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IN VITRO EVALUATION OF FUNGICIDES AND SOME PLANT EXTRACTS AGAINST RICE SHEATH ROT PATHOGEN *SAROCLADIUM ORYZAE*

Shamim Shamsi* and Pranami Chowdhury[1]

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

Sarocladium oryzae (Sawada) W. Gams & D. Hawksworth, the causal agent of sheath rot of rice was isolated from sheath rot infected rice sheaths and grains. Among ten tested fungicides Tall 25 EC completely inhibited radial growth of the fungus even at the lowest concentration 100 ppm. Similarly ethanol leaf extract of ten plants, namely *Allium sativum, Artocarpus heterophyllus*, *Asparagus racemosus, Azadirachta indica, Citrus medica, Datura metel*, *Mangifera indica, Nerium indicum, Senna alata* and *Tagetes erecta* at 5, 10 and 20% concentrations were screened for their fungicidal activity against the test fungus. All the plant extracts completely inhibited the radial growth of the test fungus at 20% concentration except *Asparagus racemosus* and *C. medica*. Ethanol extract of *Tagetes erecta* and *Mangifera indica* also completely inhibited the radial growth of the test fungus at 10% concentration.

Key words: *In vitro* evaluation, Fungicide, Plant extract, Rice sheath rot

Introduction

Rice plant (*Oryza sativa* L.) belongs to Poaceae. Sheath rot of rice is one of the major diseases of rice. Sheath rot pathogen infects the rice plant at all growth stages, but it is most destructive when infection occurs before the emergence of the panicle. The causal agent of sheath rot *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksworth was reported from India, USA and Bangladesh (Agnihothrudu 1973, Shahjahan *et al.* 1977, Miah *et al.* 1985 and Shamsi *et al.* 1995, 2010). It is externally and internally seed borne pathogen and transmitted through seed to seedling (Shamsi 1999).

The disease has caused 20 - 85% yield loss in Taiwan, and 30−80% in Vietnam, the Philippines, and India (Chen 1957). In Japan, affected areas range from 51,000 - 122,000 hectares and annual losses are estimated to be 16,000 - 35,000 tons (Rice Knowledge Bank 2016). In India Chakravarty and Biswas (1978) reported yield losses of 10 - 26% (average estimate of 14%). In the Punjab (India), averages of 30% disease incidence and 70% disease severity, with 15 - 35% grain chaffiness were reported. In severe cases, 100% seed sterility and no panicle emergence were observed (Raina and Singh 1980). In 1982, sheath rot caused losses of up to 50% (Kang and Rattan 1983). Estrada *et al.* (1984) reported significantly fewer spikelets per panicle and lower 1000-grain weight for severely diseased panicles compared with healthy and estimated total yield loss due to sheath rot at 53%. In Thailand, the yield loss for cultivar RD23, which was severely

*Author for correspondence: <Prof.shamsi@gmail.com>. [1]Present address: Directorate of Secondary and Higher Education, Dhaka, Bangladesh.

damaged by sheath rot, was estimated to be above 35% (Surin *et al*. 1988). Bigirimana *et al.* (2015) reported 85% yield loss of rice due to sheath rot. Yield loss in Bangladesh due to incidence of this disease in different seasons was found to be 18.9 - 47.4% (Shahjahan *et al*. 1994). Thus, sheath rot disease is very destructive to rice crops. Shamsi (1999) reported significant yield loss due to sheath rot in BR11, BR16 and Purbachi due to sheath rot.

Though extensive work has been done on sheath rot disease and its causal agent (Shamsi 1998, 1999, Patel and Jasrai 2012, Pareks and Chandra 2007 and Riddhi *et al.* 2013) but work on control of sheath rot disease and its causal agent is inadequate. Sheath rot disease has been spreading among the newly released varieties (Shamsi *et al.* 2010). Present research was undertaken to screen suitable fungicides and plant extracts to control the sheath rot pathogen *S. oryzae in vitro.*

Materials and Methods

Sheath rot infected panicles with flag leaf sheaths were collected from experimental field plots of BRRI (Bangladesh) and farmers field around Gazipur area, Dhaka during July, 2014 to June, 2015 at booting, panicle initiation and grain stages of rice varieties BR11, BR12, BR16, BRRI25 - BRRI34 and Kataribhog. Thirty samples with sheath rot symptom was examined to detect the causal agent. Pathogen was isolated from the infected sheaths and grains following Blotter method and Tissue Planting method on PDA medium (Shamsi *et al*. 2010). Microscopic observation of the fungus was recorded on cover slip culture of the fungus. Identification of the fungus was determined following Ou (1985).

