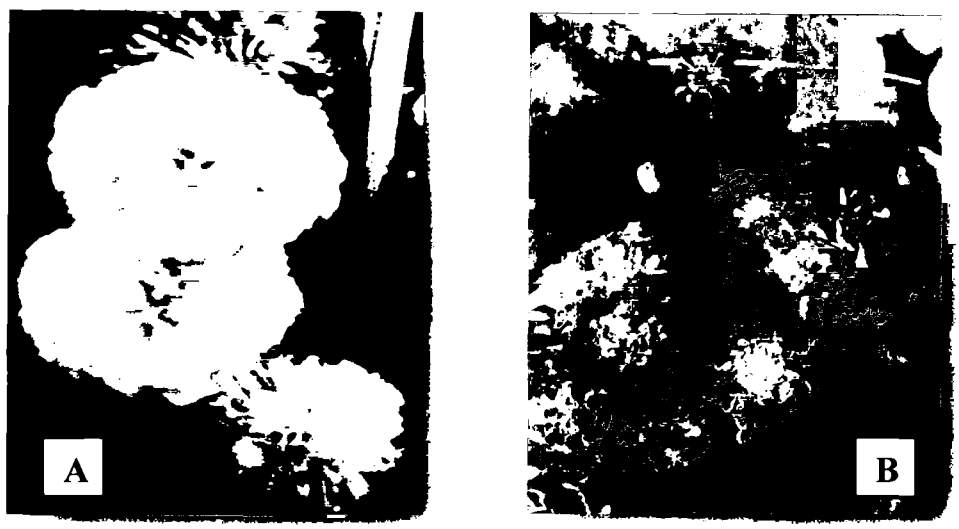

Plate 1. Healthy plants A. *Tagetes erecta* B. *T. patula*

Diseases were major constrain for marigold cultivation. In Bangladesh, due to rapid expansion of commercial marigold cultivation many diseases appear on the plants. However, reports on the occurrence of diseases of marigold in Bangladesh are scanty. Though marigold is presently a profitable cultivated crop to the farmers in Bangladesh but socioeconomic data and information of this flower are very scare. Research about its fungal diseases is inadequate so far and under Bangladesh condition no report in this regards has so far been available. Thus, it is important to find out the etiology, and identification of the associated fungi with the diseased plant.

Biotic stress is the major constrains for the *Tagetes* plant production of which fungal diseases is one of them. In Bangladesh, due to rapid expansion of commercial marigold cultivation many diseases appear on the plants. Major fungal diseases of *Tagetes* spp. are wilt, gray mold, white mold, oedema, septoria leaf spot, leaf spot, black spot, brown spot, downy and powdery mildew, rust, collar rot, root rot, stem rot, flower bud rot, damping off, Botrytis blight, anthracnose and blight etc.

From India, Mukerji and Bhasin (1986) reported different fungal diseases of marigold. From Bangladesh powdery mildew of marigold was reported by Hossain *et al.* 2010. Botrytis gray mold of marigold was reported by Sultana and Shamsi (2011) from Bangladesh. Blight of *Tagetes* spp. was reported by Aktar and Shamsi (2014, 2015, 2016 and 2018) from Bangladesh. Farmers suffer a lot due to this disease. So management of this disease is necessary to save the plant as well as the farmer. Fungicides are the most reliable strategy to achieve effective control against blight of *Tagetes* spp. (Kumar 2012). Plant extracts can be successfully exploited as safer alternatives to conventional fungicides in modern agriculture (Kuepper 2003).

They play an important role against fungi and have the potential to replace the synthetic fungicides (Tripathi and Shukla 2007). Biological control of plant diseases including fungal pathogens has been considered a viable alternative method to chemical control. In Bangladesh so far very little amount of research works related to management has been undertaken (Islam *et al.* 2015-2016, Ali *et al.* 2011-2012, Ali and Islam 2012-2013). So, we should build up an economical sustainable control measure of these diseases with the help of some fungicides, leaf extracts and biological agents.

Aims and Objectives

i. To survey and monitoring of diseases of *Tagetes* spp. in Bangladesh.

ii. Isolation, purification, characterization and identification of mycoflora associated with infected plant parts of *Tagetes* spp.

iii. Pathogenic potentiality of fungi associated with *Tagetes erecta* and *T. patula.*

iv. *In vitro* screening of some fungicides against the causal agents of blight disease of *T. erecta* and *T. patula.*

v. *In vitro* screening of leaf extracts against the causal agents of blight disease of *T. erecta* and *T. patula.*

vi. *In vitro* biological control of causal agents of blight disease of *T. erecta* and *T. patula.*

vii. *In vivo* evaluation of some selected fungicides and leaf extracts against the causal agents of blight disease of *T. erecta* and *T. patula.*

CHAPTER: 2
REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1. The Host (Marigold)

The genus *Tagetes* is mostly herbaceous plants in the sunflower family Asteraceae (Compositae). It was described as a genus by Linnaeus in 1753. It has 56 species in the sunflower family. The marigold is widely cultivated in India and Thailand. *Tagetes erecta* and *T. patula* are native to North and South America, but now has become naturalized around the world. In Bangladesh these two species are commonly grown by the gardeners as annual plants (Ahmed *et al.* 2008). Annual flower is more cheerful and easier to grow than marigolds. In Ukraine (*T. erecta*, *T. patula*, and the signet marigold, *T. tenuifolia*) are regarded as one of the national symbols, and are often mentioned in songs, poems, and tales. *Tagetes patula* is closely related to *T. erecta.* The main difference is that *T. patula* is a tetraploid plant (2n = 48) with smaller involucres and wholly or partially red-brown corollas.

2.1.1. *Tagetes erecta*:

Tagetes erecta is a medicinal herb with much segmented leaves and yellow flower heads, planted in gardens as a flower plant. The herb is locally known as "Genda", "Gada", "Genda phul" also known in English as "African Marigolds", "American Marigolds".

Description: A ribbed, hairy, annual herb, growing up to 60 cm or more. Leaves pinnately divided, pinnae serrulate. Inflorescence a capitulum, heterogamous, large, 10 cm in diameter, rayed, long peduncled, solitary on the branchlets, involucres cylindric. Flowering and fruiting: during the winter season.

Chromosome number: 2n = 24 (Fedorov, 1969).

Habitat: Gardens and nurseries.

Distribution: A native to Mexico, this species is widely cultivated in various parts of the world. In Bangladesh, it is widely cultivated in gardens and homesteads.

Economic uses/ values/ harmful aspects: There is a yellow dye quercetagetin in the flower and oil in the seeds. Leaf juice is a blood coagulant and is a good healing agent for fresh cuts. African marigold flower is especially valued for garland making, floral decoration and banquets.

Edible Uses

The petals of the flowers of some varieties can be eaten. The fresh receptacle is eaten by children. A yellow dye obtained from the flowers can be used as a saffron substitute for colouring and flavouring foods. The yellow flowers are the source of two food-colorant products: 'marigold meal' and 'marigold extract'. Marigold meal consists of the dried powdered flowers and is used mainly in poultry feed to enhance the yellow colour of the meat and of the egg-yolks. Marigold extract is a solvent extract of the flowers, used mainly in western Europe as a yellow to orange food colorant, e.g. in salad dressings, ice cream, dairy products and other foodstuffs with a high fat content, but also in soft drinks, bakery products, jams and confectionery. The leaves are sometimes used as a condiment (Ahmed *et al.* 2008).

Medicinal

The whole herb is anthelmintic, aromatic, digestive, diuretic, emmenagogue, sedative and stomachic. It is used internally in the treatment of indigestion, colic, severe constipation, coughs and dysentery. Externally, it is used to treat sores, ulcers, eczema. Sore eyes and rheumatism. The leaves are harvested as required for immediate use during the growing season, whilst the flowering plant can be dried and stored for later use. A paste of the

leaves is applied externally to treat boils, carbuncles and earaches. The flowers are carminative, diuretic and vermifuge. A decoction is used to treat colds, and mumps. It is applied externally to treat skin diseases, conjunctivitis and sore eyes. The root is laxative (Ghani 2003 and Yusuf *et al.* 2009).

Agroforestry Uses:

Secretions from the roots of growing plants have an insecticidal effect on the soil, effective against nematodes and to some extent against keeled slugs. These secretions are produced about 3 - 4 months after sowing. The flower petals also have nematacidal properties. Sometimes the plant is grown in crop fields as an insect repellent because of its sharp peculiar smell, although the plant itself is susceptible to insect pests.

Other Uses

A yellow dye is obtained from the flowers seed to dye wool, silk and cellulose fibres into shades of golden-yellow to orange and olive-green to bronze, depending on the mordanting substances used. An essential oil obtained from the flowers and leaves is used in small traces in perfumery to impart floral and 'apple' notes.

Ethnobotanical information: Fresh leaf paste is used to stop bleeding in cuts.

Propagation: By seeds and stem cuttings (Anonymous 2019).

2.1.2. *Tagetes patula*: The herb is locally known as "Genda", "Gada", "Genda phul", "Chenari" "(Manipuri)" also known in English as **"French Marigolds".**

Description: An erect, ribbed, hairy annual herb, growing up to 80 cm or more. Leaves pinnatisect, pinnae lanceolate, acute, serrulate. Inflorescence a capitulum, heterogamous, long peduncled, 18 = 10 mm, solitary, rayed, single or double, involucres cylindric. Flowering and fruiting: November-March.

Chromosome number: 2n = 48 (Fedorov, 1969).

Habitat: Gardens.

Distribution: A native of Mexio. In Bangladesh, it is cultivated throughout the country.

Economic uses/ values/ harmful aspects: There is a yellow dye quercetagetin in the flower and oil in the seeds. Leaf juice of this plant stops bleeding, if applied to cuts and wounds.

Edible Uses

The flowers are used in refreshing drinks. The leaves are used as a food flavouring. No further details are given. The essential oil is used as a food flavouring, though it is inferior to the oil obtained from *T. minuta*. The dried flowers are an adulterant of saffron (*Crocus sativus*), used for colouring foods yellow (Ahmed *et al.* 2008).

Medicinal

The whole herb is aromatic, digestive, diuretic and sedative. It is used internally in the treatment of indigestion, colic, severe constipation, coughs and dysentery. Externally, it is used to treat sore eyes and rheumatism. The leaves are harvested as required for immediate use during the growing season, whilst the flowering plant can be dried and stored for later use (Ghani 2003 and Yusuf *et al.* 2009).

Agroforestry Uses:

Secretions from the roots of growing plants have an insecticidal effect on the soil, effective against nematodes and to some extent against keeled slugs. These secretions are produced about 3 - 4 months after sowing. The growing plant repels whitefly and can be grown near tomatoes to keep that crop free of the insect.

Other Uses

The whole plant is harvested when in flower and distilled for its essential oil. The oil is used in perfumery, it is blended with sandalwood oil to produce 'attar genda' perfume about 35 kilos of oil can be extracted from 1 hectare of the plant (yielding 2,500 kilos of flowers and 25,000 kilos of herbage). The whole plant contains substances that are toxic to cockroaches. A yellow dye is obtained from the flowers. It is used to colour foods and textiles.

Ethnobotanical information: Locally used in cuts and wounds.

Propagation: By seeds and stem cuttings (Anonymous 2019).

2.2. Diseases of marigold:

Leaf spot and blight are two common diseases of *Tagetes erecta* and *T. patula.* In India Shome and Mustafee (1966) reported *A. zinniae* pape and *A. tagetica* as causal agent of blight disease of marigold. Mondal and Chaudhuri (1976) reported *Alternaria* spp. were pathogenic to marigold. *Alternaria tegetica* causes early blight of *T. erecta.* Inflorescence blight caused by *Alternaria zinnae.* Mukerji and Bhasin (1986) and Dhiman and Arora (1990) reported disease of *Tagetes* from India. Some of the important fungal diseases of marigold are flower blight (*Alternaria zinniae*), wilt and stem rot (*Phytophthora cryptogea*), Collar Rot (*Phytophthora* sp., *Pythium* sp.), damping Off (*Pythium* sp.), *Alternaria* leaf spot, *Fusarium* wilt (*Fusarium oxysporium*) and *Cercospora* leaf spot (*Cercospora megalopotamica*) (Sohi 1983, Pawar 1971). Out of these leaf spot and flower blight incited by *Alternaria tagetica* is the most serious, prevalent all over the country. Aktar and Shamsi (2015), isolated a total of 20 species of fungi from *Tagetes erecta* and *T. patula*, out of which *Aspergillus fumigatus, Alternaria alternata,* and *Curvularia lunata* were found to be pathogenic to *Tagetes erecta* and *T. patula* (Gurjar *et al.* 2019).

2.3. Occurrence of Blight disease in Bangladesh:

From Bangladesh Rahman and Rashid (2008) reported powdery mildew and Sultana and Shamsi (2011) reported gray mold of *T. erecta*, Rahman *et al.* (2015) reported white mould of *T. erecta* caused by *Sclerotinia sclerotiorum*. (Lib.) de Bary and Aktar and Shamsi (2014, 2015, 2016 and 2018) reported blight of *T. erecta* and *T. patula*.

2.4. Diseases of *Tagetes* caused by fungi:

Symptomatology and causal agents: Edward (1957) first reported leaf and inflorescence blight of marigold caused by *A. zinniae* which was characterized by elongated lesions formed on the inflorescence. The symptoms may appear as large irregular blotches on leaves which may be light tan to dark brown with zonation.

Mondal and Chaudhuri (1976) reported *Alternaria* spp. pathogenic to marigold in India and *A. zinniae* pape and *A. tagetica* Shome and Mustafee. both species cause blightening of leaves and flowers. The infection of *A. tagetica* has further been reported to be systemic. A dry rot of developing flower buds of marigold has been seen to be caused by *A. dianthi* Stevens and Halls.

Hotchkiss and Baxter (1983) characterized the blight of marigold caused by *A. tagetica* dark lesions on leaves, stems and petals.

Sen (1996) observed leaf spot and flower blight of marigold in the year 1995. A severe spotting on leaves, stems and an inflorescence including sepals and petals gives a blighted appearance to the entire plant. On the basis of pathogenicity test the fungus was identified as *A. zinniae*.

Shome and Mustafee (1966) reported *A. tagetica* nov. cause blight of marigold and the plant showed irregular brownish spots on the leaflets followed by those on stems and branches. The spots remain circular to oblong, brownish at first later turn dark brown to

blackish, enlarge coalesce to cover almost the entire leaves and part of branches giving the plant a burnt up appearance.

Wilt and Stem Rot: Wilt and Stem Rot caused by *Phytophthora cryptogea.* The fungus affects the collar portions of the plants. In nursery the infection results in damping-off and is aggravated by soil moisture. In the field the infected plants show wilting. French marigold and dwarf varieties are less susceptible whereas the African types are highly susceptible to the disease (Kumar 2012).

Collar Rot: Collar Rot caused by *Phytophthora* sp., *Pythium* sp.The symptoms are in the form of black lesions developed on the main stem. Rotting at the collar regions causes death of the plant (Kumar 2012).

Leaf Spot and Blight: Leaf Spot and Blight caused by *Alternaria, Cercospora* and *Septoria* sp. Brown necrotic spots develop on leaves, which get enlarged at the later stage of infection. The entire foliage gets damaged and results in poor vegetative growth (Kumar 2012).

Powdery Mildew: Powdery Mildew caused by *Oidium* sp., *Leveillula taurica.* The symptoms are in the form of whitish powdery growth on the aerial parts of the plant (Kumar 2012).

Flower Bud Rot: Flower Bud Rot caused by *Alternaria dianthi.* The fungus infects the young flower buds. The infected buds shrivel and become dark brown in colour. The pathogen also infects leaves causing blight. The infection is visible in the form of brown necrotic spots on margins and tips of older leaves (Kumar 2012).

Damping Off: Damping Off caused by *Pythium* sp. The disease is most prevalent at the seedling stage. Necrotic spots and rings develop on the young seedlings causing collapse of the seedlings. Considerable loss is sustained if seedlings are not properly looked after (Kumar 2012).

Gray Mold: Botrytis blight or Gray mold caused by *Botrytis cinerea.* Disease symptoms appeared as dead blotches on leaves, flowers, and stems. Rotting of stems may cause plants to collapse, flower buds may fail to open and diseased flowers that open become decayed and drop prematurely. A covering of gray fuzzy fungal growth and spores appears on infected plant tissue (Anonymous 2012).

Several microorganisms such as fungi, bacteria and virus which cause diseases in Marigold have been reported in literature. List of some diseases attacking marigold are as follows:

Table 1. List of some diseases of marigold.

Disease	Causal organism	Reference
Damping off of seedlings	*Rhizoctonia solani* Kunn.	Subrahmanyam *et al.* (1975).
Cercospora leaf blight	*Cercospora tageteserectae*	Thirumalachar and Govindu (1956)
	C. tageticola Fli.	Mitra (1935)
	C. tagetis	Kar and Mandal (1973)
Powdery mildew	*Laveillula taurica*	Sreeramulu (1953)
	Erysiphe cichoracearum	Chacko and Raghvendra Rao (I982)
Colletotrichum flower blight	*Colletotrichum capsici*	Saksena and Singh (1959)
Alternaria leaf blight	*Alternaria tagetica*	Shome and Mustafee (1966)
Alternaria bud rot	*A. dianthi*	Mondal and Chaudhuri (1976)
Intlorecence blight	*A. zinniae*	Edward (1957)
Blosom blight	*Rhizopus stolonifer*	Nair and Nassema (1999)
Leaf spot	*Pseudomonas syringae pv. tagetis*	Kudela and Zacho (1998)
Virus disease	*Cucumber mosaic virus*	Pirone *et al.* (1960)
	Aster yellow virus	Pirone *et al.* (1960)
Rust	*Puccinia tageticola*	Pirone *et al.* (1960)

2. 5. Management of blight disease of marigold:

Fungicide was used to control plant disease in 1885 for the first time in the world. Nowadays, many inorganic and organic fungicides are used frequently to control plant diseases (Mehrotra 2000). Various workers in different countries of the world evaluated the efficacy of various fungicides against *Colletotrichum* spp., *Phomopsis vexans, Macrophomina phaseolina, Rhizopus nodosus, Fusarium* spp., *Phoma* spp., *Botryodiplodia theobromae, Colletotrichum gloeosporioides, Sclerotium rolfsii* and *Alternaria* spp. under laboratory and field conditions (Backman and Rodriguez 1974, Bashar 1992, Chowdhury *et al.* 2015 and Yasmin and Shamsi 2019). Use of chemical pesticides provides excellent control of the diseases and result in improved yield.

Table 2. List of diseases of marigold and their management (Moorman 2016).

Disease	Symptoms	Pathogen/Cause	Management
Alternaria Leaf Spot	Purplish spots form on leaves and stems.	*Alternaria*	Avoid overhead irrigation. Apply fungicide registered for use on this crop.
Bacterial Leaf Spot	Small (2-5 mm) circular dead spots form on leaves and petioles. Spots have purple margins.	*Pseudomonas tagetis*	Destroy infected plants. Avoid overhead irrigation.
Botrytis Flower Blight	Flower parts brown and die. Gray masses of spores form on the infected tissue when wet.	*Botrytis cinerea*	Avoid overhead irrigation. Apply fungicide registered for use on this crop.
Fusarium Wilt	Seedlings are killed. In older plants, black streaks darken the vascular tissue up one side of the plant. Plants wilt. Roots on the greatly reduced root system are rotted. During wet weather, salmon-colored spore masses form on infected stems.	*Fusarium oxysporum*	Plant in potting mix free of pathogens. Destroy infected plants.
Leaf Burn	The tips and margins of leaves yellow and die.	Excess boron, manganese, or molybdenum	Measure and apply micronutrient solutions carefully. Manganese should not be above 55 ppm, molybdenum above 24 ppm, or boron above 3 ppm.
Septoria Leaf Spot	Oval to irregular gray to black spots with tiny dots peppering their surface (fungal fruiting structures) form first on lower leaves and then spread upward.	*Septoria tageticola*	Avoid overhead irrigation. Apply fungicide registered for use on this crop.

2.5.1. Chemical control

In vitro

Three copper fungicides (Bordeaux mixture, Blitox-50 and Fytolan) and two organic fungicides (Dithane Z-78 and Captan) were tested against *A. alternata* by poisoned food technique Captan provided superior in all the fungicides. Higher concentration of Dithane Z-78 was required for 50% inhibition (Mishra and Singh 1971).

Of the eight fungicides tested *in vitro* Chlorothalonil and Thiabendazole completely inhibited the growth of *A. helianthi* (Jeffery *et al.* 1985).

Tripathi and Lal (1989) evaluated six fungicides and found Difoltan (0.2%) and Dithane M-45 (0.2%) to be most effective to minimize the disease incidence of leaf blight of Carnation *A. dianthi*. However, systemic fungicides were observed to be less effective against the disease.

Wadiphasme *et al.* (1994) evaluated nine fungicides *in vitro* against *A. helianthi* causing leaf spot of Sunflower. The fungus was effectively controlled by all the nine fungicides, but Dithane M-45 (Mancozeb) 0.25% showed highest inhibition (86.84%) of mycelial growth by poisoned food technique.

According to Wv and Chou (1995), out of eight fungicides Flusilazole, Hexaconazole, Propiconazole and Pyrifenox at 1 ppm significantly inhibited growth of *A. carthami* on potato dextrose agar and were significantly better than Difenoconazole, Mancozeb, Phosphorus acid and Polyxin B, Phosphorus acid and Propiconazale at 1 ppm did not significantly inhibited the growth of *A. carthami* in potato dextrose agar.

Chemical control

In Field

The best control of Alternaria leaf spot of marigold in field crops was provided by Rovral at 0.25, 0.5 and 1 lb/100 gal. and by Daconil-2787 at 2 and 4 pt/100 gal (Strider 1981).

Chacko and Raghvendra Rao (1982) recommended fortnightly spray of either Dithane-M-45, Dithane Z-78, Blitox-50 for the control of Alternaria leaf spot and blight on Marigold.

Narayanappa and Jagdish Chandra (1984) reported that in the year 1979 the minimum infection was observed in plots sprayed with Blitox (21.24 %) and disease control in plots sprayed with Blitox (45.4 7%) and defoliation was controlled to the maximum extend by Dithane Z-78 (26.40 %). In the year 1980 the minimum infection was observed in plots sprayed with Dithane Z-78 (17.07%) and disease was controlled to the maximum extend in Dithane Z-78 (56.38%) and defoliation was controlled to the maximum extend by RH-2161 in Marigold caused by *Alternaria tagetica.*

According to Aurthur (1989), excellent disease control was obtained on carnation caused by a *A. dianthi* with Chipco-26019 50 W, Daconil 90 DG and tank mix combination of Benlate 50 DF with either Chipco-26019 50 W, Deconil 90 DG or Manzate 200 DF. On marigold, control of Alternaria leaf spot caused by *A. tagetes*, Daconil 90 DG at both rates (1.5 lb and 0.75 lb) provided consistently good control followed by the low rate of Chipco-26019 50 W and the tank mix of Benlate 50 DF + Chipco-26019 50 W. Poor control was obtained with Ornalin 50 W, Benlate 50 DG tank mixed with Manzate 200 DF and the low rate of Manzate 200 DF.

Chlorothalonil (the best), Mancozeb and Cuprosan-311 SD (Copper oxychloride + Maneb + Zineb) applied 5 times at 10 days interval gave good control at *A. dianthi* in Carnation (Hilal and Kamel 1990).

Raghvendra Rao and Chacko (1990) reported that the result of field experiments in 1985 and 1986 which revealed that all the treatments viz., Captaf, Rovral, Dithanez-78 and their combination spray of Rovral + Captaf and Sulfex + Rovral, except sulfex were superior in controlling *Alternaria* infection. However, spraying Sulfex + Rovral was most effective in controlling this disease.

According to Wang Kuei *et al.* (1990), the leaf spot of Carnation caused by *A. dianthi* is controlled by spray of Chlorothalonil or Difenoconazole gave good control in field and improved Carnation quality.

Of the nine fungicides evaluated for their effect on Alternaria blight of Cumin caused by *A. burnsii.* Three sprays of Mancozeb (0.2%) or Propioconazole (0.25 %) at 12 days interval after disease gave the best control (Akhbari 1992).

Wilt and Stem Rot caused by *Phytophthora cryptogea.*The disease may be controlled by soil treatment with Captan, Mancozeb, Metalaxyl and Fosetyl-Al (Kumar 2012).

Collar Rot caused by *Phytophthora* sp., *Pythium* sp. Soil sterilization and controlled watering help in reducing the disease incidence (Kumar 2012).

Leaf Spot and Blight caused by *Alternaria, Cercospora* and *Septoria* sp. The disease may be controlled by Spraying of fungicides is helpful in controlling the disease (Kumar 2012).

Powdery Mildew caused by *Oidium* sp., *Leveillula taurica.* The disease may be controlled by spraying Sulfex (3g/litre of water) can effectively control the disease (Kumar 2012).

Flower Bud Rot caused by *Alternaria dianthi.* Spraying of Mancozeb (2g/litre of water) effectively controls the flower bud and leaf infections (Kumar 2012).

Damping Off caused by *Pythium* sp. Soil sterilization by Formalin at 2% before sowing and spraying of Dithane Z-78 at 2g/ litre of water are effective in controlling the disease (Kumar 2012).

Chandel *et al.* (2010) cause of leaf spot and flower blight, and which are seed- and air borne in nature, are the major constraints in marigold cultivation. Seed treatment studies using 8 fungicides (mancozeb, foltaf, copper oxychloride, captan, zineb, chlorothalonil and thiram). All chemicals were effective although higher doses of some fungicides had an adverse effect on seed germination. Mancozeb and chlorothalonil effectively reduced the seed-borne infection in marigold with no adverse effect on seed germination even if applied in slightly higher doses (3.5 and 5.0 g/kg). In a field trial, mancozeb performed better than other chemicals by reducing the disease severity of leaf spot recorded in the control from 65.81 to 3.13% and with no incidence of flower blight even after 60 days. Captan and chlorothaonil were the next effective fungicides in management of the disease.

2.5.2. Control of these diseases with the help of some fungicides and plant extracts

In vivo

The field trials were conducted at the experimental farm of the Department of Plant Pathology, Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan during the period 2014 and 2015. A total nine fungicides and five bio formulations were screened for their efficacy in controlling the Cercospora leaf spot disease of the marigold. Bavistein and Captan gave the best disease control and the disease severity recorded were 12.37% and 17.41% respectively. Lesser disease reduction was recorded in Cabriotop (18.58%), Acrobat (21.10%), Insignia (24%), Alitte (25.28%), Metiram (26.93%), Matco (28.03%) and Antracol (30.36%). Among bio-formulations Garlic Extract + Cow urine +Soap Nut, cow urine and garlic extract were found best with the disease severity *viz.*, 15.36%,

18.07% and 19.61 respectively. While the least effective bio-formulation were field formulation (31.10%) and soap nut (32.13%) (Chandel and Kumar 2017).

Kitazin was proved to be most effective fungicide against *A. tagetica in vitro* and *in vivo* experiments. In field experiment Kitazin (0.15%) was significantly superior to all other fungicides and showed minimum per cent disease incidence, maximum per cent disease control and maximum yield per plant (Patidar 2000).

2.5.3. Herbal Control:

Fungicides are the major process to control blight disease. But most fungicides can cause acute toxicity, and some cause chronic toxicity as well (Mishra and Singh 1971). Therefore it is necessary to test the efficacy of the fungicides against the targeted pathogen.

Barnwal *et al.* (1998) reported that out of 7 plants extract tested in field experiment bulb extracts of Garlic was the most effective treatment against *A. tenussima* on *Tagetes* spp. followed by Mint and Dhatura extract.

The oil extracts of Neem and Eucalyptus at 5 per cent and leaf extracts of Neem and Eucalyptus at 20 per cent were found very effective against *A. tagetica* under *in vitro* condition (Bhadouria 2015).

Neem oil is an effective and preventive fungicide used in the control of various diseases like downy and powdery mildews, rust, leaf spot, Botrytis blight, scab and flower, twig and tip blight, anthracnose and Alternaria blight (Kuepper 2003; http:/www.attra.ncat.org.html).

Chandel *et al.* (2010) also applied a neem formulation, neemicidin against *A. zinniae.*

CHAPTER: 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1. Collection of infected samples of *Tagetes erecta* and *T. patula* showing blight symptom.

The present study is based on affected *Tagetes* spp. plant materials consisting leaves, petals, calyx and buds collected from BARI, Joydebpur, Gazipur, Dhaka, Chittagong, Comilla, Dhaka city, Khulna, Pabna, Rajshahi, Sylhet and Rangpur. A total of 184 samples were collected during the period of 2009 - 2014 and mostly showed the presence of the members of Deuteromycetes.

3.2. Incidence and severity of blight disease of *Tagetes erecta* and *T. patula.*

Disease incidence and severity were measured following Rahman and Rashid (2008). For visual estimation of severity, 0-9 point DS scale were used for rating of all foliar diseases studied (PDI=McKinney's Index, Ghosh *et al.* 2009).

No infection = 0, 0 – 10% leaf area infected = 1, 10 – 20% leaf area infected = 2, 20 – 30% leaf area infected = 3, 30 – 40% leaf area infected = 4, 40 – 50% leaf area infected = 5, 50 – 60% leaf area infected = 6, 60 – 70% leaf area infected = 7, 70 – 80% leaf area infected = 8, 80 – 90% or more leaf area infected = 9.

Effect of temperature, humidity and rainfall on disease incidence and disease severity were intensively studied. Temperature, humidity and rainfall data were recorded for the year of 2012-2014 (Collected from Bangladesh Meteorological Department, Agargaon, Dhaka).

3.3. Isolation, characterization and identification of fungi associated with blight disease of *Tagetes erecta* and *T. patula.*

The fungi were isolated from samples following the "Tissue Planting Method" following (CAB 1968).

3.3.1. Tissue planting method:

Surface sterilized inocula were used to isolate the fungi from the specimens. Fifty inocula, each measuring 2 square mm were cut with a sterilized scalpel from a particular specimen and kept in a sterile Petri plate. The inocula were washed in sterile water and then surface sterilized by dipping in 10% Clorox for 3-5 minutes. Then the inocula were transferred into a sterile Petri plate containing sterile blotting paper to remove the surface water. Thus, the surface sterilized inocula prepared and were used for isolation.

The inocula were placed in Petri plates containing sterilized Potato Dextrose Agar (PDA) medium (Potato 200 g, Dextrose 20 g, Agar 15 g and Distilled water 1000 ml). Each Petri plate contained 15 ml of PDA medium with an addition of 1 drop (ca. 0.03 ml) of lactic acid which was used for checking the bacterial growth. A total number of 30 inocula were transferred in 10 Petri plates. Then the inoculated plates were incubated at room temperature (25 ± 2°C) for seven days. The fungi growing out of the inocula were examined and identified whenever possible and transferred to PDA slants. The isolates were purified following dilution plate method (CAB 1968), maintained on PDA slants and stored at (10 ± 0.5°C) in an incubator for future studies.

Detailed morphological studies of the fungal isolates were made in order to determine their identity. For microscopic observations fungal structure like mycelia, spore bearing structures and spores were scrapped off from the surface with a scalpel or blade or picked up with a needle and was mounted in lacto phenol over a clean slide. In case of hyaline structures, a little amount of aniline blue (cotton blue) was added to the mounted fluid.

The text figures of microscopic structures were drawn with the aid of a Camera Lucida. Microscopic details of the associated fungi with *Tagetes* spp. were studied following standard techniques Khan and Shamsi 1983 and Shamsi and Sultana 2008.

Identities of the isolates were determined following the standard literatures (Barnet and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976, Ellis and Ellis 1997, Thom and Raper 1945, Raper *et al.* 1949 and Sutton 1980).

Percentage frequency of the occurrence of the fungal isolates was calculated by adopting the following formula (Spurr and Welty 1972):

$$\textbf{\% frequency} = \frac{\text{No. of inocula from which a fungal isolate was obtained}}{\text{No. of inocula cultured}} \times \mathbf{100}$$

All the specimens included in the present study were preserved in the Herbarium, Mycology and Plant pathology section, Department of Botany, University of Dhaka, Bangladesh.

3.4. Pathogenicity test:

3.4.1. Pathogenicity test following detached leaf assay *in vitro*:

The pathogenicity of all the isolated fungi was tested following modified "detached leaf technique" (Azad and Shamsi 2011). All the isolated fungi were tested for their pathogenic potentiality. Healthy matured leaves of *Tagetes* spp. were throughly washed running tap water and then surfaces disinfected in 10% chlorox for 3 minutes. Excessive chlorox was removed by placing the leaves on two layers of sterile autoclaved filter paper on petriplate. Moist chamber was prepared by placing small cotton bar at the corner of Petri plate and autoclaved. The leaves were inoculated with 2 square mm mycelial block of each fungus that were previously grown on PDA medium and incubated for seven days.

All the fungi were tested to find out their pathogenic potentiality. Six treatments with three replications for each fungi were used as follows: T_1 = (control) dorsally uninoculated leaflets, T_2 = (control) ventrally uninoculated leaflets, T_3= dorsally unpricked inoculated leaflets, T_4 = ventrally unpricked inoculated leaflets, T_5 = dorsally pricked inoculated leaflets and T_6 = ventrally pricked inoculated leaflets.

The inoculated plates were incubated at 26 - 28°C. After 5 to 7 days of inoculation lesion size and symptom was recorded and fungus was reisolated to fulfill Kochs' postulates.

3.4.2. Pathogenicity test for artificial inoculation on plants in net house:

Healthy seedlings of *Tagetes erecta* and *T. patula* were separately transplanted in earthen pots (10 inch diameter.) containing sterilized soil at three seedlings per pot and allow to grow for one month in net house providing necessary water and nutrients. Identified fungus were purified and its pathogenicity was examined by inoculating fresh healthy plants following spraying of spore suspension method. Conidia from seven days old culture of test fungus were taken in 250 ml conical flask with sterilized water. Ten ml water suspension of test fungus at 10^4 ml conc. were taken in a hand sprayer and sprayed on healthy potted plant. Control received only sterilized water without fungal inoculum. Five pots were inoculated for each treatment. The inoculated and control plants were placed in a clean bench in net house following completely randomized design.

The plants were examined daily and continued for 10 days for recording the development of symptoms. Symptom produced on artificial inoculated plants was recorded and compared with those observed on naturally infected plants. The fungus was reisolated from the inoculated plants of *Tagetes* spp. on PDA medium to fulfill Koch's postulates.

3.5. Fungitoxicity of fungicides against the test pathogens.

3.5.1 Preparation of fungicides at different concentrations.

Ten fungicides with different active ingredients, *viz.*, Bavistin 50 WP, Capvit 50 WP, Dithane M-45, Greengel 72 WP, Hayvit 80 WP, Indofil M-45, MC Sulphur 80 WDG, Ridomil Gold MZ 68WG, Salcox 50 WP and Tilt 250 EC were collected from the Krishi Upokoron BiponiKendro, Khamarbari, Framgate, Dhaka (Table 3). At first, *in vitro* fungi toxicity of these fungicides at 500 ppm concentration were evaluated against the test pathogens following poisoned food technique to screen out the effective fungicides (Hossain 1993, Hossain and Bashar 2011, Ahmed *et al.* 2014, Bashar and Chakma 2014).

For each fungicide, a stock solution having the concentration of 10000 ppm was prepared. The calculated amount of stock solution of a fungicide was supplemented with sterilized PDA medium to get the final concentration of 500 ppm. The concentration of fungicide was expressed in terms of its active ingredients. Twenty ml of the supplemented medium of a particular concentration was poured in sterilized Petri plates and allowed to solidify. In the control set, required amount of sterilized water instead of fungicide solution was added to the PDA medium. Then the solidified medium was inoculated at the centre of the Petri plate with a 5mm mycelia agar disc cut from the margin of actively growing culture of the test pathogens. Three replications were maintained in each case. The inoculated plates were incubated at 25±2°C. The radial growth of the colonies was measured at 4^{th} and 7^{th} day of incubation.

