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Effect of Fungicides and Plant Extracts on the Conidial Germination of *Colletotrichum gloeosporioides* Causing Mango Anthracnose

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In Northern Bangladesh, generally mango trees are planted as agroforest that gives higher Net Present Value (NPV) than traditional agriculture. Mango anthracnose caused by *Colletotrichum gloeosporioides* Penz. is seen as a very destructive and widely distributed disease, which results in poor market value. Five fungicides such as Cupravit, Bavistin, Dithane M-45, Thiovit and Redomil were tested against conidial germination of *C. gloeosporioides*. Dithane M-45 and Redomil were the most effective when the conidia were immersed for 10~20 minutes at 500~1000 ppm concentrations. Antifungal activities of 13 plant extracts were tested against conidial germination of *C. gloeosporioides*. Conidial germination of *C. gloeosporioides* was completely inhibited in *Curcuma longa* (leaf and rhizome), *Tagetes erecta* (leaf) and *Zingiber officinales* (rhizome) after 15 minutes of incubation respectively.

KEYWORDS: Agroforestry, Anthracnose disease, *Colletotrichum gloeosporioides*

Bangladesh is one of poor country in Southeast Asian continent. According to recent estimates, 49.8 percent population lives under the poverty line, compared to 25.0, 32.6 and 28.6 percent for Sri Lanka, Pakistan and India, respectively (UNDP, 2004). Moreover, with an annual growth rate of 1.9 percent, the total population of Bangladesh may increase from 137.5 million in 2004 to 192.22 million in 2025 (ESCAP, 2004). The poverty situation and steady population growth have raised the pressure on natural resources throughout the country. Forests are depleted by commercial timber exploitation and gradually converted into pastures, plantations and cultivated fields. By the year 2003, only 10.2 percent of the country's surface area is left under forest that, in turn, is being depleted at a rate of 3.1 percent per annum. Moreover, due to the loss of firewood resources, rural communities turn to alternative fuels such as cow dung and crop residues that previously served as organic manure on agricultural fields. In vast rural areas, agroforestry is seen as one of the very few options to lift people out of the poverty trap and break the vicious poverty cycle. Along with agroforestry, some of the rural peoples are also growing different crop species such as paddy (*Oryza sativa*), wheat (*Triticum aestivum*), sugarcane (*Saccharum officinarum*), papaya (*Carica papaya*) and banana (*Musa* sp.) to have net addition to reduce the level of poverty. In Northern Bangladesh, mainly the mango trees (*Mangifera indica*) are planted as a tree species under agroforestry project to generate high income. Mango is one of the most important fruits in Bangladesh and acclaimed as "The king of fruits". It is a good source of vitamins and minerals, which help to maintain proper health and resistance to disease (Iguina *et al.*, 1969). As a tree species under agroforestry project mango generates high income as well as improve biodiversity. But this mango crop is infected by different fungal diseases and anthracnose is one of them.

Anthracnose caused by *Colletotrichum gloeosporioides* Penz. is a very common and widely distributed disease of mango in Bangladesh. This disease is very harmful as well as spoilage through rotting, which results in less market value. As a result, the farmers fail to get good product. On the basis of this view, the present multidisciplinary research has been taken with an aim to identify the causal organism of anthracnose disease of different cultivated mango varieties under agroforestry projects in different districts in Northern Bangladesh and build up an economical sustainable control measure of this disease with the help of some fungicides and plant extracts.

Materials and Methods

Research area and data collection. This research focused on six different districts *i.e.* Chapai Nababgonj, Naogaon, Rajshahi, Natore, Pabna and Sirajgonj in Northern Bangladesh. Research methods used were primarily (1) a census and Rapid Rural Appraisal (RRA) for the

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basic social circumstances of the areas (2) observation and field-based survey using survey questionnaire and direct interviews focused especially towards experiences and actual and envisaged costs and benefits of agroforestry. Research data was collected from the period of January to July, 2004.

Fungal strain. *Colletotrichum gloeosporioides* was isolated from the upper surface of infected mango and cultured on potato dextrose agar (PDA) medium. Ten day old-culture of the pathogen was used for each experiment.

