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RESEARCH ARTICLE

BIOLOGICAL CONTROL OF THE CAUSAL ORGANISM (*Macrophomina phaseolina*) OF STEM ROT DISEASE OF JUTE

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ABSTRACT

Jute is known as the Golden fiber of Bangladesh. Pathogen poses a great problem to the cultivation of jute by inflicting severe yield losses. To control this pathogen, the antifungal effect of plant extracts of three plants species were determined by *in vitro* study using cold water, hot water, cow urine and ethanol. All concentrations of ethanol extract of *Azadirachta indica* showed 100% growth inhibition against *M. phaseolina*. The ethanol and cow urine extract of *T. patula* at 15, 20 and 25% concentrations showed 100% inhibition. *T. patula* extract showed most effectiveness than *A. indica* and *A. vasica* extract. Ethanol extracts were most effective to controlling *M. phaseolina* than cold water, hot water and cow urine extracts. The antifungal effects were also determined by *in vitro* study using three fungicides at different concentrations. Among the three fungicides, Cupravit were most effective to controlling *M. phaseolina* than Dithane M45 and Redomil.

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INTRODUCTION

Jute (*Corchorus olitorius* L., *Corchorus capsularis*) is one of the major cash crops and main stay of Bangladesh economy. It accounts for about 6% of the foreign currency earnings from exports (Islam, 2009). According to Export Promotion Bureau (EPB) data, Bangladesh exported jute and jute related goods worth nearly about $920 million in fiscal year 2015-16. Of the exports, yarn and twine was $559 million, followed by raw jute over $173 million, sacks and bags over $122 million. Among the jute growing countries of the world, Bangladesh ranks second in respect of production (Islam, 2007). The land and climatic conditions of Bangladesh are favorable for the production of high quality jute. In Bangladesh, about 0.709 million hectares of land was under jute cultivation and the total yield were 8.40 million bales (BBS, 2011). Plant diseases are caused by pathogens such as bacteria, fungi, viruses and nematodes. In compare to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. Like other crops, jute suffers from more than 13 different diseases (Fakir, 2001) and 10 of them are seed borne. Stem rot may completely kill the jute plants resulting gaps in the field (Ahmed, 1966). Baxter (1960) reported that *Macrophomina phaseolina* and *Diplodia corchori* were the most predominant and seed borne pathogens of jute. In mature stage the plant do not die but the disease badly affect the fiber quality. The market value of the fiber is 30-50% less than that of healthy plants (Khan and Strange, 1975). This disease is most common in *Corchorus capsularis* than *Corchorus olitorius* (Alam *et al.,* 1992). Large numbers of fungicides are being used in the form of dusting, slurry and soaking treatment (Agrios, 1997). Even though effective and efficient control of seed- borne fungi can be achieved by the use of synthetic chemical fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Harris *et al*., 2001). It is now realized that chemical fungicides cause serious environmental problems and are toxic to non-target organisms (Anon, 2005). Plant metabolites and plant based pesticides appear to be one of the test alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Extracts of many higher plants have been reported to exhibit antifungal

properties under laboratory trials (Parekh *et al*., 2006; Buwa and Staden, 2006; Mohana *et al*., 2008). On the basis of efficacy of plant extracts, present investigation was conducted to control the fungal pathogen of stem rot disease using the effective plant product and also study the effect of different fungicides against the isolated fungi to know the comparison between biological antifungal compounds.

MATERIALS AND METHODS

Collection of infected plant parts: During April to June 2016, sample collection of stem rot of jute have been done from the different areas of Rajshahi district. The infected stems or leaves were collected in the polythene bags, which were made airtight. Collected materials were labeled properly and then brought to the Mycology and Microbiology Laboratory, Department Botany, University of Rajshahi.

Isolation and identification of *Macrophomina phaseolina:* After the collection of the disease materials of jute plants, it was brought to the laboratory and diseased parts were then cut into small pieces, about 0.5 cm in length, in such as manner so as to include both healthy and disease tissues in each piece. The pieces were then sterilized using 0.1% $HgCl_2$ and dried between filter papers and finally transferred to PDA plate. Plates were incubated at 26 ± 2°C for 15 days. During this period the fungal colonies appeared of the PDA plates. Often the colonies were found to become contaminated due to unknown growth of bacteria and other fungi. So, fungal colonies were further transfer to new PDA media until pure culture is obtained. Single spore isolation was done using the method as described by Choi et al. (1999). Fungal colonies were isolated and identified on the basis of morphological and cultural characteristics according to the description of Ahmed et al. (1969).

