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Research Paper

In vitro control of causal agents of anthracnose of *Senna alata* L. – a herbal medicinal plant in Bangladesh

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Abstract. Efficacy of seven fungicides and seven plant extracts were evaluated against two pathogenic species of fungi isolated from *Senna alata* L. *in vitro*. The isolated fungi were *Colletotrichum gloeosporioides* (Penz.) Sacc. and *Pestalotiopsis guepinii* (Desm.) Stay. Seven fungicides Acrobate MZ, Bavistin 50 WP, Indofil M-45, MC Sulphur 80 WP, Ridomil MZ Gold, Sulcox 50 WP and Tall 25 EC at 100, 200, 300, 400 and 500 ppm concentration were evaluated against the fungi. Tall 25 EC completely inhibited the radial growth of the test fungi at all the concentrations used. Antifungal properties of ethanol leaf extracts of *Artocarpus heterophyllus* Lam., *Azadirachta indica* L., *Citrus medica* L., *Datura metel* L., *Mangifera indica* L. *Senna alata* L. and *Tagetes erecta* L. at 5, 10 and 20% concentrations was evaluated on *C. gloeosporioides* and *P. guepinii*. All the selected plant extracts completely inhibited radial growth of the test fungi at 20% concentration.

Keywords: *In vitro* control, causal agents, Athracnose*, Senna alata,* Herbal medicinal plant.

INTRODUCTION

Senna alata, the Candle Bush, is an important medicinal shrub as well as an ornamental flowering plants in the sub family Caesalpinioideae which grows well in forested areas of West Africa. It is also known as a Candela Bush, Empress Candle Plant, Ringworm Tree or "candletree". *Senna alata* is native to Mexico, and can be found in diverse habitats. In the tropics it grows up to an altitude of 1,200 m. It is an invasive species in Austronesia. In Sri Lanka this is use an ingredient of Sinhala traditional medicine. The shrub stands 3 to 4 m tall, with leaves 50 too 80 cm long. The inflorescence looks like a yellow candle. The fruit shaped like a straight pod is up to 25 cm long. Its seed are distributed by water or animals. *Cassia alata* or *Senna alata* is often called the Ringworm Bush because of its very effective fungicidal properties, for treating ringworm and other fungal infections of the skin. The leaves are ground in a mortar to obtain a kind of "green cotton wool". This is mixed with the same amount of vegetable oil then rubbed on the affected area 2 to 3 times a day. A fresh preparation is made every day. Its active ingredients include the yellow chrysophanic acid. Its laxative effect, due to its anthraquinone content, is also well proven. The plant has widely been employed for combating dysentery, helminthic infections and stomach disorders. In Ghana and Nigeria, the decoctions of the fresh leaves, roots and seeds has been used for the treatment of wound infections, bronchitis and asthma as well as ring worm and other infectious skin diseases The leaves have been reported to be useful in the treatment of convulsions, gonorrhea, heart failure, abdominal pains, oedema and also as a purgative (Joshi, 2000; Ghani, 1998; Hirt and Mpia, 2008; Yusuf et al., 2009).

Senna alata has been reported to contain anthraquinones and the methanol fractions were found to be active against *Aspergillus flavus*. This study was therefore carried out to investigate the antimicrobial activity of the root and leaf extracts of the plant against some infectious bacteria and fungi.

Aims of the study

At home and abroad, lots of research has been done on antifungal activities of *S. alata* (Rahman 2010; Agbagwa

Figure 1. *Sienna alata:* A. Flower, B. Infected pod, C. Infected leaf.

et al., 2003; Doughari and Okafora, 2007; Eunice and Osuji, 2008). Fungal diseases of *S. alata* was reported by Jillian (1990) and Shamsi et al. (2013) but there is no trace regarding the control of fungal diseases of this valuable plant. In a recent investigation two types of symptoms were recorded on *S. alata* viz. anthracnose and indistinct small scattered leaf spots (Shamsi et al., 2013). The present study was undertaken (i) to identify the fungi associated with infected leaves and pods of *S. atata*, (ii) to determine the pathogenic potentiality of the fungi associated with plant and (iii) to evaluate fungi toxic effect of fungicides and antifungal potentiality of some selected botanicals *in-vitro* against two pathogenic fungi of *S. alata* namely *Colletotrichum gloeosporioides* and *Pestalotiopsis guepinii*.