Effect of fungicides on conidial germination. Conidia of *C. gloeosporioides* were taken from 10 days old culture on PDA. Conidial suspension was made separately in different concentrations (0.05, 0.10, 0.15, 0.20 and 0.25%) of each fungicide {Cupravit (copper oxychloride), Bavistin (melhyl-2 benzimidazole carbamate), Dithane M-45 (complex of zinc and maneb containing 20% manganese and 2.5% zinc), Thiovit (poly sulphide) and Redomil (melhyl-2 benzimidazole carbamate-N-2-methobis acetyl, alaninite; zinc manganese ethylene bis (dithiocarbamate)}. These suspensions (5 *ml*) were taken in sterilized watch glass and kept at 25°C for 5~30 minutes. A drop of conidial suspension treated by fungicides was taken on separate slides at 5 minutes intervals and kept in moisture chamber at 25°C for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed over conidial suspension on the slides. The slides were examined under the microscope of high power (×400) for recording the inhibition percentage of conidial germination of *C. gloeosporioides.*

Extraction of plants. Leaf extraction of *T. erecta, C. procera, B. calycinum, B. lasera, C. roseus, D. metel, A. aspera, C. antiquarum, M. olefera, C. longa, C. cajans, C. alata, O. sanctum, A. vasica, P. hydropiper, Azadirachta indica, Acalypha indica, V. rosea, C. longa*; and rhizome extraction of *C. longa, Z. officinales*; and bulb extraction of *A. sativum* tissues in sterilized distilled water was done by following the method described by Sridhar and Mahadevan (1982). Five gram tissues were cut into pieces and immediately plunged in sterilized distilled water in a beaker and allowed to boil for 5~10 minutes using five to ten ml of sterilized distilled water for each gram of tissues. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of cheesecloth. Re-extracted the ground tissues for 3 minutes in sterilized distilled water and 2~3 *ml* of sterilized distilled water was used for every gram of tissues. The extracts were cooled and passed through cheesecloth and filtered through Whatman's no. 1 filter paper. Extracts were evaporated on a stream bath to dryness and made 5% concentration with sterilized distilled water for experiment.

Inhibition of conidial germination by plant extracts. Conidia from the culture on PDA plates were taken and conidial suspensions (10^3 conidia/*ml*) were made separately with different concentration of plant extracts. These treated suspensions were taken in small sterilized petri dishes (65 mm) and kept at 25°C for 5~10 minutes. A drop of treated conidia suspension (10^3 conidia/*ml*) from different plant extracts was taken on separate depression slides at 5 minutes interval and kept at 25°C in a moisture chamber for 24 hours of incubation. After that a drop of lactophenol cotton blue was put over the conidial suspension on the slides. The slides were examined under the microscope of high power (×400) for recording the percentage of conidial germination inhibition of *C. gloeosporioides.*

Statistical analysis. Cost-benefit analysis is applied to assess the agroforestry. The net present value (NPV), internal rate of return (IRR), benefit-cost ratio (B/C) and pay back period of agroforestry were calculated and compared. Statistical analysis of data given as percentage was carried out from angular transform and data were analyzed by using Microsoft Excel Software. LSD were determined, wherever, the calculated 'F' value were significant at 5% level (Snedecor and Cochram, 1980). Disease incidence was recorded by adopting the grading formula of Siddarmaiah *et al.* (1978) and mean values were obtained.

Agroforestry model in the study area. The model of agroforestry project in the study area is classified as alley cropping system with the mixture of crops and trees. The mango (*Mangifera indica*) trees are planted as a multipurpose tree species in this project. Crop species, i.e., paddy (*Oryza sativa*), wheat (*Triticum aestivum*), papaya (*Carica papaya*), banana (*Musa* sp.), ginger (*Zingiber officinale*) and turmeric (*Curcuma domestica*) are intercropped with mango trees for the purpose of income increase. Paddy, wheat, papaya, banana etc., are intercropped in the first 10 years. After that time the shade-tolerant ginger and turmeric are commonly planted under mango trees with the purpose of income increase and rational utilization of sunlight and improvement of soil protection and erosion control.

