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IN VITRO SCREENING OF FUNGICIDES AND PLANT EXTRACTS AGAINST *COLLETOTRICHUM GLOEOSPORIOIDES* (PENZ.) SACC. THE CAUSAL AGENT OF ANTHRACNOSE DISEASE OF *RAUWOLFIA SERPENTINA* (L.) BENTH EX KURZ

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Abstract

Ten fungicides *viz*., Amcogin 50 WP, Autostin 50 WDG, Capvit 77 WP, Dithane M 45, Greengel 72 WP, Haybit 80 WG, Oxivit 40 SC, Ridomil Gold MZ, Sulcox 50 WP and Tilt 250 EC at 100, 200, 300, 400 and 500 ppm concentrations were tested against, C*olletotrichum gloeosporioides* (Penz.) Sacc., the causal agent of anthracnose disease of *Rauwolfia serpentina* (L.) Benth ex Kurz following poisoned food technique. Amcogin, Autostin and Tilt showed complete growth inhibition of *C. gloeosporioides* at 100 ppm concentration. Ethanol extracts of ten plants *viz., Adhatoda vesica, Azadirachta indica, Citrus limon, Curcuma longa, Lantana camara, Moringa oleifera, Ocimum sanctum, Psidium guajava, Thuja orientalis* and *Vitex negundo* were selected to evaluate their efficacy at 5, 10, 15 and 20% concentrations against the same pathogenic fungus. Out of the ten plant extracts, *Azadirachta indica, Citrus limon, Moringa oleifera* and *Psidium guajava* showed complete radial growth inhibition of *C. gloeosporioides* at 20% concentration. Amcogin, Autostin, and Tilt are suggested as best inhibiting chemical fungicides for *C. gloeosporioides*. Extracts of *A. indica, C. limon, M. oleifera* and *Psidium guajava* were also found to be superior to other plant extracts tested in controlling the test pathogen.

Key words: In vitro screening, Fungicides, Plant extracts, C*olletotrichum gloeosporioides*, Anthracnose, *Rauwolfia serpentina*

Introduction

Rauwolfia serpentina (L.) Benth ex Kurz is a medicinal shrub belonging to Apocynaceae. The shrub is locally known as 'Sarpagandha' also known as Indian snakeroot. It grows in India, Thailand, South America and Africa. It is widely distributed in the sub-Himalayan tract from Punjab to Nepal, Sikkim and Bhutan (Ahmed *et al*. 2008). In Bangladesh it grows in Chittagong, Sylhet and Mymensingh (Chowdhury 1995). Khan *et al.* (2001) reported *R. serpentina* in red data book as endangered plant. International Union For conservation of nature (IUCN) has placed this plant under endangered status (Mabberley 2008). Root of this shrub is mostly used for insomnia

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(sleeplessness), mental disorders characterized by symptoms like convulsions, excessive talking, maniacal behavior, aggressive behavior etc.. Seventeen different alkaloids have been extracted from the bark of the root of this shrub. Serpentine is one of those alkaloids (Ghani 2003). Anthracnose is one of the destructive diseases of *R. serpentina.* C*olletotrichum gloeosporioides* (Penz.) Sacc. is the causal agents of this disease. The other diseases include Target leaf blotch, Cercospora leaf spot, Die-back, Powdery mildew and Fusarium wilt, Root-knot disease, etc. Mukerji and Bhasin (1986) and Yasmin and Shamsi (2015) reported diseases of *R. serpentina* from India and Bangladesh, respectively. Most of the research work carried out on *R. serpentina* falls under Phytochemical, Pharmacological, Biochemical and Antimicrobial disciplines, but research about its fungal diseases and control is inadequate (Yusuf *et al*. 2009 and Azmi and Qureshi 2012).