The fungicides which were effective at 500 ppm concentration for controlling the test pathogens were further tested in different concentrations. The five fungicides i.e., Bavistin DF, Capvit 50 WP, Dithane M-45, Greengel 72 WP and Tilt 250 EC showed effective result against *C. gloeosporioides*. But in case of *S. rolfsii* three fungicides i.e., Dithane M-

45, Greengel 72 WP and Tilt 250 EC showed effective result. So these were again tested in 100, 200 and 400 ppm concentrations to find out their minimal requirement of concentration. This procedure was the same as mentioned above. At first, for each fungicide a stock solution having 10,000 ppm concentration was prepared. Then calculated amount of stock solution of fungicide as supplemented with sterilized PDA medium to get the concentration of 100, 200 and 400 ppm, respectively. In control set, required amount of water was supplemented instead of a fungicide. Then 15 ml of medium was poured in each Petri plate and allowed them to solidify. Thereafter at the center of the plate 5 mm agar disk of test pathogens was inoculated. The plates were incubated at 25±2°C in an incubator. The radial growth of control and treatment plates was measured at 4^{th} and 7^{th} day of incubation.

Table 3. Particulars of the fungicides used in the present study.

Sl. No.	Trade name	Formulation	Recommended Dose (ppm)	Ten times lesser of Recommended Dose (ppm)	Manufacturer
1.	Bavistin	50 WP	1000	100	BASF Bangladesh Ltd.
2.	Capvit	50 WP	2000	200	Asia Trade International
3.	Dithane M	M-45	2000	200	Bayer Crop Science Ltd.
4.	Greengel	72 WP	2000	200	Green Care Bangladesh
5.	Hayvit	80 WP	2000	200	Syngenta (BD) Ltd.
6.	Indofil	M-45	2000	200	Auto Crop care Ltd.
7.	MC Sulphur	80 WDG	2000	200	Agri Search (India) Private Limited, Nashik, Maharashtra.
8.	Ridomil Gold	68 WG	2000	200	Syngenta (BD) Ltd.
9.	Salcox	50 WP	2000	200	Haychem (Bangladesh)
10.	Tilt	250 EC	500	50	Syngenta (BD) Ltd.

3.6. *In vitro* effect of leaf extracts on the radial growth of the test pathogens.

3.6.1. Preparation of ethanol leaf extract of selected plants at different concentrations.

A total of ten plant parts were selected for evaluating their effect on the radial growth of *Alternaria alternata, Aspergillus fumigatus* and *Curvularia lunata*, the causal agents of blight disease of *Tagetes erecta* and *T. patula*. Particulars of all these plant parts are given in Table 4. The leaves 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% concentrations (Khatun and Shamsi 2016). Three replications were maintained for both the experiments and control sets. The inoculated Petri plates were incubated at 25±2°C. The radial growth of the colonies of the test pathogens was measured after 5 days of incubation.

Table 4. Particulars of plant's leaf extracts used in the present study.

Sl. No.	Plant species	Family	Used part
1.	***Artocarpus heterophyllus* Lam.**	Moraceae	Leaves
2.	***Azadirachta indica* A. Juss.**	Meliaceae	Leaves
3.	***Cassia sophera* L.**	Caesalpiniaceae	Leaves
4.	***Citrus medica* L.**	Rutaceae	Leaves
5.	***Datura metel* L.**	Solanaceae	Leaves
6.	***Houttuynia cordata* Thunb.**	Saururaceae	Leaves
7.	***Lantana camara* L.**	Verbenaceae	Leaves
8.	***Mangifera indica* L.**	Anacardiaceae	Leaves
9.	***Moringa oleifera* Lam.**	Moringaceae	Leaves
10.	***Vitex negundo* L.**	Verbenaceae	Leaves

3.6.2. Inoculation of the test pathogens

The medium thus prepared was poured into sterilized Petri plates and was allowed to solidify. Each Petri plate was inoculated centrally with a 5 mm agar disc cut from the margin of actively growing culture of the test pathogens. In the control set, a Petri plate containing PDA medium with the requisite amount of distilled water instead of a plant extract was also inoculated with agar disc of the test pathogen in the same as described above. Three replications were maintained for both the experiments and control sets. The inoculated Petri plates were incubated at 25±2°C. The radial growth of the colonies was measured at 5th day of incubation.

The fungi toxicity of the fungicides and plant parts extracts in terms of percentage inhibition of mycelial growth was calculated by using the following formula followed by Bashar and Rai (1991):

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

Where, I = Per cent growth inhibition, C = Growth in control, T = Growth in treatment.

Data on different parameters were analyzed following computer package MSTAT-C and means were compared using DMRT. The data were collected and evaluated by analysis of variance (ANOVA) by using STAR statistical program followed by Yasmin and Shamsi (2019)

3.7. Evaluation of antagonistic potential of some soil fungi against the test pathogens.

Twenty species of fungi were isolated from infected parts of *T. erecta* and *T. patula* during the period of 2009 to 2014. Among the isolated fungi three were found pathogenic to both the species of *Tagetes* Aktar and Shamsi (2018). The pathogenic fungi were *Alternaria*

alternata, A. fumigatus and *C. lunata.* These three fungi were selected as test pathogen against three antagonistic fungi.

Serial dilution method was used to isolate antagonistic fungi from rhizosphere soil of the host plant (Krieg 1981). Among the isolated soil fungi, *Aspergillus flavus, A. niger* and *T. viride* were selected to test their antagonistic potential against the test fungi *A. alternata, A. fumigatus* and *C. lunata* following dual culture technique (Bashar and Rai 1994). The parameter used for the assessment of the colony interaction and per cent inhibition of radial growth was calculated (Fokkema 1976). Effects of volatile and non-volatile metabolites of the selected soil fungi against the test pathogens were also studied.

At first 1 gm soil was added with 99 ml of distilled water in a conical flask and mixed it very well with a glass rod and marked as mother suspension. Then five test tubes each containing 9 ml sterilized distilled water were taken. 1 ml of mother suspension was added into the 1st test tube and made it 10 ml. So, into the first test tube the mother suspension was diluted 10 times. After mixed it well, 1 ml of suspension from the 1st test tube was added into the 2nd test tube and made it 10 ml. So, into the 2nd test tube the mother suspension was diluted 100 times. This process was performed for rest of the test tubes and diluted the mother suspension 10, 100, 1000, 10000 and 100000 times.

For each dilution, 1 ml of suspension was poured into a sterilized Petri plate and then about 15 ml of sterilized melted PDA medium (about 50°C) was added. The plate was moved gently on the Laminar air flow table to get a homogenous distribution of the suspension. Five replications were maintained for each dilution. All the Petri plates were incubated into 25±2°C temperature. After 3 days, individual fungal colonies belonging to the genera *Aspergillus* and *Trichoderma* were sub-cultured on PDA slants randomly, from the culture plates and stored at 4°C in an incubator for future studies. Identities of soil

fungi were determined following the standard literature (Gilman 1967, Ellis 1971, 1976, Barnett and Hunter 2000, Sutton 1980). Cultures were maintained by sub-culturing after four weeks intervals. From the isolated soil fungi, *Aspergillus flavus, A. niger.* and *Trichoderma viride.* were selected randomly to study colony interactions with the test pathogens.

3.7.1. Colony interactions.

Colony interactions between the test pathogens and the selected soil fungi were studied in dual cultures on Potato dextrose agar medium. A Petri plate with 15ml solidified PDA medium was inoculated with 5 mm mycelial agar disc of a pathogen and a soil fungus, 30 mm apart from each other. Three replications were maintained in each case. The inoculated plates were incubated at 25± 2°C temperatures for 5 days. The colony growth of the pathogen was measured at the both sides, that is, towards and opposing each other from their central loci. The radial growth was measured after 3 and 5 days. Intermingled and inhibition zone was also measured during the same period.

Assessments of colony interaction between the test pathogens and the soil fungi were done in terms of grades which were determined by the model of Skidmore and Dickinson (1976) (Appendix 2). The grades and types are as follows:

Grade 1 (Type A): Mutually intermingling growth were both fungi grew into one another without any microscopic sign of interaction.

Grade 3 (Type Bi): Intermingling growth where the fungus being observed into the opposed fungus either above or below its colony.

Grade 2 (Type Bii): Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony

Grade 4 (Type C): Slight inhibition with a narrow demarcation line (1-2mm).

Grade 5 (Type D): Mutual inhibition at a distance more than 2 mm.

The parameter used for the assessment of the colony interaction were the width of inhibition zone, intermingled zone and per cent inhibition of radial growth was calculated by the formula of Fokkema (1976).

$$\text{Antagonistic effect} = \frac{R1-R2}{R1} \times 100$$

Where, R1 denotes the radial growth of the pathogens towards the opposite side and R2 denotes the radial growth of the pathogen towards the antagonist. The same method was followed for all possible combinations amongst the pathogens and selected three soil fungi.

3.7.2. Effect of volatile substances emanating from the cultures of the soil fungi on the radial growth of the test pathogens.

The soil fungi *Aspergillus flavus, A. niger* and *Trichoderma viride* were selected to evaluate their antagonistic effect of volatile metabolites against test pathogens *A. alternata, A. fumigatus and C. lunata.*

The method described by Dennis and Webster (1971 b) was followed for this study. The soil fungi were grown in 9 cm Petri plates on PDA medium for 3-4 days. After the inoculation at 25±2°C, the lid of each Petri plate was replaced by the same sizes bottom plate, containing 15 ml PDA medium, centrally inoculated with a test pathogen. Then Petri plates were covered by scotchtape so that no volatile substances can be moved from the inside of the Petri plates. Control was also prepared in the same way but the test pathogen at the bottom. Three replications were maintained in each test pathogens. These sets were incubated at 25°C. Colony diameters of the test fungi, in all sets, were measured and the per cent inhibition or stimulation in the colony diameter of the test fungi was calculated

after 4^{th} and 7^{th} day of incubation. The formula of per cent growth inhibition is given below:

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

Where, I = Per cent growth inhibition, C = Growth in control, T = Growth in treatment.

3.7.3. Effect of culture filtrates (Non-volatile metabolites) of the soil fungi on the radial growth of the test pathogens.

The soil fungi *Aspergillus flavus, A. niger* and *Trichoderma viride* were selected to evaluate their antagonistic effect of non-volatile metabolites against test pathogens *A. alternata, A. fumigatus and C. lunata.*

Three equal size blocks each of individual fungus, cut from the actively growing margins of 5 days old cultures, were inoculated separately into the 250 ml conical flasks each containing 100 ml sterilized Potato dextrose broth medium. After 10 days of incubation at 25±2°C, the culture of a soil fungus filtered first through a Whatman filter paper and then centrifuged at 3000 ppm for 20 minutes.

5, 10 and 20 ml metabolites of each fungus were added in 95, 90 and 80 ml sterilized PDA medium separately. The conical flask containing the PDA medium and culture filtrates was moved in different directions gently on the Laminar air flow table to get the homogenous distribution of the supplemented medium. This concentration was found to be most suitable for such studies by (Singh and Kumar 2011)). Each Petri plate contained 15 ml of PDA medium and metabolites with an addition of 1 drop (ca 0.03) of lactic acid which was used to check the bacterial growth. 5, 10 and 20 ml of supplemented medium was poured in a sterilized Petri plate and was allowed to solidify. Each Petri plate was inoculated centrally with a 5 mm agar disc, cut from the margin of actively growing culture of a test pathogen. In the control, Petri plate containing PDA medium without

culture filtrates were inoculated with a test pathogen as described above. In control set, equal amount of sterilized water was added with the PDA medium instead of culture filtrate. Three replications of each treatment were maintained. All the Petri plates were incubated at 25±2°C. The radial growth of the colonies was measured after 4 and 7 days of incubation. The per cent inhibition of each test pathogen was calculated with the formula given below:

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

Where, I = Percent growth inhibition, C = Growth in control, T = Growth in treatment

Data on different parameters were analyzed following computer package MSTAT-C and means were compared using LSD. The data were collected and evaluated by analysis of variance (ANOVA) by using STAR statistical program followed by Yasmin and Shamsi (2019)

3.8. Evaluation of selected fungicides and ethanol leaf extracts in controlling blight of *T. erecta* and *T. patula* in field condition

The experiment was conducted in the field plots of Botanical Garden, Dhaka University during the tenure of 2015-2017 to evaluate two fungicides and two ethanol leaf extracts against blight of *T. erecta* and *T. patula.* The unit plot size were 1.5 x 1 m and spacing between sub- plots 1 m. Two fungicides Bavistin 50 WP and Tilt 250 EC at 100 ppm concentration and ethanol leaf extracts of *Azadirachta indica* and *Citrus medica* at 10 % concentration were used in the experiment. A total of four sprays were done at 15 days interval. Experimental design was RBD, having three replications. Data were recorded after 15 days of each spray. Final data were recorded after 15 days of last spray. Per cent disease index (PDI) was calculated (Rahman and Rashid 2008).

Analysis of data

Data on different parameters were analyzed following computer package MSTAT-C and means were compared using LSD. The data were collected and evaluated by analysis of variance (ANOVA) by using STAR statistical program followed by Yasmin and Shamsi (2019)

CHAPTER: 4
RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1. Incidence and severity of blight disease of *Tagetes erecta* and *T. patula.*

In Bangladesh *Tagetes erecta* and *T. patula* are commonly grown by the gardeners as annual ornamental plants. A total of 184 samples were examined to record the diseases of *Tagetes* spp. in Bangladesh. Diseased samples were collected from BARI, Joydebpur, Gazipur, Dhaka, Chittagong, Comilla, Dhaka city, Khulna, Pabna, Rajshahi, Sylhet and Rangpur. Blight symptom was recorded on leaves, calyx, buds and petals of *T. erecta* and *T. patula* during the period of 2009-2014 (Plates 2-5).

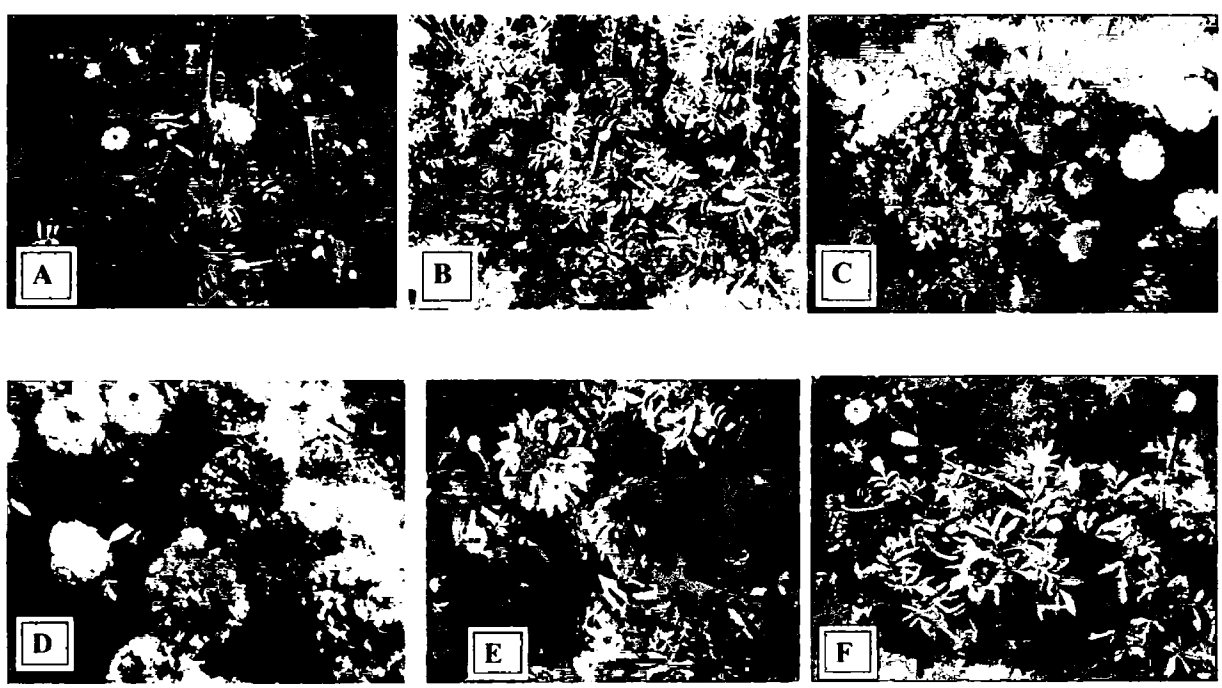

Plate 2. *Tagetes erecta*: Diseased samples collected from different locations: A-B. BARI, Joydebpur, Gazipur, C. Bangabandhu Sheikh Mujibur Rahman Novo Theatre, D. Mohakhali, E. Mirpur and F. Curzon Hall, Dhaka.

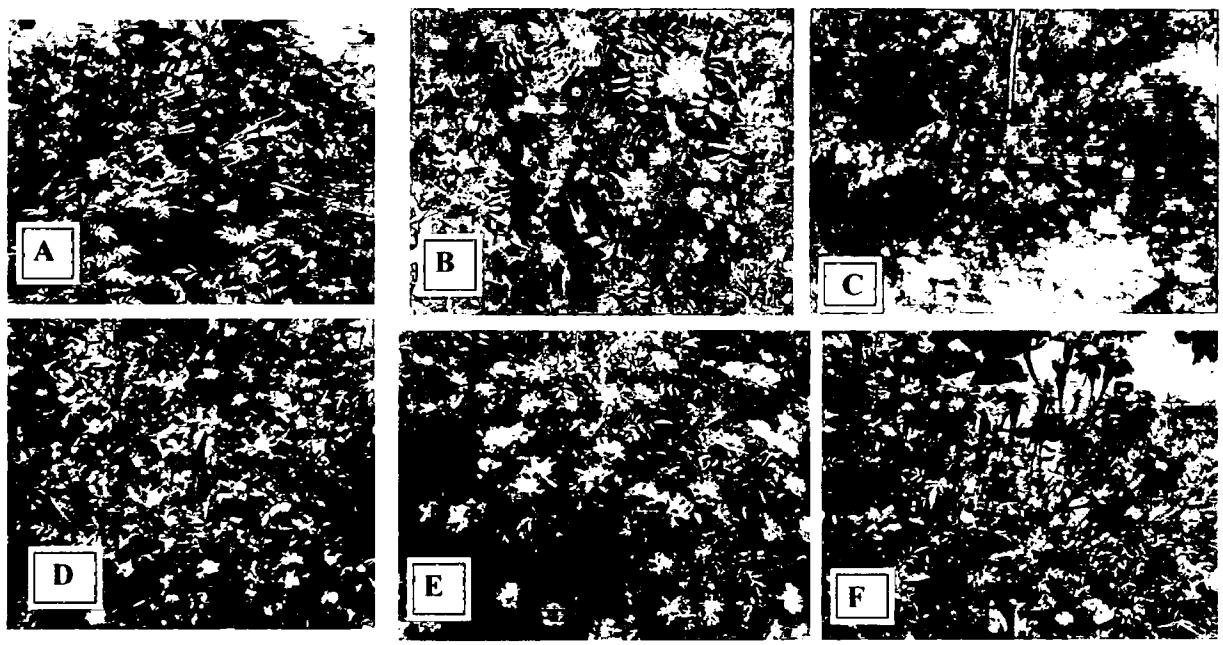

Plate 3. *Tagetes patula*: Diseased plant collected from different locations A. Curzon Hall, B. Mirpur, C. Bangabandhu Sheikh Mujibur Rahman Novo Theatre, D. Joydebpur, Gazipur, E. Mohakhali and F. Gulshan, Dhaka.

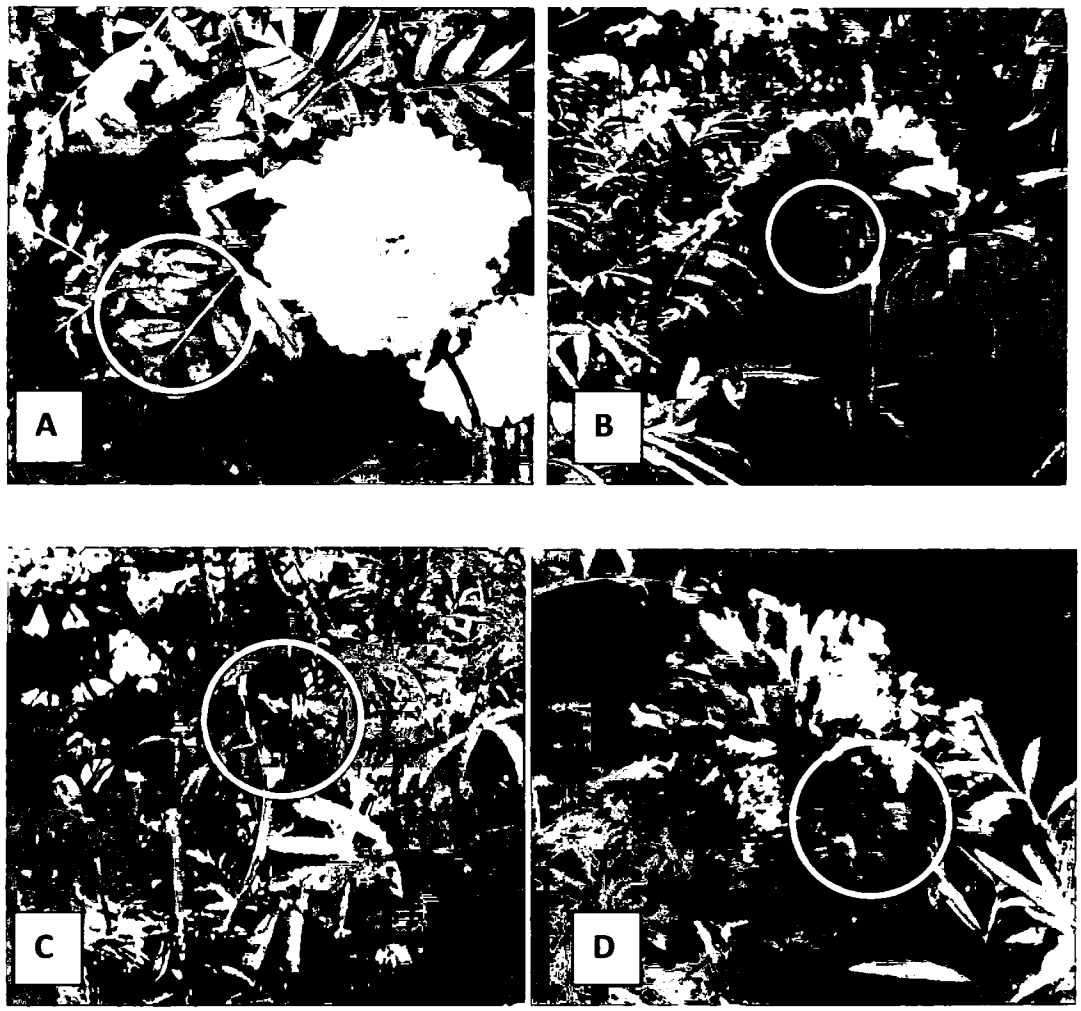

Plate 4. Diseased plant parts of *Tagetes erecta*: A. Leaves, B. Calyx, C. Bud and D. Petals.

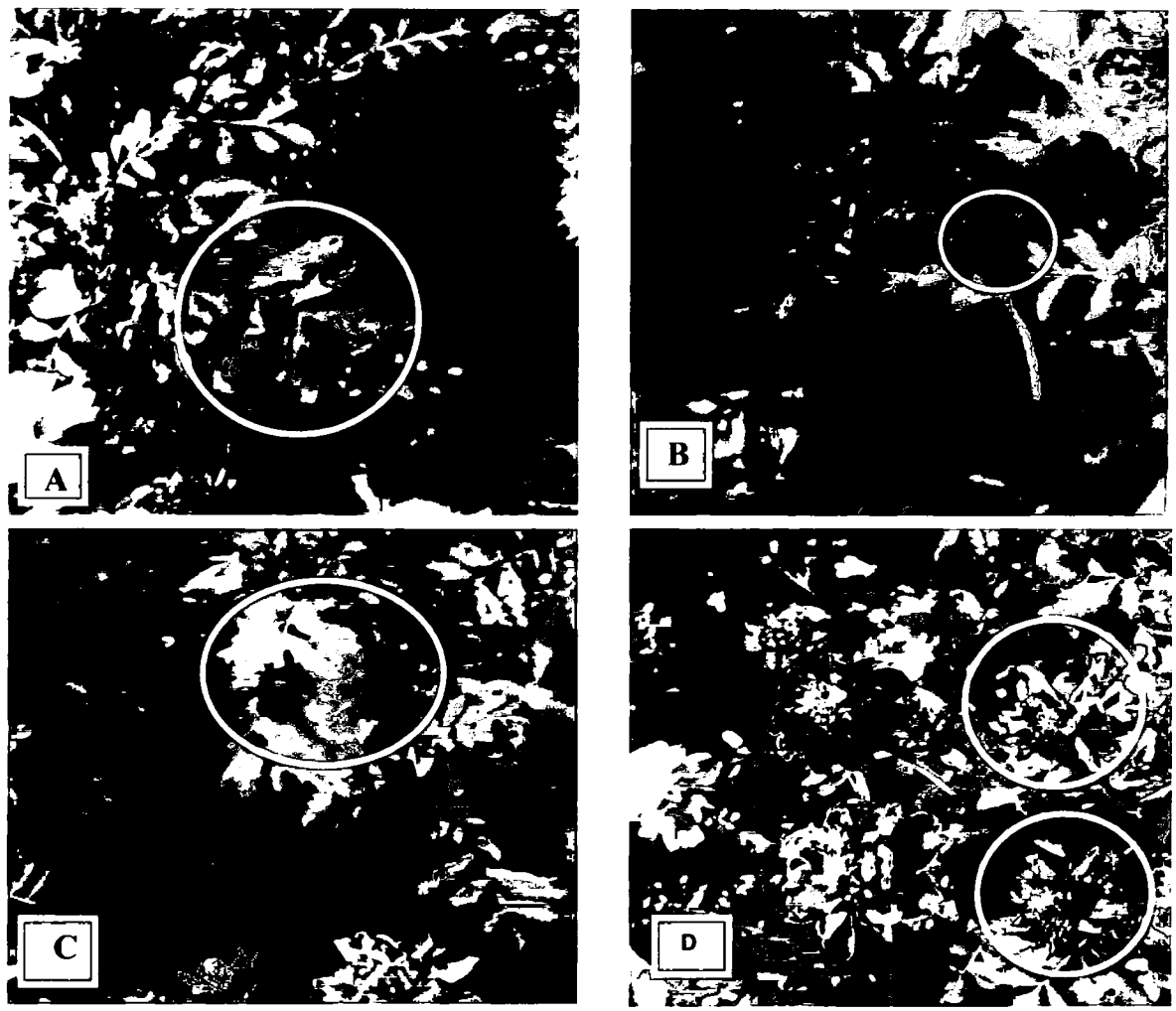

Plate 5. Diseased plant parts of *Tagetes patula*: A. Leaves, B. Calyx, C. Bud and D. Petals.

Disease incidence and severity of *T. erecta* and *T. patula* were recorded from 2012 to 2014. Figure 1 shows that in *T. erecta* blight incidence was started from January and gradually increased up to May during all the years studied. Lowest disease incidence was recorded (5.4–5.8%) in *T. erecta* in the month of January (2012-2014) where highest disease incidence was recorded (32.4 - 45.6%) in the month of May 2012-2014. Figure 2 shows that in case of *T. patula,* lowest disease incidence was recorded (2.8–5.2%) in January (2012-2014) where highest disease incidence was recorded (28.0 - 40.0%) in May (2012-2014). Figure 3 shows that rainfall was 1 mm in January 2012 and May 2012-2014. 65-72% humidity was recorded in January (2012–2014) and 68-78% humidity was recorded in May (2012–2014) (Fig. 4). Temperature recorded in the month of January was 17.6-18.9 ^{0}C and it was 28-30.2 ^{0}C in the months of May (2012-2014) (Fig. 5).

Disease severity of *Tagetes* spp. owing to blight was recorded at DS scale 0-9 during all the years studied. Highest disease severity (9) was recorded in the year 2013 and 2014 in the month of May in both the species examined. Lowest DS (1) was recorded in the year 2012 in case of *T. erecta* and 2013-2014 in case of *T. patula* (Figs. 6-8).

Blight symptom was recorded on different parts of *Tagetes erecta* and *T. patula* during the tenure of 2009 to 2014. 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.

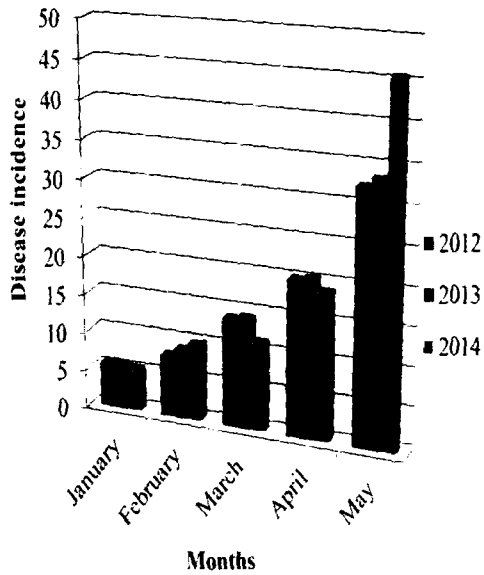

Fig. 1. Disease incidence of *Tagetes erecta* from January - May (2012-2014).

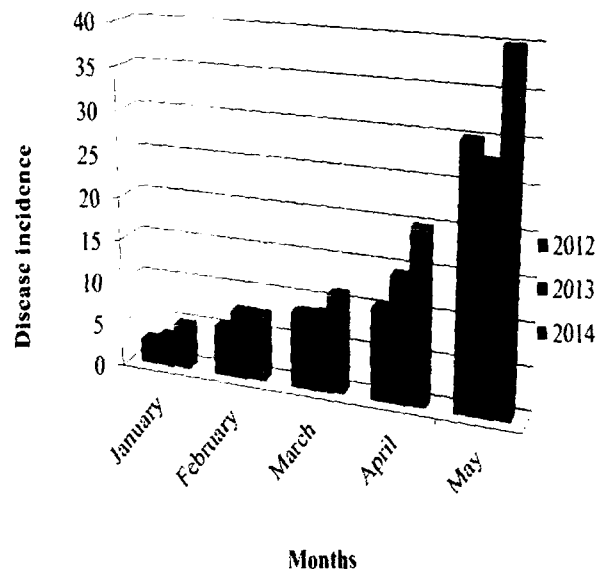

Fig. 2. Disease incidence of *T. patula* from January - May (2012-2014).

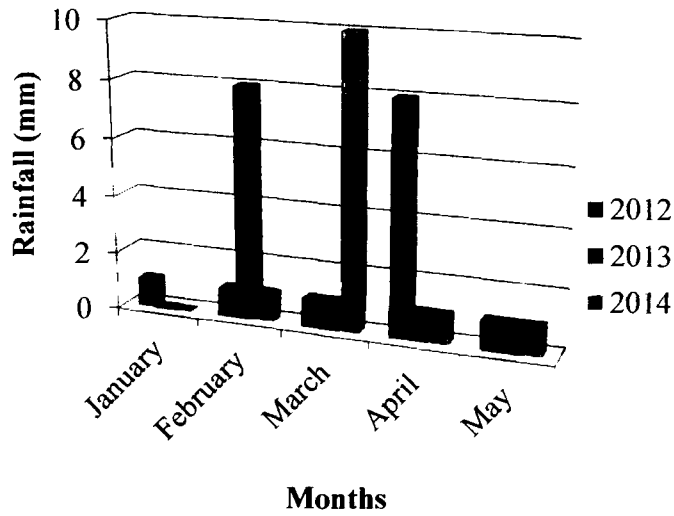

Fig. 3. Rainfall from January-May (2012-2014) in Dhaka city.

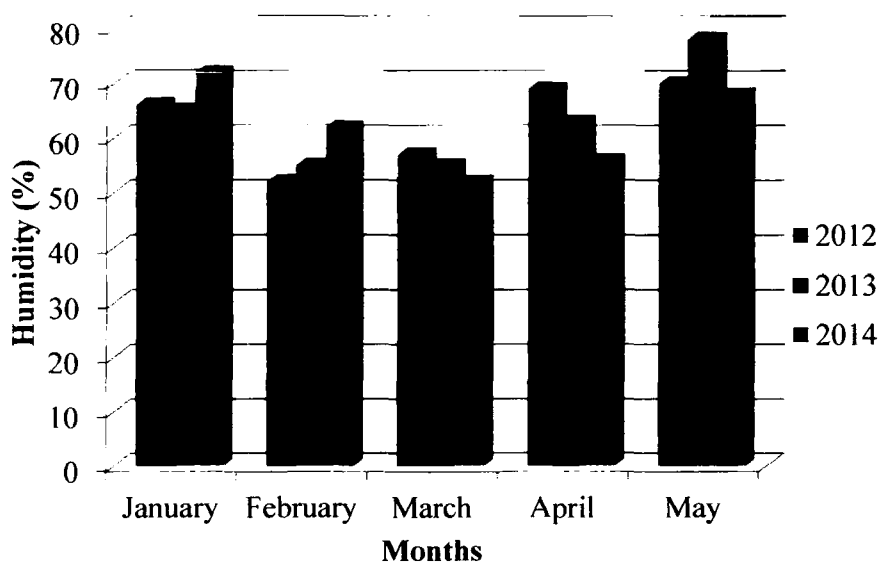

Fig. 4. Humidity from January-May (2012-2014) in Dhaka city.

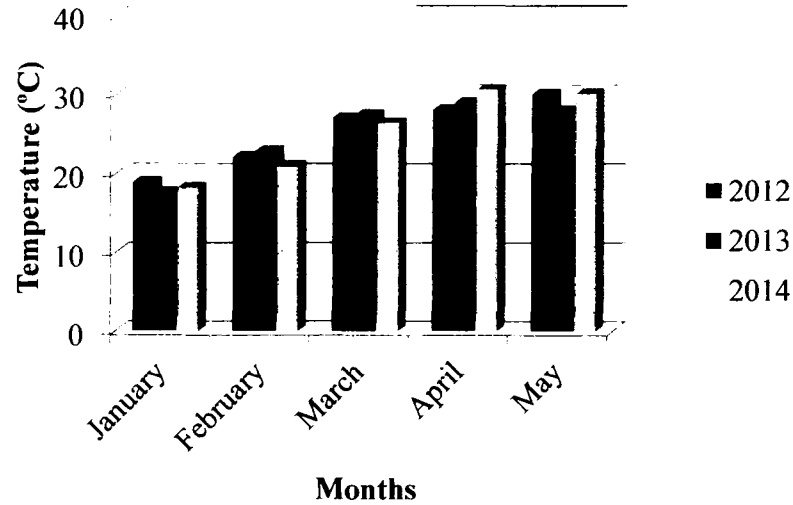

Fig. 5.Temperature from January-May (2012-2014) in Dhaka city.

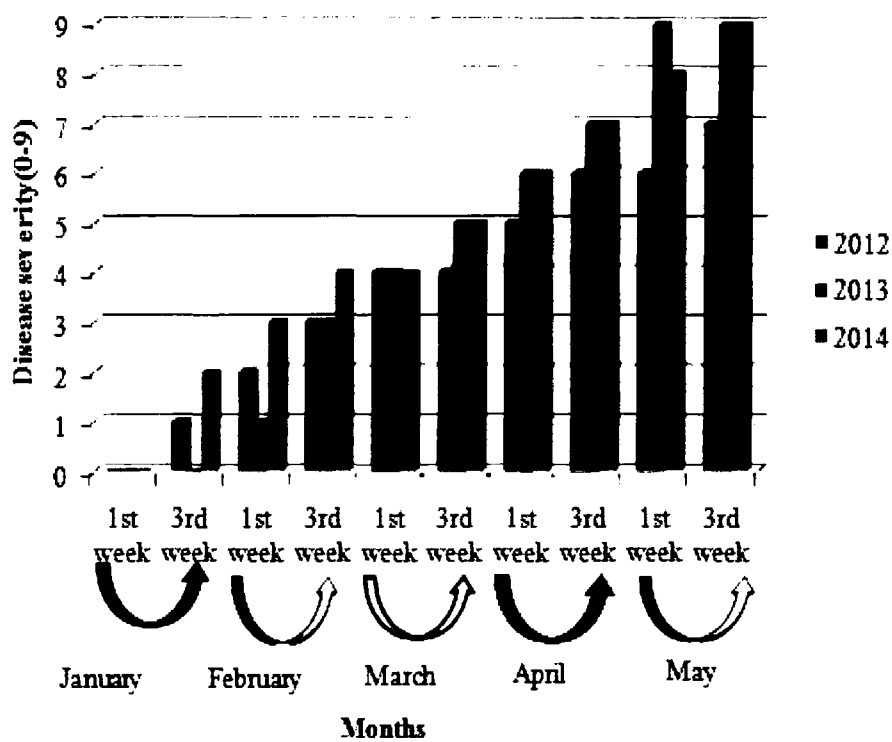

Fig. 6. Disease severity of *Tagetes erecta* in different years.