Results and Discussions

Financial result. Agroforestry is the main income earning options of the farmers, and it is more benefited if we compare it with traditional agriculture. The calculations of NPV, IRR, B/C and payback period of traditional agriculture and agroforestry model are illustrated in Table 1. The

Table 1. Financial result of agroforestry versus agriculture (acre)

	Agroforestry	Agriculture
NPV (at 10%) (taka)	588,493.53	234,190.69
IRR (at 10%)	55%	70%
B/C	4.07	2.29
Payback period (years)	3	2

Note : US$ 1.00 = Taka 62 (as of July 2004), time horizon for this project is 30 years.

agroforestry system gives positive and much higher NPV than traditional agriculture. The B/C of agroforestry is nearly about twice than agriculture. But the IRR of agriculture is higher than agroforestry because in the beginning years the return of agriculture is high. The calculation clearly makes that the agroforestry is more attractive, which gives well higher positive yearly net cash flow than traditional agriculture. The whole agroforestry system is financially viable and contributes significantly towards land use efficiency, employment generation, environmental stability and social equity.

Anthracnose disease incidence. Field investigation results of anthracnose disease of mango in different districts i.e. Chapai Nababgonj, Naogaon, Rajshahi, Natore, Pabna and Sirajgonj in Northern Bangladesh were showed in Table 2. Five different mango varieties were investigated in the present study. The highest (37.49, 37.40, 37.01, 34.89, 35.40 and 34.90 per cent) disease incidence were recorded in *Langra* variety at Chapai Nababgonj, Naogaon, Rajshahi, Natore, Pabna and Sirajgonj districts, respectively and the lowest was in Gopalbhog (27.27%) at Chapai Nababgonj district. In case of fazli variety, the lowest disease incidence was recorded at Naogaon (28.27%), Rajshahi (28.85%) and Natore (28.22%) districts. On the other hand, in Ashwina variety the lowest disease incidence was counted 30.44 and 30.35 per cent at Pabna and Sirajgonj districts respectively. Khirsapat variety showed intermediary disease incidence in the investigated district in Northern Bangladesh.

Table 2. Anthracnose incidence on some mango varieties as observed and recorded at the different districts in Northern Bangladesh

Name of the districts	Local name of varieties	Total no. of plants	Total no. of fruits	Total no. of infected fruits	Percentage of diseased fruits
Chapai Nababgonj	Fazli	15[a]	3208	965	30.08
	Ashwina	17	5255	1684	32.04
	Langra	25	6890	2583	37.49
	Gopalbhog	23	5237	1428	27.27
	Khirsapat	18	5461	1807	33.09
Naogaon	Fazli	26	5638	1594	28.27
	Ashwina	19	6778	2279	33.62
	Langra	28	7925	2964	37.40
	Gopalbhog	21	4683	1436	40.66
	Khirsapat	17	5246	1767	33.68
Rajshahi	Fazli	14	3892	1123	28.85
	Ashwina	26	9682	3059	31.59
	Langra	20	5987	2216	37.01
	Gopalbhog	19	5835	1684	28.86
	Khirsapat	22	6598	2385	36.14
Natore	Fazli	28	7562	2134	28.22
	Ashwina	15	6125	1994	32.55
	Langra	24	7156	2497	34.89
	Gopalbhog	13	3847	1161	30.18
	Khirsapat	16	4913	1563	31.81
Pabna	Fazli	16	5326	1623	30.47
	Ashwina	29	9726	2961	30.44
	Langra	22	6954	2462	35.40
	Gopalbhog	14	4165	1284	30.83
	Khirsapat	18	5637	1815	32.19
Sirajgonj	Fazli	24	7506	2365	31.51
	Ashwina	23	9136	2773	30.35
	Langra	14	4569	1595	34.90
	Gopalbhog	13	3815	1236	32.39
	Khirsapat	17	5369	1782	33.19

[a]Mean of five replications.