Inhibitory effect of cow dung and cow urine against radial mycelial growth of *Macrophomina phaseolina:* Control measures were done by using cow urine, cow dung, medicinal plant extracts and fungicides.

Effect of cow urine: In this test, cow urine potato dextrose agar (CUPDA) medium was used. Urine was added with PDA in different 10, 20, 30, 40 and 50%) concentrations. Mycelial blocks of 5 mm diameter of *M. phaseolina*, taken from 7 days old culture were used. Every after 24 ours mycelial growth from the disc was measured and it was continued till 10 days. Percentage of inhibition of mycelial growth was calculated (Ashrafuzzaman, 1976).

Effect of cow dung: For this test, cow dung potato dextrose agar (CDPDA) was used. Cow dung was added with PDA in different (0.5, 1, 1.5, 2 and 2.5%) concentrations. The medium were inoculated and mycelial growth was recorded.

Inhibitory effect of different concentrations of plant extracts on radial mycelial growth of *Macrophomina phaseolina:* Plant parts were collected from different areas of the Rajshahi University. Plant specimens were brought to the laboratory of Plant Pathology, Mycology and Microbiology Lab, Department of Botany, University of Rajshahi and stored in refrigerator at 4°C for further use. Leaves of three plants (Table 1) were washed thoroughly under running tap water and soaked in 1% solution of sodium hypochlorite for 2 minutes, rinsed thoroughly with sterilized distilled water and air dried at room temperature for 2h. For testing efficacy of plant extracts cold water, hot water, and cow urine extracts of these plant parts were prepared by weighing 100 gm of each plant part and 100 ml of cold water, hot water and cow urine respectively were added. Hot water extract was prepared by heating extract in a container at 80 °C temperature on water bath for 20 minutes. Extract concentration of 100% were thus obtained. The extracts were sieved through four layers of sterile muslin cloth and centrifuged at 3000 rpm for 20 min. The 5 ml of each extract concentration was added with 95 ml of molten PDA for 5% concentration. Thus we obtained 5, 10, 15, 20 and 25% extract concentrations to prepare a PDA extract mixture (Sangoyomi, 2004). The Petri plates were gently swirled to ensure even distribution of the extracts. The agar extract mixture was allowed to solidify. After the solidification of the medium, 1 cm diameter plug from 7-day-old colony of *Macrophomina phaseolina* was inoculated aseptically in the center of each Petri plate and incubated at 27ºC. The colony diameter of *M. phaseolina* was measured after 7 days of incubation. Three replicates in a completely randomized design were used within each treatment. The efficacy of medicinal plant products were expressed and percent of radial mycelial growth over the control which was calculated by using the following formula (Vincent, 1947).

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

Where, I = Percent inhibition, C = Radial growth in control, T = Radial growth in treatment

The pathogen *M. phaseolina* was treated by the plant extract on PDA medium. After 5 days of inoculation data on mycelial growth was recorded.

Inhibitory effect of fungicides against radial mycelial growth of *Macrophomina phaseolina:* Three chemical fungicides viz. Cupravit 50WP (50% copper oxychloride), Rodomil WG (40g Metalexil+640g Mancozeb/kg) and Dithane M-45 (Manganese ethylene bisdithio carbamate plus zinc) were collected from local market authorized agrochemical shops at Rajshahi in Bangladesh. Three fungicides viz., Dithane M-45, Cupravit and Ridomil were used against *M. phaseoilna* at the rate of 500, 1000, 1500, 2000 and 2500 ppm concentrations and each fungicide poured into sterilized glass Petri plates at 20 m1/plate. Each plate was inoculated with a 1 cm diameter plug of agar that had been colonized by the *M. phaseoilna* and plates were sealed with parafilm to prevent dehydration. Inoculated plates were incubated for 4 days at 27±2°C. Three replicates in a completely randomized design were used within each treatment. The percent inhibition of mycelial growth over control was calculated using the formula given by Vincent, 1947.

Statistical analysis: All the above experiments of the present study were conducted in triplicate consistency of results and statistical purpose. The data were expressed as mean and standard error (M±SE) using Microsoft Excel software 2013. $P<0.05$ was considered statistically significant in ANOVA test.

RESULTS

Inhibitory effect of cow dung and cow urine against radial mycelial growth of *Macrophomina phaseolina*: Effects of cow urine and cow dung on mycelial growth of *M. phaseolina* varied widely.