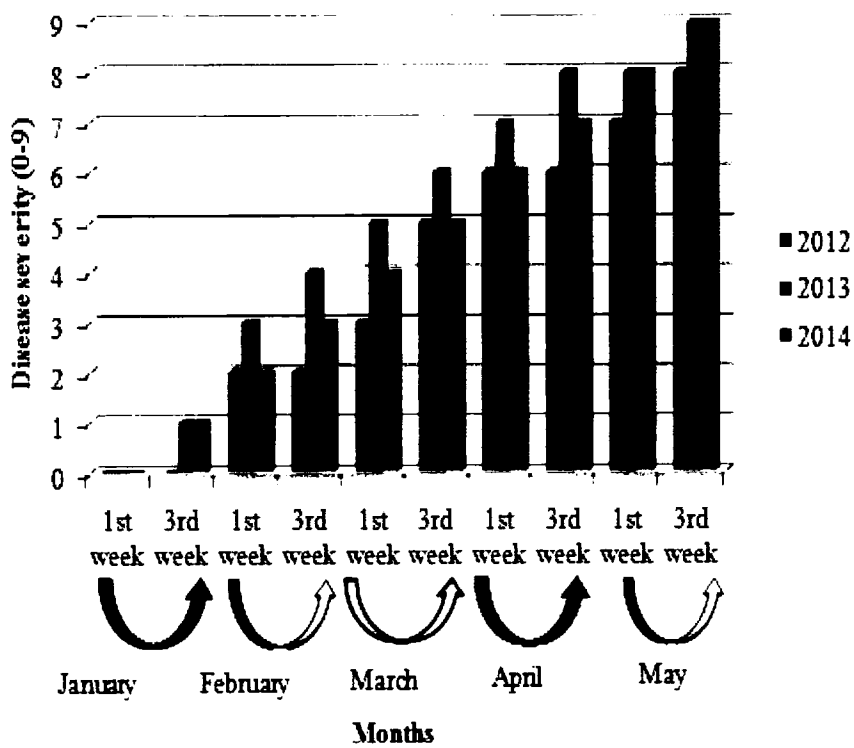

Fig. 7. Disease severity of *Tagetes patula* in different years.

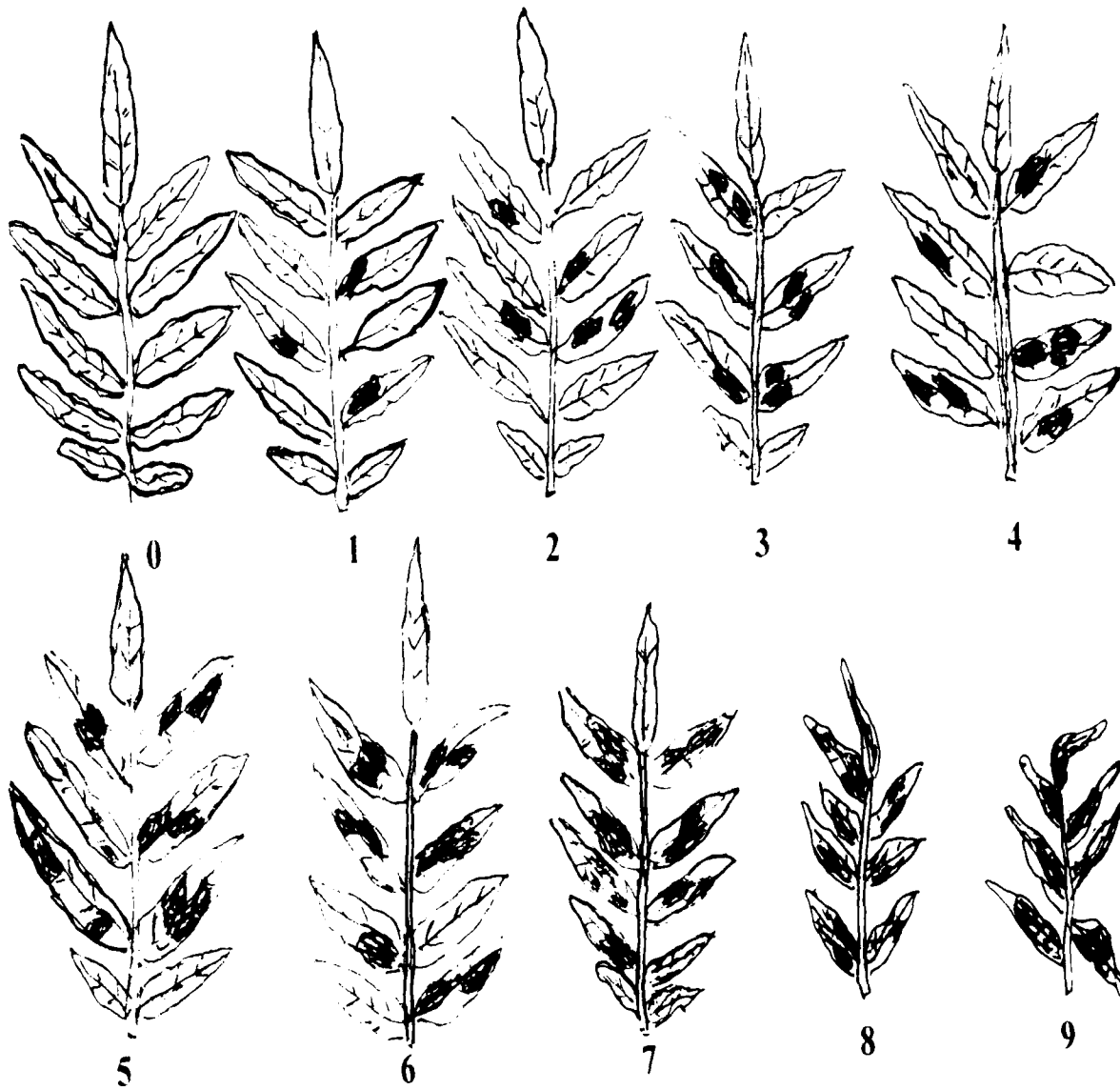

Fig. 8. Diagrammatic presentation of disease severity scale 0-9.

4.2. Isolation, characterization and identification of fungi associated with blight disease of *Tagetes erecta* and *T. patula*

A total of twenty fungi were isolated from infected plant parts of *T. erecta* and *T. patula*. The isolated fungi were *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem, *Bipolaris australiensis* (M.B. Ellis) Tsuda & Ueyama, *Chaetomium globosum* Kunze, *Cladosporium elatum* (Harz) Nannf., *Corynespora cambrensis* M. B. Ellis, *Curvularia brachyspora* Boedijn, *C. fallax* Boedijn , *C. lunata* (Wakker) Boedijn, *C. stapeliae* (du Plessis) Hughes & du Plessis, *Epicoccum purpurascens* Ehrenb. ex Schlecht., *Fusarium semitectum* Berk. & Rav., *Monochaetia ceratoniae* (Sousa da Camera) Sutton, *Nigrospora panici* Zimm., *Penicillium italicum* Wehmer, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill, *Trichoderma viride* Pers. and *Trichothecium roseum* Link (Table 5).

A total of 20 species of fungi were isolated from *Tagetes erecta* and *T. patula*. *Corynespora cambrensis, Monochaetia ceratoniae* and *Nigrospora panici* are new record for Bangladesh.

Among the isolated fungi *Alternaria alternata, Aspergillus fumigatus* and *Curvularia lunata* were found to be pathogenic to *Tagetes erecta* and *T. patula* (Aktar and Shamsi 2014, 2015 and 2016).

Table 6 shows that incase of *Alternaria alternata* frequency percentage of association of fungi 97.50 was the highest in the year 2013 and 8.33 was the lowest in the year 2014. In case of *Aspergillus fumigatus* frequency percentage of association of fungi 77.50 was the highest in the year 2014 and 0.83 was the lowest in the year 2009. In case of *Curvularia lunata* frequency percentage of association of fungi 29.16 was the highest in the year 2010 and 4.16 was the lowest in the year 2013.

Table 5. List of fungi associated with infected plants of *Tagetes erecta* and *T. patula* during 2009-2014.

Name of isolates	*Tagetes erecta*	*T. patula*
Alternaria altrenata	+	+
Aspergillus flavus	+	+
A.fumigatus	+	+
A. niger	+	+
Bipolaris australiensis	-	+
Chaetomium globosum	+	+
Cladosporium elatum	+	+
Corynesora cambrensis	+	+
Curvularia brachyspora	+	+
C. fallax	+	+
C. lunata	+	+
C. stapeliae	-	+
Epicoccum purpurascens	-	+
Fusarium semitectum.	+	+
Monochaetia ceratoniae	+	+
Nigrospora panici	+	+
Penicillium italicum	+	+
Rhizopus stolonifer	+	+
Trichoderma viride	+	+
Trichothecium roseum	+	-

'-' = No fungal isolates

Table 6. Frequency percentage of association of fungi with *Tagetes erecta* and *T. patula* from 2009 to 2014.

Name of the Fungi	Years											
	2009		2010		2011		2012		2013		2014	
	T. erecta	*T. patula*	*T. erecta*	*T. patula*	*T. erecta*	*T. patula*	*T. erecta*	*T. patula*	*T. erecta*	*T. patula*	*T. erecta*	*T. patula*
Alternaria alternata	42.49	34.99	48.33	45.83	52.49	-	10.00	-	**97.50**	60.83	8.33	37.50
Aspergillus flavus	-	-	-	4.16	3.33	-	27.49	25.83	-	1.66	1.66	30.00
A. fumigatus	20.00	**0.83**	-	50.00	8.33	-	-	-	-	27.50	**77.50**	-
A. niger	8.33	4.99	65.83	20.00	1.66	10.83	10.83	16.66	42.50	13.33	-	-
Bipolaris australiensis	-	7.50	-	-	-	-	-	-	-	-	-	-
Chaetomium globosum	-	-	27.49	12.49	-	-	-	1.66	-	-	-	-
Cladosporium elatum	19.99	1.66	57.49	29.99	-	-	-	1.66	12.50	1.66	-	9.16
Corynespora cambrensis	-	-	16.66	20.83	-	-	-	-	-	-	-	-
Curvularia brachyspora	-	-	0.83	4.16	-	25.00	-	4.16	8.33	-	-	-
C. fallax	-	-	-	-	5.00	41.66	8.33	2.50	5.00	-	-	-
C.lunata	20.83	4.99	6.66	**29.16**	-	-	12.50	-	8.33	**4.16**	16.66	-
C. stapeliae	-	-	-	-	-	-	-	10.83	-	-	-	-
Epicoccum purpurascens	-	-	-	4.16	-	-	-	-	-	-	-	-
Fusarium semitectum	18.33	-	24.99	8.33	54.99	20.00	8.33	-	14.99	0.83	-	-
Monochaetia ceratoniae	10.00	-	-	4.16	-	-	-	-	-	-	-	-
Nigrospora panici	-	-	5.83	8.33	13.33	-	-	-	-	-	-	-
Penicillium italicum	5.00	3.33	9.16	2.50	8.33	40.83	15.00	16.66	17.49	0.83	-	-
Rhizopus stolonifer	-	-	-	-	1.66	-	9.99	11.66	-	-	-	-
Trichoderma viride	-	-	16.66	8.33	7.50	6.66	6.66	-	-	-	-	-
Trichothecium roseum	-	-	-	-	1.66	-	-	-	-	-	-	-

-' = No fungal isolates

4.3. Taxonomic treatment of fungal taxa

Alternaria alternata (Fr.) Keissler, 1912, *Beih. Bot. Zbl.*, **29** : 434. **(Plate 6A, Fig. 9A)**

Colonies greenish black velvety. Reverse blackish. Hyphae pale to mid brown, smooth septate, 1-5 µm in diameter. Conidiophores flexous, septate, pale to mid brown, up to 85 µm long, but usually much shorter (14-60) µm and 4-7(9) µm in diameter. Conidia straight, muriform, oblong, rounded at the base, pale to mid brown, 2-7 (mostly 5) septate, 20-55 (76) × 8-18 (13) µm. Beak 2-5 µm thick.

Specimen examined: Isolated from infected leaves of *Tagetes erecta* L., Botanical Garden, University of Dhaka, Dhaka, 10 February 2009, M. Aktar 1.

Aspergillus flavus Link, in Obs. p.16. 1809; also in Sp. Plant. **6:** 66. 1824, **(Plate 6B)**

Colonies effuse, greenish. Reverse greenish. *Mycelium* well-developed, septate, profusely branched and hyaline. Conidiophores long, greenish brown variable mostly 440 -560 × 4-8 µm. Vesicles globose or subglobose, thick walled, commonly 20–30 µm, usually fertile on the upper half only. Sterigmata in one series, 5-10 × 2-3 µm. Conidia dark green in mass, one celled globose, spinose, catenulate, mostly 2.5-3 µm in diameter.

Specimen examined: Isolated from the infected bud of *Tagetes patula* L., Botanical Garden, University of Dhaka, Dhaka, 14 February 2010, M. Aktar 38.

Aspergillus fumigatus Fresenius, in Beitrage zur Mykologie, p.81, pl.10, figs.1-11. Frankfurt, 1850-53. **(Plate 6C)**

Colonies of the fungus on PDA plates were grey-green, cottony and reverse side was off white. Conidiophores are aseptate, smooth, greenish up to 500µm in length and 2-8 µm width. Vesicles flask shaped, 20–30 µm, typically fertile over the upper half. Sterigmata in one series are crowed. Conidia are grey-green, one celled, globose, echinulate, catenate, 2-3 µm diameter.

Specimen examined: Isolated from infected petals of *Tagetes erecta* L., Botanical Garden, University of Dhaka, 7 April 2009, M. Aktar 19.

Aspergillus niger van Tieghem, Ann. Sci. Nat. Bot., Ser. 5, **8:** 240. (1867). **(Plate 6D)**

Colonies effuse, black. Reverse brownish. Mycelium well-developed, septate, profusely branched and brownish. Conidiophores brown 200–459 × 8–11µm. Vesicles globose or subglobose, thick walled, commonly 22–54 µm. Sterigmata typically in two series, closely packed covering the vesicle usually 20–30 × 7–8 µm. Conidia dark brown, one celled globose, spinose, cattenulate. 2–4 (5.5) µm in diameter.

Specimen examined: Isolated from infected leaves of *Tagetes erecta* L., Botanical Garden, University of Dhaka, Dhaka, 10 February 2009, M. Aktar 3.

Bipolaris australiensis (Bugnicourt) Subram. & Jain ex M. B. Ellis; Subram. & Jain, 1966, *Curr. Sci.*, **35**: 354. **(Plate 7A, Fig. 9B)**

Colonies effuse, grey to dark blackish brown, velvety. Reverse blackish. Hyphae pale to dark brown, smooth, septate, 2-4 µ thick. Conidiophores solitary flexuous or geniculate, septate, reddish brown, sometimes up to 150 µ long but usually shorter, width 3-7 µm. Conidia straight, ellipsoidal or oblong, rounded at the ends, pale brown to mid reddish brown, 11.2- 26 ×6.6-9.8 µm.

Specimen examined: Isolated from the infected petals of *Tagetes patula* L., Botanical Garden, University of Dhaka, Dhaka, 18 March 2009, M. Aktar 9.

Chaetomium globosum Kunze ex Fr., Systema Mycologicum 3: 255 (1829).

(Plate 7B, Fig. 9C)

Colony cottony, greyish black, mycelia septate profusely branched. Perithecia globose with appendages. Ascospores lemon shaped and 8-14.8 ×3.4- 6.8 µm.

Specimen examined: Isolated from infected leaves of *Tagetes erecta* L., Bangabandhu Sheikh Mujibur Rahman novo theatre, 17 January 2010, M. Aktar 28.

Cladosporium elatum (Harz) Nannf., 1934, *Svenska SkogsvFor . Tidskr.* **32**: 397.

(Plate 7C, Fig. 10A)

Colonies on medium effuse, olivaceous grey, reverse blackish olive. Conidiophores straight or flexuous, pale to mid brown or olivaceous brown, smooth, up to 87.6 µm long, 5-6.8 µm thick. Conidia in very long branched chains forming wide, loose heads, fusiform, limoniform or subspherical tapered into a narrow tube or tubes at one or both ends, mostly 0 septate, very pale brown or olivaceous brown, smooth, 8–16 × 4.2–5.4, *ramo–conidia* present.

Specimen examined: Isolated from infected leaves of *Tagetes erecta* L., Botanical Garden, University of Dhaka, Dhaka, 16 February 2009, M. Aktar 5.

Corynespora cambrensis M. B. Ellis, 1971. **(Plate 7D, Fig. 10B)**

Colonies on medium effuse, reverse grayish, Hyphae brown, smooth, septate, 2-4 µm thick. Conidiophores arising singly or sometimes in fascicles, often proliferating terminally through the apical conidial scar, straight or flexuous, unbranched, brown or olivaceous brown, smooth. Conidia solitary often connected with the conidiophore with a hyaline isthmus or catenate, mostly obclavate, cylindrical., subhyaline, pale to darkly pigmented, pseudoseptate, smooth, 24.3- 110.7 × 5.4-10.8 µm.

Specimen examined: Isolated from infected petals of *Tagetes erecta* L., Chittagong road, 13 February 2010, M. Aktar 33.

The fungus is new record for Bangladesh.

Curvularia brachyspora Boedijn. Ellis, M.B., Mycol. Pap., **106**:2-43, 1966.

(Plate 8A, Fig. 11A)

Colonies effuse, black, reverse blackish. Hyphae brown, smooth, septate. Conidiophores solitary, branched, straight, mostly flexuous geniculate, mid brown, septate, up to 62 µm long and 6.8–7.2 µm thick. Conidia mostly 3-septate, dark brown, mostly curved, smooth, 22–28 × 9–13 µm.

Specimen examined: Isolated from infected leaves of *T*agetes *erecta* L., Botanical Garden, University of Dhaka, Dhaka, 14 February 2010, M. Aktar 34. ***Curvularia fallax*** Boedijn. . Ellis, M.B., Mycol. Pap., **106**:2-43, 1966.

(Plate 8B, Fig. 11B)

Colonies effuse, black, hairy. Reverse blakish. Hyphae brown, smooth, septate .Conidiophores solitary, branched, straight, mostly flexuous geniculate, mid brown, septate, up to 62 µm long and 6.8–7.2 µm thick. Conidia mostly 4-septate, dark brown, mostly curved, smooth, (16) 22–40 × (7) 9–15 µm.

Specimen examined: Isolated from infected petals of *Tagetes patula* L., BARI, 24 February 2011, M. Aktar 47.

Curvularia lunnata (Wakker) Boedijn. Ellis, M.B., Mycol. Pap., **106**:2-43, 1966.

(Plate 8C, Fig. 12A)

Colonies effuse, dark black. Reverse blakish Hyphae brown, smooth, septate, branched, straight or slightly undulating, often geniculate, pale to dark brown, septate, 20-64.4 (83.6) µm long, 3-5.4 µm thick, often swollen at the base. *Conidia* 3 septate, olivaceous black to

dark brown, almost always curved at the third cell from the base which is larger and darker than the others, end cells subhyaline or pale brown, smooth, 20–34 × 9-15 µm.

Specimen examined: Isolated from infected buds of *Tagetes erecta* L., Botanical Garden, University of Dhaka, Dhaka, 16 February 2009, M. Aktar 6.

Curvularia stapeliae (du Plessis) Hughes & du Plessi. Ellis, M.B., Mycol. Pap., **106**:2-43, 1966. **(Plate 8D, Fig. 12B)**

Colonies effuse, grayish black. Reverse blakish. Hyphae brown, smooth, septate, branched, straight or slightly undulating, often geniculate, pale to dark brown, septate, 20-64.4 (83.6) µm long, 3-5.4 µm thick, often swollen at the base. *Conidia* 3 septate, olivaceous black to dark brown, almost always curved at the third cell from the base which is larger and darker than the others, end cells subhyaline or pale brown, smooth, 30-45×11-17 µm.

Specimen examined: Isolated from the infected petals of *Tagetes patula* L., Khagrachari, 24 May 2012, M. Aktar 61.

Epicoccum purpurascens Ehrenb. ex Schlecht., 1824, Synop. Pl. crypt.: 136.

(Plate 9A, Fig. 13A)

Colonies grayish green, reverse blakish green. Hyphae brown, septate, profusely branched. *Sporodochia* up to 2.5 mm. diam. *Conidiophores* 5-16 × 3-6 µm. Conidia brown muriform, 14 - 27 (51) µm diam.

Specimen examined: Isolated from the infected bud of *Tagetes patula* L., Botanical Garden, University of Dhaka, Dhaka, 14 February 2010, M. Aktar 36.

Fusarium semitectum Berk. & Rav. in Berkeley, *Grevillea***3**: 98, 1875.

(Plate 9B, Fig. 13B)

Colonies at first white and gradually becomes pink in colour. Mycelia hyaline, septate, profusely branched. Sporodochia absent, Phialide present. Microconidia 6-11.2 × 1.6-2.8 µm and macroconidia 17.2-25.2 × 1.8-3.4 µm.

Specimen examined: Isolated from infected leaves of *Tagetes erecta* L., Botanical Garden, University of Dhaka, Dhaka, 16 February 2009, M. Aktar 7.

Monochaetia ceratoniae (Sousa da Camera) Sutton, *Mycol. Pap.* **88:** 42 (1963).

(Plate 9C, Fig. 13C)

Colonies white, cottony reverse off white. Mycelium immersed, branched, pale brown, septate. Acervulus black shinning. Conidiophores hyaline short, cylindrical, straight or curved, sparsely branched, septate only at the base. Conidia brown, 4 septate, end cells hyaline, 19-23 × 5.6-7.4 µm, median cells thin-walled, smooth, 13-14µm long; apical appendage 5-6 µm long, basal appendage 2.5-4 µm long.

Specimen examined: Isolated from the infected petals of *Tagetes erecta* L., Botanical Garden, University of Dhaka, Dhaka, 16 February 2009, M. Aktar 8.

The fungus is new record for Bangladesh.

Nigrospora panici Zimm. M.B. Ellis 1971. **(Plate 9D, Fig. 14A)**

Colonies brownish black with shinning black conidia on the surface. Mycelium partially superficial, branched, brown, septate. Conidiophores semi-macronemtous, mostly unbrached, hyaline. Conidia solitary, spaerical or broadly ellipsoidal, compressed dorsiventrally, aseptate, black shinning, smooth 0-septate. 22.4-33.6 µm diam.

The fungus is new record for Bangladesh.

Specimen examined: Isolated from the infected petals of *Tagetes erecta* L., Chittagong road, 27 January 2010, M. Aktar 30.

Penicillium italicum Wehmer, Hedwigia **33:** 211-214. 1894. **(Plate 10A)**

Colonies on PDA medium growing restrictedly, often marked by a few shallow furrows, with margins usually its inner surface pale gray-green shades. Penicilli asymmetric, often comparatively long up to 50-70 μm and 3-5 μm width, bearing tangled chains of conidia. Strigmata 3-6 in a whole 8-12 × 3.5 μm. Conidia one celled with greenish tinge, 4-5 × 25-35 μm.

Specimen examined: Isolated from the infected calyx of *Tagetes erecta* L., Botanical Garden, University of Dhaka, Dhaka, 12 April 2009, M. Aktar 25.

Rhizopus stolonifer (Ehrenb.) Vuill., Revue Mycologique Toulouse **24**: 24: 54 (1902).

(Plate 10B)

Mycelium coenocytic, well-developed, branched and fluffy. *Mycelium* produces many aerial stolones that develop rhizoids at certain points. Directly above the rhizoids one or more *sporangiophores* are produced. The top of each *sporangiophores* becomes swollen as the latter reaches maturity, and a *sporangium* is developed. The central portion of *sporangium* becomes highly vaculated and it eventually surrounded by a wall that separates it's from the peripheral zone. The central portion in the columella. Sporangium produces non motile sporangiospores.

Specimen examined: Isolated from the infected petal of *T. erecta* L. Mushroom Centre, Savar, 21 April 2011, M. Aktar 51.

Trichoderma viride Pers., Neues Magazin für die Botanik **1**: 92 (1794). **(Plate 10C)**

Colony effuse, light green. *Conidiophores* hyaline, much branched, bearing phiallides single or in groups. *Conidia* hyaline, powdery mass, 1-celled, ovoid, borne in small terminal clusters 3-4 µm.

Specimen examined: Isolated from the infected petal of *Tagetes erecta* L., Chittagong road, 8 February 2010, M. Aktar 32.

Trichothecium roseum (Pers.) Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin **3** (1): 18, t. 1:27 (1809). **(Plate 10D, Fig. 14B)**

Colonies effuse, at first white but soon turning rosy pink. Conidiophores up to 147× 3–4.5 µm hyaline, often slightly swollen at their tips. Conidia hyaline, pink in mass, 1-septate, thick walled, each with a flattened protuberance at the base, 12- 18.4 × 5.4–7.2 µm, often clustered.

Specimen examined: Isolated from the infected petal of *T. erecta* L., BARI, 14 March 2011, M. Aktar 49.

A detailed survey of literature revealed *Corynespora cambrensis, Monochaetia ceratoniae* and *Nigrospora panici* has not been reported previously in any relevant literature of Bangladesh (Siddiqui *et al.* 2007, Shamsi and Hosen 2016, Shamsi 20 17 and Momtaz *et al.* 2018).

Hence, *Corynespora cambrensis, Monochaetia ceratoniae* and *Nigrospora panici* are reported here as new fungal record for Bangladesh.

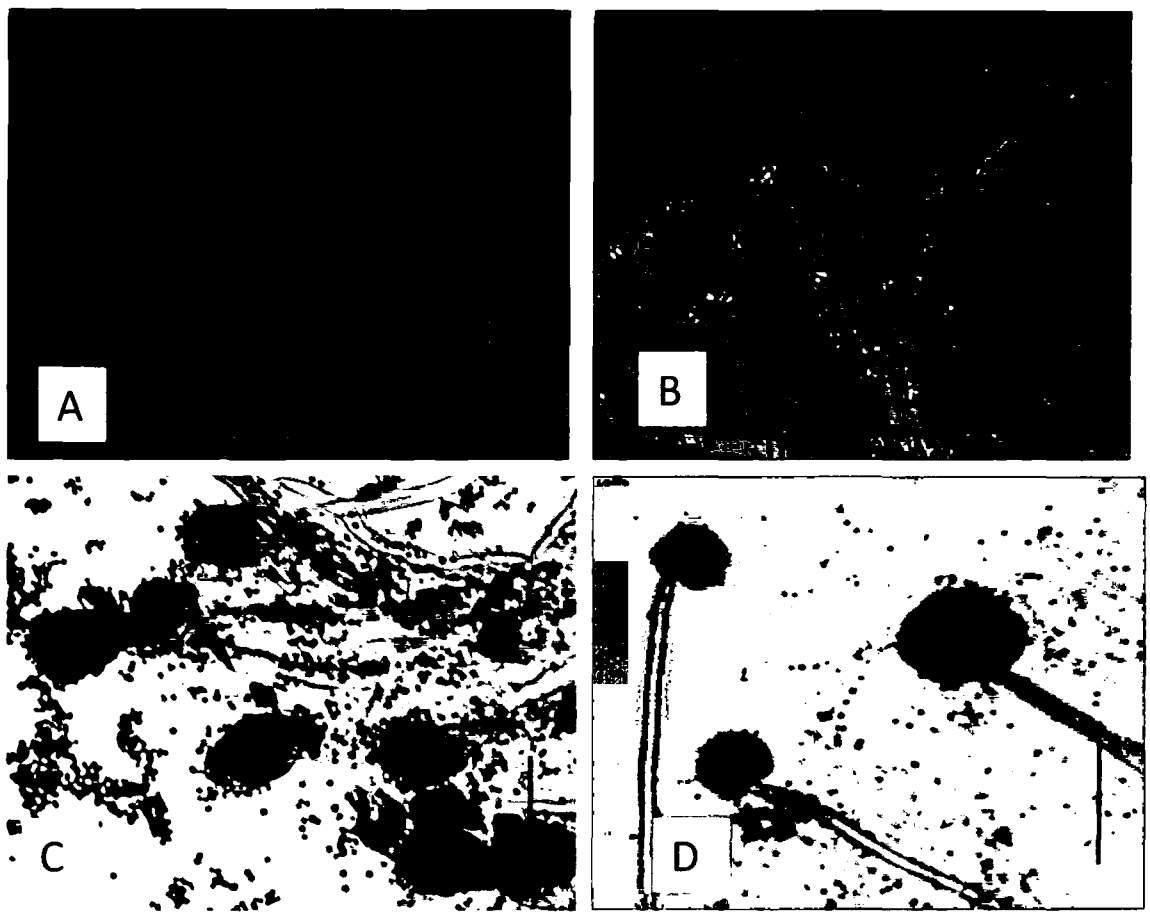

Plate 6. Conidiophores and conidia of A. *Alternaria alternata,* B. *Aspergillus flavus,* C. *A. fumigatus* and D. *A. niger* (Bar = 50 µm).

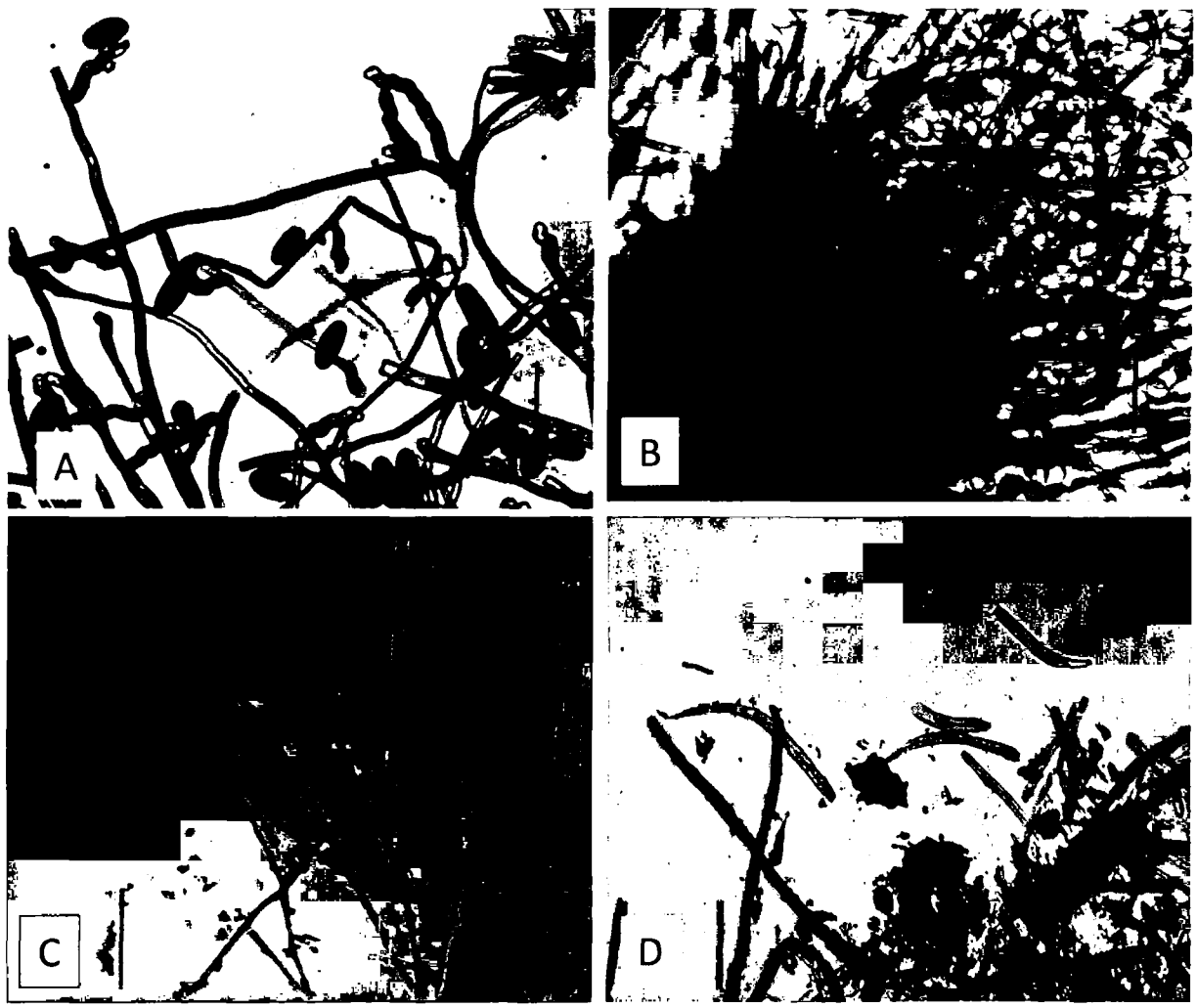

Plate 7. Conidiophores and conidia of A. *Bipolaris australiensis,* B. Peritheceum, appendages and ascospores of *Chaetomium globosum*, C. Conidiophores and conidia of *Cladosporium elatum* and D. Conidiophores and conidia of *Corynespora cambrensis* (Bar = 50 µm).

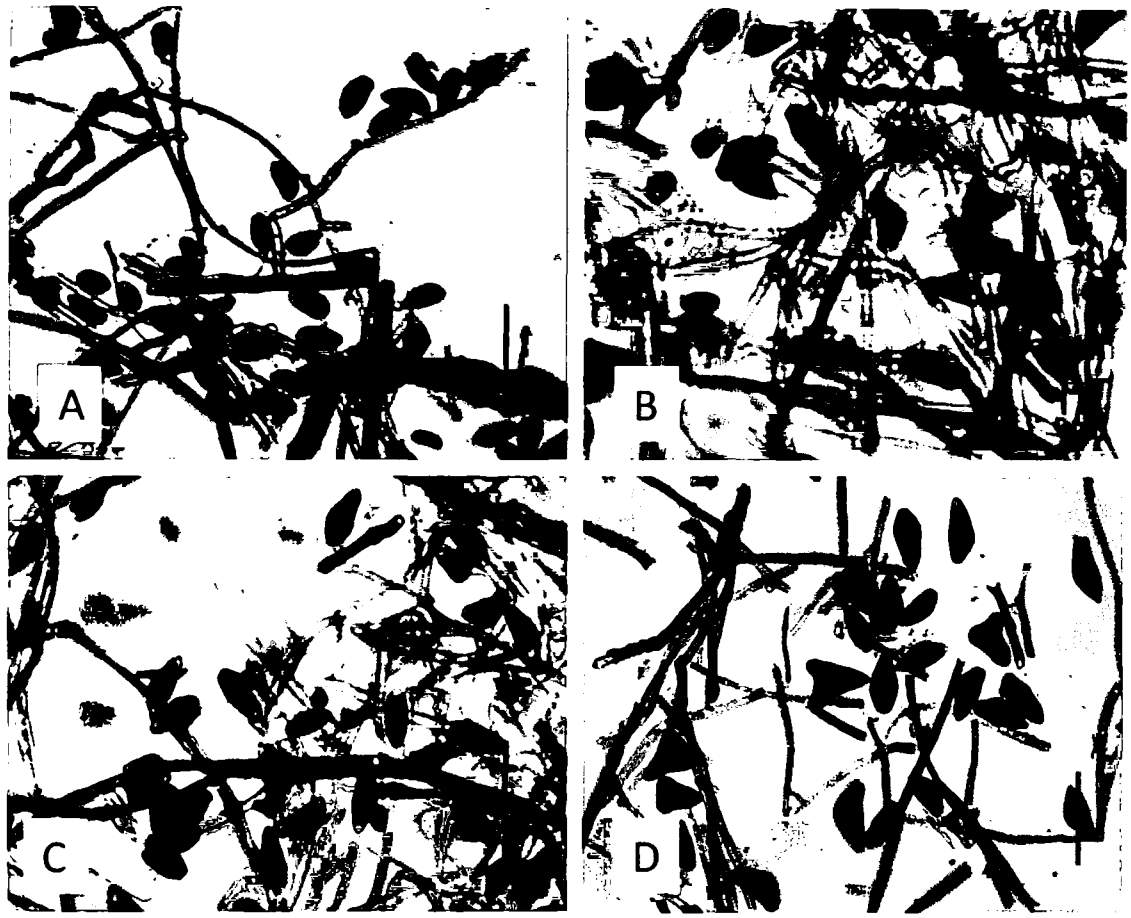

Plate 8. Conidiophores and conidia of A. *Curvularia brachyspora*, B. *C. fallax*, C. *C. lunata* and D. *C. stapeliae* (Bar = 50 µm).