Table 3. Inhibition percentage of several fungicides on conidial germination of *C. gloeosporioides* according to incubation period

Fungicide	Concentration (ppm)	Immersed period (minutes)						$LSD_{(0.05)}$
		5	10	15	20	25	30	
Cupravit	500	10[a]	17	30	42	57	72	1.6111
	1000	30	37	46	53	69	80	
	1500	38	46	57	66	77	87	
	2000	60	68	73	80	85	91	
	2500	75	81	85	90	94	98	
Bavistin	500	19	36	40	48	53	63	1.0741
	1000	35	54	61	67	75	82	
	1500	57	65	72	75	81	87	
	2000	75	83	88	91	95	98	
	2500	88	90	93	97	100	100	
Dithane M-45	500	91	97	100	100	100	100	1.9078
	1000	96	100	100	100	100	100	
	1500	100	100	100	100	100	100	
	2000	100	100	100	100	100	100	
	2500	100	100	100	100	100	100	
Thiovit	500	32	38	46	53	59	67	1.1593
	1000	44	49	58	64	69	76	
	1500	60	64	69	75	81	85	
	2000	72	75	81	87	92	97	
	2500	81	86	92	96	100	100	
Redomil	500	90	95	99	100	100	100	1.9710
	1000	96	99	100	100	100	100	
	1500	100	100	100	100	100	100	
	2000	100	100	100	100	100	100	
	2500	100	100	100	100	100	100	

a = Inhibition percentage of conidial germination.
Treatments significant at 5% level of probability.

Effect of fungicides. Antifungal activity of five fungicides such as Cupravit, Bavistin, Dithane M-45, Thiovit and Redomil were tested on conidial germination of *C. gloeosporioides*. Among them, Dithane M-45 and Redomil completely inhibited conidial germination of *C. gloeosporioides* when the fungus was immersed for 10~20 minutes at 500~1000 ppm concentrations. Bavistin and Thiovit were also 100 percent effective against conidial germination of *C. gloeosporioides* when the fungus was immersed for 25 minutes at 2500 ppm concentrations. Cupravit was moderately effective against conidial germination of the fungus (Table 3). LSD values indicate significant difference on percentage of conidial germination in different concentration of fungicides.

Hossain *et al.* (2001) reported the efficacy of different fungicides in controlling the purple blotch of onion seed-crop and observed that combined application of Rovral 50 wp @ 0.2% + Redomil MZ-72 @ 0.2% gave the best control of purple blotch and maximum seed yield of onion followed by individual application of Rovral 50 wp @ 0.2% and Score 250 EC @ 0.05% when sprayed at an interval of 15 days. Alam *et al.* (2000) reported the effect of fungicides on the inhibition of *Bipolaris sorokiniana* and found Bavistin, Dithane M-45 tilt to be the most effective fungicides. They stated that concentrations of 500 to 2500 ppm and 1/10 to 1/1000 *ml* were the most effective after 5 to 30 minutes immersion. Singh *et al.* (1990) reported that controlling of leaf spot, caused by *A. brassicicola*, under field conditions by 7 fungicides namely Emisa-6, Bavistin, Captafol, Cuman-1, Difolatam, Dithane M-45 and Dithane Z-78 and suggested economically viable control measures acceptable to farmers. In the present study, it is found that Dithane M-45 and Redomil are the most effective fungicides against *C. gloeosporioides*, which is in complete agreement with the findings of Singh *et al.* (1990).

