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Original Article

In vitro evaluation of fungicides and plant extracts against pathogenic fungi of jute seeds

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ABSTRACT: Two pathogenic fungi *viz*. *Curvularia lunata* (Wakker) Boedijn and *Fusarium oxysporum* Schlecht were isolated from different accessions of *Corchorus capsularis* L. collected from Bangladesh Jute Research Institute (BJRI). "Blotter", "Paper towel" and "Agar plate" methods were used to isolate the fungi. Antifungal properties of six extracts obtained from six higher plants namely *Allium sativum* L., *Azadirachta indica* A. Juss., *Citrus grandis* Merr., *C. limon* (L.) Burm.f., *Datura metel* L. and *Zingiber officinale* Rosc. at 5, 10 and 20% concentrations were evaluated against the test pathogens. As regards the plant extracts, the most promising fungitoxic effect was recorded in case of *Allium sativum* L. against *Curvularia lunata* and *Fusarium oxysporum*. Five fungicides with different active ingredients *viz*., Bavistin DF, Capvit 50 WP, Dithane M-45, Green gel and Tilt 250 EC were selected to evaluate their *in vitro* efficacy at 100, 200, 400 and 500 ppm concentrations against the test pathogens. Dithane M-45 and Tilt 250 EC completely inhibited the radial growth of *Curvularia lunata* at all the concentrations while Bavistin DF completely inhibited the radial growth of *Fusarium oxysporum* at all the concentrations. The present investigation revealed that Tilt 250 EC, Dithane M-45, and Capvit were the best inhibiting agent against the *in vitro* growth of *C. lunata* and *F. oxysporum.*

KEYWORDS: Fungicides, Pathogenic fungi, Plant extracts, Jute seed

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INTRODUCTION

Jute is the most important cash crop of Bangladesh and plays an important role in the economy of Bangladesh. Among the jute growing countries of the world, Bangladesh ranks second in respect of production[1,2]. Nearly 12-15% of the jute products are exported to about 20 countries of the world earning foreign exchange to the tune of Rs. 2000 crores per annum, and the trend is on the increase[3,4].

Like all other commercially important plants, one of the major bottlenecks of jute production is its constant exposure to different biotic and abiotic stresses. The severe yield loss of jute depends on certain factors of which diseases play a major role. In Bangladesh, yield loss due to diseases is about 8-20% depending on the severity of the diseases[5].

Nowadays, many inorganic and organic fungicides are used frequently to control plant diseases[6]. So far an appreciable amount of work has been done on the control of seed borne pathogens of jute by fungicidal seed treatment[7]. Very few works have been done for the control of seed-borne diseases of jute by plant extracts[8]. In recent years, some researches on the fungitoxicity of extracts of various parts of higher plants have indicated the possibility of their exploitation as natural fungitoxicants for controlling plant diseases[9-13]. Plant extracts are cheap, can be easily prepared and whenever required. Ahmed and Sultana[5] and Miah *et al*.[14] also showed resistant effect of plant extract to the disease without any phytotoxicity to the host. A lot of researches have been done home and abroad on jute but information on storage mycoflora of jute seeds and its control is inadequate. The present investigation was undertaken to achieve the aim of controlling seed borne pathogens of jute and recommend the best inhibiting agent to control them.

MATERIALS AND METHODS

The research work was undertaken with the collaboration of Genetic Resource and Seed Division of Bangladesh Jute Research Institute (BJRI) situated at Manik Mia Avenue, Farmgate, Dhaka 1207. Twenty one different accessions of seeds of *Corchorus capsularis* L. were taken from the Genetic Resource and Seed Division of BJRI. The collected seed samples were kept in brown paper bag and stored immediately in a dry safe place in the laboratory until used for the experiments.

For the study 400 seeds were taken from each sample following Tissue planting method, Blotter method and Paper towel method. Blotter moist chamber were made by placing 3 layers of filter paper at the bottom of the Petri plate and covered with its upper part. Each Petri plate was moistened by adding distilled water and sterilized under 15 lb pressure, 120ºC temperature for 30 minutes in an autoclave. Seeds were surface sterilized with 10% chlorox for 3 minutes and washed three times with sterilized water and placed on the filter paper (inside the Petri plate) and kept in room condition.

In tissue planting method surface sterilized seeds as mentioned above were placed on sterilized potato dextrose agar medium in Petri plate. Each Petri plate contained 15 ml of PDA with 1 drop of lactic acid (0.03 ml) which was used to check the bacterial growth. Inoculated Petri plates were incubated for 5-7 days at 25 ± 2º C.

Fungal population growing from the plated seed were sub-cultured and maintained on PDA slants. Identification of the isolates was determined following

standard literatures[15-21]. Percentage of prevalence of fungi in different specimens was also recorded. Pathogenicity of the test fungi was done following seed inoculation technique[22].