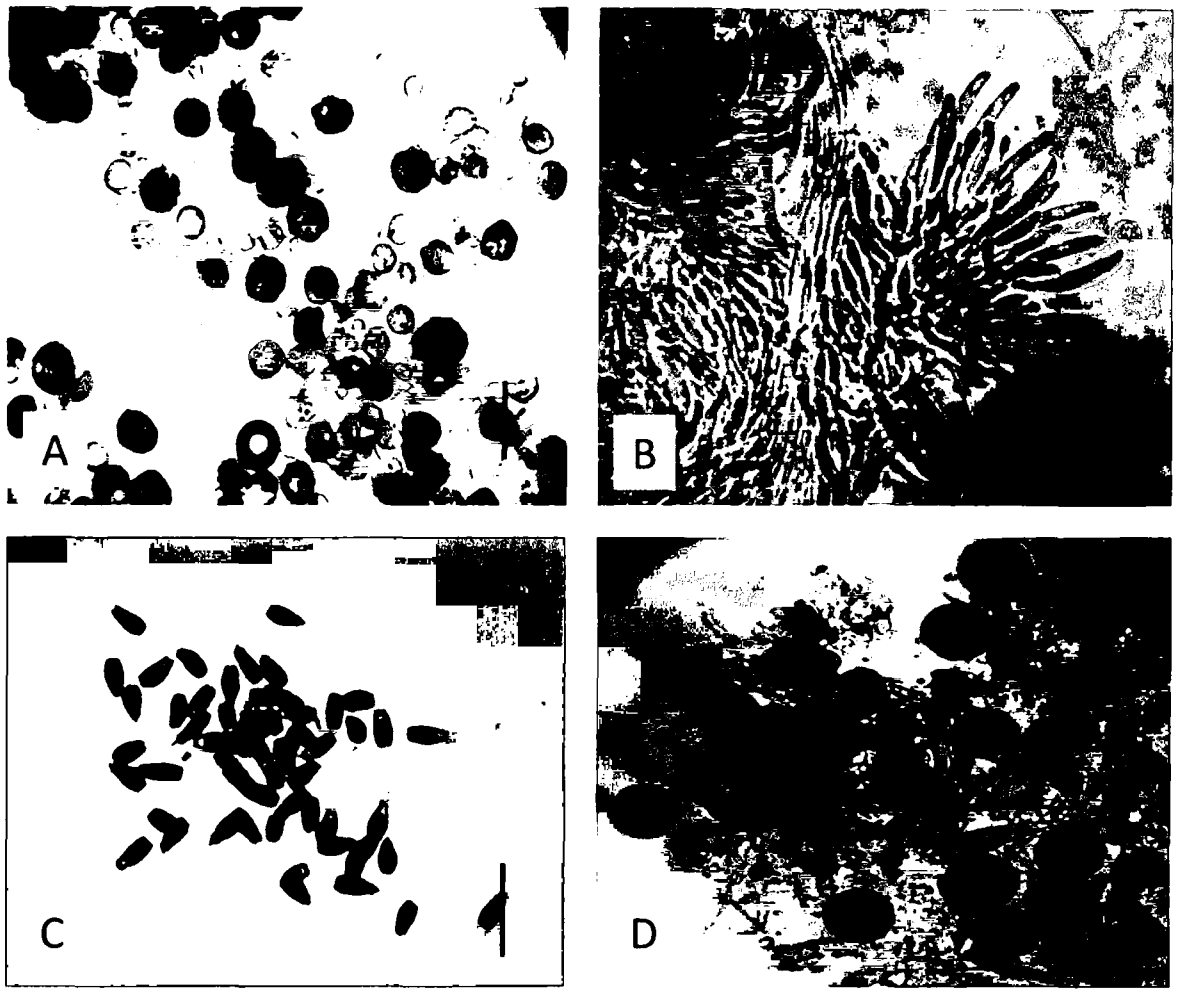

Plate 9. Sporodochia, conidiophores and conidia of A. *Epicoccum purpurascens,*, B. *Fusarium semitectum,* C. Acervulus, conidiophores and conidia of *Monochaetia ceratoniae* and D. Conidiophores and conidia of *Nigrospora panici* (Bar = 50 μm).

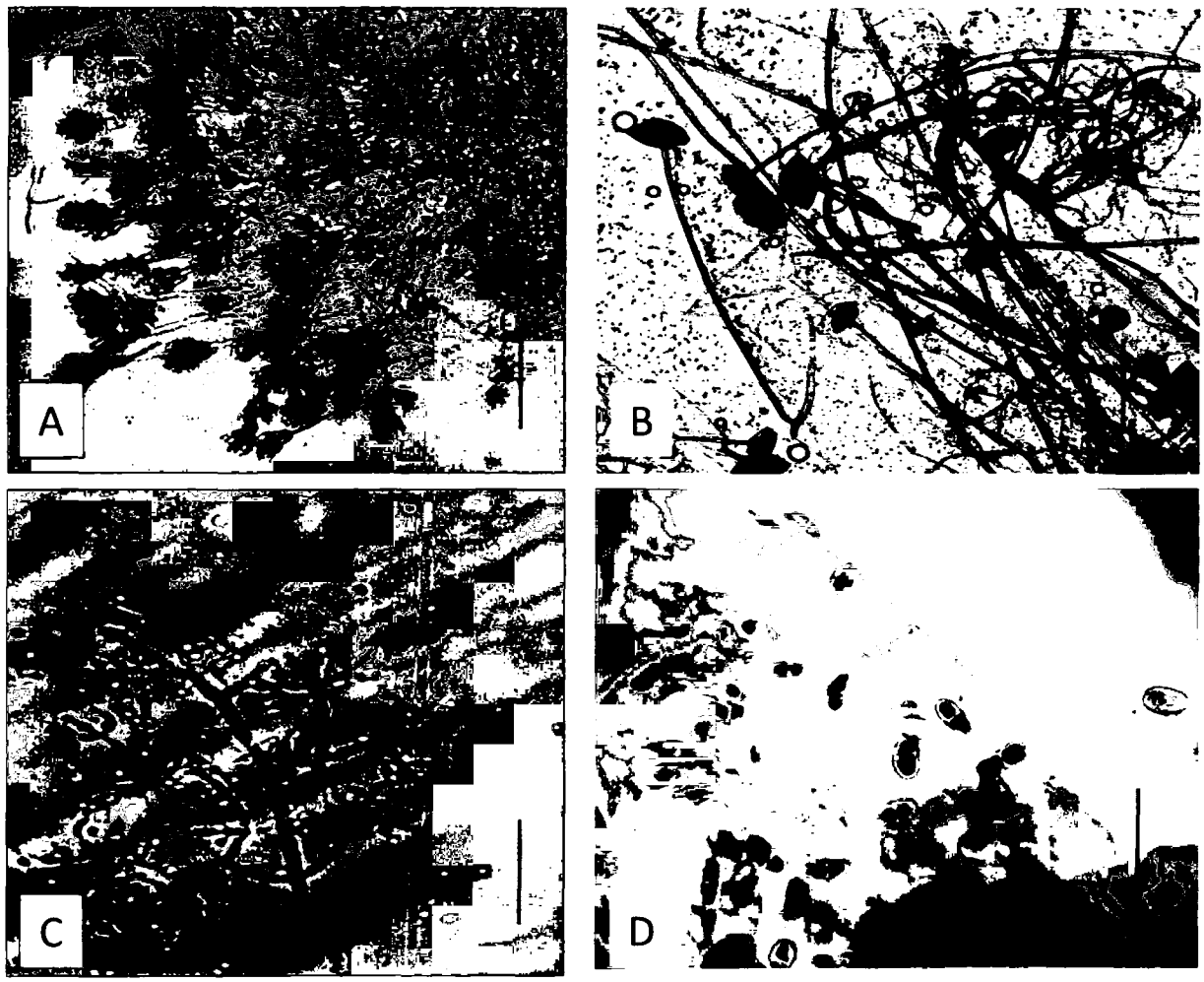

Plate 10. Conidiophores and conidia of A. *Penicillium italicum*, B. *Rhizopus stolonifer*, C. *Trichoderma viride* and D. *Trichothecium roseum* (Bar = 50 µm).

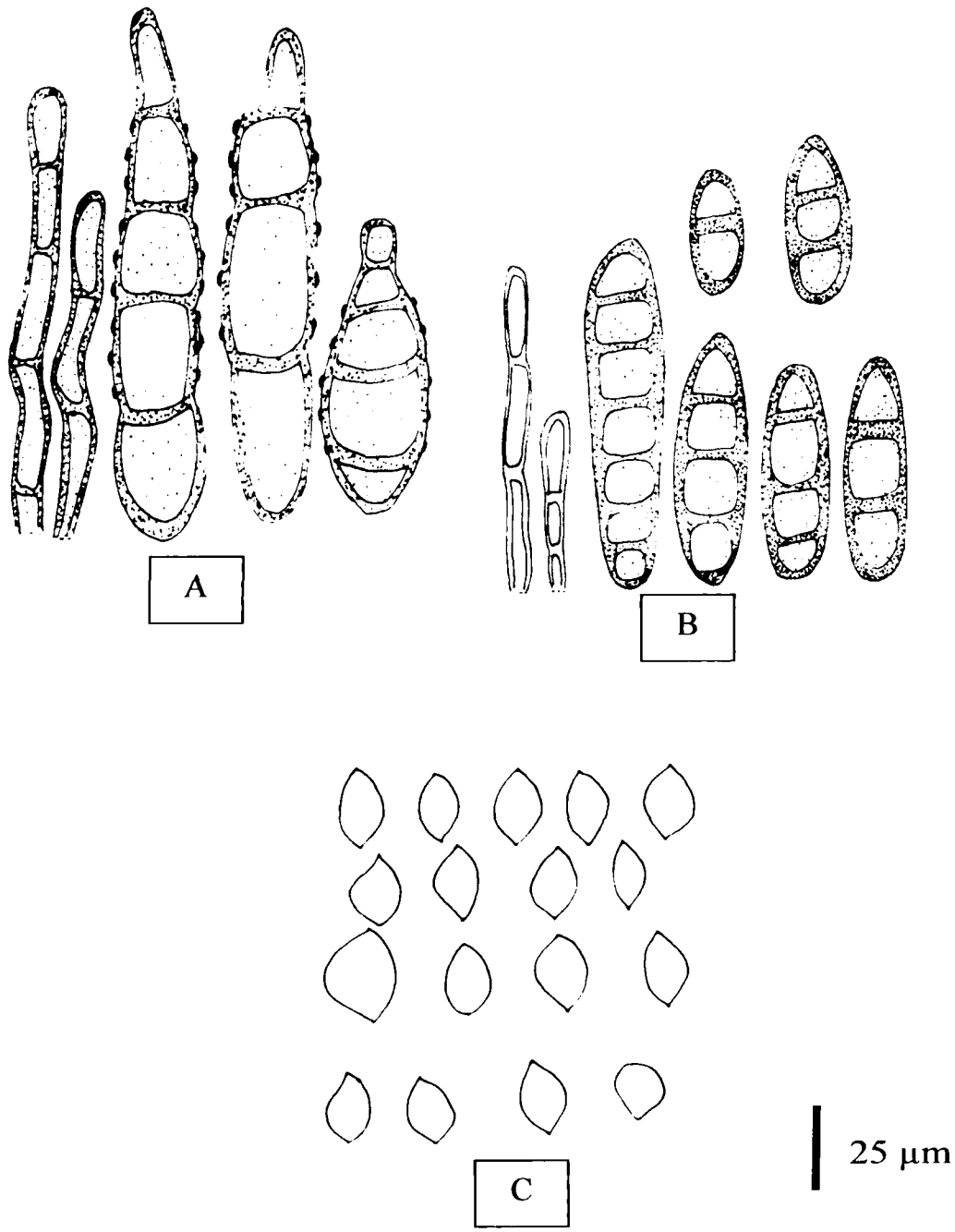

Fig. 9. A. Conidiophores and conidia of *Alternaria alternata,* B. Conidiophores and conidia of *Bipolaris australiensis,* C. Ascospores of *Chaetomium globosum* on *Tagetes* spp. (Bar = 25 µm).

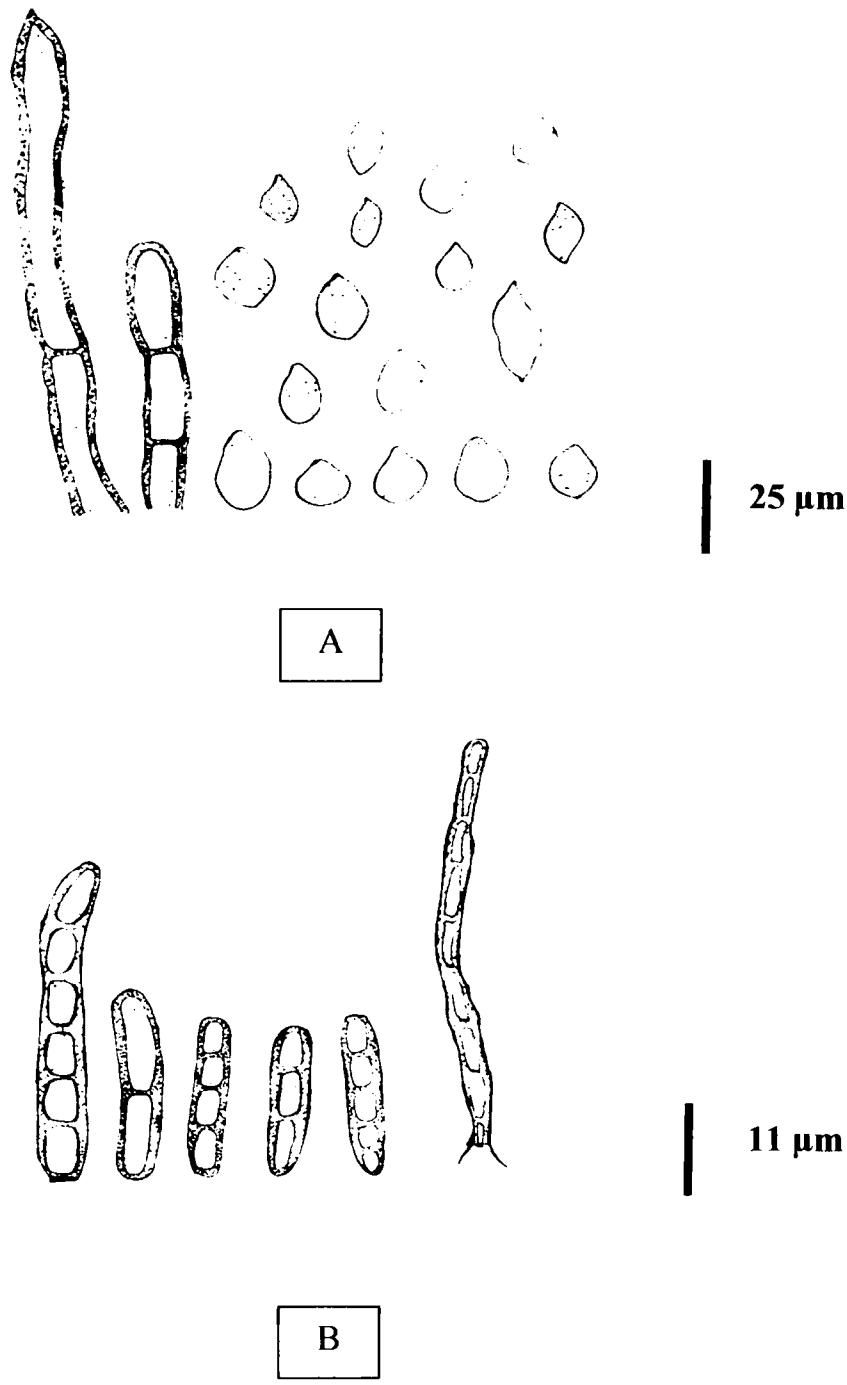

Fig. 10. Conidiophores and conidia of A. *Cladosporium elatum* and B. *Corynespora cambrensis* on *Tagetes* spp. (Bar = A 25 µm, B 11 µm).

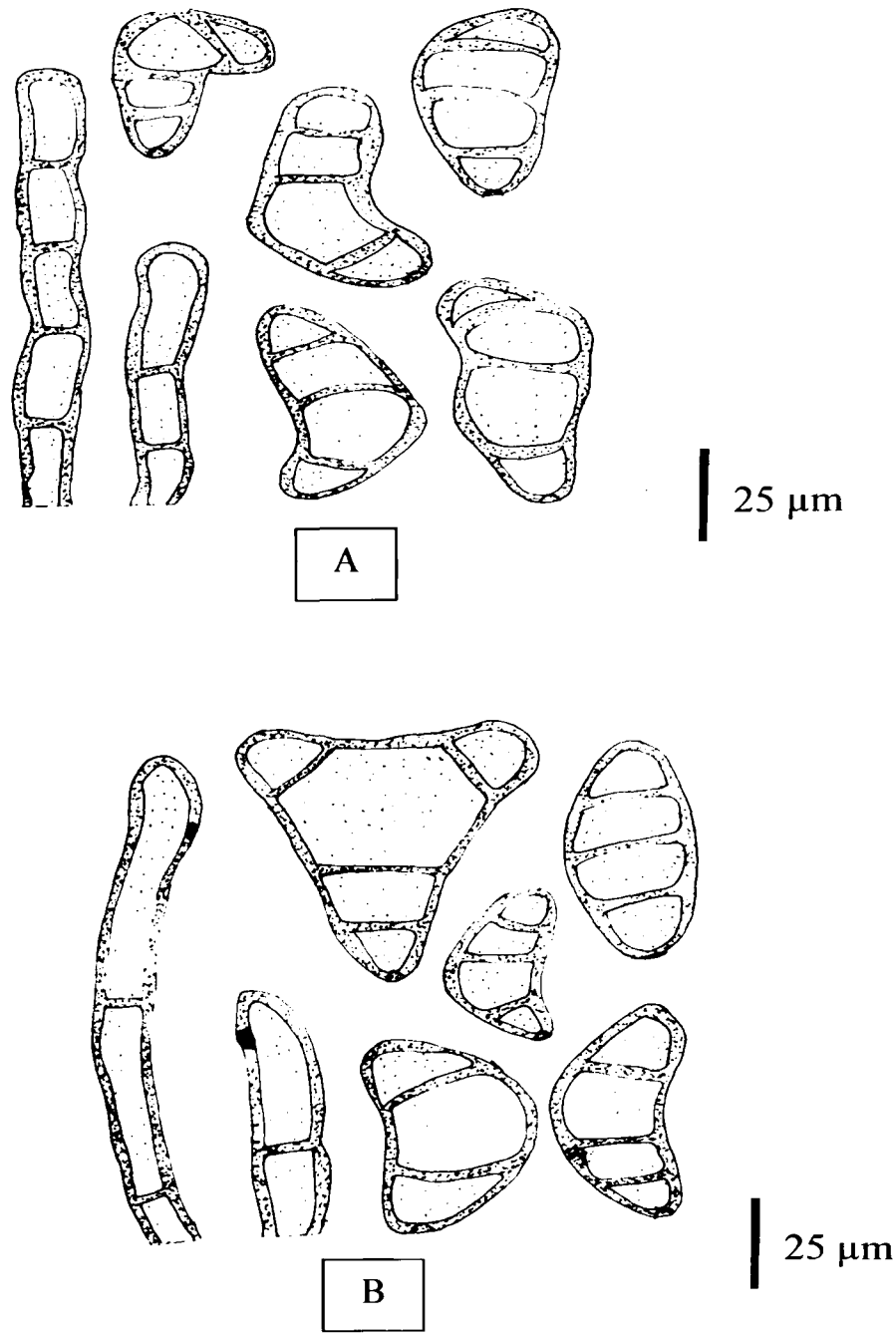

Fig.11. A. *Curvularia brachyspora,* B. *C. fallax* on *Tagetes* spp. (conidiophores and conidia. Bar = 25 µm).

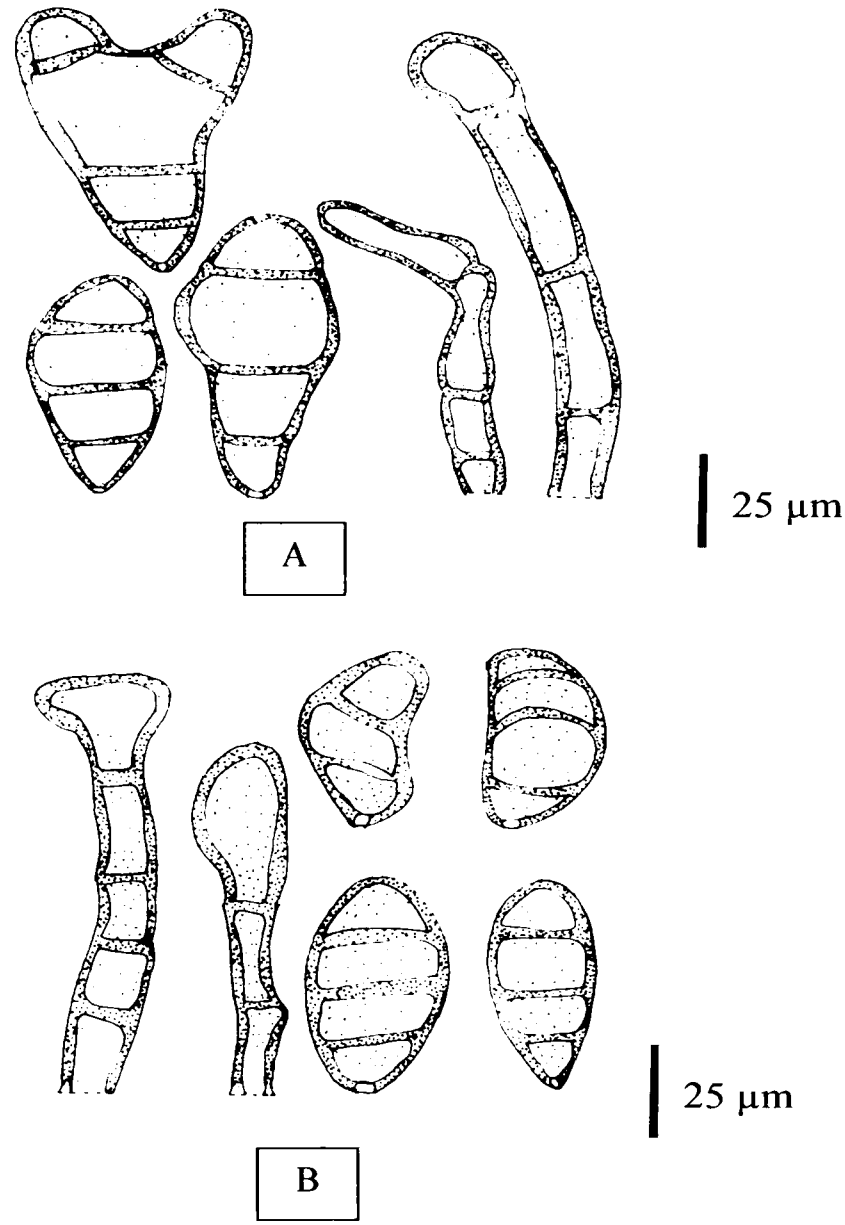

Fig.12. A. *Curvularia lunata* B. *C. stapeliae* on *Tagetes* spp. (conidiophores and conidia. Bar = 25 µm).

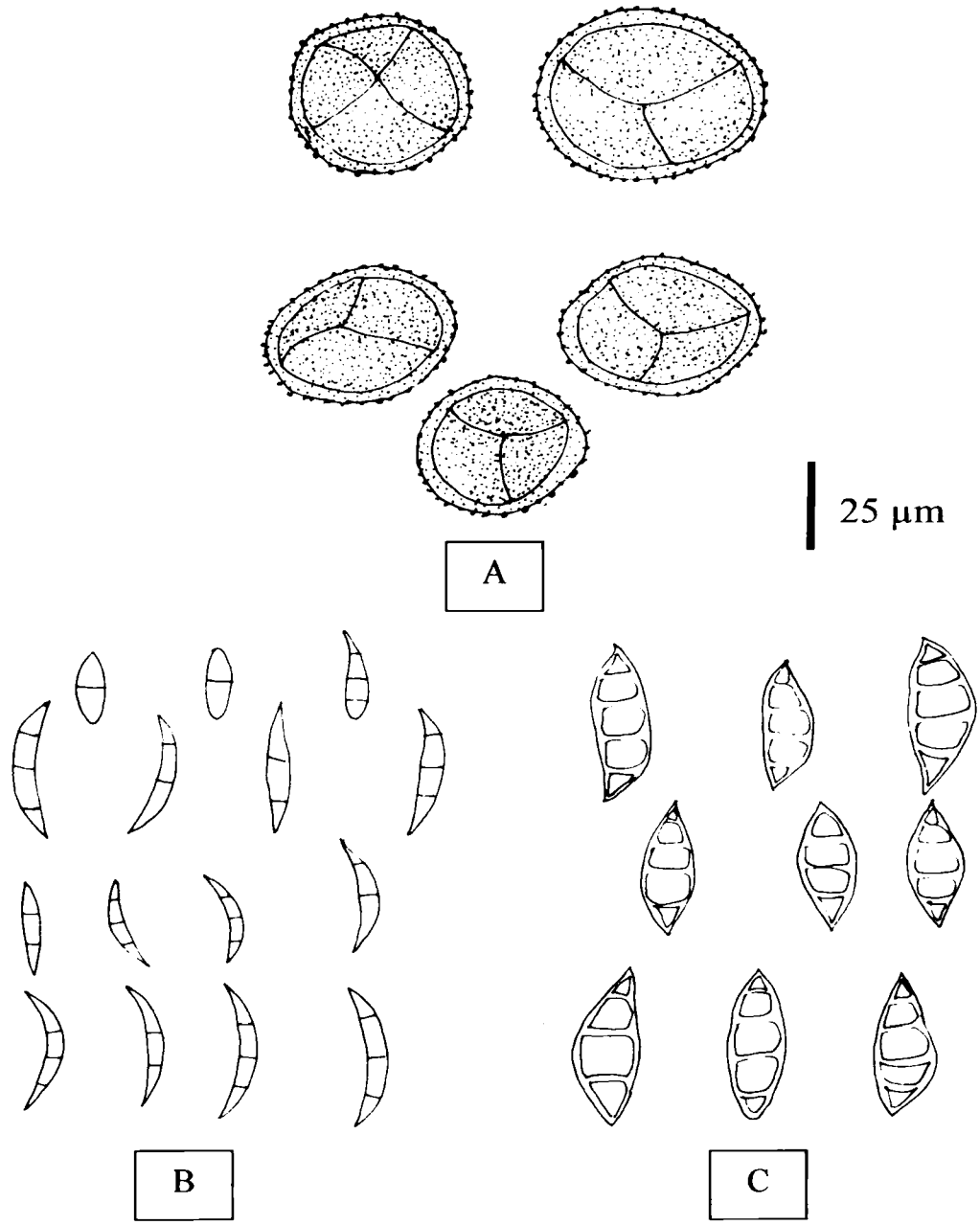

Fig.13. Conidia of A. *Epicoccum purpurascens,* B. *Fusarium semitectum,* C. *Monochaetia ceratoniae* on *Tagetes* spp. (Bar = 25 µm).

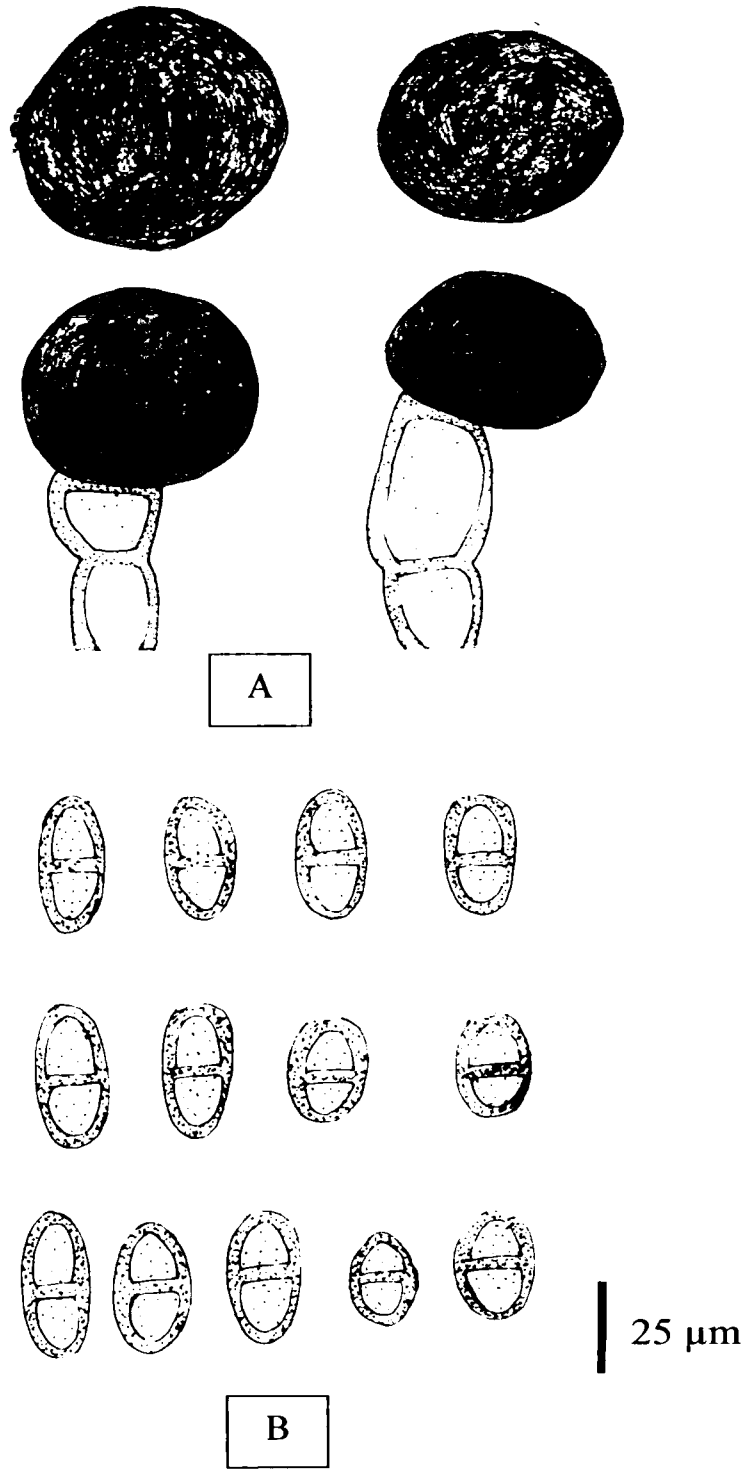

Fig.14. A. Conidiophores and conidia of *Nigrospora panici,* B. Conidia of *Trichothecium roseum* on *Tagetes* spp. (Bar = 25 µm).

4.4. Pathogenicity test

All the fungi isolated from infected plant parts of *Tagetes* spp.were tested for their pathogenic potentiality following Detached leaf technique. *Alternaria alternata, Aspergillus fumigatus* and *Cuvularia lunata* showed symptom on all the inoculated leaflets and plants of *Tagetes* spp. *in vitro* and *in vivo* except control leaflets and plants. The fungi was successfully reisolated from inoculated leaflets and plants.

Pathogenicity test of *Alternaria alternata, Aspergillus fumigatus* and *Cuvularia lunata* following detached leaf technique and spraying of spore suspension are presented in plate 11-18.

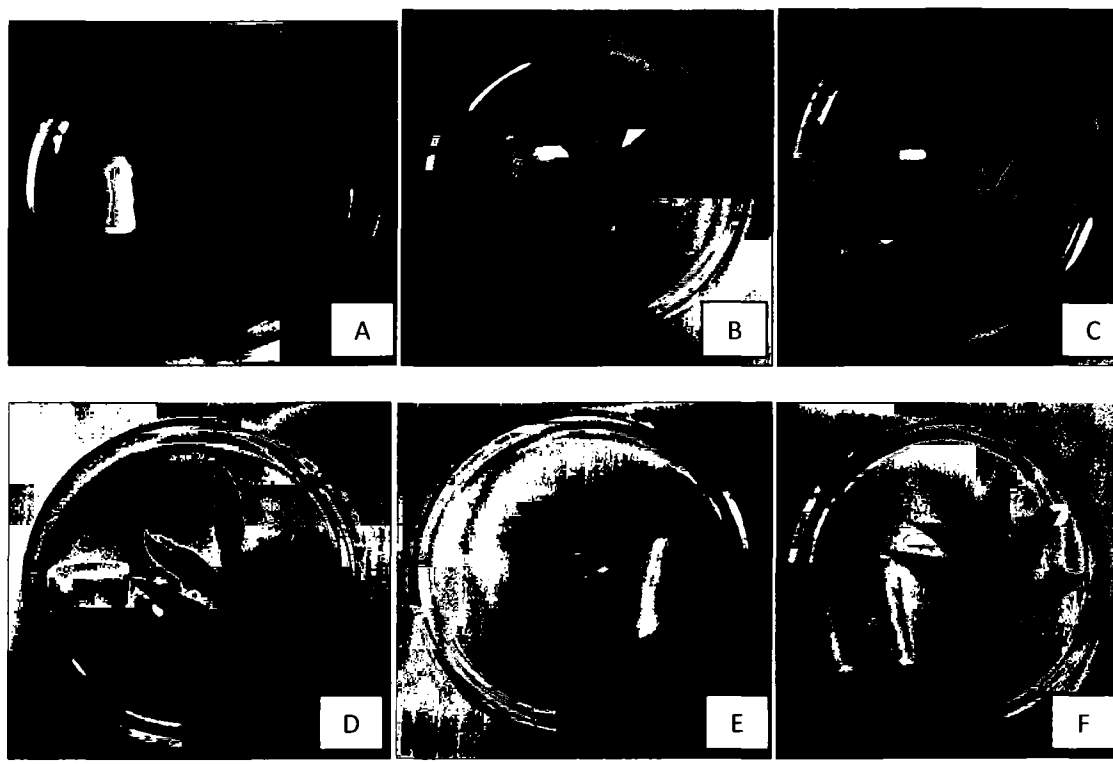

Plate 11. *Tagetes erecta* inoculated by *Alternaria alternata*: A. T_1 = (control) dorsally uninoculated leaflets, B. T_2 = (control) ventrally uninoculated leaflets, C. T_3 = dorsally unpricked inoculated leaflets, D. T_4 = ventrally unpricked inoculated leaflets, E. T_5 = dorsally pricked inoculated leaflets and F. T_6 = ventrally pricked inoculated leaflets.