Effect of plant extracts on conidial germination. Thirteen plant extracts such as *Curcuma longa*, *Ocimum sanctum*, *Adhatoda vasica*, *Polygonum hydropiper*, *Azadirachta indica*, *Tagetes erecta*, *Zingiber officinales*, *Acalypha indica*, *Datura metel*, *Allium sativum* and *Vinca rosea*) tested as biopesticides and the inhibition percentage of conidial germination of *C. gloeosporioides* are presented in Table 4. One hundred per cent conidial germination was found in C. *longa* (leaf and rhizome), *A. indica*

Table 4. Inhibitory effect of different plant extracts on conidial germination of *C. gloeosporioides* after immersing for 5 to 30 minutes

Name of plants	Used parts	Immersed period (minutes)						Length of germ tube (mm)
		5	10	15	20	25	30	
Curcuma longa	Leaf	83[a]	95	100	100	100	100	2.15~22.55
Curcuma longa	Rhizome	88	97	100	100	100	100	1.35~9.80
Ocimum sanctum	Leaf	8	13	18	27	33	40	9.63~144.56
Adhatoda vasica	Leaf	10	15	22	28	34	42	6.42~128.5
Polygonum hydropiper	Leaf	32	35	40	48	55	62	3.21~89.95
Azadirachta indica	Leaf	50	58	67	75	85	94	3.60~48.18
Azadirachta indica	Bark	70	75	81	87	95	100	1.60~32.12
Tagetes erecta	Leaf	85	97	100	100	100	100	1.30~17.20
Zingiber officinales	Rhizome	96	100	100	100	100	100	1.15~13.25
Acalypha indica	Leaf	10	18	24	35	42	48	12.85~112.43
Datura metel	Leaf	15	28	35	49	62	78	9.63~89.95
Allium sativum	Bulb	10	17	25	29	34	39	12.85~128.5
Vinca rosea	Leaf	7	12	19	23	30	37	16.06~160.62

a = Inhibition percentage of conidial germination.
$LSD_{(0.05)}$1.2518.
Treatment significant at 5% level of probability.

(bark), *T. erecta* (leaf) and *Z. officinales* (rhizome) after 15, 15, 30, 15 and 10 minutes of treatment, respectively. The lowest conidial germination inhibition was recorded in *V. rosea* (leaf), *A. sativum* (bulb), *O. sanctum* (leaf), *A. vasica* (leaf) and *A. indica* (leaf). 94, 78 and 62 per cent conidial germination inhibition was occurred in *A. indica* (leaf), *D. metel* (leaf) and *P. hydropiper* (leaf) after 30 minutes of immersion, respectively. The longest germ tube formation was found in *V. rosea* (leaf) and shortest was in *Z. officinales* (rhizome). LSD value indicates significant difference on percentage of conidial germination of *C. gloeosporioides* in different plant extracts. Alam *et al.* (2002) reported that inhibition of conidial germination of four fungi such as *Bipolaris sorokiniana*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizopus artocarpi* and *Botryodiplodia theobromae* was tested using the extracts of different parts of *Vinca rosea* and *Azadirachta indica* and showed good results in their inhibition. *Vinca rosea* root extract completely inhibited spore germination of *Bipolaris sorokiniana* and *Rhizopus artocarpi* when it was immersed from 5~30 minutes at 5 : 1.25 (g/*ml*) concentration. *A. indica* (leaf, root and seed) extracts also showed good (100%) inhibition results on *R. artocarpi*. Natarajan and Lalithakumari (1987) reported that the antifungal activity of the leaf extract of *Lawsonia inermis* on *D. oryzae* was tested at 1 : 40 dilution (EC_{50} concentration) by measuring the growth. The antifungal substance contained in leaf of Lawsone identified as 2-dhydroxy-1, 4 naphthoquinone. Under *in vivo* condition, foliar spray of the leaf extract effectively controlled disease than the seed treatment with fungicides.

Therefore, this study assesses the effect of 5 fungicides and 13 plant extracts against mango anthracnose fungus under agroforestry project in Northern Bangladesh, which is one of the poorest countries in South Asia. The fungicides Dithane M- 45 and Redomil are most effective against *C. gloeosporioides* that causes decrease in yields. Plant extracts such as *Curcuma longa*, *Tagetes erecta* and *Zingeber officinales* were also most effective against the pathogen. The tested plant extracts have more or less antifungal activity due to having different chemical compounds. These results are important for practical implication of the poor farmers and policy makers to promote mango based agroforestry in northern Bangladesh. Because, cost-benefit of agroforestry is financially viable and higher compared to traditional agricultural projects.

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