Table 1. Name of the plants and their used part for making plant extracts

Local name	Scientific name	Family	Used part
Basak	*Adhatoda vasica*	Acanthaceae	Leaf
Neem	*Azadirachta indica*	Meliaceae	Leaf
Gada	*Tagtes patula*	Asteraceae	Leaf & stem

Table 2. Effects of different concentration of cow dung on the mycelia growth against *Macrophomina phaseolina* after different days of incubation

Incubation days	PDA	Mycelial growth(mm) in CDPDA					% of inhibition of mycelia growth				
		0.5	1	1.5	2	2.5	0.5	1	1.5	2	2.5
1	20	15	14	10	0	0	25	24	50	100	100
2	40	30	30	20	0	0	25	25	50	100	100
3	60	50	40	40	0	0	16.66	33.3	33.3	100	100
4	70	65	60	50	0	0	7.14	14.28	28.5	100	100
5	90	80	70	60	0	0	11.11	22.2	33.3	100	100
F value ($LSD_{p\leq0.05}$)		9.46 (13.91)					3.02 (6.12)				
Dry wt. (mg)	3.2	3	2.5	2	0	0	6.25	37.5	37.5	100	100

CDPDA: Cow dung potato dextrose agar, PDA: Potato Dextose Agar

Table 3. Effects of different concentrations of cow urine on the mycelia growth against *Macrophomina phaseolina* after different days of incubation

Incubation days	PDA	Mycelial growth (mm)* in CUPDA					Inhibition of mycelia growth* (%)				
		10	20	30	40	50	10	20	30	40	50
1	20	15	12	10	8	7	25	40	50	60	65
2	40	35	32	20	18	12	15	20	50	55	70
3	60	60	50	45	40	16	0	16.6	25	33.3	73.3
4	75	75	70	70	50	18	0	6.6	6.6	33.3	73.3
5	90	90	85	80	70	22	0	5.45	11.1	22.5	75.5
F value ($LSD_{p\leq0.05}$)		24.62 (11.92)					6.22(11.07)				
Dry wt. (mg)	3.2	3.2	3	3	2	1	0	6.25	6.25	37.5	68.5

CDPDA: Cow dung potato dextrose agar, PDA: Potato Dextose Agar

Table 4. Antifungal activity cold water, hot water, cow urine and ethanol extracts of *Adhatoda vasica* against *Macrophomina phaseolina*

Extracts	Concentrations (%)	Mycelial growth (mm)	Inhibition of growth (%)	Dry weight (mg)
Cold water	5	88	2.22	35
	10	81	10.00	30
	15	65	27.78	28
	20	50	44.44	25
	25	22	75.56	16
Hot water	5	85	5.56	32
	10	43	52.22	27
	15	31	65.56	22
	20	18	80.00	15
	25	08	91.11	12
Cow urine	5	59	34.44	25
	10	49	45.56	22
	15	46	48.89	21
	20	41	54.44	20
	25	38	57.78	18
Ethanol	5	20	77.78	15
	10	10	88.89	07
	15	00	100.00	00
	20	00	100.00	00
	25	00	100.00	00
Control		90		50

After 5 days of incubation, the mycelial growth was recorded as 80, 70 and 60 mm at 0.5, 1 and 1.5% concentrations, respectively, whereas in PDA the growth was 90 mm (Fig. 1A). After 5 days of incubation the maximum mycelial dry weight was found 3 mg at 0.5% concentration and the minimum was 2.0 mg at 1.5% concentration (Table 2).Results showed that, cow urine have a very effective role on suppression of mycelial growth than that of untreated (control). After 5 days of incubation, the mycelial growth was recorded as 90, 85, 80, 70 and 30 mm at 10, 20, 30, 40 and 50% concentrations (Fig. 1B). The maximum mycelial dry weight was observed 3.3 mg at 10% concentration and the minimum was 1.0 mg at 50% concentration (Table 3).

Inhibitory effect of different concentrations of plant extracts on radial mycelial growth of *Macrophomina phaseolina:* The effect of different concentrations (5, 10, 15, 20 and 25%) of three plants extracts with cold water, hot water, cow urine and alcohol namely. *Adhatoda vasica, Azadiracta indica*, *Tagetes patula* were considered as fungal

Table 5. Antifungal activity cold water, hot water, cow urine and ethanol extracts of *Azadirachta indica* against *Macrophomina phaseolina*

Extracts	Concentrations (%)	Mycelial growth* (mm)	Inhibition of growth (%)	Dry weight (mg)
Cold water	5	88.0	2.22	35
	10	83.0	7.78	30
	15	79.0	12.22	28
	20	72.5	19.22	25
	25	40.0	55.55	10
Hot water	5	76.3	15.00	30
	10	75.5	16.11	26
	15	74.5	17.22	24
	20	66.0	26.67	23
	25	510	43.33	18
Cow urine	5	80.0	11.11	30
	10	62.0	31.11	28
	15	50.0	44.22	25
	20	45.0	50.00	20
	25	41.0	54.44	18
Ethanol	5	00.0	100	00
	10	00.0	100	00
	15	00.0	100	00
	20	00.0	100	00
	25	00.0	100	00
Control		90.0		50