For evaluating *in vitro* effect of plant parts extracts on the radial growth of the test pathogens, six higher plants namely *Allium sativum* L., *Azadirachta indica* A. Juss., *Citrus limon* (L.) Burm. f., *Citrus grandis* Merr., *Datura metel* L. and *Zingiber officinale* Rosc. were selected. The plant parts were collected from the Botanical Garden, Curzon Hall, Dhaka University Campus, Dhaka. The desired parts of each plant were thoroughly washed in tap water, air dried and then used for fresh extract preparation. Leaf extracts were prepared by crushing known weight of fresh materials with distilled water in ratio of 1:1 (w/v). The pulverized 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 and the filtrate was collected in 250 ml Erlenmeyer flasks. In this method, the requisite amount of the filtrate of each plant extract was mixed with PDA medium to get 5, 10 and 20% concentration.

Five fungicides with different active ingredients *viz*., Bavistin DF, Capvit 50 WP, Dithane M-45, Greengel and Tilt 250 were collected from the Krishi Upokoron Biponi Kendro, Khamarbari, Farmgate, Dhaka. At first, *in vitro* fungitoxicity of these fungicides at 500ppm concentration were evaluated against the test pathogens following poison food technique. 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 100, 200, 400 and 500 ppm. The concentrations of fungicides were 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 5 mm mycelial agar disc cut from the margin of actively growing culture of the test pathogen.

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 the 5-7th day of incubation. The growth inhibition of each test fungi was calculated by using the following formula:

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

Where, I = Percent growth inhibition

C = Growth in control

T = Growth in treatment

The results were statistically analyzed by "t" test following Steel and Torrie (1960)[23].

RESULTS AND DISCUSSION

It was revealed that two pathogenic fungi were found to be associated with the seeds of twenty one jute accessions. Isolated pathogens were *Curvularia lunata* (Wakker) Boedijn and *Fusarium oxysporum* Schlecht.

All the six extracts obtained from six higher plants showed fungitoxicity against both the test pathogens at 5, 10 and 20% concentrations. As regards the plant extracts, the most promising fungitoxic effect was recorded in case of *Allium sativum* L. against *Fusarium oxysporum* (100%) and *Curvularia lunata* (100%) (Tables 1-2). Efficacy of garlic extracts in controlling seed-borne fungal infection in different crops has also been reported by several workers[24-27].

The order of effectiveness of plant parts extracts against *Fusarium oxysporum* at 20% concentration was *Allium sativum* (100%) > *Citrus limon* (85.70%) > *Zingiber officinale* (78.94%) > *Azadirachta indica* (55.26%) > *Datura metel* (50.94%) > *C. grandis* (45.45%) (Table 1).

The order of effectiveness of plant parts extracts against *Curvularia lunata* at 20% concentration was *Allium sativum* (100%) > *Zingiber officinale* (69.38%) > *Azadirachta indica* (63.26%) > *Citrus limon* (58.33%) > *C. grandis* (56.75%) > *Datura metel* (43.10%) (Table 2).

Table 1. Effects of plant extracts on the radial growth of *Fusarium oxysporum* at different concentrations.

Sl. No.	Name of plant extracts	% inhibition of radial growth at different concentrations (%)		
		5	10	20
1.	*Allium sativum*	100^{a}	100^{a}	100^{a}
2.	*Azadirachta indica*	47.36^{a}	50.00^{a}	55.26^{a}
3.	*Citrus grandis*	3.03^{NS}	15.15^{c}	45.45^{b}
4.	*C. limon*	14.28^{b}	71.42^{a}	85.70^{a}
5.	*Datura metel*	47.16^{a}	49.05^{a}	50.94^{a}
6.	*Zingiber officinale*	26.31^{b}	31.57^{b}	78.94^{a}

a, b and c indicate significance at 0.1, 1 and 5% level, respectively. In a row, figures with same letter do not differ significantly whereas figures with dissimilar lettet differ significantly (as per DMRT), NS = Not significant.

Extracts of *Azadirachta indica* (neem) and *Zingiber officinale* were moderately effective while extracts of *Datura metel* and *Citrus grandis* were not so effective in controlling the test pathogens (Tables 1-2). Rahman *et al.* (1999) also found moderate effect of neem extract against fungi associated with wheat seeds[25]. Using the plant extracts of six higher plants at 5, 10, 20% concentrations, it was found that fungitoxic effect of these plant extracts increased with increasing concentrations (Tables 1-2). It was found that although not promising but still the fungitoxic effect of these plant extracts persisted even at 5% concentration (Tables 1-2).

Table 2. Effects of plant extracts on the radial growth of *Curvulari lunata* at different concentrations.