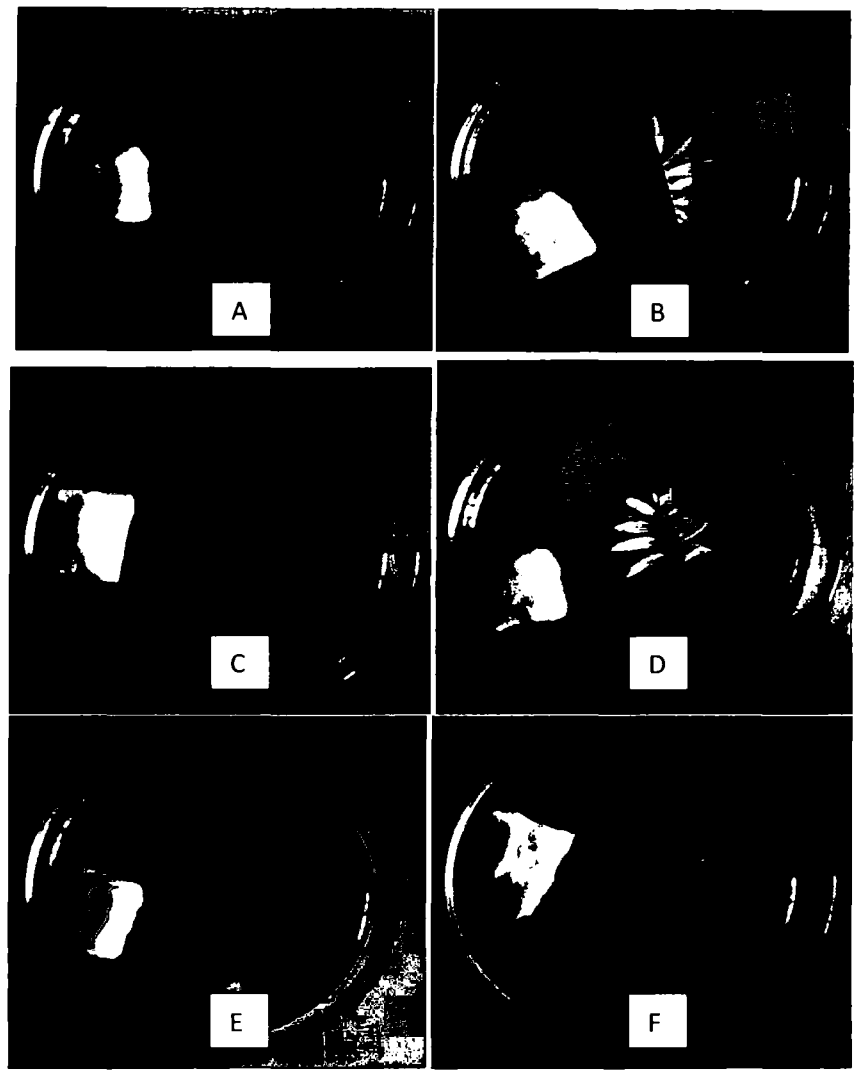

Plate 12. *Tagetes patula* inoculated by *Alternaria alternata:* A. T_1 = (control) dorsally uninoculated leaflets, B. T_2 = (control) ventrally uninoculated leaflets, C. T_3 = dorsally unpricked inoculated leaflets, D. T_4 = ventrally unpricked inoculated leaflets, E. T_5 = dorsally pricked inoculated leaflets and F. T_6 = ventrally pricked inoculated leaflets.

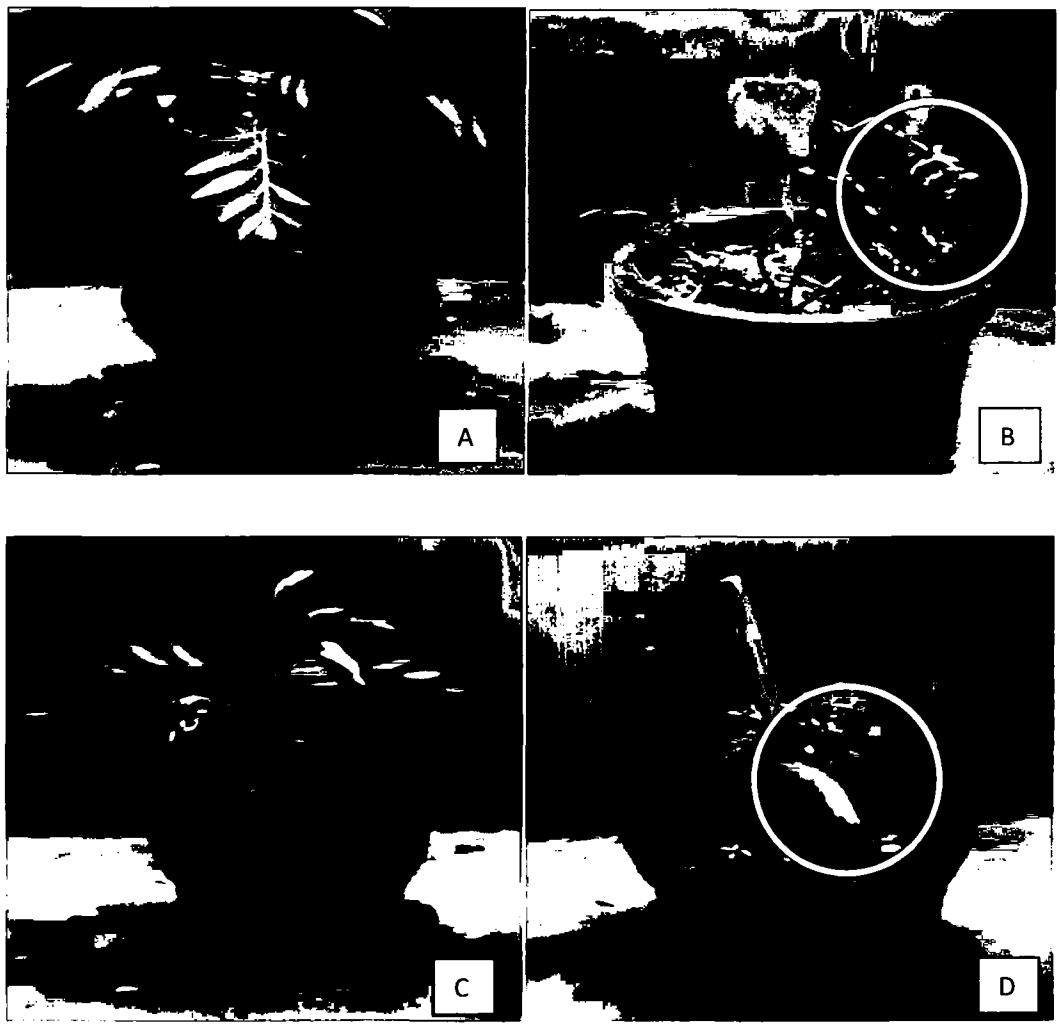

Plate 13. *Tagetes erecta* inoculated by *Alternaria alternata*: A. control, B. inoculated plant and *Tagetes patula*: C. control, D. inoculated plants.

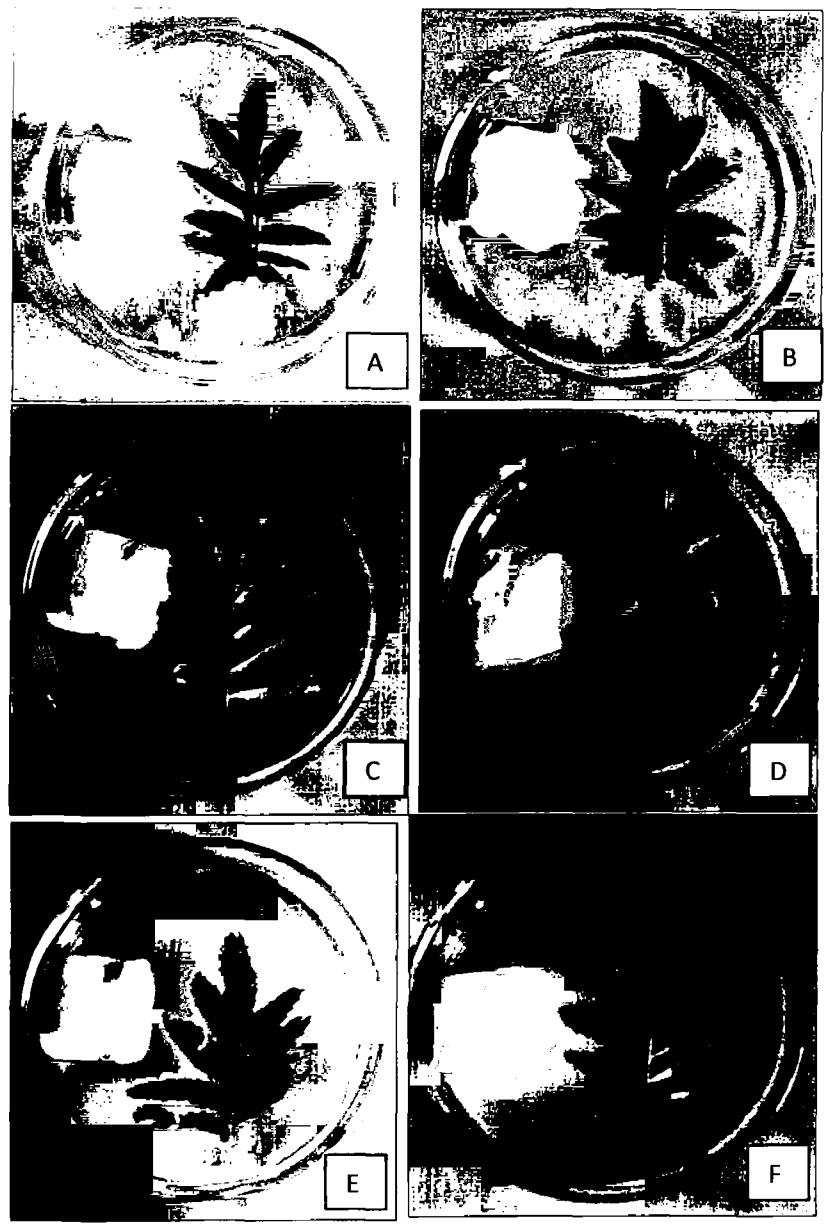

Plate 14. *Tagetes erecta* inoculated by *Aspergillus fumigatus*: A. T_1 = (control) dorsally uninoculated leaflets, B. T_2 = (control) ventrally uninoculated leaflets, C. T_3 = dorsally unpricked inoculated leaflets, D. T_4 = ventrally unpricked inoculated leaflets, E. T_5 = dorsally pricked inoculated leaflets and F. T_6 = ventrally pricked inoculated leaflets.

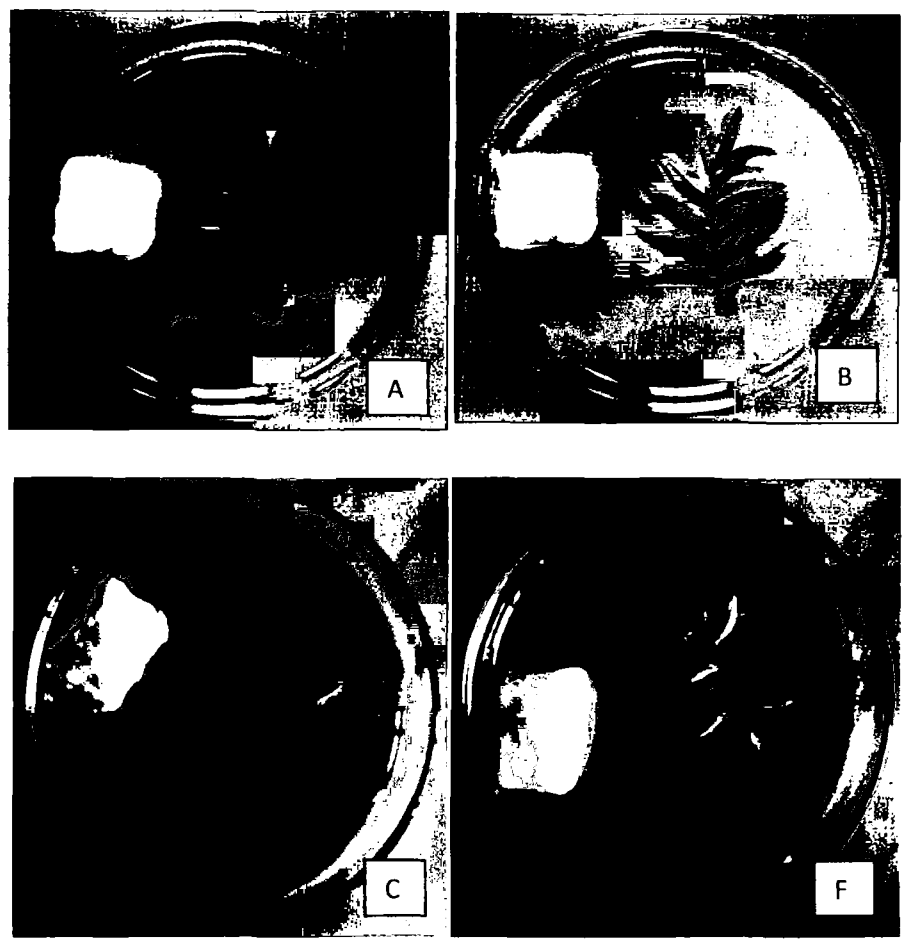

Plate 15. *Tagetes patula*: inoculated by *Aspergillus fumigatus*: A. T_1 = (control) dorsally uninoculated leaflets, B. T_2 = (control) ventrally uninoculated leaflets, C. T_5 = dorsally pricked inoculated leaflets and F. T_6 = ventrally pricked inoculated leaflets.

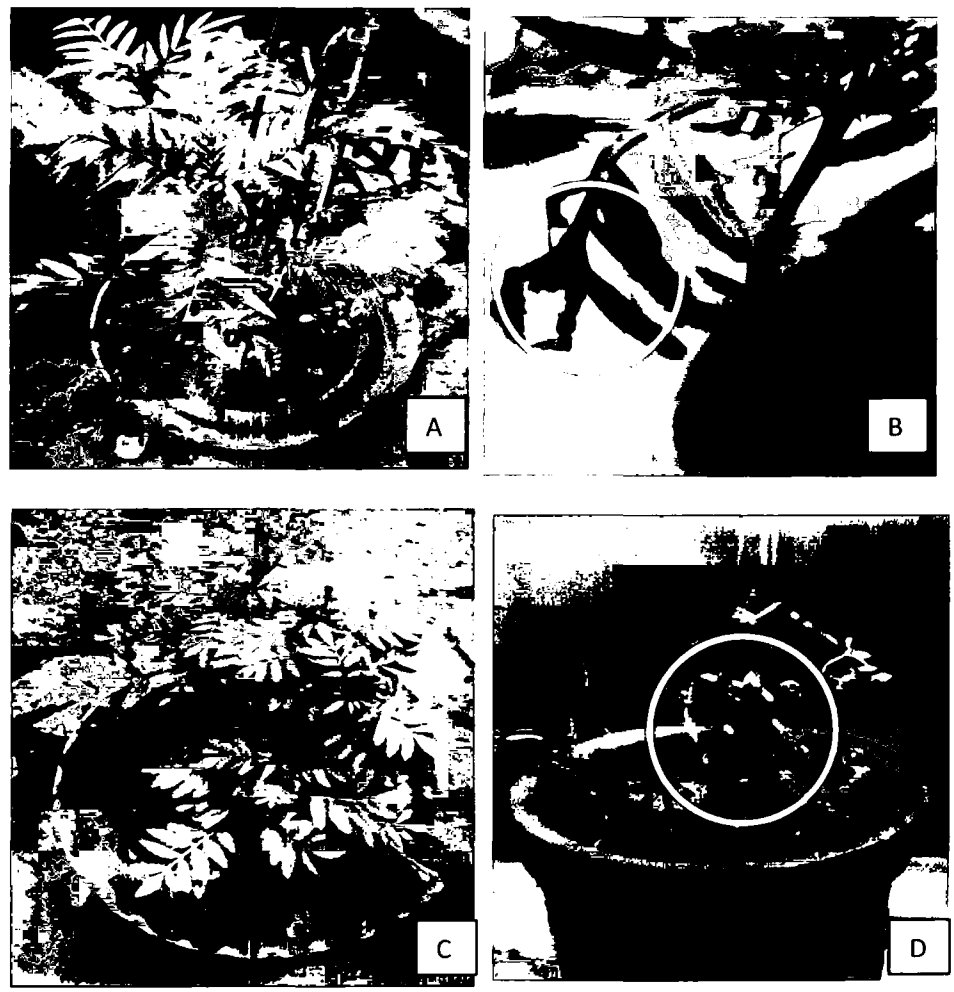

Plate 16. *Tagetes erecta* inoculated by *Aspergillus fumigatus*: A. control, B. inoculated Plant. *Tagetes patula*: C. control, D. inoculated Plant.

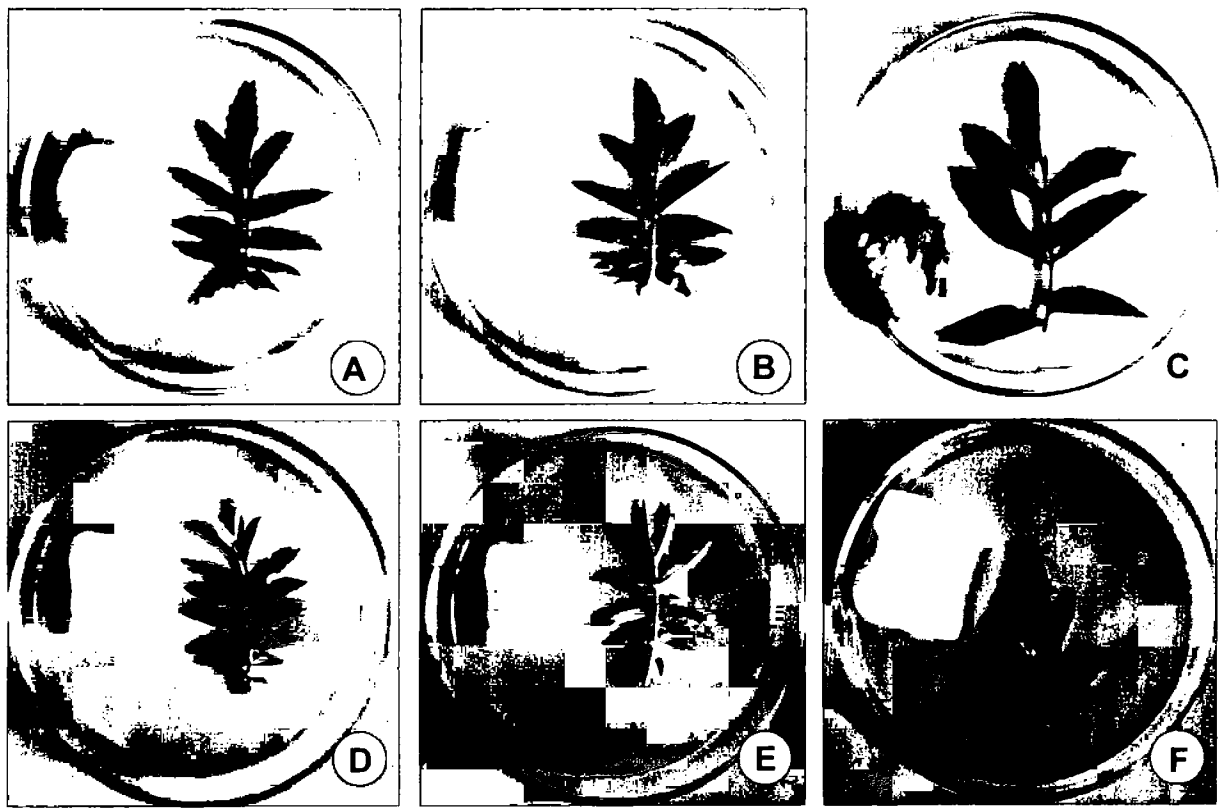

Plate 17. *Tagetes erecta* inoculated by *Curvularia lunata*: A. T_1 = (control) dorsally uninoculated leaflets, B. T_2 = (control) ventrally uninoculated leaflets, C. T_3 = dorsally unpricked inoculated leaflets, *Tagetes patula*: D. T_1 = (control) dorsally uninoculated leaflets, E. T_2 = (control) ventrally uninoculated leaflets, F. T_3 = dorsally unpricked inoculated leaflets.

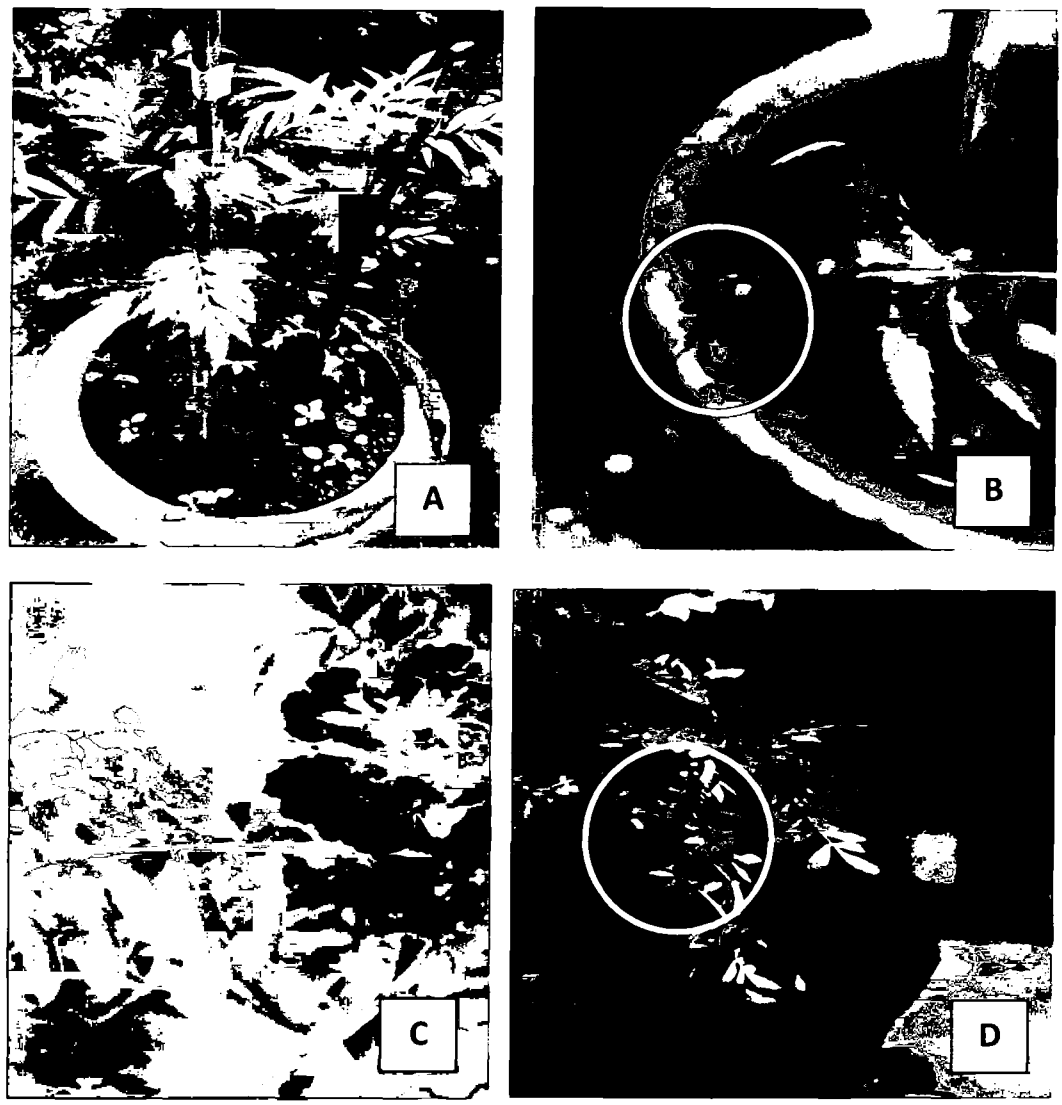

Plate 18. *Tagetes erecta* inoculated by *Curvularia lunata*: A. control, B. plant inoculated with *C. lunata* and *T. patula*: C. control, D. plant inoculated with *C. lunata*.

4. 5. Management

4.5.1 Fungi toxicity of fungicides against the test pathogens.

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 Bavistin 50 WP and Tilt 250 EC at all the concentrations used.

On the radial growth of *A. alternata* Bavistin 50 WP, Salcox 50 WP and Tilt 250 EC were responsible for complete inhibition at all the concentration tested. Capvit 50 WP also inhibited the growth completely at 300, 400 and 500 ppm. Capvit showed 53.77 and 79.88 % inhibition at 100 and 200 ppm respectively. Dithane M-45 and MC Suilphur 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 completely inhibited 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 Gold showed 14.46, 24.22, 32.70, 39.62 and 52.52 % inhibition at 100, 200, 300, 400 and 500 ppm respectively (Table 7, Fig. 15, Plate19, Append. 3).

Table 7: *In vitro* screening of fungi toxicity of fungicides against *Alternaria alternata* at different concentrations.

Name of Fungicides	% inhibition of radial growth of test fungi at different concentrations				
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Bavistin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Capvit	53.77^{b}	79.88^{b}	100^{a}	100^{a}	100^{a}
Dithane	48.74^{bc}	54.09^{c}	55.97^{b}	100^{a}	100^{a}
Greengel	18.72^{d}	28.77^{de}	35.62^{d}	42.47^{c}	48.41^{b}
Hayvit	2.75^{e}	18.35^{f}	22.93^{e}	26.91^{d}	41.90^{c}
Indofil	42.81^{c}	48.93^{c}	52.90^{b}	83.79^{b}	100^{a}
Ridomil	14.46^{d}	24.22^{ef}	32.70^{d}	39.62^{c}	52.52^{b}
Salcox	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Sulphur	18.24^{d}	31.45^{d}	40.25^{c}	100^{a}	100^{a}
Tilt	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV%	7.28	4.31	2.56	2.81	1.88

Mean followed by the same letter (s) within a column did not differ significantly at 5% level by DMRT.

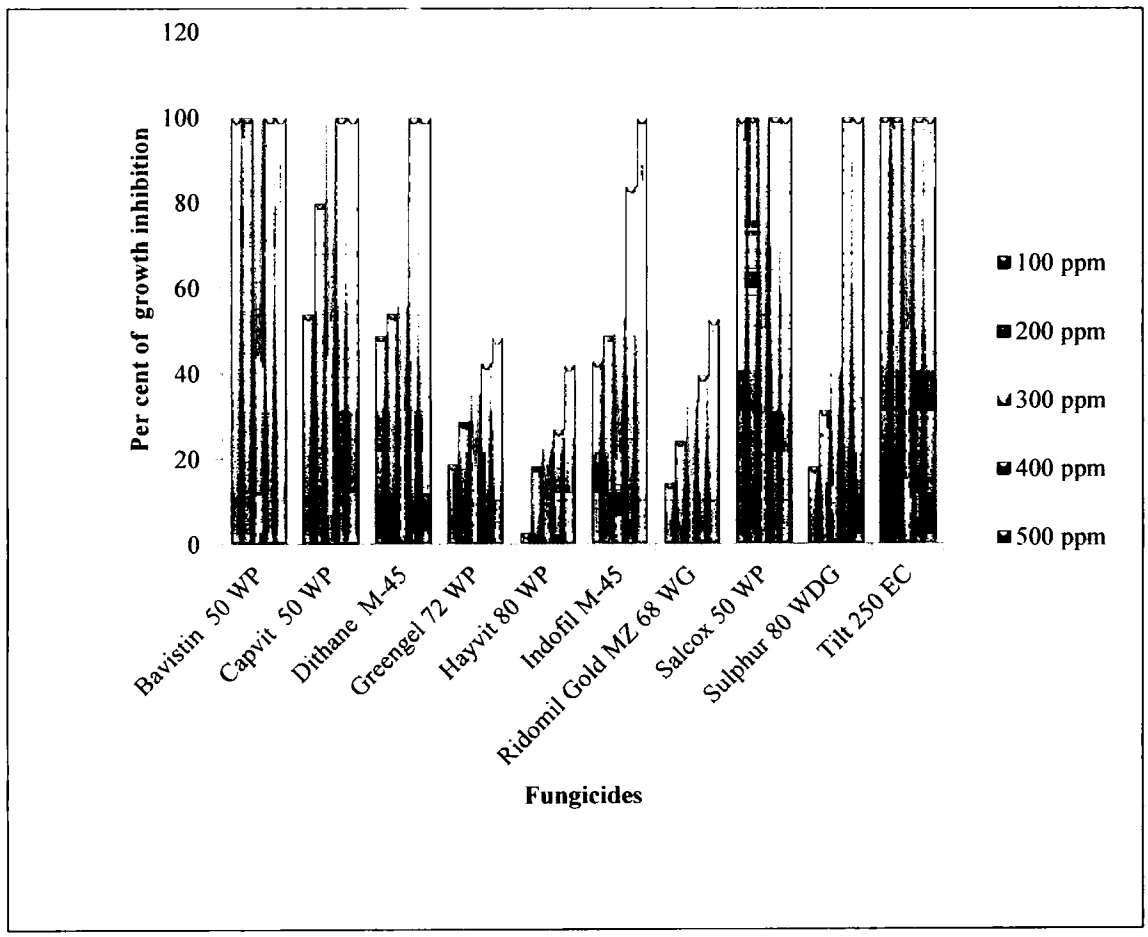

Fig.15. Per cent inhibition of radial growth of *Alternaria alternata* owing to fungicides at different concentrations.

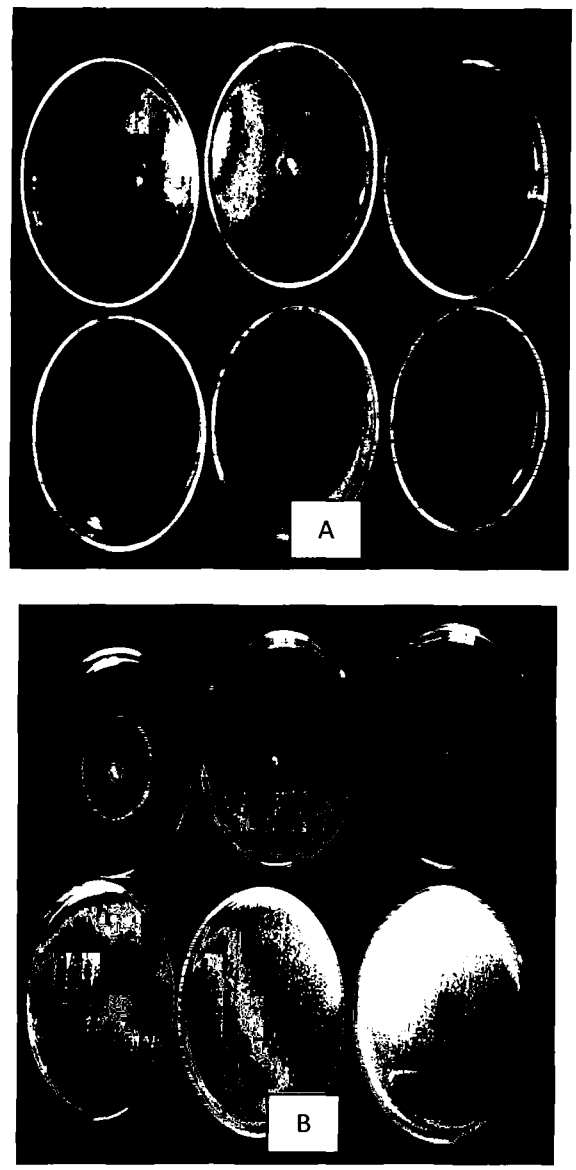

Plate 19. Fungi toxicity of fungicides against *Alternaria alternata* at different concentrations: (A) Bavistin and (B) Tilt.

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 61.42 % inhibition at 100, 200, 300, 400 and 500 ppm respectively. Ridomil 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 (Table 8, Fig. 16, Plate 20 and Append. 4).

Table 8. *In vitro* screening of fungi toxicity of fungicides against *Aspergillus fumigatus* at different concentrations.

Name of Fungicides	% inhibition of radial growth of test fungi at different concentrations				
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Bavistin	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Capvit	46.41^{b}	62.09^{b}	100^{a}	100^{a}	100^{a}
Dithane	6.04^{d}	11.35^{ef}	15.46^{g}	21.98^{d}	30.92^{e}
Greengel	24.64^{c}	32.46^{cd}	39.71^{e}	45.80^{c}	48.70^{d}
Hayvit	24.84^{c}	35.29^{c}	46.41^{d}	100^{a}	100^{a}
Indofil	3.37^{d}	21.35^{de}	27.34^{f}	44.94^{c}	61.42^{e}
Ridomil	3.08^{d}	6.67^{f}	15.64^{g}	21.03^{d}	27.44^{e}
Salcox	42.94^{b}	66.10^{b}	73.45^{b}	100^{a}	100^{a}
Sulphur	1.49^{d}	39.30^{c}	57.71^{c}	67.66^{b}	76.12^{b}
Tilt	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV%	16.34	10.48	3.34	4.42	2.51

Mean followed by the same letter (s) within a column did not differ significantly at 5% level by DMRT

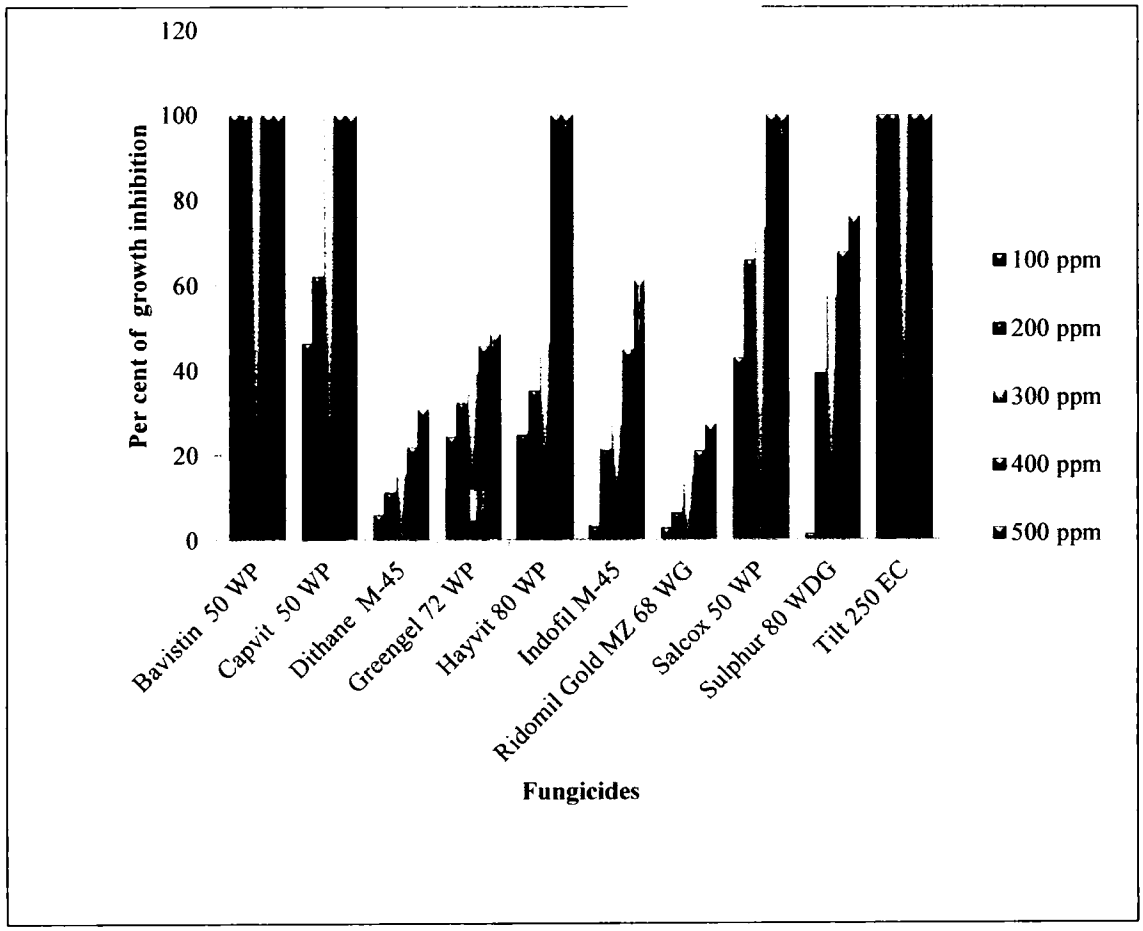

Fig.16. Per cent inhibition of radial growth of *Aspergillus fumigatus* owing to fungicides at different concentrations

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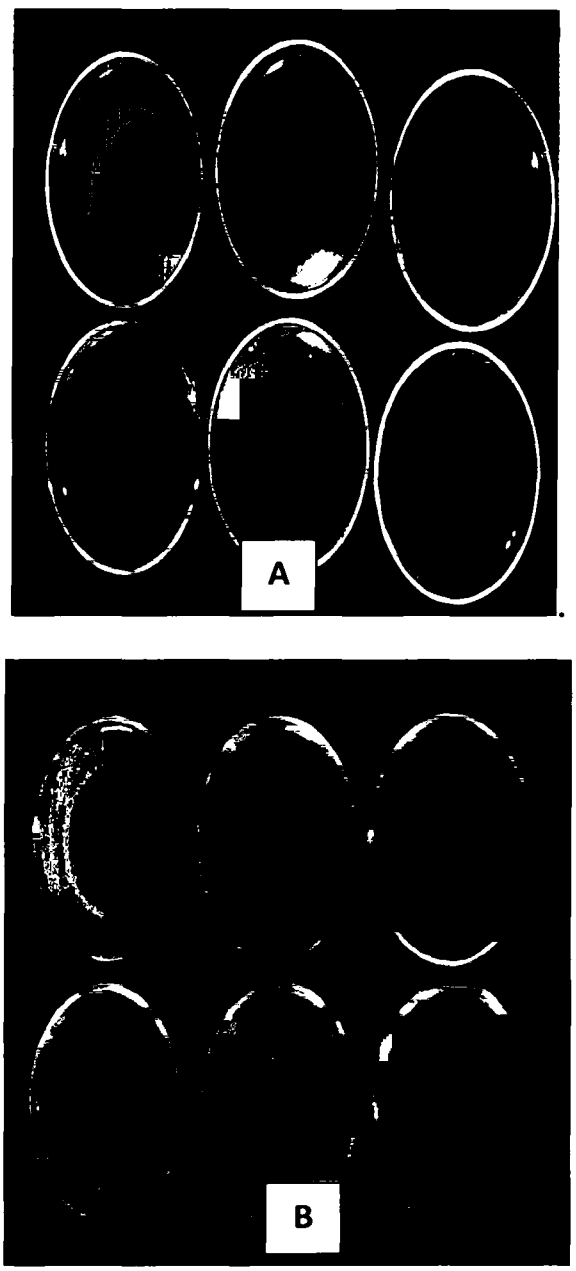

Plate 20. Fungi toxicity of fungicides against *Aspergillus fumigatus* at different concentrations: (A) Bavistin and (B) Tilt.