Fungicides are chemical compounds used to kill parasitic fungi or their spores. Fungi can cause serious damage in agriculture, resulting in critical losses of yield, quality and profit. Some fungicides are dangerous to human health. Plants and other organisms have chemical defenses that give them an advantage against microorganisms such as fungi. Some of these compounds can be used as fungicides. Due to environmental concerns of controlling fungal disease by toxic chemicals, researchers have focused their efforts on developing alternative methods of controlling fungal diseases. Plant extracts can be successfully exploited in modern agriculture which has recently attracted the attention of several workers. Plant constituents have been reported to be successful fungitoxicants because of low phytotoxicity, easy biodegradability and favorable effects for the growth of the host (Fawcett and Spencer 1970, Panday *et al*. 1983 and Nene and Thapliyal 1993). Extracts obtained from many plants have recently studied for their antifungal activities (Monoharachary and Reddy 1978, Miah *et al*. 1990, Hosen *et al*. 2016).

The present investigation has been undertaken to evaluate the fungi toxicity of some fungicides and plant leaves extracts *in vitro* against test pathogen *C. gloeosporioides* isolated from *R. serpentina.*

Materials and Methods

Infected leaves of *R. serpentina* were collected from field of Botanical garden, Curzon Hall Campus, Dhaka University, during the period of April, 2007 to August, 2012. In the year 2013 in addition to Botanical garden, Curzon Hall campus Dhaka University, infected leaves of *R. serpentina* were collected from Gazipur, Dhaka, Lawachara, Sylhet, Botanic garden, Chittagong University campus and Bangladesh Agricultural University

campus Mymensingh. Collected samples were examined and associated fungi were isolated.

Isolation of fungi: The associated fungi were isolated following 'Tissue planting' method on PDA medium. Identification of the fungi were done following standard methods. All the isolated fungi were tested for their pathogenic potentiality.

Sub-culturing of test pathogen: Pure culture of *C. gloeosporioides* from culture slant was inoculated on PDA plates and incubated for seven days in an incubator at 25° C. Five mm mycelial block from Petri plates was used for *in vitro* control of test pathogen.

The experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka, Bangladesh.

Preparation of fungicides at different concentrations: Ten fungicides with different active ingredients, *viz*., Amcogin 50 WP (50% Carbendazim, MCO Pesticide Ltd. India ACI Formulations), Autostin 50 WDG (50% Carbendazim, ISACAM Co., Hong Kong), Capvit 77 WP (Copper oxychloride, Asia Trade International), Dithane M 45 (80% Mancozeb, Bayer Crop Science Ltd.), Greengel 72 WP (64% Mancozeb, 8% Metalaxil, Green Care Bangladesh), Haybit 80 WG (Abamactin, Syngenta (BD) Ltd), Oxivit 40 SC (Copper Oxichloride, Chemet Wets & Flows Pvt. Ltd.), Ridomil Gold MZ (Metalaxil-Mancozeb, Syngenta (BD) Ltd.), Sulcox 50 WP (Copper Oxichloride, Haychem, Bangladesh) and Tilt 250 EC (Propiconazole, Syngenta (BD) Ltd.) were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. The selected fungicides were evaluated at 100, 200, 300, 400 and 500 ppm concentrations for their *in vitro* efficacy against *C. gloeosporioides* the causal agent of anthracnose of *R. serpentina.* Concentration of fungicides was prepared following Chowdhury *et al.* (2015).

Preparation of plant extracts at different concentrations: Ethanol extracts of leaves of ten plants *viz., Adhatoda vesica* Nees, *Azadirachta indica* A. Juss, *Citrus limon* (L.) Burm.f., *Curcuma longa* L., *Lantana camera* L., *Moringa oleifera* Lam., *Ocimum sanctum* L., *Psidium guajava* L., *Thuja orientalis* L. and *Vitex negundo* L. were selected to evaluate their efficacy at 5, 10, 15 and 20% concentrations against the same pathogenic fungus. The leaves were thoroughly washed in tap water, air dried and were 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, 15 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.

The fungitoxicity 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.

The results were statistically analyzed following computer package MSSTAT-C and means were compared using DMRT.

Results and Discussion

A total of 12 fungal species, namely *Alternaria alternata* (Fr.) Keissler, *Aspergillus niger* van Tieghme, *Colletotrichum gloeosporioides* (Penz.) Sacc., *Fusarium* spp., *Macrophoma* sp., *Nigrospora* sp., *Penicillium* spp., *Pestalotiopsis guepinii*, *Rhizopus stolonifer* (Ehrenb. ex. Fr.) Lind, and *Trichoderma viride* were isolated from anthracnose symptom of *R. serpentina*. Among the isolated fungi *C. gloeosporioides* were found to be pathogenic to *R. serpentina* (Yasmin and Shamsi 2015).