Collection of samples

Healthy and infected leaf and pod samples were collected from the Botanical Garden, University of Dhaka, during the period of January to December 2013 (Figure 1A to C). Symptom was recorded from fresh collection and microscopic observations of the associated fungi were listed.

Isolation of the fungi

Fungi were isolated from healthy and diseased samples following the “Blotter” and “Tissue planting” method on PDA medium (Tuite, 1969). The isolated fungi were identified following standard Literature (Ellis, 1971, 1976; Ellis and Ellis, 1997; Sutton, 1980).

Pathogenicity of the fungi

Pathogenicity of the isolated fungi were done following “Detached leaf technique” (Azad and Shamsi, 2011).

In vitro control of test pathogen with fungicides

Seven fungicides with different active ingredients viz. Acrobate MZ (Dimethorph + mancozeb), Bavistin 50 WP (50% carbendazin methyl benzimidazol-2-yl carbamate), Indofil M-45(80% mancozeb manganese ion + ethylene bisdithio carbamate), MC Sulphur 80 WP (80% Sulphur), Ridomil MZ Gold, Sulcox 50 WP (copper oxychloride) and Tall 25 EC (propiconazole) were collected from Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. Efficacy of fungicides was evaluated against two fungi *C. gloeosporioides* and *Pestalotiopsis guepinii*.

For each fungicide, a stock solution having the concentrations of 10000 ppm was prepared. The calculated amount of the stock solution of a fungicide was supplemented with sterilized PDA medium to get the conc. of 100, 200, 300, 400 and 500 ppm. In the control set required amount of sterile water instead of fungicide solution was added to the PDA medium. Five mm mycelial agar disc cut from the margin of actively growing 7 days culture of test fungi and then it was inoculated at the centre of the plate. Three replications were

maintained in both cases.

In vitro control of test pathogen with plant extracts

Source of plant extract

Seven plants were selected for *in vitro* evaluation of their ethanol leaf extracts on the vegetative growth of two test pathogens. The plants were *Artocarpus heterophyllus* Lam., *Azadirachta indica* L., *Citrus medica* L., *Datura metel* L., *Mangifera indica* L., *Senna alata* L. and *Tagetes erecta* L. Ethanol leaf extracts at 5, 10 and 20% concentration were evaluated against two pathogenic fungi following poison food techniques (Grover and Moore, 1962).

The desired parts of each plant were thoroughly washed in tap water, air dried and were prepared by crushing known weight of fresh materials with ethanol in ratio 1:1(w/v). The mass of a plant part was squeezed through four folds of fine cloth and the extracts were centrifuged at 3000 rpm for 20 min to remove particulate matter. The supernatants were filtered through Whatman filter paper No.1 and the filtrate was collected in 250 ml Erlenmeyer flasks. The requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10 and 20% concentration.

The radial growth of the colonies was measured at the 5th day of incubation. The percent growth inhibition of the test fungus was calculated by using the following formula:

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

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

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

RESULTS AND DISCUSSION

Fungi associated with *S. alata*

A total of 5 species of fungi belonging to 5 genera of Deuteromycetes namely *Aspergillus niger* Van Tiegh, *Cladosporium cladosporioides* (Fresen.) de Vries, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia lunata* (Wakker) Boedijn and *Pestalotiopsis guepinii* (Desm.) Stay, were found to be associated with *S. alata.*

Results of present study slightly differ from the previous research regarding the association of fungi. Shamsi et al. (2013) reported 8 species of fungi belonging to eight genera of Deuteromycetes and one unidentified Hyphomycetes were associated with *S. alata* during the period of January, 2009 to December, 2010. The fungi were *Acromoniella* sp., *Arthrinium saccharicola* Stevenson, *Aspergillus niger* Van Tiegh, *Cladosporium cladosporioides* (Fresen.) de Vries, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Curvularia lunata* (Wakker) Boedijn, *Nigrospora sphaerica* (Sacc.) Mason, *Pestalotiopsis guepinii* (Desm.) Stay and an unidentified Hyphomycetes.