On the radial growth of *Curvularia lunata* Bavistin 50 WP and Tilt 250 EC were responsible for complete inhibition at all the concentration tested. Dithane and Sulphur also completely inhibited growth of the fungus at 400 and 500 ppm. Dithane also inhibited the growth completely at 300 ppm. Dithane showed 47.52 and 65.96 % inhibition at 100 and 200 ppm respectively. Sulphur showed 39.05, 51.91 and 71.90 % inhibition at 100, 200 and 300 ppm respectively. Capvit showed 35.80, 46.91, 64.82, 75.00 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 respectively. Ridomil showed 25.31, 41.05, 58.33, 66.98 and 73.45% inhibition at 100, 200, 300, 400 and 500 ppm respectively. 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, Bavistin and Tilt showed best result (Table 9, Fig. 17, Plate 21 and Append. 5).

Table 9: *In vitro* screening of fungi toxicity of fungicides against *Curvularia lunata* at different concentrations.

Name of Fungicides	% inhibition of radial growth of test fungi at different concentrations				
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Bavistin	100[a]	100[a]	100[a]	100[a]	100[a]
Capvit	35.80[bc]	46.91[de]	64.82[bc]	75[b]	80.25[b]
Dithane	47.52[b]	65.96[b]	100[a]	100[a]	100[a]
Greengel	17.65[de]	31.04[f]	53.27[d]	64.71[c]	72.55[c]
Hayvit	5.40[ef]	14.41[g]	21.62[e]	27.03[d]	30.63[d]
Indofil	18.07[de]	56.22[c]	63.45[bc]	69.08[c]	100[a]
Ridomil	25.31[cd]	41.05[e]	58.33[cd]	66.98[c]	73.45[c]
Salcox	5.41[ef]	9.91[g]	16.22[e]	20.72[e]	26.13[d]
Sulphur	39.05[bc]	51.91[cd]	71.90[b]	100[a]	100[a]
Tilt	100[a]	100[a]	100[a]	100[a]	100[a]
CV%	16.38	5.46	5.81	3.03	2.37

Mean followed by the same letter (s) within a column did not differ significantly at 5% level by DMRT

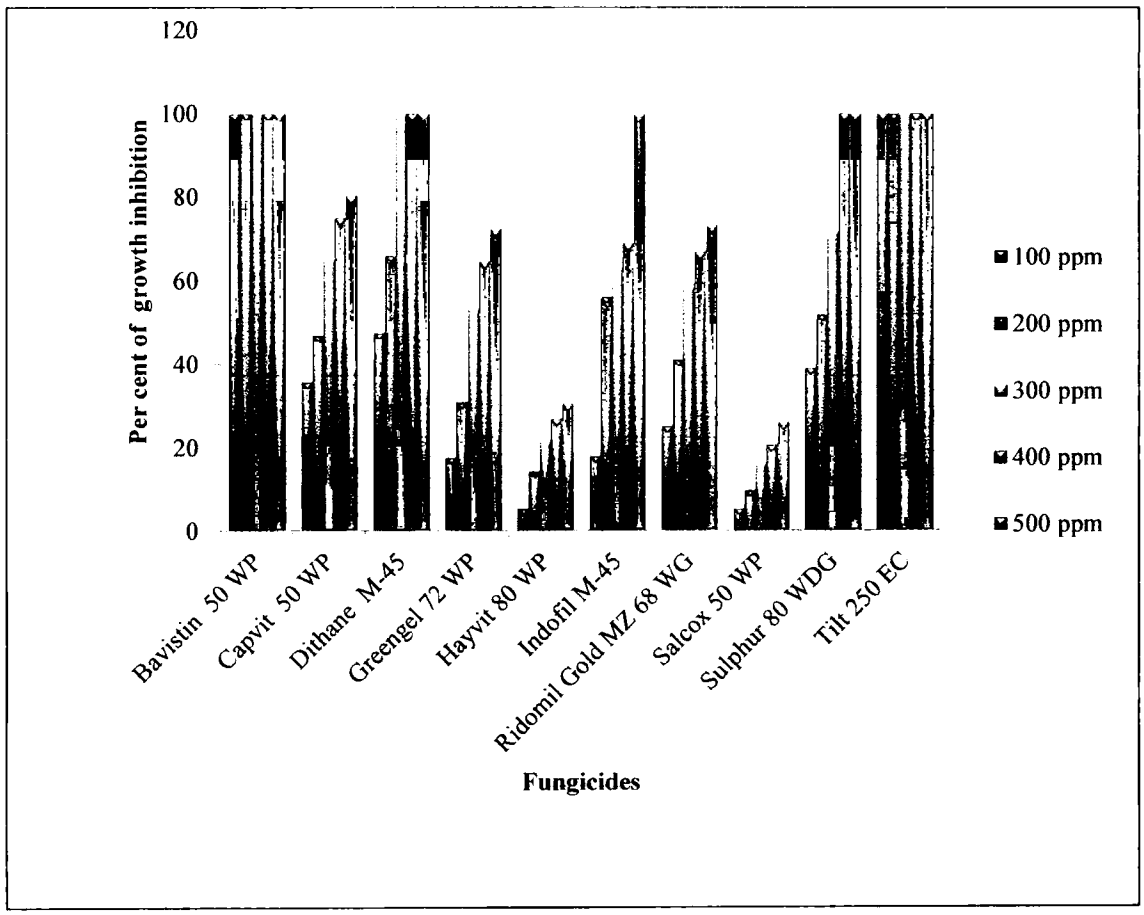

Fig. 17. Per cent inhibition of radial growth of *Curvularia lunata* owing to fungicides at different concentrations.

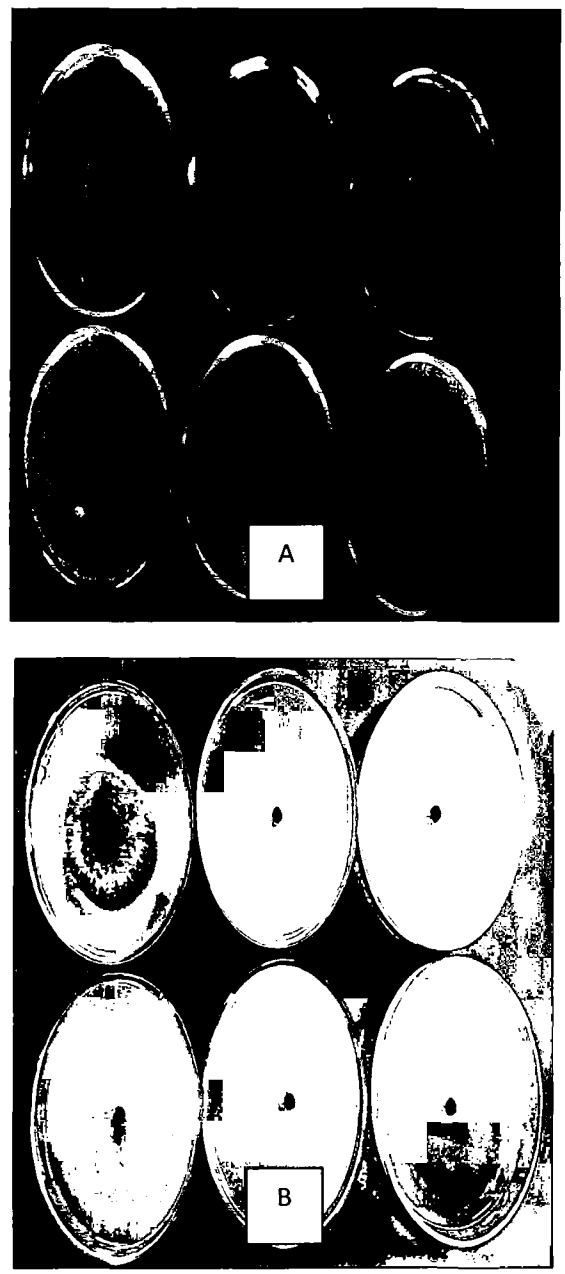

Plate 21. Fungi toxicity of fungicides against *Curvularia lunata* at different concentrations: (A) Bavistin and (B) Tilt.

4.5.2 Effect of leaf extracts on the growth of the test pathogens.

Antifungal properties of ethanol leaf 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 leaf extracts completely inhibited the radial growth of the test fungi at 20% concentrations except *Lantana camara, Mangifera indica* and *Vitex negundo.*

Twenty per cent ethanol leaf extract of *Artocarpus heterophyllus, A. indica, C. sophera, C. medica, D. metel, H. cordata, L. camara, M. indica, M. oleifera* and *V. negundo* were responsible for complete inhibition of radial growth of *Alternaria alternata* at 20% concentration. Ten per cent ethanol leaf 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 per cent ethanol leaf extract of *A. indica, C. medica* and *M. oleifera* were responsible for complete inhibition of radial growth of *A. alternata.* At the same concentration *A. heterophyllus, C. sophera, D. metel, H. cordata, L. camara, M. indica* and *V. negundo* showed 54.09%, 42.67%, 67.55%, 38.15%, 20.49%, 44.33% and 28.72% growth inhibition against the fungus (Table 10, Fig. 18, Plate 22 and Append. 6).

Table 10: Effect of leaf extracts against the radial growth of *Alternaria alternata* at different concentrations.

Name of the plants	% inhibition of radial growth of test pathogen		
	5%	10%	20%
Artocarpus heterophyllus	54.09^{bc}	100^{a}	100^{a}
Azadirachta indica	100^{a}	100^{a}	100^{a}
Cassia sophera	42.67^{cd}	59.56^{c}	100^{a}
Citrus medica	100^{a}	100^{a}	100^{a}
Datura metel	67.55^{b}	100^{a}	100^{a}
Houttuynia cordata	38.15^{cd}	100^{a}	100^{a}
Lantana camara	20.49^{e}	34.25^{d}	100^{a}
Mangifera indica	44.33^{cd}	78.41^{b}	100^{a}
Moringa oleifera	100^{a}	100^{a}	100^{a}
Vitex negundo	28.72^{de}	58.07^{c}	100^{a}
CV%	11.08	1.97	-

Mean followed by the same letter (s) within a column did not differ significantly at 5% level by DMRT

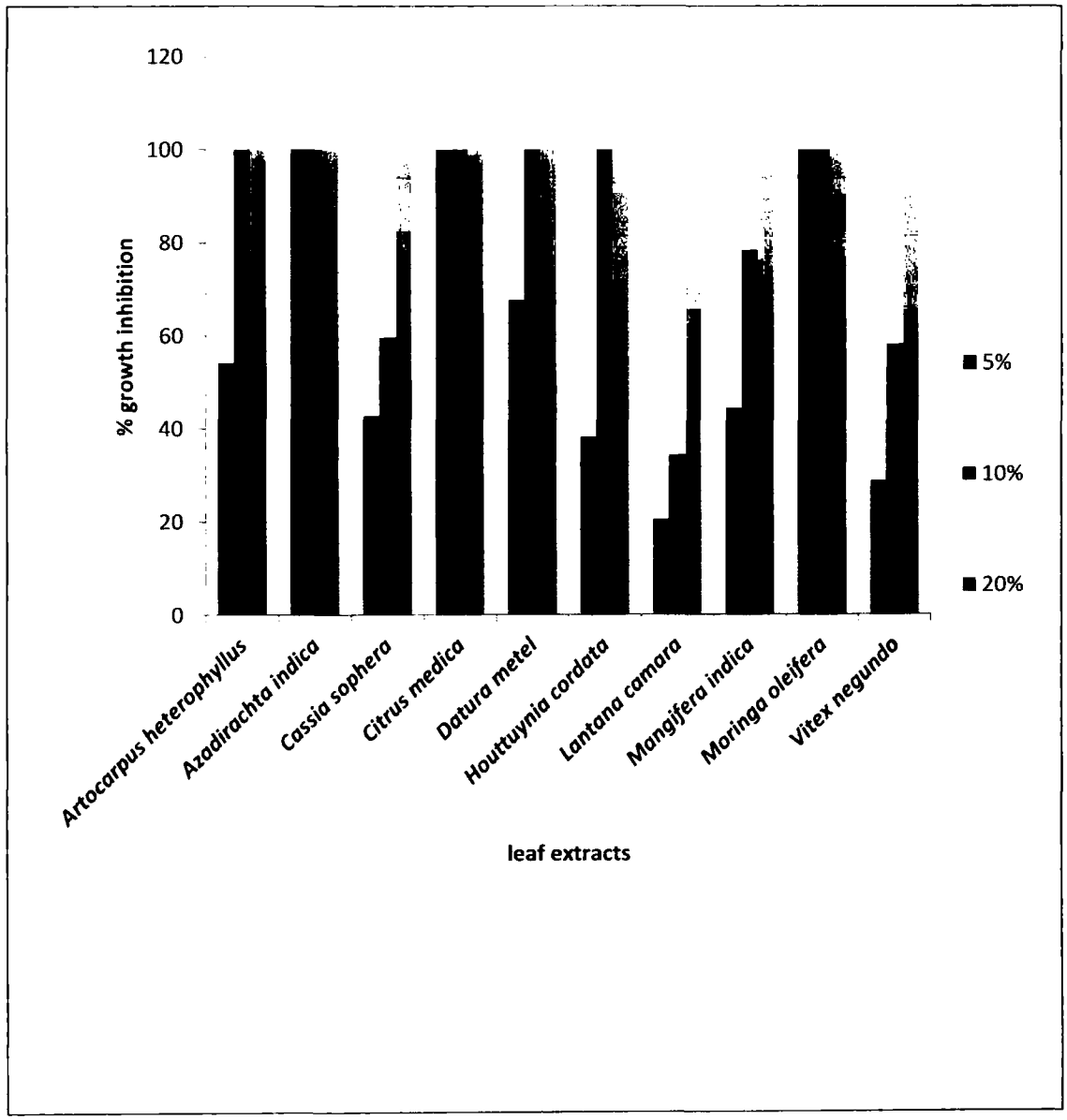

Fig.18. Per cent inhibition of radial growth of *Alternaria alternata* owing to leaf extracts at different concentrations.

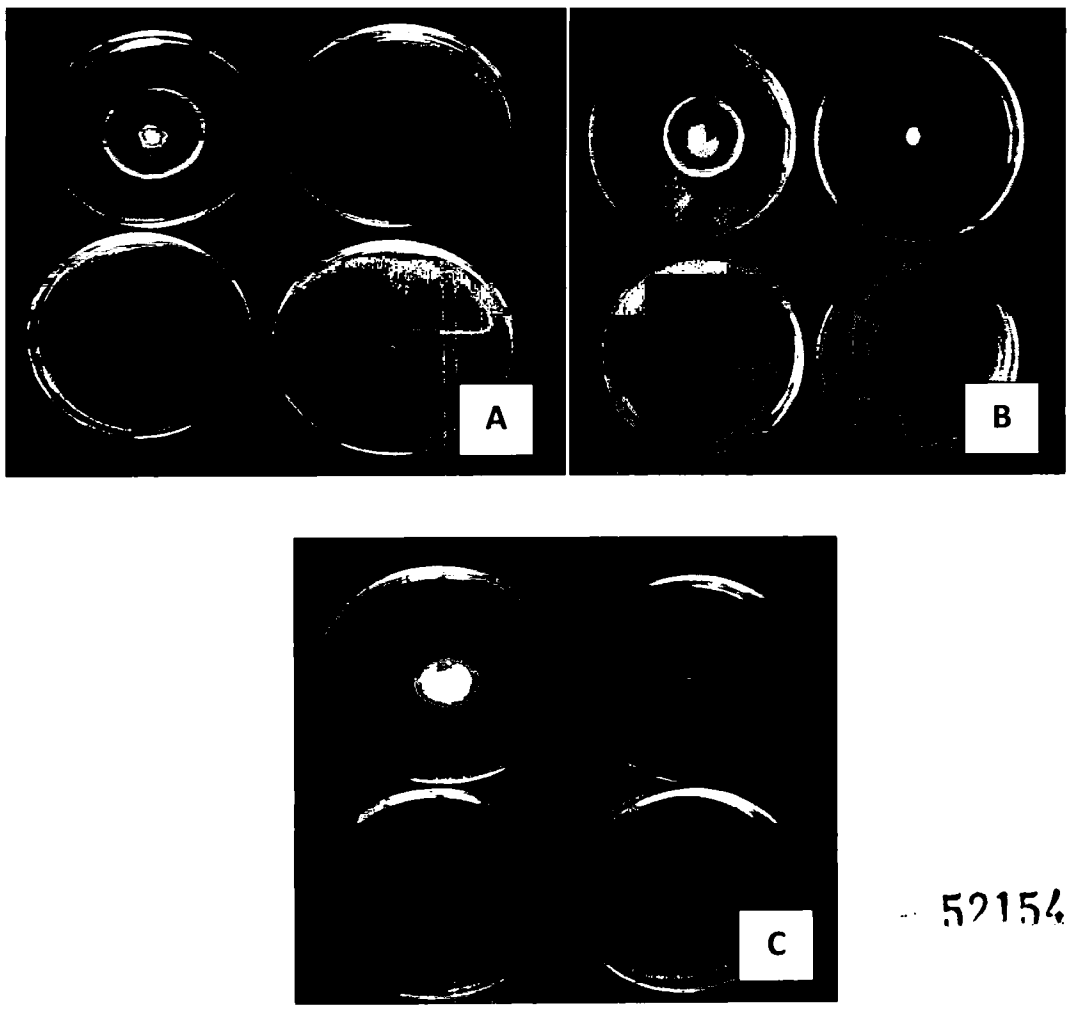

Plate 22. Fungi toxicity of leaf extracts against *Alternaria alternata* at different concentrations: (A) *Azadirachta indica* (B) *Citrus medica* and (C) *Moringa oleifera.*

Twenty per cent ethanol leaf 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 per cent ethanol leaf 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 per cent ethanol leaf extract of *A. indica, C. medica* and *D. metel* were responsible for complete inhibition of radial growth of *A. fumigatus. Artocarpus heterophyllus*, *C. sophera, H. cordata*, *L. camara*, *M. indica, M. oleifera* and *V. negundo* showed 41.27%, 48.44%, 31.11%, 43.48% 28.50% 43.65% and 42.51% growth inhibition against *Aspergillus fumigatus* at 5% concentration respectively (Table 11, Fig. 19, Plate 23 and Append.7).

Table 11: Effect of leaf extracts against the radial growth of *Aspergillus fumigatus* at different concentrations.

Name of the plants	% inhibition of radial growth of test pathogen		
	5%	10%	20%
Artocarpus heterophyllus	41.27[b]	66.67[bc]	100[a]
Azadirachta indica	100[a]	100[a]	100[a]
Cassia sophera	48.44[b]	56.77[c]	100[a]
Citrus medica	100[a]	100[a]	100[a]
Datura metel	100[a]	100[a]	100[a]
Houttuynia cordata	31.11[c]	61.48[bc]	100[a]
Lantana camara	43.48[b]	71.50[b]	100[a]
Mangifera indica	28.50[c]	38.65[d]	54.59[c]
Moringa oleifera	43.65[b]	69.05[b]	100[a]
Vitex negundo	42.51[b]	62.32[bc]	75.36[b]
CV%	4.88	5.72	1.30

Mean followed by the same letter (s) within a column did not differ significantly at 5% level by DMRT

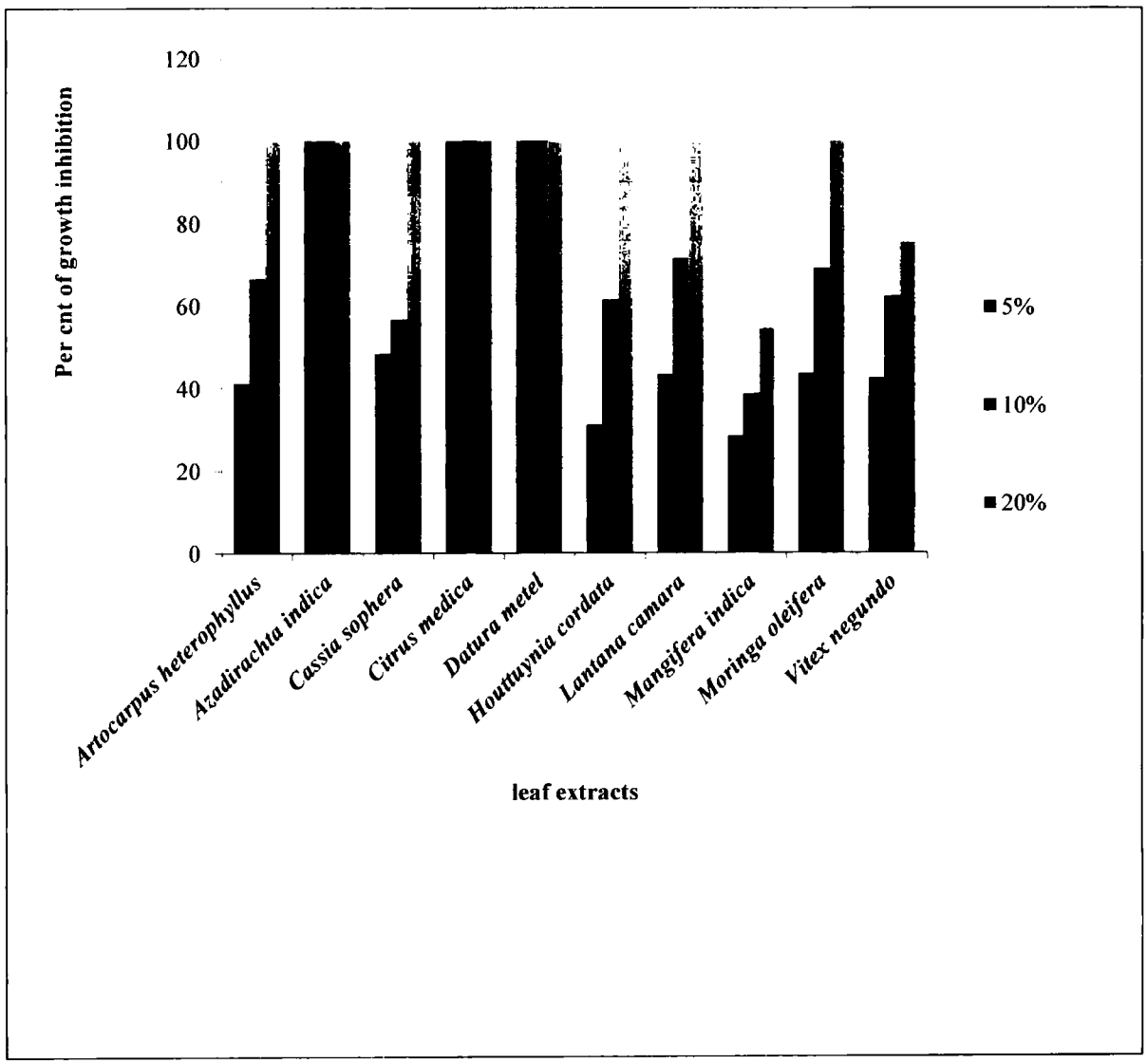

Fig. 19. Per cent inhibition of radial growth of *Aspergillus fumigatus* owing to leaf extracts at different concentrations.

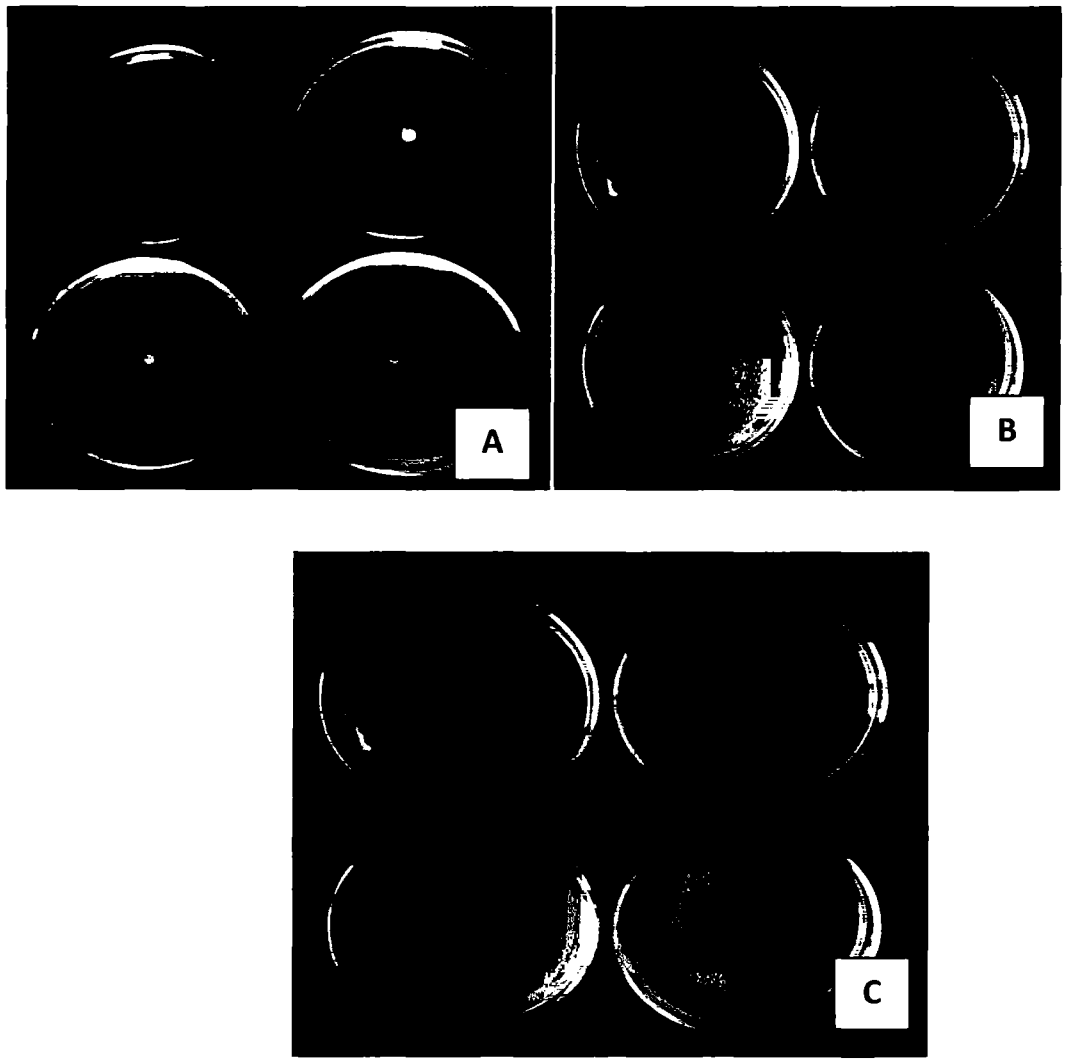

Plate 23. Fungi toxicity of leaf extracts against *Aspergillus fumigatus* at different concentrations: (A) *Azadirachta indica* (B) *Citrus medica* and (C) *Datura metel.*

Twenty per cent ethanol leaf 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 per cent ethanol leaf 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 per cent ethanol leaf extract of *A. indica* and *C. medica* were responsible for complete inhibition of radial growth of *C. lunata. Artocarpus heterophyllus*, *C. sophera, D. metel, H. cordata, L. camara*, *M. indica, M. oleifera* and *V. negundo* showed 65.97%, 32.93%, 78.31 %, 25.56%, 30.23%, 53.91%, 54.17% and 30.87 growth inhibition against *C. lunata* respectively (Table 12, Fig. 20, Plate 24 and Append.8).

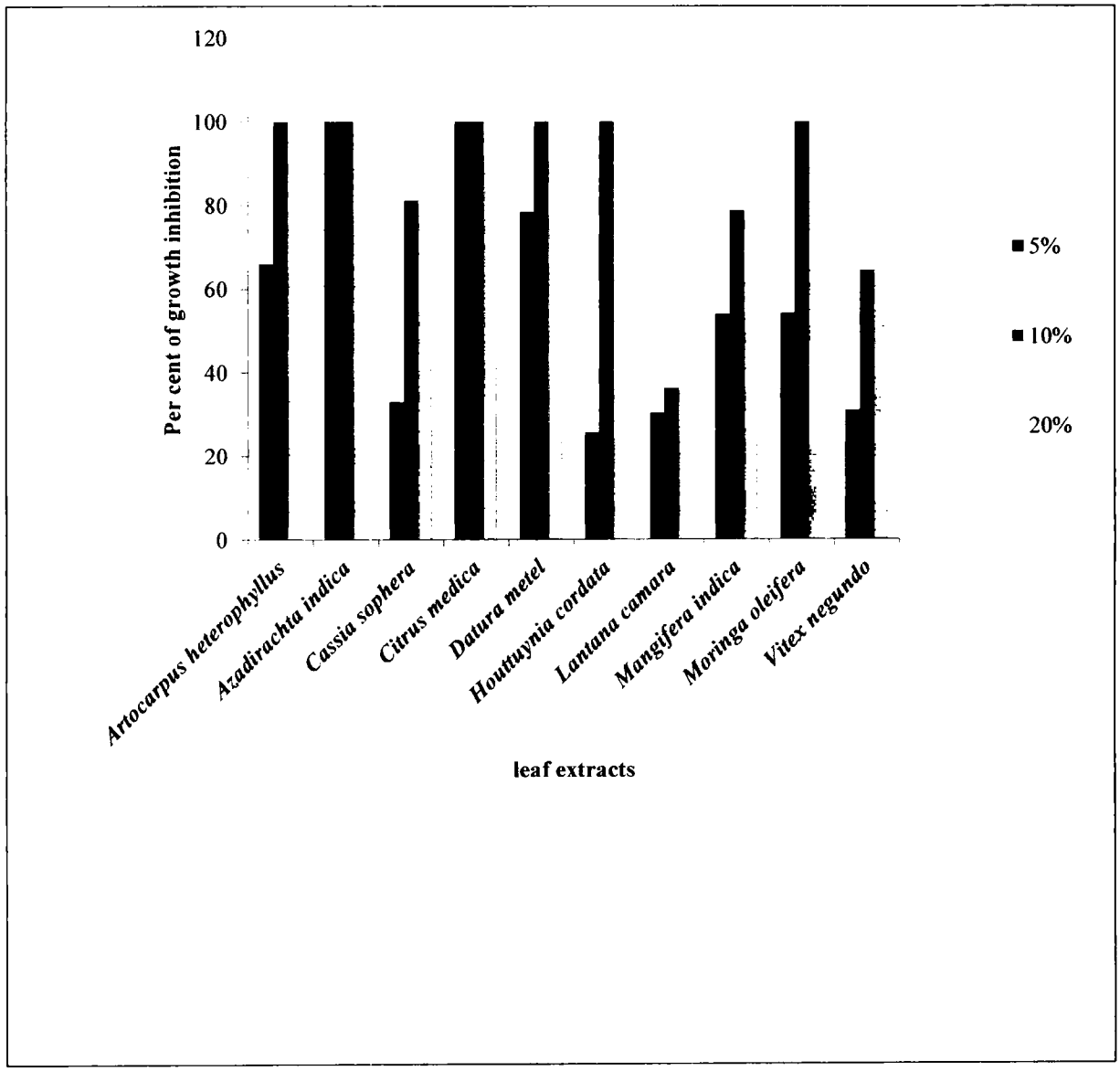

Fig. 20. Per cent inhibition of radial growth of *Curvularia lunata* owing to leaf extracts at different concentrations.

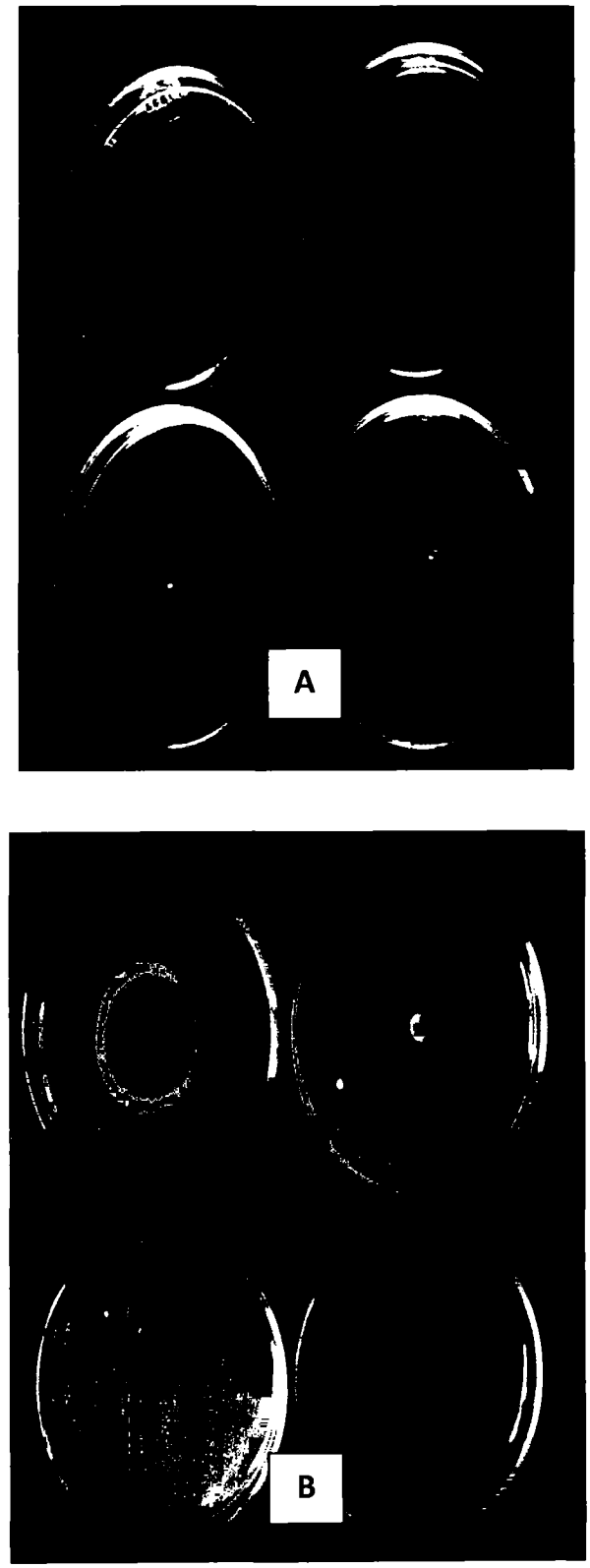

Plate 24. Fungi toxicity of leaf extracts against *Curvularia lunata* at different concentrations: (A) *Azadirachta indica* and (B) *Citrus medica.*

In vitro experiment showed that Bavistin 50 WP and Tilt 250 EC completely inhibited radial growth of *Alternaria alternata, A. fumigatus* and *C. lunata* at 100 ppm concentration. Ethanol leaf extract of *A. indica* and *C. medica* also inhibited the radial growth of the causal agents of blight of *T. erecta* and *T. patula* at 5% concentration.