In the present investigation, among 10 fungicides tested complete inhibition of the radial growth of *C. gloeosporioides* was observed with Amcogin 50 WP, Autostin 50 WDG and Tilt 250 EC at all the concentrations used (Table1 and Plate 1. A-C.).

Capvit showed complete inhibition of the test fungus at 300, 400 and 500 ppm concentrations. Maximum 30.92% inhibition of the test fungus was recorded by Dithane M-45 at 500 ppm concentration. Diathane M-45 was not so good fungicide against the test fungus. Greengel showed maximum 48.70% radial growth of test fungus at 500 ppm concentrations. Haybit and Sulcox showed complete inhibition of the test fungus at 400 and 500 ppm concentrations. Oxivit showed 46.63 and 60.67% growth inhibition against the test fungi at 400 and 500 ppm concentration, respectively.

Performance of Ridomil MZ Gold is better than Diathane, Greengel and Oxivit. This fungicides completely inhibited the radial growth of the test fungus at 300, 400 and 500 ppm concentrations.

Table 1. Fungi toxicity of fungicides against *Colletotrichum gloeosporioides* at different concentrations.

Name of fungicides	% inhibition of radial growth of test fungus at different concentrations (ppm)				
	100	200	300	400	500
Amcogin	100 [a]	100[a]	100[a]	100[a]	100[a]
Autostin	100[a]	100[a]	100[a]	100[a]	100[a]
Capvit	46.41[b]	62.09[b]	100[a]	100[a]	100[a]
Diathane	6.04[d]	11.35[e]	15.46[f]	21.98[c]	30.92[d]
Greengel	24.64[c]	32.46[c]	39.71[d]	45.80[b]	48.70[c]
Haybit	24.84[c]	35.29[c]	46.41[c]	100[a]	100[a]
Oxivit	3.93[f]	20.79[d]	28.09[e]	46.63[b]	60.67[b]
Ridomil	3.08[f]	6.67[f]	100[a]	100[a]	100[a]
Sulcox	42.94bc	66.10b	73.45[b]	100[a]	100[a]
Tilt	100[a]	100[a]	100[a]	100[a]	100[a]
CV%	12.48	4.44	2.32	2.65	1.91

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT. Efficiency gradient of fungicides against *Colletotrichum gloeosporioides* at 100 ppm concentration: Amcogin >Autostin 50 WDG/ / Tilt 250 EC > Capvit > Ridomil Gold MZ 68 WG > Sulcox > Hayvit > Oxivit > Greengel > Diathane M 45.

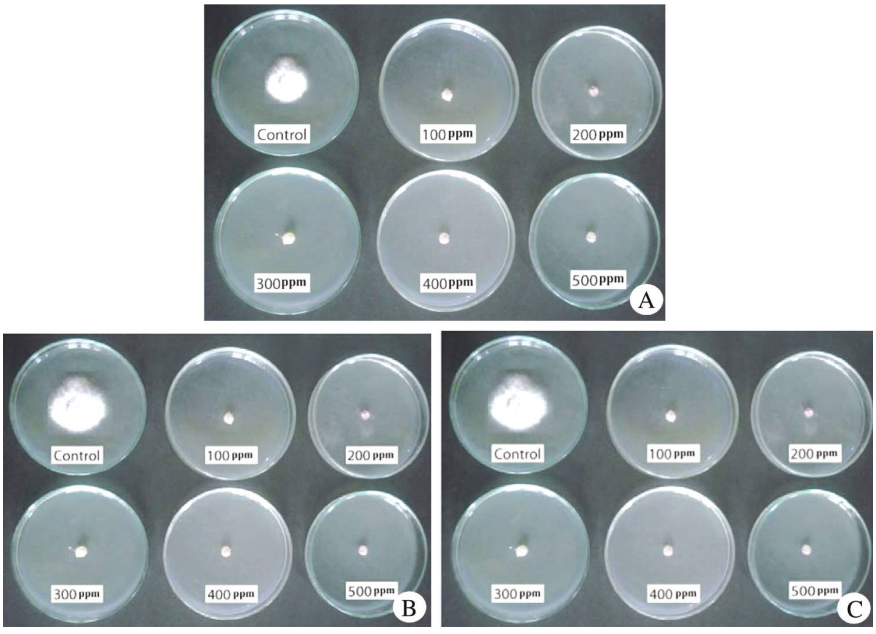

Plate 1. Fungi toxicity of fungicides against *Colletotrichum gloeosporioides* at different concentrations: A. Amcogin, B. Autostin and C. Tilt.