Pathogenic potentiality of the fungi isolated from *S. alata*

Isolated fungi were tested for their pathogenic potentiality following modified “detached leaf technique” (Azad and Shamsi 2011). *Colletotrichum gloeosporioides* and *P. guepinii* were found to be pathogenic to *S. alata* in both the experiment (Figure 2A to D).

Efficacy of fungicides against *Colletotrichum gloeosporioides*

Seven fungicides Acrobate MZ, Bavistin 50 WP, Indofil M-45, MC Sulphur 80 WP, Ridomil MZ Gold, Sulcox 50 WP, and Tall 25 Ec at 100, 200, 300, 400 and 500 ppm concentrations were evaluated against the pathogenic fungi *C. gloeosporioides* and *P. guepinii.* Tall 25 EC completely inhibited the radial growth of the test fungi at all the concentrations used (Figures 3 to 4).

Indofil M-45 completely inhibited the radial growth of *C. gloeosporioides* at all concentrations used. Acrobate MZ also showed 100% inhibition of radial growth of the fungus at 300, 400 and 500 ppm concentration. Bavistin 50 WP, Ridomil MZ Gold and Sulcox 50 WP also completely inhibited radial growth of test fungus at 400 and 500 ppm concentrations. MC Sulphur caused 50 and 68.75% inhibition of radial growth of the fungus at 400 and 500 ppm concentrations respectively. Acrobate showed 37.5 and 64% inhibition of radial growth of fungus at 100 and 200 ppm concentrations. Bavistin showed 40, 43.75, 63.3% and Sulcox showed 37.5, 43.75 and 50% inhibition of radial growth of fungus at 100, 200 and 300 ppm concentrations respectively. Ridomil showed 20, 26.66, 33.33% and MC Sulphur showed 25, 26 and 40% inhibition of radial growth of the fungus at 100, 200 and 300 ppm concentrations respectively. Amongst the fungicides, Indofil and Tall 25 EC showed best result and completely checked the vegetative growth of the fungus and MC Sulphur 80 WP showed lowest percentage of inhibition of radial growth of the fungus (Figure 3).

Efficacy of fungicides against *Pestalotiopsis guepinii*

In case of *P. guepinii* Tall 25 EC completely inhibited the radial growth of the test fungi at all the concentrations used. Indofil M-45 showed complete inhibition of radial