4.5.3. Evaluation of antagonistic potential of some soil fungi against the test pathogens.

Three antagonistic fungi namely *Aspergillus flavus, A. niger* and *T. viride* were isolated from field soli of *Tagetes erecta* and *T. patula* following serial dilution method.

4.5.3.1. Antagonism between the test pathogens and soil fungi.

The results of colony interactions have been summarized in Table 13. In dual culture colony interaction *A. flavus, A. niger* and *T. viride* showed 53.06, 54.49 and 71.03% growth inhibiting effect on *A. alternata.* The same antagonistic fungi showed 29.9, 32.26 and 38.49% growth inhibition on *A. fumigatus.* The same antagonistic fungi also showed 41.06, 43.94 and 60.71 % growth inhibition on *C. lunata* (Table 13).

Table 13. Effect of antagonists on the radial growth of *Alterenaria alternata, A. fumigatus and C. lunata.*

Name of antagonists	Type	% inhibition of radial growth and intermingled zone of the test pathogens					
		A. alternata		*A. fumigatus*		*C. lunata*	
		% inhibition of growth	intermingled zone (cm)	% inhibition of growth	intermingled zone (cm)	% inhibition of growth	intermingled zone (cm)
Aspergillus flavus	Bii	53.06	0.2	29.9	0.17	41.06	0.1
Aspergillus niger	Bii	54.49	0.2	32.26	0.2	43.94	0.17
Trichoderma viride	Bii	71.03	0.17	38.49	0.25	60.71	0.2

Bii = Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony (2).

In contrast to the present study, Aktar *et al.* 2014 reported that in dual culture colony interaction *Aspergillus niger*, *Trichoderma viride*, *A. flavus* and *A. fumigatus* showed 75.87, 75.5, 51.78 and 45.52% growth inhibition on *C. lunata*. This variation might be due to selection of different test pathogens. In dual culture technique, significantly maximum inhibition was recorded in *T. viride* (66.40%) according to Patel and Joshi (2001).

4.5.3. 2. Effect of volatile substances emanating from the cultures of the soil fungi on the growth of the test pathogens.

The results of the effect of volatile metabolites on antagonistic fungi against marigold pathogens are presented in Table 14. The maximum inhibition of radial growth of *A. alternata* was observed with volatile metabolites of *T. viride* which was 74.55% followed by *A. flavus* 61.82% and. *A. niger* 28.49%. The maximum inhibition of radial growth of *A. fumigatus* was observed with volatile metabolites of *A. flavus* that was 37.43% followed by *T. viride* 28.07% and *A. niger* 16.38%. The complete inhibition of radial growth of *C. lunata* was observed with volatile metabolites of *A. niger* and that was 100% followed by *T. viride* 83% and *A. flavus* 81.37% after 6 days of incubation at 25±2°C (Table.14, Fig.21 and Append. 9-11).

In contrast to the present study, Aktar *et al.* (2014) reported that volatile metabolites produced by an isolate of *A. niger*, *A. flavus*, *A. fumigatus* and *T. viride* inhibited the mycelial growth of *Colletotrichum* sp. by 14.68, 11.78, 11 and 11%, respectively. Further the volatile metabolites produced by isolates of *T.viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelia growth of *Fusarium semitectum* by 13.5, 9.5, 8 and 7.75%, respectively. Differences in per cent inhibition with the present study might be due to the difference in organism involved in the interaction. Thakur and Harsh (2014) reported that volatile metabolites produced from the culture of *A. niger* showed 42.43% inhibition of mycelia growth of *C. gloeosporioides.*

Table 14. Per cent inhibition of radial growth of the test pathogens by volatile metabolites of antagonistic fungi.

Name of antagonist	% inhibition of radial growth of the test pathogens		
	A. alternata	*A. fumigatus*	*C. lunata*
Aspergillus flavus	61.82[b]	37.43[a]	81.37[b]
Aspergillus niger	28.49[c]	16.38[c]	100[a]
Trichoderma viride	74.55[a]	28.07[b]	83.00[b]

Mean followed by the same letter (s) within a column did not differ significantly at 5% level by LSD.

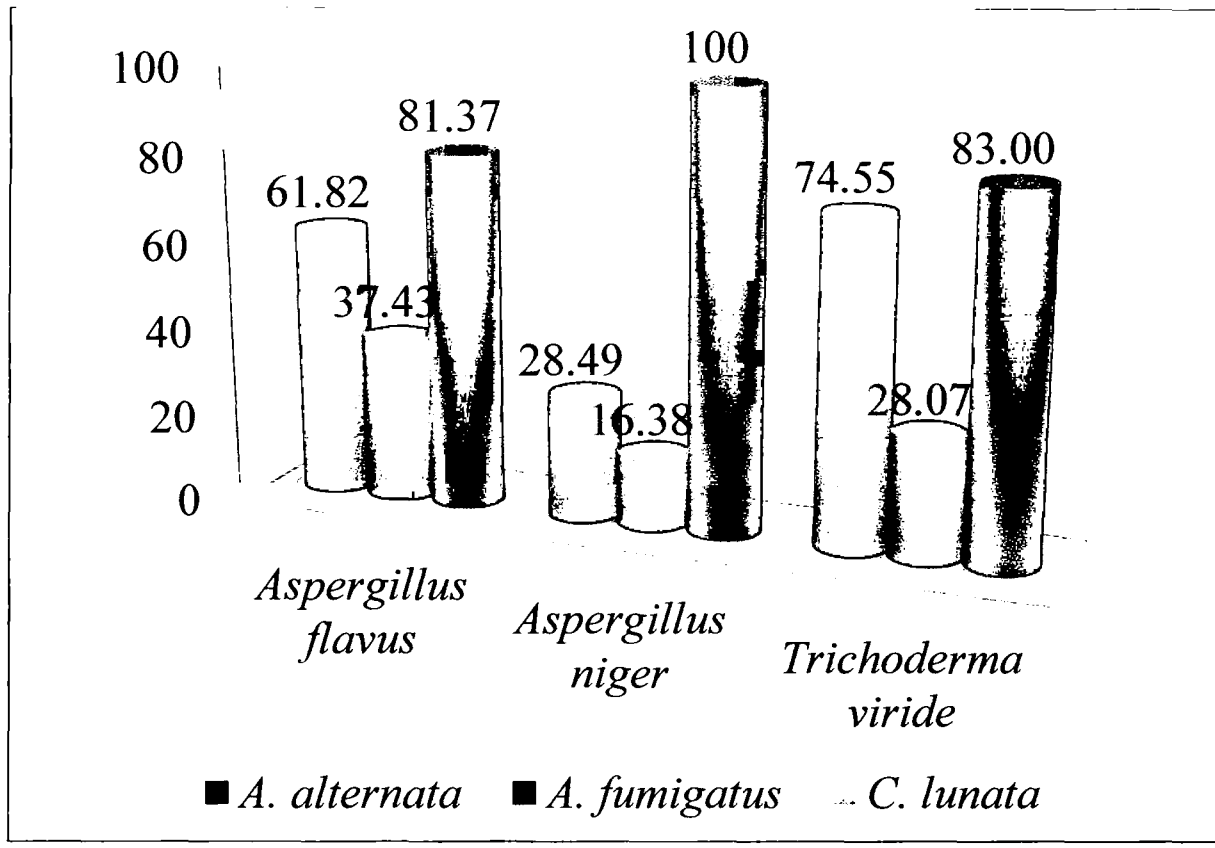

Fig. 21. Growth inhibition of test pathogens owing to volatile metabolites of antagonists.

4.5.3.3. Effect of culture filtrates (Non-volatile metabolites) of the soil fungi on the growth of the test pathogens.

Table 15 and Fig. 22-24 show the effect of non-volatile metabolites on the growth of *A. alternata*, *A. fumigatus* and *C. lunata*. Non-volatile metabolites of *A. niger* and *T. viride* showed complete radial growth inhibition of *A. alternata* at all concentrations used followed by *A. flavus* 56.10% (Table 15, Fig. 22 and Append.12). The complete inhibition of the radial growth of *Aspergillus fumigatus* was observed with non-volatile metabolites of *A. niger* at all concentrations used (Table 15, Fig. 23 and Append.13). Non-volatile metabolites of *A. niger* showed maximum 47.44% radial growth inhibition of *C. lunata* at 20% concentration followed by *A. flavus* 37.82% (Table 15, Fig. 24 and Append.14).

In contrast to the present study, Aktar *et al.* (2014) reported that non-volatile metabolites produced by an isolate of *A. niger*, *Trichoderma viride*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *Colletotrichum* sp. by 52.56, 44.72, 40.0 and 37.2% , respectively. Further, the non-volatile metabolites produced by an isolate of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelia growth of *F. semitectum* by 50, 45, 8 and 7.75%, respectively. Differences in per cent inhibition with the present study might be due to the difference in organism strain involved in the interaction. Madhanraj *et al.* (2010) reported that culture filtrates of *T. viride* and *A. niger* inhibited the mycelial growth of *F. solani* by 85 and 70% at 20% concentration, respectively. Tran (2010) used *T. viride* to control *S. rolfsii* and found effective result.

Tiwari *et al.* (2011) tested two biocontrol agents *viz.*, *Aspergillus niger* and *Trichoderma viride* were tested against ten white rot and one brown rot wood decay fungi (WDF) by dual culture technique under laboratory conditions. The result showed that both *A. niger and T. viride* inhibit growth of all WDF tested. The percentage inhibition of radial

growth values of *T. viride* and *A. niger* are almost the same (ranging from 29.2 to 66.7%) and the average mean value of *T. viride* (51.7%) is 13.3% more than that of *A. niger* (45.5%).

In Bangladesh *Tagetes erecta* and *T. patula* are commonly grown by the gardeners as annual plants. The essential oil from this plant is being investigated for antifungal activity, including treatment of candidiasis and treating fungal infections in plants.The plant is used in companion planting for many vegetable crops. Both the species are used in Ayurvedic treatment. Plant has also mosqutocidal potentiality.Ninety five per cent farmers in Jessore and Jhenaidah district cultivate marigold as commercial basis. Due to rapid expansion of commercial marigold cultivation many diseases appear on the plants. Though marigold is presently a profitable cultivated crop to the farmers in Bangladesh but socioeconomic data and information of this flower are very scarce.

Present research suggested that *A. niger* and *T. viride* may be exploited commercially as a biocontrol agent against blight pathogens of *T. erecta* and *T. patula.* Moreoverthe present investigation will be helpful for designing an ecofriendly management of blight disease of *Tagetes* spp.

The present investigation suggests that *A. niger* and *T. viride* may be exploited commercially as a biocontrol agent against blight pathogens of *T. erecta* and *T. patula*

Table 15. Per cent inhibition of radial growth of test pathogens by non- volatile metabolites of antagonistic fungi.

Name of antagonist	Concentrations (%)	% inhibition of radial growth of test pathogens		
		A. alternata	*A. fumigatus*	*C. lunata*
Aspergillus flavus	5	21.95b	33.34[b]	10.26[ab]
	10	46.34b	45.46[b]	30.77[a]
	20	56.10b	52.12[b]	37.82[b]
Aspergillus niger	5	100a	100a	7.69a
	10	100a	100a	18.59b
	20	100a	100a	47.44a
Trichoderma viride	5	100a	13.04[c]	13.46a
	10	100a	25.36[c]	20.51b
	20	100a	36.96[c]	35.90b

Mean followed by the same letter (s) within a column did not differ significantly at 5% level by LSD.

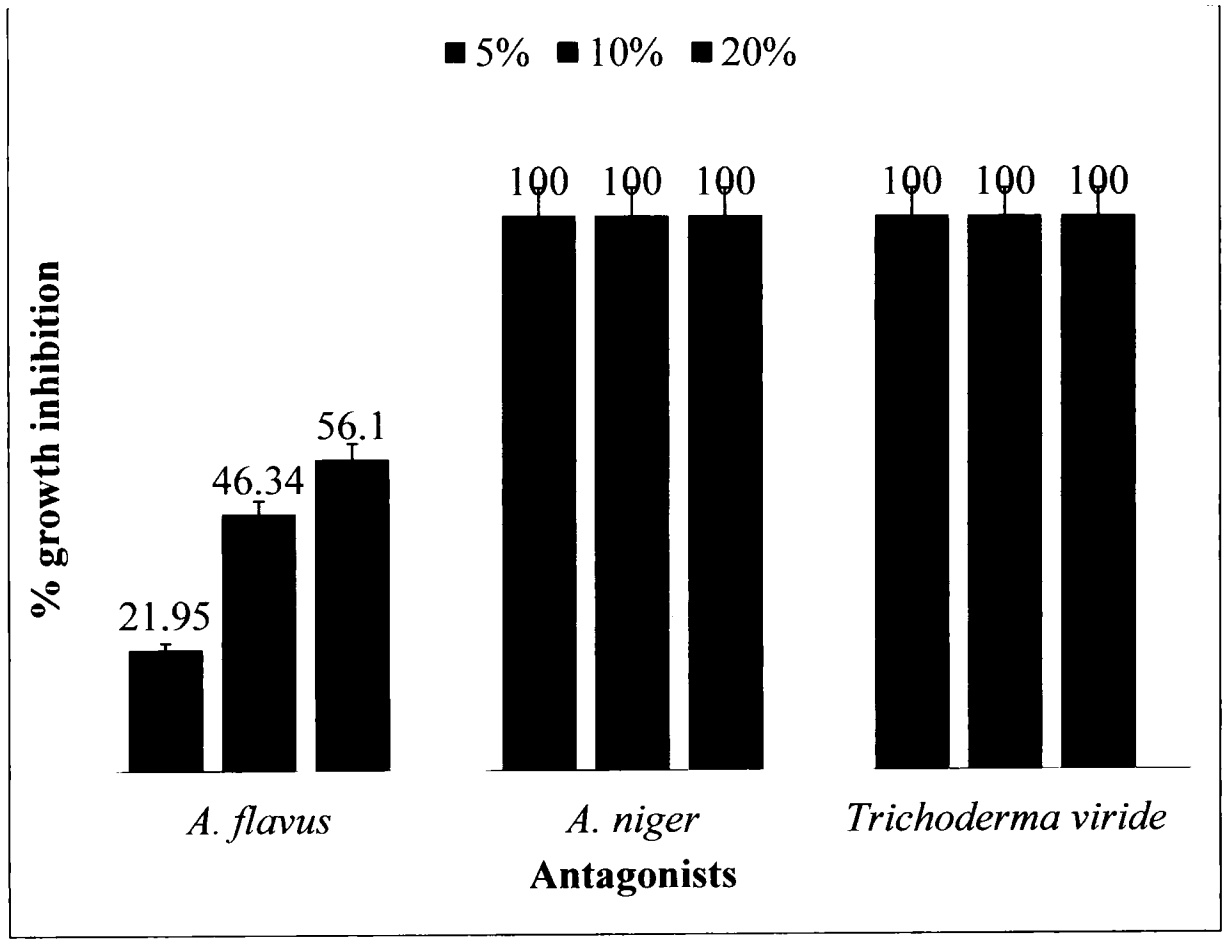

Fig. 22. Per cent growth inhibition of radial growth of *Alternaria alternata* owing to non-volatile metabolites of antagonistic fungi.

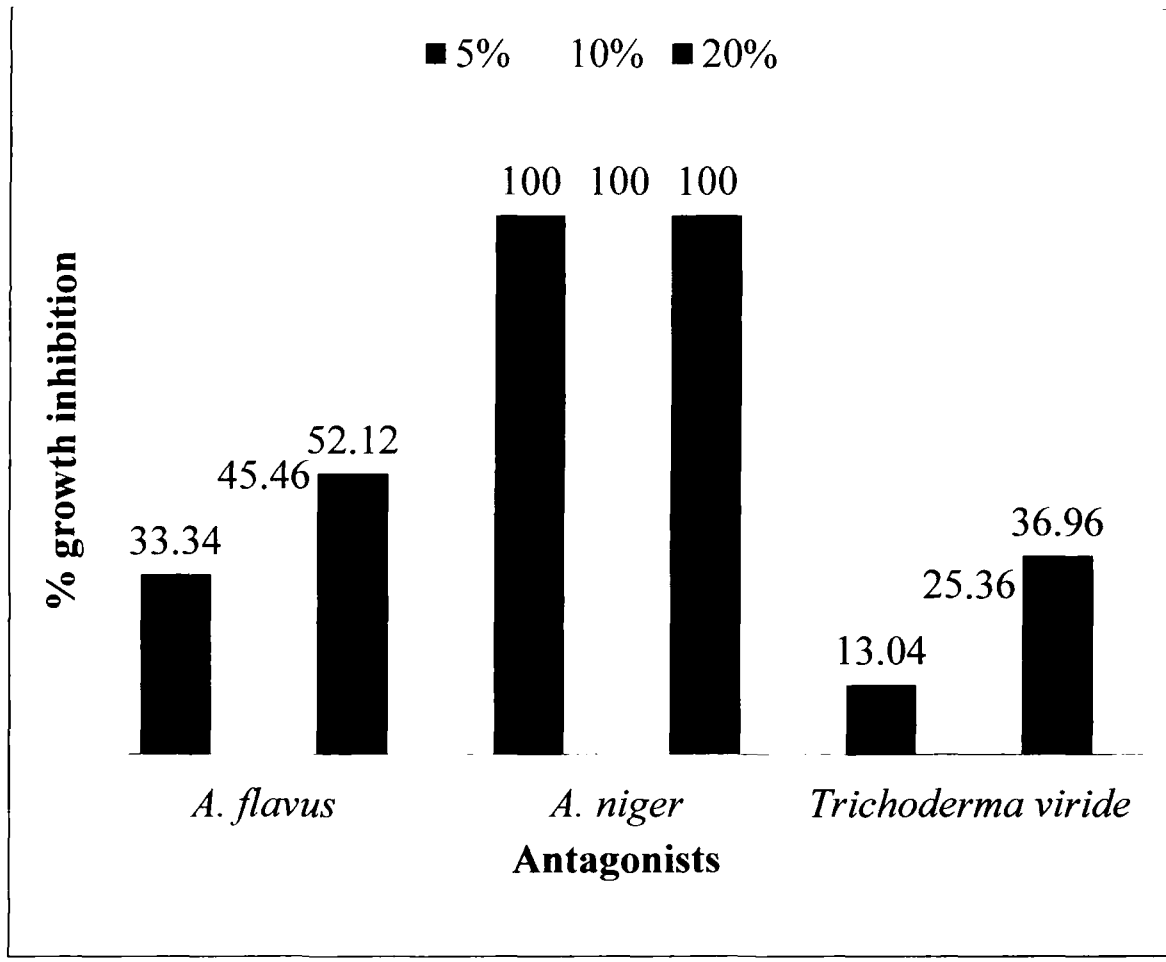

Fig. 23. Per cent inhibition of radial growth of *Aspergillus fumigatus* owing to non-volatile metabolites of antagonistic fungi.

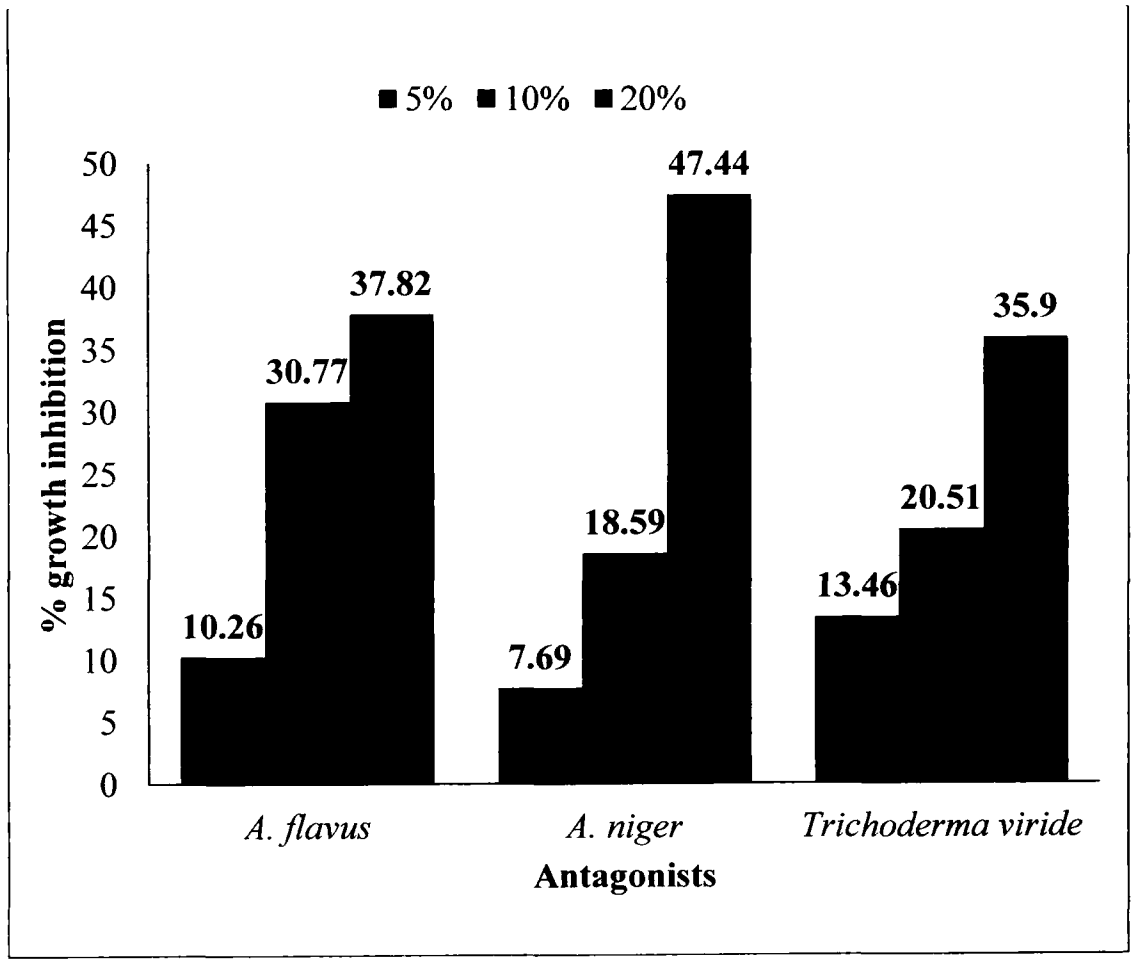

Fig. 24. Per cent inhibition of *Curvularia lunata* owing to non-volatile metabolites of antagonistic fungi.

4.5.4. Field Experiment

In 2015-2017, out of four treatments, Tilt 250 EC showed the promising result in controlling blight of *Tagetes erecta.* Per cent disease index (PDI) value was lowest 3.27 in 2015, 3.27 in 2016 and 3.41 in 2017. Number of infected flowers/plant was lowest 1.57 in 2015, 2.93 in 2016 and 5.50 in 2017. Number of healthy flowers/plant was maximum 10.13 in 2015, 14.93 in 2016 and 17.13 in 2017 (Table 16, 17, 18, Plate 25 and Append.15-17).

Bavistin 50 WP showed 6.08 in 2015, 5.65 in 2016 and 6.04 in 2017 PDI in blight infected plants. Infected flowers per plant were 4.13 in 2015, 4.53 in 2016 and 7.23 in 2017. Healthy flowers per plant were 9.17 in 2015, 9.40 in 2016 and 10.10 in 2017 (Table 16, 17, 18, Plate 25 and Append.15-17).

Leaf extract of *Azadirachta indica* showed 4.76 in 2015, 4.77 in 2016 and 4.58 in 2017 PDI in blight infected plants. Infected flowers per plant were 3.17 in 2015, 3.33 in 2016 and 6.57 in 2017. Healthy flowers per plant were 8.40 in 2015, 12.67 in 2016 and 16.53 in 2017 (Table 16, 17, 18, Plate 25 and Append.15-17).

Citrus medica showed 6.04 in 2015, 5.03 in 2016 and 5.18 in 2017 PDI in blight infected plants. Infected flowers per plant were 3.30 in 2015, 4.07 in 2016 and 6.13 in 2017. Healthy flowers per plant were 6.30 in 2015, 11.07 in 2016 and 13.67 in 2017 (Table 16, 17, 18, Plate 25 and Append.15-17).

Whereas Control plant showed 7.54 in 2015, 6.37 in 2016 and 6.27 in 2017 PDI in blight infected plants. Infected flowers per plant were 4.53 in 2015, 4.83 in 2016 and 7.83 in 2017. Healthy flowers per plant were 5.23 in 2015, 7.80 in 2016 and 8.60 in 2017 (Table 16, 17, 18, Plate 25 and Append.15-17).

Table 16. Screening of fungicides and leaf extracts to control blight disease of *T. erecta* in 2015.

Treatment	PDI	No. of Infected flowers/ plant	No. of healthy flowers/ plant
Bavistin 50 WP	6.08 b	4.13 ab	9.17 ab
Tilt 250 EC	3.27 d	1.57 d	10.13 a
Azadirachta indica	4.76 c	3.17 c	8.40 b
Citrus medica	6.04 b	3.30 bc	6.30 c
Control	7.54 a	4.53 a	5.23 c
CV (%)	10.18	13.27	8.96

Means followed by the same letter within a column did not differ significantly at 5% level by LSD.

Table 17. Screening of fungicides and leaf extracts to control blight disease of *T. erecta* in 2016.

Treatment	PDI	No. of Infected flowers/ plant	No. of healthy flowers/ plant
Bavistin 50 WP	5.65 ab	4.53 ab	9.40 d
Tilt 250 EC	3.27 d	2.93 c	14.93 a
Azadirachta indica	4.77 c	3.33 c	12.67 b
Citrus medica	5.03 bc	4.07 b	11.07 c
Control	6.37 a	4.83 a	7.80 e
CV (%)	7.58	7.30	4.72

Means followed by the same letter within a column did not differ significantly at 5% level by LSD.

Table 18. Screening of fungicides and leaf extracts to control blight disease of *T. erecta* in 2017.

Treatment	PDI	No. of Infected flowers/ plant	No. of healthy flowers/ plant
Bavistin 50 WP	6.04 a	7.23 ab	10.10 c
Tilt 250 EC	3.41 d	5.50 d	17.13 a
Azadirachta indica	4.58 c	6.57 bc	16.53 a
Citrus medica	5.18 b	6.13 cd	13.67 b
Control	6.27 a	7.83 a	8.60 d
CV (%)	3.17	8.06	5.89

Means followed by the same letter within a column did not differ significantly at 5% level by LSD.

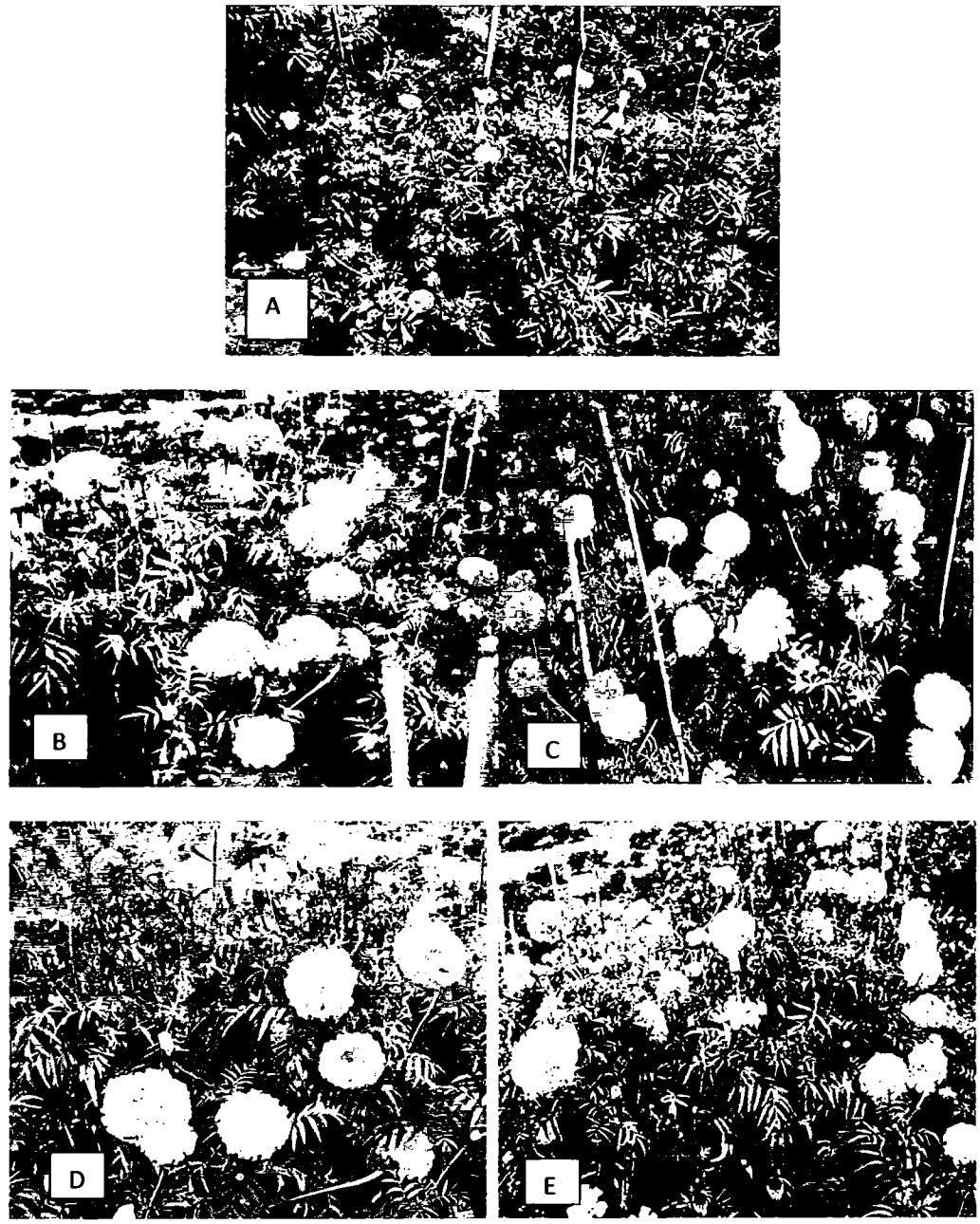

Plate 25. Effects of fungicides and leaf extracts on yield of flowering of *T. erecta*: A. Control, B. Bavistin, C. Tilt, D. *A. indica* and E. *C. medica.*

In 2015-2017, out of four treatments, Tilt showed the promising result in controlling blight of *Tagetes patula,* Per cent disease index (PDI) value was lowest 2.91 in 2015, 4.27 in 2016 and 3.23 in 2017. Number of infected flowers/plant was lowest 3.30 in 2015, 1.70 in 2016 and 2.80 in 2017. Number of healthy flowers/plant was maximum 15.30 in 2015, 25.00 in 2016 and 23.17 in 2017(Table 19, 20, 21, Plate 26 and Append.18-20).

Whereas Bavistin showed 5.28 in 2015, 5.31 in 2016 and 5.29 in 2017 PDI in blight infected plants. Infected flowers per plant were 4.40 in 2015, 3.67 in 2016 and 3.63 in 2017. Healthy flowers per plant were 12.97 in 2015, 11.07 in 2016 and 15.73 in 2017 (Table 19, 20, 21, Plate 26 and Append.18-20).

Azadirachta indica showed 3.48 in 2015, 4.96 in 2016 and 4.36 in 2017 PDI in blight infected plants. Infected flowers per plant were 4.10 in 2015, 6.33 in 2016 and 2.90 in 2017. Healthy flowers per plant were 15.17 in 2015, 23.47 in 2016 and 21.43 in 2017 (Table 19, 20, 21, Plate 26 and Append.18-20).

Whereas *Citrus medica* showed 4.45 in 2015, 5.08 in 2016 and 5.03 in 2017 PDI in blight infected plants. Infected flowers per plant were 4.57in 2015, 6.50 in 2016 and 3.30 in 2017. Healthy flowers per plant were 12.17 in 2015, 20.10 in 2016 and 15.53 in 2017 (Table 19, 20, 21, Plate 26 and Append.18-20).

Whereas Control plant showed 5.44 in 2015, 6.20 in 2016 and 5.80 in 2017 PDI in blight infected plants. Infected flowers per plant were 6.17 in 2015, 6.80 in 2016 and 4.63 in 2017. Healthy flowers per plant were 9.53 in 2015, 8.33 in 2016 and 7.77 in 2017 (Table 19, 20, 21, Plate 26 and Append.18-20).

Table 19. Screening of fungicides and leaf extracts to control blight disease of *T. patula* in 2015.

Treatment	PDI	No. of Infected flowers/ plant	No. of healthy flowers/plant
Bavistin 50 WP	5.28 a	4.40 b	12.97 b
Tilt 250EC	2.91 d	3.30 c	15.30 a
Azadirachta indica	3.48 c	4.10 b	15.17 a
Citrus medica	4.45 b	4.57 b	12.17 b
Control	5.44 a	6.17 a	9.53 c
CV (%)	6.02	21.62	3.87

Means followed by the same letter within a column did not differ significantly at 5% level by LSD.

Table 20. Screening of fungicides and leaf extracts to control blight disease of *T. patula* in 2016.

Treatment	PDI	No. of Infected flowers/ plant	No. of healthy flowers/plant
Bavistin 50 WP	5.31 b	3.67 b	11.07 b
Tilt 250 EC	4.27 c	1.70 c	25.00 a
Azadirachta indica	4.96 b	6.33 a	23.47 a
Citrus medica	5.08 b	6.50 a	20.10 a
Control	6.20 a	6.80 a	8.33 b
CV (%)	6.39	10.67	20.89

Means followed by the same letter within a column did not differ significantly at 5% level by LSD.

Table 21. Screening of fungicides and leaf extracts to control blight disease of *T. patula* in 2017.

Treatment	PDI	No. of Infected flowers/ plant	No. of healthy flowers/plant
Bavistin 50 WP	5.29 ab	3.63 b	15.73 b
Tilt 250 EC	3.23 d	2.80 c	23.17 a
Azadirachta indica	4.36 c	2.90 c	21.43 a
Citrus medica	5.03 b	3.30 bc	15.53 b
Control	5.80 a	4.63 a	7.77 c
CV (%)	6.68	10.50	8.46

Means followed by the same letter within a column did not differ significantly at 5% level by LSD.