Laboratory evaluation of fungicides revealed that all the fungicides causes partial or complete inhibition of *C. gloeosporioides*, at all the concentration tested. Imtiaj *et al.* (2005) reported that Diathane M-45 and Ridomil MZ Gold were effective in controlling conidial germination of *C. gloeosporioides*, the causal agents of mango at 500~1000 ppm concentrations.

Table 1 presented that amongst the ten fungicides, Amcogin 50 WP, Autostin 50 WDG and Tilt 250 EC showed best result in controlling the test pathogen *in vitro*. Shamsi *et al.* (2014) reported that radial growth of *C. gloeosporioides* isolated from *Senna alata* completely inhibited by Tilt 25 EC at all the concentrations used. Sharma and Verma (2007) reported that Bavistin check the growth of *C. gloeosporioides* causal agent of anthracnose of mango (*Mangifera indica* L.), completely at 100 ppm. Hosen *et al.* (2016) observed complete inhibition of the growth of the jute pathogen *C. gloeosporioides* with Bavistin DF, Greengel 72 WP and Tilt 250 EC at 100, 200 and 400 ppm, respectively.

Results of plant extracts on the radial growth of *C. gloeosporioides* are presented in Table 2. All the plant extracts showed varied degree of growth inhibition of the pathogen at different concentrations. Out of the ten plant extracts, *A. indica, C. limon, M. oleifera* and *P. guajava* completely inhibited radial growth of test pathogen *C. gloeosporioides* at 5, 10, 15 and 20% concentrations. Leave extracts of *A. vasica, C. longa, L. camara, O. sanctum, T. orientalis* and *V. nigundo* showed 80.66, 84, 67.94, 88.60, 71 and 42.92% radial growth of test fungus at 20% concentration. The per cent inhibition of the pathogens increases with the increase of the concentration of the plant extracts in culture medium.

Prasad and Anamika (2015) reported that the extracts of *L. camara* were found to be most effective for the control of the *C. gloeosporioides.* Imtiaj *et al.* (2005) found that plant extracts such as *C. longa* was also most effective against *C. gloeosporioides* the causal agents of anthracnose of mango. Hosen *et al.* (2016) also observed inhibition of the growth of the jute pathogen *C. gloeosporioides* with *C. limon, D. metel* and *A. indica* at 20% concentration. Plant parts and their constituents of some higher plants have already been reported to be of successful nature of fungitoxicants, lesser phytotoxicity, systemicity, easily biodegradability and favourable effects of the growth of the host (Fawcett and Spencer 1970, Pandey *et al.*1983). Chakraborty *et al.* (2009) reported the efficacy of various cell free extracts of the plants against the growth inhibition of the pathogen. The effectiveness of extracts varied significantly with dosage, where 100% inhibition of the pathogen was achieved both with neem and garlic extracts.

Table 2. Fungi toxicity of plant extracta against *Colletotrichum gloeosporioides* at different concentrations.