Pathogenicity of the fungus was done following seed inoculation technique (Chowdhury *et al.* 2015). Rahman and Hossain (2009) reported that in Bangladesh Homai, followed by Bavistin, Tilt, Topsin-M and Tecto caused minimum DI (Disease intensity) in sheath rot. In the present investigation ten fungicides *viz.,* Bavistin 50WP (50% carbendazin methyl benzimidazo-2-yl carbamate), Capvit 50 WP, Dithane M-45, Greengel, Hayvit 80 WP, Indofil M-45 (80% mancozeb manganese ion + ethylene bisdithio carbamate), Ridomil MZ Gold, Salcox 50WP (copper oxychloride), MC sulphur 80WP (80% sulphur) and Tall 25 EC (propiconazole) were selected due to their effectiveness against major rice diseases (Chowdhury *et al.* 2015). Fungicides were collected from Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka.

For each fungicide, a stock solution having the concentrations of 10,000 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 fungus and then it was inoculated at the centre of the plate. Three replications were maintained in both cases.

Ten angiospermic plants *viz. Allium sativum* L. *Artocarpus heterophyllus* Lam., *Asparagus racemosus* Willd., *Azadirachta indica* L., *Citrus medica* L., *Datura metel* L., *Mangifera indica* L., *Nerium indicum* Mill., *Senna alata* L. and *Tagetes erecta* L. with antifungal properties were

selected for *in vitro* evaluation of their ethanol leaf extracts on the vegetative growth of the test pathogen. Ethanol leaf extracts at 5, 10 and 20% concentration were evaluated against the pathogenic fungi following poison food techniques (Shamsi *et al*. 2014).

Bulb of *A. sativum* and fresh leaves of rest of the nine plants was separately washed in tap water, air dried and was 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 minutes 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.

Sarocladium oryzae was also grown on PDA plates with 5, 10 and 20% of ethanol. PDA plate without addition of plant extract served as control.

The radial growth of the colonies was measured at the fifth day of incubation. The per cent growth inhibition of the test fungus was calculated by using the following formula:

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

where, I = per cent growth inhibition, C = growth in control and T = growth in treatment.

Results and Discussion

The sheath rot pathogen *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksworth mainly attacks the uppermost leaf sheaths enclosing the young panicles. The lesions start as oblong or somewhat irregular spots 0.5 - 1.5 cm long, with brown margins and grey centre, or they may be greyish-brown throughout (Ou 1985). They enlarge and often coalesce and may cover most of the leaf sheath. The young panicles remain within the sheath, do not develop further and rot, or only partial panicle emergence may be observed. Young lesions show whitish, powdery growth both outside and inside affected sheaths. Old lesions have less or no powdery growth of the fungus and appear as dry, brownish lesions with the enclosed panicle rotted. Spikelets extended outside the rotted sheath normally develop, but are usually highly discoloured (brown) and may be partially filled and infected by the pathogen.

Sarocladium oryzae (Sawada) W. Gams & D. Hawksworth (**Fig. 1. A-B**)

Colony appears white compact or cottony, reverse yellowish pink. The fungus produces whitish, sparsely branched, septate mycelia. Conidiophores arising from mycelia slightly thickened from hyphae, branched once or twice, each times with 3 - 4 branches in a whorl. The main axis 14 - 23 × 1.5 - 2.0 µm. The terminal branches are tapering towards the apex. Conidia hyaline, smooth, aseptate, cylindrical, 2 - 0 - 14 × 1.5 - 1.8 µm (Fig. 1. A-B).

Amongst the ten fungicides used in the present investigation, Bavistin, Dithane M-45, Greengel, Ridomil and Indofil were systemic while Capvit, Hayvit, Salcox, Sulphur and Tall 25 EC were protective fungicides. Tall 25 EC completely inhibited the radial growth of *S. oryzae* at all the concentrations used. Bavistin and Salcox showed complete inhibition of radial growth at 300, 400 and 500 ppm concentrations. Capvit showed 100% inhibition of radial growth at 400 and 500 ppm concentrations. Rest of the fungicides showed 46.42 - 70% radial growth of the fungus at 500 ppm concentration (Fig. 2).