Sl. No.	Name of plant extracts	% inhibition of radial growth at different concentrations (%)		
		5	10	20
1.	*Allium sativum*	100[a]	100[a]	100[a]
2.	*Azadirachta indica*	48.97[a]	57.14[a]	63.26[a]
3.	*Citrus grandis*	2.70[NS]	13.50[c]	56.75[a]
4.	*C. limon*	37.50[b]	47.90[a]	58.33[a]
5.	*Datura metel*	39.65[a]	41.37[a]	43.10[a]
6.	*Zingiber officinale*	34.69[a]	53.06[a]	69.38[a]

Abbreviations are same in Table T.1.

This observation suggested that fungitoxicity of the plant extracts that have been found to be promising against both the test pathogens could be increased further by using these plant extracts at higher concentrations.

Amongst the five fungicides used in the present investigation, Bavistin and Dithane M-45 are systemic while Green gel, Tilt and Capvit are protective fungicides. All the fungicides inhibited the radial growth of the test pathogens but complete inhibition of *Curvularia lunata* was observed with Dithane M-45 and Tilt 250 EC at all the concentrations (Table 4) and complete inhibition of *Fusarium oxysporum* was observed with Bavistin DF at all the concentrations (Table 3).

In case of *Curvularia lunata*, the growth was completely checked with Tilt 250 EC, Dithane M-45, Green gel and Capvit at 500 ppm concentration (Table 4). Bavistin was found to be responsible for 92.10 % inhibition of *C. lunata* at the same concentration (Table 4). In contrast to the present study Chowdhury *et al*. (2015) observed complete inhibition of radial growth of *Curvularia*

Table 3. Per cent inhibition of radial growth of *Fusarium oxysporum* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)			
	100	200	400	500
Bavistin DF	100[a]	100[a]	100[a]	100[a]
Green gel	20.58a	100[a]	100[a]	100[a]
Capvit	2.43[NS]	31.7[0b]	100[a]	100[a]
Dithane M-45	62.50[b]	75.00[b]	100[a]	100[a]
Tilt 250 EC	58.73[a]	71.42[a]	100[a]	100[a]

Abbreviations are same in Table T.1.

Table 4. Per cent inhibition of radial growth of *Curvularia lunata* at different concentrations of fungicides.

Name of fungicides	% inhibition of radial growth at different concentrations (ppm)			
	100	200	400	500
Bavistin DF	17.70[b]	27.08[a]	50.00[a]	92.10[a]
Green gel	52.20[a]	59.70[a]	100[a]	100[a]
Capvit	75.40[a]	65.50[a]	93.44[a]	100[a]
Dithane M-45	100[a]	100[a]	100[a]	100[a]
Tilt 250 EC	100[a]	100[a]	100[a]	100[a]

Abbreviations are same in Table T.1.

lunata with Dithane at 500 ppm concentration and 80% inhibition with Bavistin at the same concentration[13]. Dithane M-45, Green gel and Tilt 250 EC were responsible for complete inhibition at 400 ppm concentration whereas Bavistin DF and Capvit showed 50 and 93.44% inhibition, respectively (Table 4).
The complete inhibition of radial growth of *Fusarium oxysporum* was observed with all the six fungicides at 400 and 500 ppm concentrations (Table 3). Bavistin DF and Green gel were responsible for complete inhibition at 200 ppm concentration whereas Capvit, Dithane M-45 and Tilt 250 EC showed 31.7, 75 and 71.42% inhibition, respectively (Table 3).

Efficacy of various fungicides against the two test fungi *in vitro* indicates that Dithane M-45, Tilt 250 EC and Capvit showed promising results as compared to others (Table 3-4). The same fungicides also showed different effects on different test pathogens in the present investigation. This variation might be due to selection of different test pathogens. Singh and Singh (1970) observed that reaction of *Fusarium* spp. to fungicides varies from species to species and sometimes even from isolate of the same species[28].

Efficiency gradients observed in the present study expressed that Tilt 250 EC, Dithane M-45, and Capvit were the best inhibiting agent against the *in vitro* growth of the test pathogens.