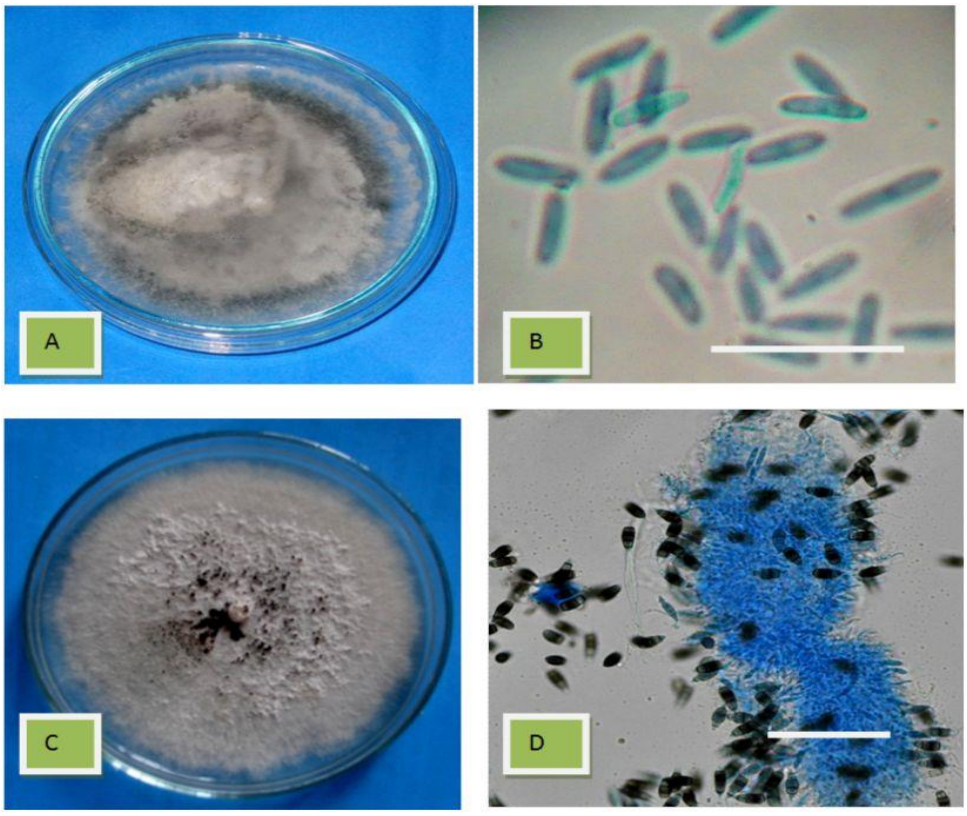

Figure 2. A-B. Culture plate and conidia of *Colletotrichum gloeosporioidse,* C-D Culture plate and acevuli of *Pestalotiopsis guepinii* {B. Bar = 25 µm, D. Bar = 50 µm).

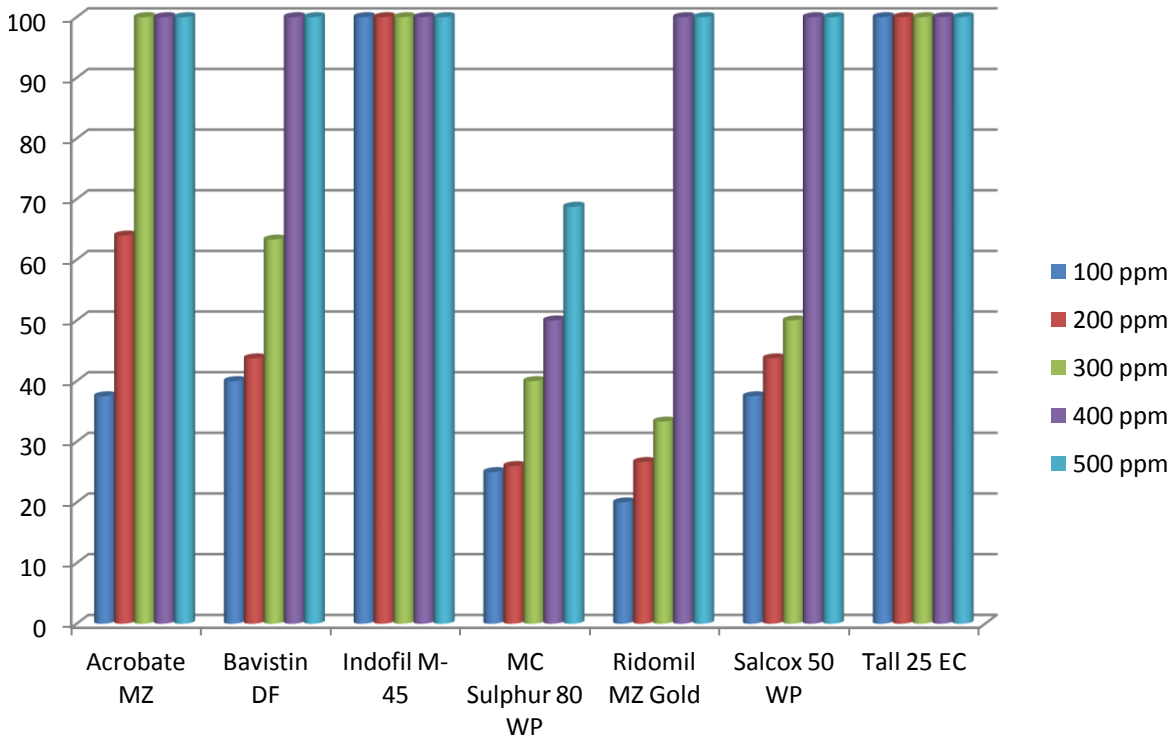

Figure 3. Fungitoxicity of fungicides against the radial growth of *Colletotrichum gloeosporioides* at different concentrations.