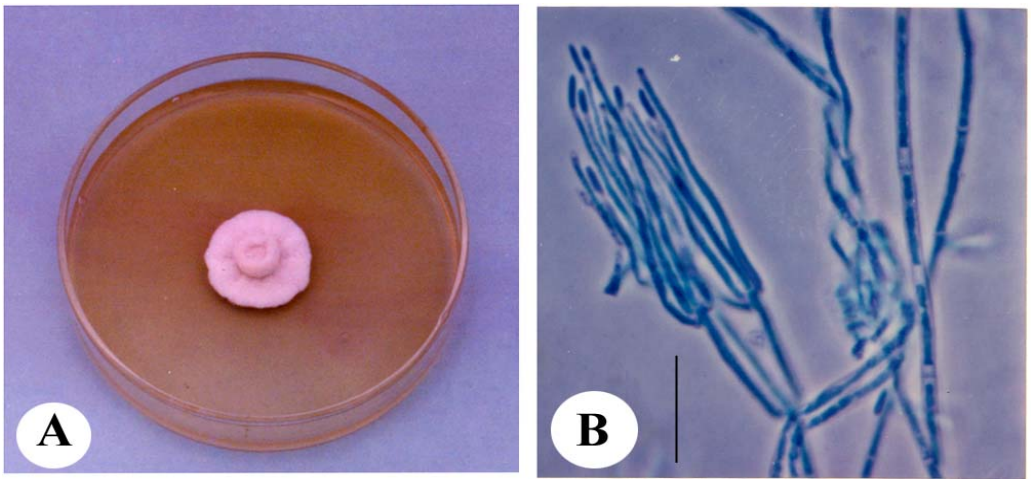

Fig. 1. *Sarocladium oryzae* : A. Ten days colony on PDA plate and B. Hyphae, conidiophores and conidia of the fungus. (Bar = 25 µm).

Farid *et al*. (2002) reported that four fungicides *viz.* Bavistin, Hinosan, Tilt 250 EC and Dithane M-45 were effective against *Bipolaris oryzae*. Dithane M-45 was the best with 100% reduction of the prevalence of the pathogen and inhibited the mycelial growth at 0.3% of the seed weight as seed treatments and 500 ppm as mycelial growth inhibition test followed by Tilt 250 EC, Hinosan and Bavistin. All the test fungicides were effective against *Bipolaris oryzae* at higher concentration.

The plant world is a rich store house of natural chemicals. Most of the higher plants contain rich diversity of bioactive PSMS like phenols, flavanoids, quinones, tannins, alkaloids, saponins, sterols and terpenoids responsible to play a defensive role in the plants. Such plant chemicals contribute to diverse biological activities such as antimicrobial, allelopathic, antioxidant and bio-regulatory properties and these natural products thus can certainly substitute harmful synthetic fungicides for plant disease control (Patel and Jasrai 2010, 2012, Riddhi *et al*. 2013 and Ahmed *et al*. 2013).

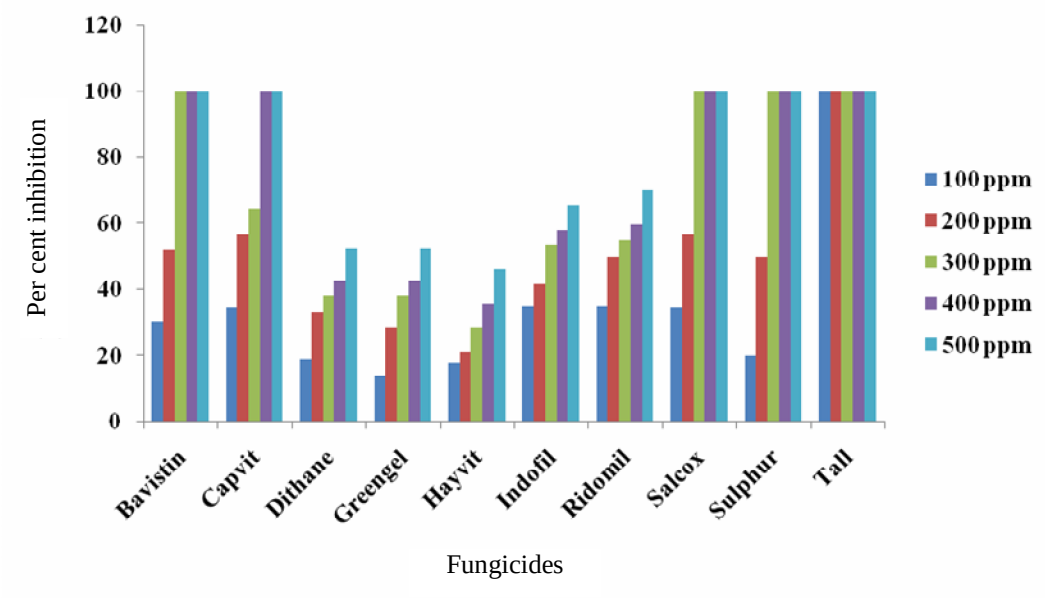

Fig. 2. Per cent inhibition of radial growth of *Sarocladium oryzae* owing to fungicides at different concentrations.