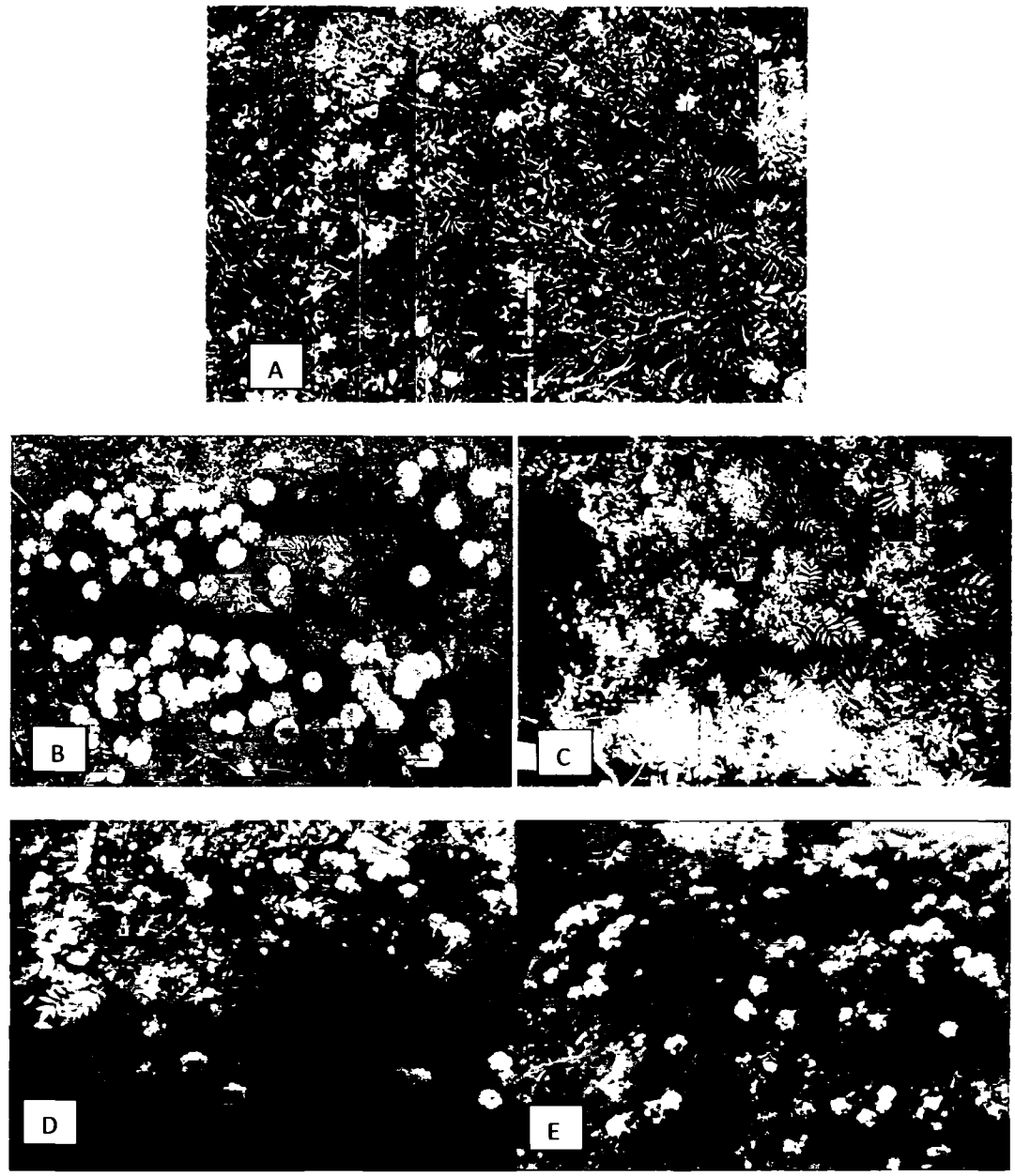

Plate 26. Effects of fungicides and leaf extracts on yield of flowering of *T. patula*: A. Control, B. Bavistin, C. Tilt, D. *A. indica* and E. *C. medica.*

From India Chandel and Kumar (2017) reported Bavistein and Captan gave the best disease control of Cercospora leaf spot of marigold and showed the disease severity (12.37% and 17.41%) respective. Among bioformulations Garlic Extract + Cow urine +Soap Nut, cow urine and garlic extract were found best with the disease severity viz. 15.36%, 18.07%. Whereas Control plantshowed 5.44 in 2015, 6.20 in 2016 and 5.80 in 2017 PDI in blight infected plants. Infected flowers per plant were 6.17 in 2015, 6.80 in 2016 and 4.63 in 2017. Healthy flowers per plant were 9.53 in 2015, 8.33 in 2016 and 7.77 in 2017.

Ali *et al.* (2011-2012) reported that application of Score in *Botrytis* grey mold infected fields of marigold showed (0.2%) lowest number of infected flowers (43.77), PDI 1.6 and produced highest number of healthy flowers (56.11). Whereas control plots showed highest number of infected flowers (69.32), PDI (3.93) and lowest count of healthy flowers per plant (30.68).

Ali and Islam (2012-2013) reported that owing to application of Score (0.05%) in *Botrytis* blight field of marigold lowest number of infected per plant was 17.20, lowest DS was 1.72 and highest number of healthy flowers were 82.80. Control plants showed highest infected flowers 25.37, DS 2.54 and lowest number of healthy flowers 74.63%.

Islam *et al.* (2015-2016) applied Tilt 250EC (0.5ml/L) and Secure 600 WG (2gm/L) and observed that Disease severity of Botrytis blight was reduced (13.48%0 and (15.30%) respectively. Moreover control plots showed highest 55% disease severity.

CHAPTER: 5
SUMMARY

SUMMARY

Two species of marigold *viz., Tagetes erecta* and *Tagetes patula* belongs to Asteraceae (Compositae) family are native to North and South America. Presently these species become naturalized around the world. *Tagetes* spp. are important for their antifungal properties. Plant is also used against fever dysenteries, indigestions, ulcers and eczemas. Leaves are used as blood clotting agents in Ayurbedhic treatment. It is most effective against the nematode species *Pratylenchus penetrans* (Olabiyi and Oyedunmade 2000 and Politi *et al.* 2012). Plant has also mosqutocidal potentiality (Rajasekaran *et al.* 2004). Seeds of *T. erecta* is a natural pesticide. *Tagetes erecta*, the Mexican marigold, also called Aztec marigold, is a species of the genus *Tagetes* native to Mexico. Despite its being native to the Americas, it is often called African marigold. In Mexico, this plant is found in the wild in the State of México, Puebla, and Veracruz.

Tagetes patula is called French marigold. French marigolds are commonly planted in butterfly gardens as a nectar source. The essential oil from this plant is being investigated for antifungal activity, including treatment of candidiasis and treating fungal infections in plants. The plant is used in companion planting for many vegetable crops. Its root secretions are believed to kill nematodes in the soil and it is said to repel harmful insects, such as white flies on tomatoes.

Marigold is now a profitable cultivated crop to the farmers of Bangladesh but socioeconomic data and information of this flower are very scare in this regards Hoque *et al.* (2012). Leaf spot and blight are two common diseases of *Tagetes erecta* and *T. patula.* Mukerji and Bhasin (1986) reported disease of *Tagetes* from India. From Bangladesh Hossain *et al.* (2010) reported powdery mildew and Sultana and Shamsi (2011) reported

gray mold of *T. erecta*. Aktar and Shamsi (2018) reported blight disease of *T. erecta* and *T. patula* from Bangladesh.

In the present investigation blight symptom was recorded on different parts of *T. erecta* and *T. patula* during the tenure of 2009 to 2014. 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. Temperature shows noticeable effect on disease development but rainfall and humidity did not show any effect on disease development.

A total of 20 species of fungi were associated with blight symptom of different plant parts of *T. erecta* and *T. patula*. The associated fungi with blight symptom were *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Bipolaris australiensis*, *Chaetomium globosum*, *Cladosporium elatum*, *Corynespora cambrensis*, *Curvularia brachyspora*, *C. fallax*, *C. lunata*, *C. stapeliae*, *Epicoccum purpurascens*, *Fusarium semitectum*, *Monochaetia ceratoniae*, *Nigrospora panici*, *Penicillium italicum*, *Rhizopus stolonifer*, *Trichoderma viride* and *Trichothecium roseum*. *Corynespora cambrensis*, *Monochaetia ceratoniae* and *Nigrospora panici* are new record for Bangladesh.

Among the isolated fungi *Alternaria alternata*, *Aspergillus fumigatus* and *Curvularia lunata* were found to be pathogenic to both the species of *Tagetes* (Aktar and Shamsi, 2014, 2015 and 2016).

Ten fungicides, ethanol leaf extracts of ten angiospermic plants and antagonistic potentiality of three soil fungi *viz.*, *A. flavus*, *A. niger* and *T. viride* were screened *in vitro* against three pathogenic fungi of *T. erecta* and *T. patula* in the laboratory of Mycology and Plant Pathology In the Department of Botany, University of Dhaka.

Ten fungicides namely Bavistin 50 WP, Capvit 50 WP, Dithane M-45, Greengel 72 WP, Hayvit 80 WP, Indofil M-45, MC Sulphur 80 WDG, Ridomil Gold MZ 68 WG, Salcox 50 WP and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm were evaluated against the above mentioned pathogenic fungi of *Tagetes*. Bavistin 50 WP and Tilt 250 EC completely inhibited the radial growth of the test fungi at all concentrations. Antifungal properties of ethanol leaf extracts of *Artocarpus heterophyllus*, *Azadirachta indica*, *Cassia sophera*, *Citrus medica*, *Datura metel*, *Houttuynia cordata*, *Lantana camara*, *Mangifera indica*, *Moringa oleifera* and *Vitex negundo* at 5, 10 and 20% concentrations were evaluated against the three test pathogens. Leaf extracts of *A. indica* and *C. medica* also completely inhibited the radial growth of the test fungi at all concentrations. Except *Lantana camara*, *Mangifera indica* and *Vitex negundo,* all the seven plant extracts completely inhibited radial growth of the test fungi at 20% concentrations. Three antagonistic fungi were isolated from the field soil of blight infected *Tagetes* spp. by 'serial dilution method'. The fungi were identified as *Aspergillus flavus, A. niger* and *Trichoderma viride.* Antagonistic potentiality of aforesaid fungi were evaluated against the pathogenic fungi of *Tagetes erecta* and *T. patula* following 'dual culture colony interaction' and volatile and non-volatile metabolites. The pathogenic fungi were *A. alternata*, *Aspergillus fumigatus* and *Curvularia lunata.* In dual culture colony interaction, out of three soil fungi *T. viride* showed the highest growth inhibition on *A. alternata* (71.03%), *A. fumigatus* (38.49%) and *C. lunata* (60.71%). The maximum inhibition of radial growth of *A. alternata* (74.55%) was observed with the culture filtrates of *T. viride* owing to volatile metabolites. The maximum inhibition of radial growth of *A. fumigatus* (37.43%) was also observed with the culture filtrates of *A. flavus* owing to volatile metabolites. The complete inhibition of radial growth of *C. lunata* was observed with the culture filtrates of *A. niger* owing to volatile metabolites. The complete inhibition of radial growth of *A. alternata* was

observed with non-volatile metabolites of *A. niger* and *T. viride* at all concentrations used. The complete inhibition of radial growth of *A. fumigatus* was also observed with non-volatile metabolites of *A. niger* at all concentrations used. The maximum inhibition of radial growth of *C. lunata* was observed with non-volatile metabolites of *A. niger* at 20% concentrations used. *Aspergillus niger* and *T. viride* may be exploited commercially as a biocontrol agent against blight pathogens of *T. erecta* and *T. patula.*

Field experiment was conducted in Botanical research garden, Department of Botany, University of Dhaka during the tenure of 2015, 2016 and 2017 to evaluate the efficacy of two fungicides and two plant extracts against blight disease of *T. erecta* and *T. patula.* Both the fungicides Bavistin 50 WP and Tilt 250 EC and leaf extracts of *A. indica* and *C. medica* show effective management of the disease over untreated check. However, among the treatments Bavistin 50 WP and Tilt 250 EC at 100 ppm concentration and *A. indica* and *C. medica* at 10% concentration was found significantly superior in controlling PDI (Per cent disease index) and increasing number of healthy flowers. Number of healthy flowers was highest per plant 17.13 in *T. erecta* in the year 2017 and 25.00 in *T. patula* in the year 2016.

CHAPTER: 6
CONCLUSION AND RECOMMENDATION

Conclusion and Recommendation

Based on the findings of the present investigation the following conclusions are drawn:

1. Blight symptom was recorded on different parts of *Tagetes erecta* and *T. patula* during the tenure of 2009 to 2014.
2. Disease incidence was started from January and gradually increased up to May.
3. The Lowest disease severity (DS 1) was recorded in the month of January and the highest disease severity (DS 9) was recorded in the month of May.
4. Rainfall and humidity did not show any effect on disease development but temperature shows noticeable effect on disease development.
5. A total of 20 species of fungi were isolated from *Tagetes erecta* and *T. patula.*
6. *Corynespora cambrensis, Monochaetia ceratoniae* and *Nigrospora panici* are new record for Bangladesh.
7. Among the isolated fungi *Alternaria alternata, Aspergillus fumigatus* and *Curvularia lunata* were found to be pathogenic to *Tagetes erecta* and *T. patula.*

***In vitro* investigation:**

8. Bavistin 50 WP and Tilt 250 EC identified as best inhibiting chemical fungicides against blight disease of *Tagetes erecta* and *T. patula.*
9. The present investigation suggests that *Aspergillus niger* and *Trichoderma viride* showed promising inhibitory effect on test pathogens.

10. Out of 10 ethanol leaf extracts, *Azadirachta indica* and *Citrus medica* showed complete inhibition of test pathogens.

***In vivo* experiment:**

11. Bavistin 50 WP and Tilt 250 EC at 100 ppm concentration and leaf extracts of *A. indica* and *C. medica* at 10% concentration significantly reduced PDI of blight of *Tagetes erecta* and *T. patula* and subsequently increase the healthy flower production in the field in the years of studied.

Recommendations:

1. Blight disease damaged this plant.
2. Present investigation is the first approach of disease management of this plant in Bangladesh.
3. Findings of this research work will be helpful for designing a proper management of blight disease of *Tagetes erecta* and *T. patula.*
4. Application of Tilt at 100 ppm concentration may be commercially used for managing blight disease of *Tagetes erecta* and *T. patula.*
5. For more confirmation the above mentioned fungicides also need to 2-3 years trial in field condition.
6. In small scale gardening or those person who want to maintain the plants in the yard as medicinal purposes or as ornamental plants, *Azadirachta indica* at 10% concentration can be used for controlling diseases and production of healthy flower.

CHAPTER: 7
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CHAPTER: 8
APPENDICES

APPENDICES

Appendix-1

(a). Preparation of Lacto Phenol

Lacto phenol solution used as mounting medium consisted of the following composition (Anisworth 1963).

Substances	Amount
Phenol crystals	20 gm
Lactic acid	20 ml
Glycerol	40 ml
Distilled water	20 ml

After weighting the constituents were taken in a conical flask to which distilled water added. The flask was taken well till a homogenous solution was obtained.

(b). Preparation of Cotton Blue Stain

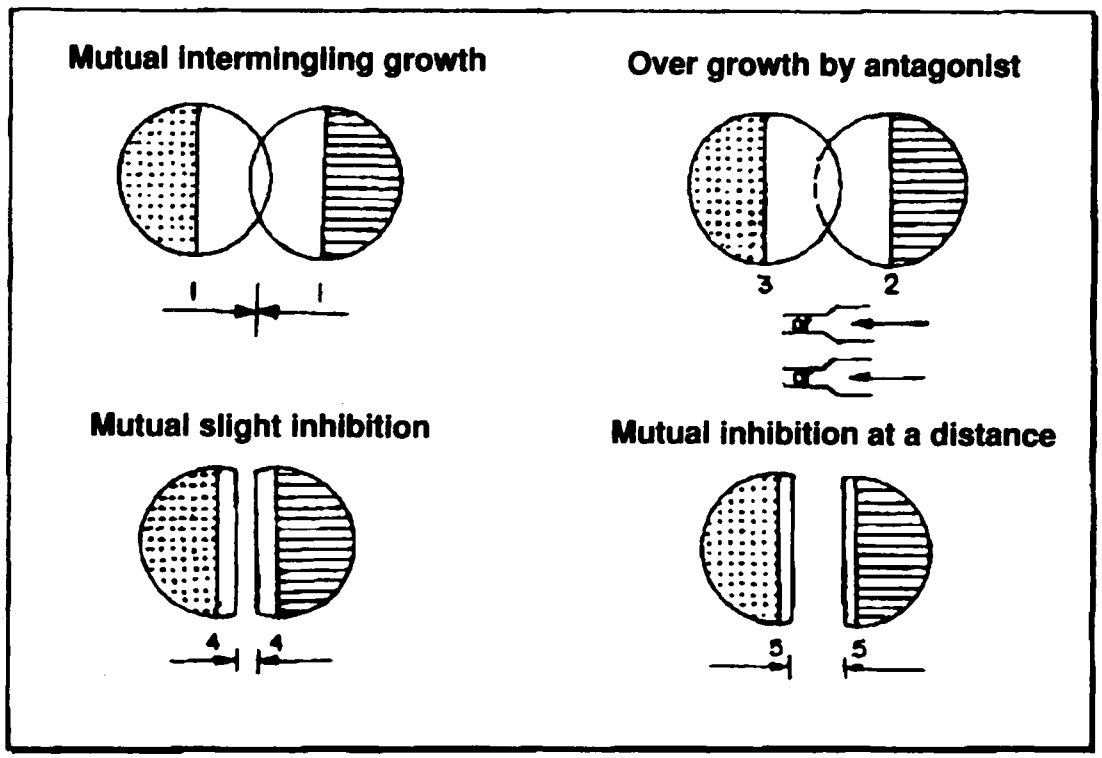
One gram of cotton blue was added to 100 ml of lacto phenol and shaken well it was dissolved. The solution of lacto phenol and cotton blue were stored in cool dark place and it was stored in an amber colored bottle.

(c). Potato Dextrose Agar (PDA) Medium

Substances	Amount
Peeled and sliced potato	200.00 gm
Dextrose	20.00 gm
Agar	15.00 gm
Distilled water	1000.00 ml
pH	6.0

Appendix- 2

The colony interaction model of Skidmore and Dickinson (1976)



* = Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), based on Skidmore and Dickinson (1976).

** **A** = Mutually intermingling growth were both fungi grew into one another without any microscopic sign of interaction (1).

Bi = Intermingling growth where the fungus being observed into the opposed fungus either above or below its colony (3).

Bii = Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony (2).

C = Slight inhibition with a narrow demarcation line, 1-2 mm (4).

D = Mutual inhibition at a distance more than 2 mm (5).

Appendix 3. ANOVA for screening fungicides against *Alternaria alternata in vitro.*

Per cent Inhibition of radial growth of *Alternaria alternata* against different fungicides

Table 3A. ANOVA TABLE
Response Variable: X100ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	45954.0912	4595.4091	420.43	0.0000
Error	22	240.4635	10.9302		
Total	32	46194.5547			

CV (%) 7.28

Table 3B. ANOVA TABLE
Response Variable: X200ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	39768.4882	3976.8488	755.47	0.0000
Error	22	115.8093	5.2641		
Total	32	39884.2976			

CV (%) 4.31

Table 3C. ANOVA TABLE
Response Variable: X300ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	39404.8998	3940.4900	1778.73	0.0000
Error	22	48.7373	2.2153		
Total	32	39453.6371			

CV (%) 2.56

Table 3D. ANOVA TABLE
Response Variable: X400ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	41940.3045	4194.0304	1026.07	0.0000
Error	22	89.9244	4.0875		
Total	32	42030.2289			

CV (%) 2.81

Table 3E. ANOVA TABLE
Response Variable: X500ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	36839.0038	3683.9004	1772.80	0.0000
Error	22	45.7162	2.0780		
Total	32	36884.7200			

CV (%) 1.88

Appendix 4. ANOVA for screening fungicides against *Aspergillus fumigatus in vitro.* Per cent Inhibition of radial growth of *Aspergillus fumigatus* against different fungicides.

Table 4A. ANOVA TABLE
Response Variable: X100ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	41896.7581	4189.6758	152.55	0.0000
Error	22	604.2299	27.4650		
Total	32	42500.9880			

CV (%) 16.34

Table 4B. ANOVA TABLE
Response Variable: X200ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	36658.2883	3665.8288	179.44	0.0000
Error	22	449.4559	20.4298		
Total	32	37107.7443			

CV (%) 10.48

Table 4C. ANOVA TABLE
Response Variable: X300ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	40665.0366	4066.5037	1334.01	0.0000
Error	22	67.0632	3.0483		
Total	32	40732.0998			

CV (%) 3.34

Table 4D. ANOVA TABLE
Response Variable: X400ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	44686.7103	4468.6710	562.64	0.0000
Error	22	174.7323	7.9424		
Total	32	44861.4426			

CV (%) 4.42

Table 4E. ANOVA TABLE

Response Variable: X500ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	39735.5448	3973.5545	1375.95	0.0000
Error	22	63.5328	2.8879		
Total	32	39799.0776			

CV (%) 2.51

Appendix 5. ANOVA for screening fungicides against *Curvularia lunata in vitro.* Per cent Inhibition of radial growth of *Curvularia lunata* against different fungicides.

Table 5A. ANOVA TABLE
Response Variable: X100ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	36821.7971	3682.1797	106.80	0.0000
Error	22	758.5267	34.4785		
Total	32	37580.3238			

CV (%) 16.38

Table 5B. ANOVA TABLE
Response Variable: X200ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	33069.5850	3306.9585	501.27	0.0000
Error	22	145.1389	6.5972		
Total	32	33214.7239			

CV (%) 5.46

Table 5C. ANOVA TABLE
Response Variable: X300ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	36014.5079	3601.4508	306.11	0.0000
Error	22	258.8373	11.7653		
Total	32	36273.3452			

CV (%) 5.81

Table 5D. ANOVA TABLE
Response Variable: X400ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	37924.4044	3792.4404	955.83	0.0000
Error	22	87.2894	3.9677		
Total	32	38011.6938			

CV (%) 3.03

Table 5E. ANOVA TABLE
Response Variable: X500ppm

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
name	10	38948.7272	3894.8727	1370.55	0.0000
Error	22	62.5202	2.8418		
Total	32	39011.2474			

CV (%) 2.37

Appendix 6. ANOVA for screening leaf extracts against *Alternaria alternata in vitro*. Per cent Inhibition of radial growth of *Alternaria alternata* against different leaf extracts.

Table 6A. ANOVA TABLE
Response Variable: X 5 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	10	35047.5368	3504.7537	97.26	0.0000
Error	22	792.7384	36.0336		
Total	32	35840.2752			

CV (%) 11.08

Table 6B. ANOVA TABLE
Response Variable: X 10 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	10	34709.2552	3470.9255	1567.96	0.0000
Error	22	48.7003	2.2137		
Total	32	34757.9555			

CV (%) 1.97

Appendix 7. ANOVA for screening leaf extracts against *Aspergillus fumigatus in vitro.*

Per cent Inhibition of radial growth of *Aspergillus fumigatus* against different leaf extracts.

Table 7A. ANOVA TABLE
Response Variable: X 5 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	10	32880.2454	3288.0245	498.36	0.0000
Error	22	145.1477	6.5976		
Total	32	33025.3932			

CV (%) 4.88

Table 7B. ANOVA TABLE
Response Variable: X 10 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	10	26193.9421	2619.3942	183.36	0.0000
Error	22	314.2746	14.2852		
Total	32	26508.2167			

CV (%) 5.72

Table 7C. ANOVA TABLE
Response Variable: X 20 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	10	30121.7369	3012.1737	2488.31	0.0000

Error 22 26.6317 1.2105
Total 32 30148.3686

CV (%) 1.30

Appendix 8. ANOVA for screening leaf extracts against *Curvularia lunata in vitro.* Per cent Inhibition of radial growth of *Curvularia lunata* against different leaf extracts.

Table 8A. ANOVA TABLE
Response Variable: X 5 per cent

Source DF Sum of Square Mean Square F Value Pr(> F)
---
treat 10 30572.7366 3057.2737 163.80 0.0000
Error 22 410.6323 18.6651
Total 32 30983.3689

CV (%) 8.31

Table 8B. ANOVA TABLE
Response Variable: X 10 per cent

Source DF Sum of Square Mean Square F Value Pr(> F)
---
treat 10 32793.3316 3279.3332 808.32 0.0000
Error 22 89.2530 4.0570
Total 32 32882.5846

CV (%) 2.57

Table 8C. ANOVA TABLE
Response Variable: X.20 per cent

Source DF Sum of Square Mean Square F Value Pr(> F)
---
treat 10 28116.1443 2811.6144 677.53 0.0000
Error 22 91.2950 4.1498
Total 32 28207.4393

CV (%) 2.31

Appendix 9. ANOVA for screening of volatile metabolites of soil fungi against *Alternaria alternata*.

Per cent inhibition of *Alternaria alternata* owing to volatile metabolites of soil fungi

Table 8. ANOVA TABLE
Response Variable: inhibition per centage

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	10188.3460	3396.1153	397.87	0.0000
Error	8	68.2866	8.5358		
Total	11	10256.6326			

CV (%) 7.09

Appendix 10. ANOVA for screening of volatile metabolites of soil fungi against *Aspergillus fumigatus*.

Per cent inhibition of *Aspergillus fumigatus* owing to volatile metabolites of soil fungi.

Table 10. ANOVA TABLE
Response Variable: inhibition per centage

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	2343.5336	781.1779	132.39	0.0000
Error	8	47.2037	5.9005		
Total	11	2390.7373			

CV (%) 11.87

Appendix 11. ANOVA for screening of volatile metabolites of soil fungi against *Curvularia lunata* .

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Per cent inhibition of *Curvularia lunata* owing to volatile metabolites of soil fungi.

Table 11. ANOVA TABLE
Response Variable: inhibition per centage

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	18111.9462	6037.3154	7543.50	0.0000
Error	8	6.4027	0.8003		
Total	11	18118.3489			

CV (%) 1.35

Appendix 12. ANOVA for screening of non-volatile metabolites of soil fungi against *Alternaria alternata*.

Per cent inhibition of *Alternaria alternata* owing to non-volatile metabolites of soil fungi.

Table 12 A.ANOVA TABLE
Response Variable: X 5 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	24499.0556	8166.3519	5486.66	0.0000
Error	8	11.9072	1.4884		
Total	11	24510.9628			

CV (%) 2.20

Table 12 B.ANOVA TABLE
Response Variable: X 10 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	20929.6401	6976.5467	4687.28	0.0000
Error	8	11.9072	1.4884		
Total	11	20941.5473			

CV (%) 1.98

Table 12 C.ANOVA TABLE
Response Variable: X 20 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	20251.2225	6750.4075	4535.35	0.0000
Error	8	11.9072	1.4884		
Total	11	20263.1297			

CV (%) 1.91

Appendix 13. ANOVA for screening of non-volatile metabolites of soil fungi against *Aspergillus fumigatus*.

Per cent inhibition of *Aspergillus fumigatus* owing to non-volatile metabolites of soil fungi.

Table 13 A. ANOVA TABLE
Response Variable: X 5 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	17774.0574	5924.6858	4061.64	0.0000
Error	8	11.6695	1.4587		

Total	11	17785.7269			

CV (%) 3.30

Table 13 B. ANOVA TABLE
Response Variable: X 10 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	16244.5642	5414.8547	2255.78	0.0000
Error	8	19.2035	2.4004		
Total	11	16263.7677			

CV (%) 3.63

Table 13 C. ANOVA TABLE
Response Variable: X 20 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	15434.4765	5144.8255	2249.80	0.0000
Error	8	18.2943	2.2868		
Total	11	15452.7708			

CV (%) 3.20

Appendix 14. ANOVA for screening of non-volatile metabolites of soil fungi *against Curvularia lunata.*

Per cent inhibition of *Curvularia lunata* owing to non-volatile metabolites of soil fungi.

Table 14 A. ANOVA TABLE
Response Variable: X 5 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	296.8457	98.9486	14.60	0.0013
Error	8	54.2338	6.7792		
Total	11	351.0795			

CV (%) 33.15

Table 14 B. ANOVA TABLE
Response Variable: X 10 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	1477.8215	492.6072	145.50	0.0000
Error	8	27.0849	3.3856		
Total	11	1504.9064			

CV (%) 10.53

Table 14 C. ANOVA TABLE
Response Variable: X 20 per cent

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
treat	3	3899.0343	1299.6781	234.09	0.0000
Error	8	44.4162	5.5520		
Total	11	3943.4505			

CV (%) 7.78

Appendix 15. ANOVA for screening of fungicides and leaf extracts in controlling blight disease of *Tagetes erecta* in 2015.

Table 15 A. PDI

ANOVA TABLE
Response Variable: PDI

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	1.9444	0.9722	3.06	0.1029
treat	4	30.8791	7.7198	24.31	0.0002
Error	8	2.5400	0.3175		
Total	14	35.3635			

CV (%) 10.18

Table 15 B. DF (Disease Flower)

ANOVA TABLE
Response Variable: DF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.5160	0.2580	1.31	0.3210
treat	4	15.6893	3.9223	19.98	0.0003
Error	8	1.5707	0.1963		
Total	14	17.7760			

CV (%) 13.27

Table 15 C. HF (Healthy Flower)

ANOVA TABLE
Response Variable: HF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	1.4453	0.7227	1.46	0.2877
treat	4	49.4973	12.3743	25.03	0.0001
Error	8	3.9547	0.4943		

Total	14	54.8973
CV (%)	8.96	

Appendix 16. ANOVA for screening of fungicides and leaf extracts in controlling blight disease of *Tagetes erecta* in 2016.

Table 16 A. PDI

ANOVA TABLE
Response Variable: PDI

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.0976	0.0488	0.34	0.7212
treat	4	17.1101	4.2775	29.86	0.0001
Error	8	1.1459	0.1432		
Total	14	18.3537			
CV (%)	7.58				

Table 16 B. DF (Disease Flower)

ANOVA TABLE
Response Variable: DF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.4320	0.2160	2.61	0.1339
treat	4	7.6427	1.9107	23.11	0.0002
Error	8	0.6613	0.0827		
Total	14	8.7360			
CV (%)	7.30				

Table 16 C. HF (Healthy Flower)

ANOVA TABLE
Response Variable: HF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.7373	0.3687	1.33	0.3179
treat	4	92.7093	23.1773	83.42	0.0000
Error	8	2.2227	0.2778		
Total	14	95.6693			
CV (%)	4.72				

Appendix 17. ANOVA for screening of fungicides and leaf extracts in controlling blight disease of *Tagetes erecta* in 2017.

Table 17 A. PDI

ANOVA TABLE
Response Variable: PDI

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.1111	0.0556	2.13	0.1813
treat	4	16.0772	4.0193	154.10	0.0000
Error	8	0.2087	0.0261		
Total	14	16.3970			
CV (%)	3.17				

Table 17 B. DF (Disease Flower)

ANOVA TABLE
Response Variable: DF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	5.5453	2.7727	9.64	0.0074
treat	4	10.0107	2.5027	8.70	0.0052
Error	8	2.3013	0.2877		
Total	14	17.8573			
CV (%)	8.06				

Table 17 C. HF (Healthy Flower)

ANOVA TABLE
Response Variable: HF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	9.4813	4.7407	7.84	0.0130
treat	4	172.7093	43.1773	71.39	0.0000
Error	8	4.8387	0.6048		
Total	14	187.0293			
CV (%)	5.89				

Appendix 18. ANOVA for screening of fungicides and leaf extracts in controlling blight disease of *Tagetes patula* in 2015.

Table 18 A. PDI

ANOVA TABLE
Response Variable: PDI

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.1732	0.0866	1.28	0.3287
treat	4	14.7361	3.6840	54.58	0.0000

Error	8	0.5400	0.0675	
Total	14	15.4493		
CV (%)	6.02			

Table 18 B. DF (Disease Flower)

ANOVA TABLE
Response Variable: DF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.9373	0.4687	0.49	0.6279
treat	4	13.1760	3.2940	3.47	0.0633
Error	8	7.5960	0.9495		
Total	14	21.7093			
CV (%)	21.62				

Table 18 C. HF (Healthy Flower)

ANOVA TABLE
Response Variable: HF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	1.4893	0.7447	2.92	0.1114
treat	4	68.0827	17.0207	66.84	0.0000
Error	8	2.0373	0.2547		
Total	14	71.6093			
CV(%)	3.87				

Appendix 19. ANOVA for screening of fungicides and leaf extracts in controlling blight disease of *Tagetes patula* in 2016.

Table 19 A. PDI

ANOVA TABLE
Response Variable: PDI

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.1331	0.0666	0.61	0.5659
treat	4	5.8133	1.4533	13.36	0.0013
Error	8	0.8703	0.1088		
Total	14	6.8167			
CV (%)	6.39				

Table 19 B. DF (Disease Flower)

ANOVA TABLE
Response Variable: DF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	1.3960	0.6980	2.45	0.1477
treat	4	59.8067	14.9517	52.52	0.0000
Error	8	2.2773	0.2847		
Total	14	63.4800			
CV (%)	10.67				

Table 19 C. HF (Healthy Flower)

ANOVA TABLE
Response Variable: HF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	35.5693	17.7847	1.32	0.3205
treat	4	671.9493	167.9873	12.43	0.0016
Error	8	108.1107	13.5138		
Total	14	815.6293			
CV (%)	20.89				

Appendix 20. ANOVA for screening of fungicides and leaf extracts in controlling blight disease of *Tagetes patula* in 2017.

Table 20 A. PDI

ANOVA TABLE
Response Variable: PDI

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.2662	0.1331	1.33	0.3182
treat	4	11.7835	2.9459	29.34	0.0001
Error	8	0.8032	0.1004		
Total	14	12.8530			
CV (%)	6.68				

Table 20 B. DF (Disease Flower)

ANOVA TABLE
Response Variable: DF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	0.8813	0.4407	3.35	0.0877
treat	4	6.5440	1.6360	12.44	0.0016
Error	8	1.0520	0.1315		
Total	14	8.4773			

CV (%)	10.50

Table 20 C. HF (Healthy Flower)

ANOVA TABLE
Response Variable: HF

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
rep	2	40.1613	20.0807	10.02	0.0066
treat	4	438.9560	109.7390	54.76	0.0000
Error	8	16.0320	2.0040		
Total	14	495.1493			

CV (%)	8.46

Appendix 21

LIST OF ABBREVIATIONS

Abbreviation	Full word
ANOVA	Analysis of Variance
Append.	Appendix
@	At the rate
°C	Degree Celsius
Fig.	Figure
g	Gram (s)
Kg	Kilogram
m	Meter
ml	milliliter
mm	millimeter
cm	Centimeter
µm	Milli micron
viz.	Namely
pH	Negative logarithm of hydrogen ion concentration
ppm	Parts per million
PDA	Potato dextrose agar
PDI	Per cent Disease Index
DS	Disease Severity
%	Per cent
sp.	Species
et al.	With others
Vol.	Volume

Published paper from this research work

1. Aktar M and Shamsi S 2014. Report on Alternaria blight of *Tagetes erecta* and *Tagetes patula* caused *by Alternaria alternata* (Fr.) Keissler. J. Asiat. Soc. Bangladesh. Sci. **40**(1):133-140.
2. Aktar M and Shamsi S 2015. Blight of two species of marigold (*Tagetes*) caused by *Aspergillus fumigatus* Frsenius. Bangladesh J. Plant Pathol. **31**(1&2): 1-6.
3. Aktar M and Shamsi S 2016. Report on blight of *Tagetes* spp. caused by *Curvularia lunata* (Wakker) Boedijn. Bangladesh J. Bot. **45**(1):167-173.
4. Aktar M and Shamsi S 2018. Incidence and severity of blight disease of *Tagetes erecta* and *T. patula* Journal of Bioresearch Communications **4** (1): 464-469.

Published abstract

1. Aktar, M. and S. Shamsi. 2012. Report on Alternaria blight of *Tagetes* spp. caused By *Alternara alternata* (Fr.) Keissier. Paper presented in Annual plant Taxonomy Conference, 22 December 2012, Dhaka, Bangladesh.

2. Aktar, M. and S. Shamsi. 2018. *In vitro* evaluation of fungicides and plant extracts Against pathogenic fungi of *Tagetes erecta and T. patula.* 7th International Botanical Conference, 3-4 February. 2018. Department of Botany, University of Dhaka. Bangladesh.

3. Aktar, M. and S. Shamsi. 2018. Mycoflora associated with infected plant parts of *Tagetes erecta* L. and *Tagetes patula* L. 7th International Botanical Conference, 3-4 February. 2018. Department of Botany, University of Dhaka. Bangladesh.