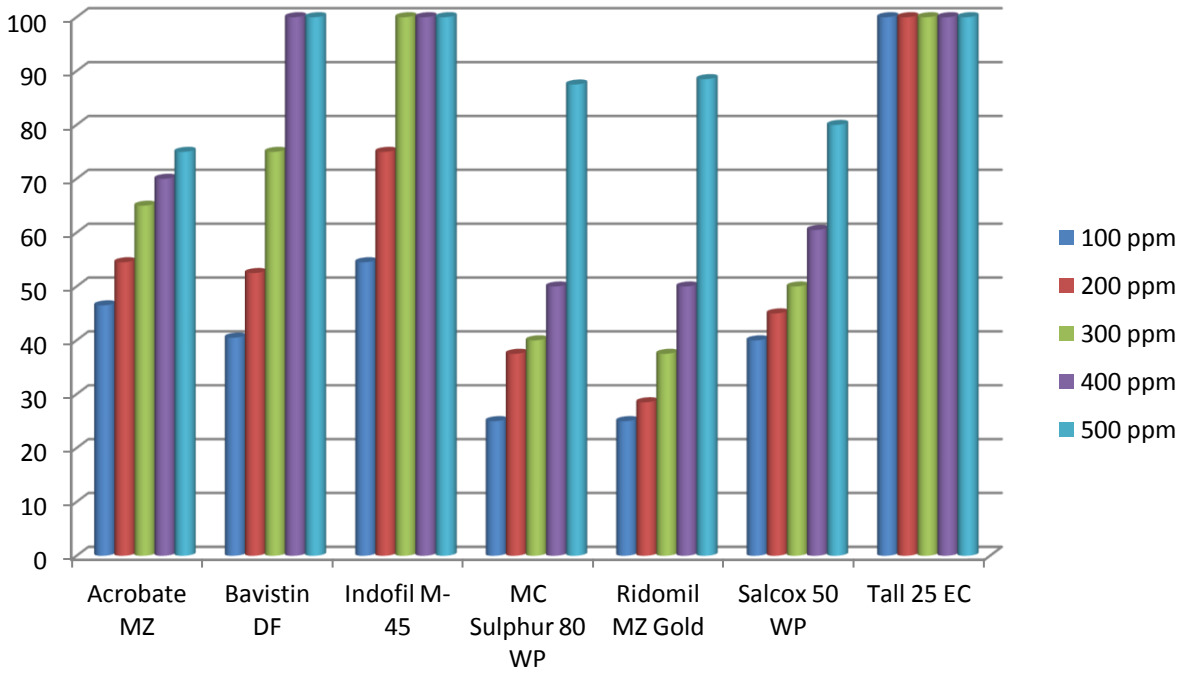

Figure 4. Percent inhibition of radial growth of *Pestalotiopsis guepinii* owing to fungicides at different concentrations.

growth of the fungus at 300, 400 and 500 ppm concentrations. Bavistin showed complete inhibition of radial growth of the fungus at 400 and 500 ppm concentration. Bavistin was also responsible for 40.5, 52.5 and 75% inhibition of the fungus at 100, 200 and 300 ppm concentration .respectively. Acrobate also showed 46.5, 54.5, 65.5, 70 and 75% inhibition of growth of test fungus at 100, 200, 300, 400 and 500 ppm concentration, respectively. Ridomil MZ Gold showed 25, 28.5, 37.5, 50 and 88.5% inhibition of the fungus at 100, 200, 300, 400 and 500 ppm concentrations.

Sulcox showed 40, 45, 50, 60.5, 80% and MC Sulphur 80 WP showed 25, 37.5, 40, 50 and 87.5% inhibition of radial growth of the fungus at 100, 200, 300, 400 and 500 ppm concentration respectively. Amongst fungicides tested, Tall 25 EC showed best growth inhibition and Ridomil MZ Gold and MC Sulphur 80 WP showed lowest percentage of inhibition of radial growth of *P. guepinii* at 100 ppm concentration (Figure 4).