Name of plants	% inhibition of radial growth of the pathogen at different concentrations (%)			
	5	10	15	20
Adhatoda vasica	52.11[b]	63.33[b]	78.31[a]	80.66[a]
Azadirachta indica	100[a]	100[a]	100[a]	100[a]
Citrus limon	100[a]	100[a]	100[a]	100[a]
Curcuma longa	50.00[b]	53.0[b]	67.50[a]	84.00[a]
Lantana camara	40.67[c]	49.44[b]	56.87[b]	67.94[b]
Moringa oleifera	100[a]	100[a]	100[a]	100[a]
Ocimum sanctum	62.77[b]	50.71[b]	71.21a	88.60[a]
Psidium guajava	100[a]	100[a]	100[a]	100[a]
Thuja orientalis	27.67[c]	32.22[b]	64.89[a]	71.00[a]
Vitex negundo	18.85[NS]	37.37[c]	39.92[b]	42.92[b]
CV%	1.63	1.26	1.37	1.51

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT. NS = Not significant. Efficiency gradient of plant extracts against *Colletotrichum gloeosporioides* at 20% concentration: *Azadirachta indica* > *Citrus limon* > *Moringa oleifera* > *Psidium guajava* > *Ocimum sanctum* > *Curcuma longa* > *Adhatoda vasica* > *Thuja orientalis* > *Lantana camara* > *Vitex negundo.*

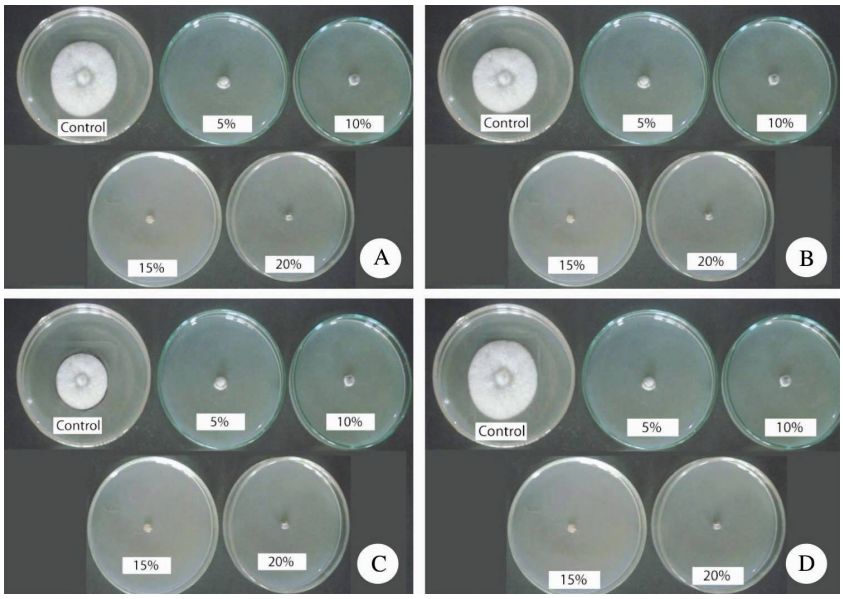

Plate 2. Fungi toxicity of plant extracts against *Colletotrichum gloeosporioides* at different concentrations: A. *Azadiracta indica* B. *Citrus lemon*, C. *Moringa oleifera* and D. *Psidium guajava.*

Ethanol extract of *Azadirachta indica* showed complete inhibition of radial growth of *C. gloeospoiroides* the causal agents of anthracnose of *Senna alata* at all concentratios used. *Citrus medica*, *Datura metel*, *Mangifera indica*, *Senna alata* and *Tagetes erecta* at 10 and 20% concentrations, were also capable of complete inhibition of radial growth of the fungus *C. gloeosporoides* isolated from *S. alata* (Shamsi *et al*. 2014). Rahul and Anamika (2015) reported that ethanol leaf extract of *Lantana camara* has the potential antifungal compound to control *C. gloeosporioides* the casual organism of papaya anthracnose. They also found the significant inhibitory effect of lemon leaf in controlling the redial growth of *C. gloeosporioides.*

Conclusion

Fungicide Amcogin 50 WP, Autostin 50 WDG and Tilt 250 EC were found to be significantly effective against *C. gloeosporioides* anthracnose of *R. serpentine*. These fungicides may be used in field trial to confirm their efficacy in controlling anthracnose of *R. serpentina.* Effect of ethanol plant extract of *Azadirachta indica, Citrus limon, Moringa oleifera* and *Psidium guajava* showed promising prospect in controlling radial growth of *C. gloeosporioides* the causal agents of anthracnose of *R. serpentina.* These ecofriendly plant extract should be studied in field experiment to evaluate their efficacy in controlling anthracnose of *R. serpentina* in field condition.

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