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REFERENCES

1. Islam, M.M. and Rahman, M.M. 2008. Hand Book on Agricultural Technologies on Jute, Kenaf and Mesta Crops. Bangladesh Jute Research Institute (BJRI) Dhaka. pp. 2.
2. Sadi, S. 2007. Pat Hotter Nepotthe (Factors Behind the Destruction of Jute) Vol. **1**, Dhaka, Bangladesh, Dhaka printers.
3. Hossain, M.A., Mannan, S.A., Sultana, K. and Khandakar, A.L. 1994. Survey on the constrains of quality jute seed at farm level. Agril. Support Service Project (GOB/WORLD BANK/ODA). Dhaka, Bangladesh.
4. Basu, A., Ghosh, M., Meyer, R., Powell, W., Basak, S.L. and Sen, S.K. 2004. Analysis of genetic diversity of jute determined by means of SSR markers and AFLP profiling. Crop Sci. **44**, 678-685.
5. Ahmed, N. and Sultana, K. 1985. Survey on the production and quality jute seed at farm level. Annual Report, Bangladesh Jute Research Institute, Dhaka, pp. 296-323.
6. Mehrotra, R.S. 2000. Plant Pathology. Tata McGraw Hill Publishing Co., New Delhi. pp. X+771.
7. Imam-Fazli, S.F. and Ahmed, Q.A. 1959. Fungus organisms associated with jute seeds and their effect on germinating seeds and seedlings. Agric. Pak. **11**, 393-406.
8. Fakir, G.A. and Khan, A.A. 1992. Control of some selected seed-borne fungal pathogens of jute by seed treatment with garlic extract. Proc. BAU Res. Prog. **6**, 176-180.
9. Bashar, M.A. and Rai, B. 1991. Antifungal activity of extracts of some plant parts against *Fusarium oxysporum* f. sp. *ciceri*. Bangladesh J. Bot. **20**, 219-222.
10. Hossain, K.S. 1993. A study on the fungi associated with damping-off infected seedling of cabbage, cauliflower and kohlrabi of nursery beds of the Dhaka city and the effect of some fungicides, antibiotics and plant extracts on the growth of three isolates. M.S. Thesis, Dhaka University. Dhaka, pp. 4+58.
11. Anwar, M.N., Singh, P., Begum, J. and Chowdhury, J.U. 1994. Antifungal activity of some selected plants on phytopathogenic fungi. Bangladesh J. Life Science. **6**, 23-26.
12. Salma, U. 1995. Fungitoxicity of extracts of twenty higher plants and four fungicides on three fungal plant pathogens. M. Sc. Thesis, Dhaka University, Dhaka. pp. v+42.
13. Chowdhury, P., Bashar, M.A. and Shamsi, S. 2015. *In vitro* evaluation of fungicides and plant extracts against pathogenic fungi of two rice varieties. Bangladesh J. Bot. **44**(2), 251-259.
14. Miah, M.A., Ahmed, T., Sharma, N.R., Ali, A. and Miah, S.A. 1990. Antifungal activity of some plant extracts. Bangladesh J. Bot. **19**, 5-10.
15. Barnett, H.L. and Hunter, B. 2000. Illustrated Genera of Imperfect Fungi. 4th edn., Burgess Pub. Co. Minneapolis. pp. 185.
16. Benoit, M.A. and Mathur, S.B. 1990. Identification of species Curvularia on Rice Seed, Proc. Inst. Seed Test. Ass. **35**(1), 1-23.
17. Booth, C. 1971. The Genus Fusarium. Commonwealth Mycological Institute. Kew, Surrey, England.
18. Ellis, M.B. 1971. Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England. pp. 608.
19. Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England. pp. 326-328.
20. Subramanian, C.V. 1971. Hypomycetes, Indian Council of Agriculture Research, New Delhi, pp. 930+xi.
21. Sutton, B.C. 1980. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stroma. The Commonwealth Mycological Institute, England. pp. 696.
22. Reddy, K.S. and Subbayya, J. 1989. Evaluation of different inoculation method for screening rice cultivers for sheath rot resistance. (Eng.) *Oryza* **26**(4), 416-417.
23. Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics, with special reference to the Biological Sciences. McGraw-Hill Book Co., New York, pp. xvi + 481.
24. Dubey, R.C. and Dwivedi, R.S. 1991. Fungitoxic properties of some plant extracts against vegetative growth and sclerotial viability of *Macrophomina phaseolina*. Indian Phytopathol., **44**(3), 411-413.
25. Rahman, G.M.M., Islam, M.R. and Wadud, M.A. 1999. Seed treatment with plant extracts and hot water: a potential biophysical method of controlling seed-borne infection of wheat. Bangladesh J. Train. Dev., **12**(1&2), 185-190.
26. Meah, M.B., Islam, M.R. and Islam, M.M. 2004. Development of an integrated approach of management of Phomopsis blight and fruit rot of eggplant in Bangladesh. Annual Research Report, Department of Plant Pathology, BAU, Mymensingh, Bangladesh, pp. 57.
27. Rahsid, M.M., Khan, M.M.R., Hossain, M.A. and Hossain, M.M. 2007. Management of seed borne fungi of jute in Mymensingh region. Bangladesh J. Crop Sci. **18**(1), 209-214.
28. Singh, N. and Singh, R.S. 1970. Development of wilt causing species of *Fusarium* in fungicide treated soils. Indian Phytopath. **23**, 545-552.