Efficacy of plant extracts against *Colletotrichum gloeosporioides*

Antifungal properties of ethanol extract of *Artocarpus heterophyllus*, *Azadirachta indica*, *Citrus medica*, *Datura metel*, *Mangifera indica*, *Senna alata* and *Tagetes erecta* at 5, 10 and 20% concentrations were evaluated against the fungi *P. guepinii* and *C. gloeosporioides*. All the plant extracts completely inhibited the radial growth of the test fungi at 20% concentration (Figure 5 to 6).

Ethanol extract of *Azadirachta indica* showed 100% inhibition of radial growth of *C. gloeospoiroides* at all concentration used. *Citrus medica*, *Datura metel*, *Mangifera indica*, *Senna alata* and *Tagetes erecta* at 10 and 20% concentration, were also capable of complete inhibition of radial growth of the fungus *C. gloeosporoides. Artocarpus heterophyllus* completely inhibited the radial growth of the fungus at 20% concentration but at 10% concentration the same plant extract showed 80% inhibition of radial growth of the fungus. Plant extract of all six plants, that is, *T. erecta, D. metel, S. alata*, *C. medica*, *M. indica* and *A. heterophyllus* at 5% concentration were capable of 50, 75, 52, 67, 42, 44% inhibition of radial growth of the fungus. *Artocarpus heterophyllus* and *M. indica* showed lowest percent inhibition of the fungus (40%) at 5% concentration (Figure 5).

Efficacy of fungicides against *Pestalotiopsis guepinii*

Ethanol leaf extract of *Azadirachta indica, Citrus medica, Datura metel* and *Mangifera indica* completely inhibited the radial growth of the fungus at 10 and 20% concentrations. *Artocarpus heterophyllus, Senna alata,* and *Tagetes erecta* als ocompletely inhibited the radial growth of the fungus at 20% concentration. 10% ethanol extracts of *Artocarpus hetrophyllus, Senna alata and Tagetes erecta* showed 75, 85% and 74% inhibition of radial growth of the fungus respectively. Lowest inhibition of radial growth of the fungus was 48% at 5% plant

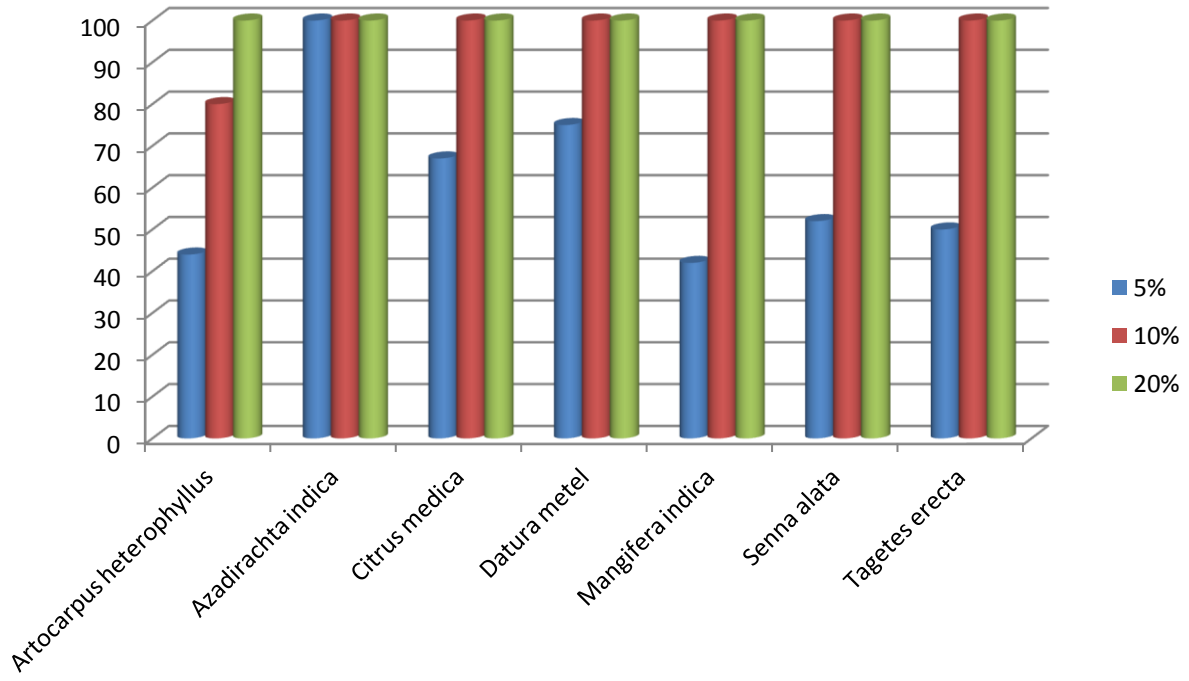

Figure 5. Percent inhibition of radial growth of *Colletotrichum gloeosporioides* at different concentrations of plant extracts.