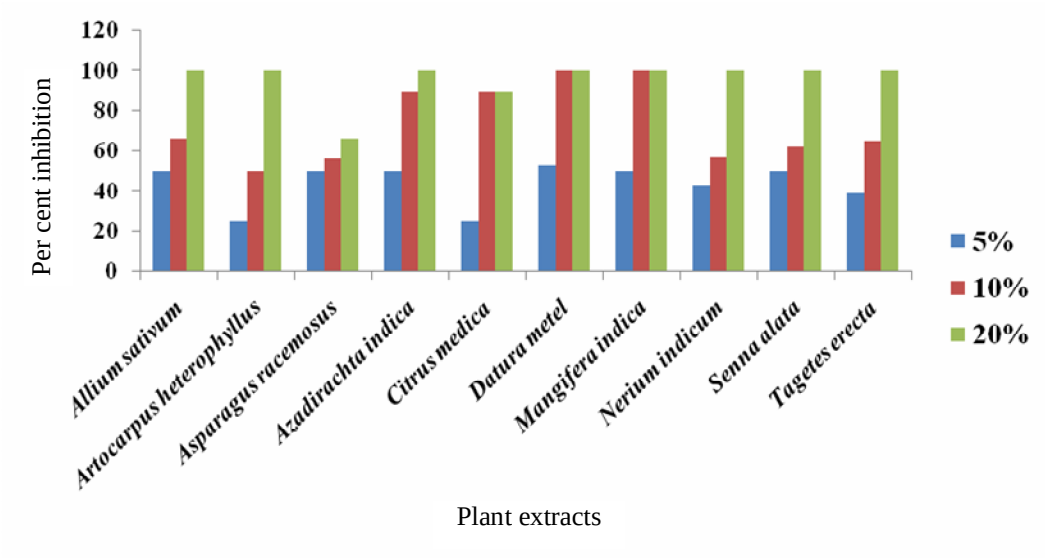

Fig. 3. Per cent inhibition of radial growth of *Sarocladium oryzae* owing to plant extracts at different concentrations.

Twenty per cent ethanol extract of *Allium sativum*, *Artocarpus heterophyllus*, *Azadirachta indica*, *Datura metel*, *Mangifera indica*, *Nerium indicum*, *Senna alata* and *Tagetes erecta* completely inhibited the radial growth of *S. oryzae.*

Ten per cent ethanol extract of *Datura metel* and *M. indica* also completely inhibited the radial growth of test pathogen. Ten per cent ethanol extract of eight plants i.e. *A. indica, A. sativum, A. heterophyllus, A. racemosus, C. medica, N. indicum, S. alata* and *Tagetes erecta* showed 90, 66.66, 50, 56.66, 90, 66.66, 57.14, 62.5 and 65.22 % inhibition of radial growth of the test fungus, respectively.

Five per cent ethanol extract of *D. metel* showed maximum 52.94 % inhibition of radial growth of the test fungus. *Citrus medica* and *A. heterophyllus* showed minimum 25% inhibition of radial growth of the test fungus at the same concentration (Fig. 3).

The test pathogen was also grown on PDA medium with ethanol excluding plant extracts. Twenty per cent inhibition of radial growth of the test fungus was recorded at 10% concentration of ethanol whereas ethanol extract of *D. metel* and *M. indica* completely inhibited the radial growth of the test fungus at the same concentration.

Mohana *et al.* (2011) reported that methanol extract of *Acacia nilotica, Caesalpinia coriaria, Decalepis hamiltonii, Emblica officinalis,* and *Lawsonia inermis* showed significant antifungal activity at 3500 µg/ml concentration on seed pathogens *viz. Alternaria alternata, Aspergillus flavus, Curvularia lunata, Drechslera oryzae, D. halodes, Fusarium moniliforme, Pyricularia oryzae* and *Trichoconis padwickii* by poisoned food technique.

Yeasmin *et al.* (2012) reported seed borne fungi of rice, namely *Aspergillus flavus, A. niger, Bipolaris oryzae, Curvularia oryzae, Fusarium oxysporum, F. moniliforme, Nigrospora oryzae,* and *Penicillium* sp., where prevalence of *Bipolaris oryzae* (7.5%) and *F. moniliforme* (8.3%) were the maximum. They treated the fungi with Provax and garlic extract and observed that due to treatment the seed borne fungi was reduced up to 100% over the control. They also reported that Provax was found to be best and similar to garlic (1 : 1) extract against seed borne pathogens of rice.

Present investigation indicates that Bavistin 50 WP, Salcox 50 WP and MC sulphur 80 at 300 ppm concentration are capable of complete inhibition of the radial growth of *S. oryzae*. Tall 25 EC at 100 ppm is capable of complete inhibition of the radial growth of *S. oryzae*. Similarly 10% ethanol extract of *D. metel, M. indica* also completely inhibited the radial growth of the fungus.

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