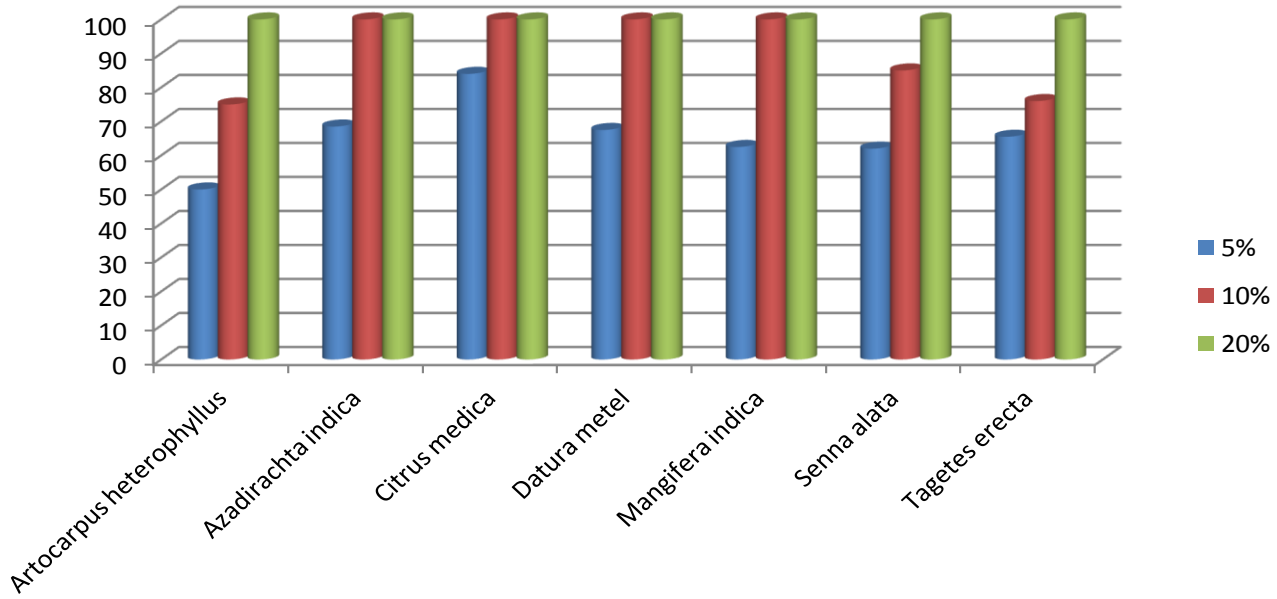

Figure 6. Percent inhibition of radial growth of *Pestalotiopsis guepinii* owing to plant extracts at different concentrations.

extracts of *A. heterophyllus* (Figure 6).

Present study clearly indicates that the fungicide Tall 25 EC completely inhibited the radial growth of both the pathogenic fungi *Colletotrichum gloeosporioides* and *Pestalotiopsis guepinii* at 100 ppm concentration. Indofil M-45 Ridomil MZ Gold completely inhibited the radial growth of *C. gloeosporioides* at the same concentration of the fungicide. Though *Senna alata* sometimes is attacked by *Colletotrichum gloeosporioides* and *Pestalotiopsis guepinii* but its healthy ethanol plant extract is already capable of exhibiting antifungal properties. All the selected fungicides and ethanol plant extracts with antifungal properties were first time used against the *C. gloeospoiroides* and *P. guepinii* the causal

agents of anthracnose of *S. alata.* The results of present investigation is a new addition in the field of Mycology and Plant Pathology.

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