Table 6. Antifungal activity cold water, hot water, cow urine and ethanol extracts of *Tagetes patula* against *Macrophomina phaseolina*

Extracts	Concentrations (%)	Mycelial growth (mm)	Inhibition of growth (%)	Dry weight (mg)
Cold water	5	45.0	50.00	15
	10	39.5	56.11	13
	15	35.0	61.11	11
	20	15.0	83.33	9
	25	10.0	88.89	8
Hot water	5	41.0	54.44	14
	10	27.0	70.00	11
	15	26.0	71.11	12
	20	08.0	91.11	6
	25	00.0	100.00	00
Cow urine	5	87.0	3.33	34
	10	54.5	39.44	25
	15	00.0	100.00	00
	20	00.0	100.00	00
	25	00.0	100.00	00
Ethanol	5	11.5	87.22	08
	10	00.0	100.00	00
	15	00.0	100.00	00
	20	00.0	100.00	00
	25	00.0	100.00	00
Control		90.0		40

Table 7. Effect of Dithan M 45 on the radial mycelial growth of *M. phaseolina* after different incubation period

Concentration (ppm)	Radial growth of mycelium (mm)[a] in different incubation period (days)[b]				% of inhibition of mycelia growth in different incubation period (days)[b]				Dry wt. (mg)
	1	2	3	4	1	2	3	4	
500	00	10	18	22	100	75	72.30	75.55	16
1000	00	00	8	11	100	100	87.69	87.77	10
1500	00	00	00	00	100	100	100	100	00
2000	00	00	00	00	100	100	100	100	00
2500	00	00	00	00	100	100	100	100	00
PDA (Control)	20	40	65	90					32
F value ($LSD_{p\leq0.05}$)	12.60 (12.06)				7.39 (6.94)				327.74 (1.74)

[a]Radial growth of mycelium (mm) , [b]Incubation period (days)

growth inhibitor and the effect of mycelia growth of *Macrophomina phaseolina* were tested (Alam et al., 2004). *A. indica* extracts with cold water, the maximum mycelial growth inhibition was found 55.55% at 25% concentration and the minimum was 2.22% at 5% concentration. In case of hot water extract of *A. indica*, the maximum mycelial growth inhibition was found 43.33% at 25% concentration and the minimum was 15% at 5% concentration.

Cow urine extract of *A. indica* showed the highest 54.44% growth inhibition in 25% concentration and the lowest was 11.11% in 5% concentration (Fig. 2). There were no growth of *M. phaseolina* occurred at all concentrations of ethanol extract of *A. indica* (Table 5). *A. vasica* extracts with cold water, the highest growth inhibition of the fungus was recorded 75.56% at 25% concentration and the lowest was 2.22% at 5% concentration.

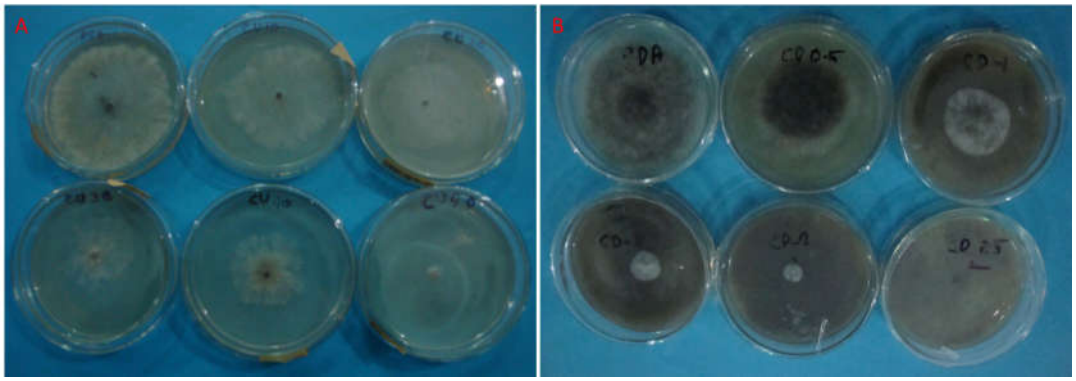

Fig. 1. Mycelial growth of *Macrophomina phaseolina* in different concentrations of (A) cow urine and (B) cow dung

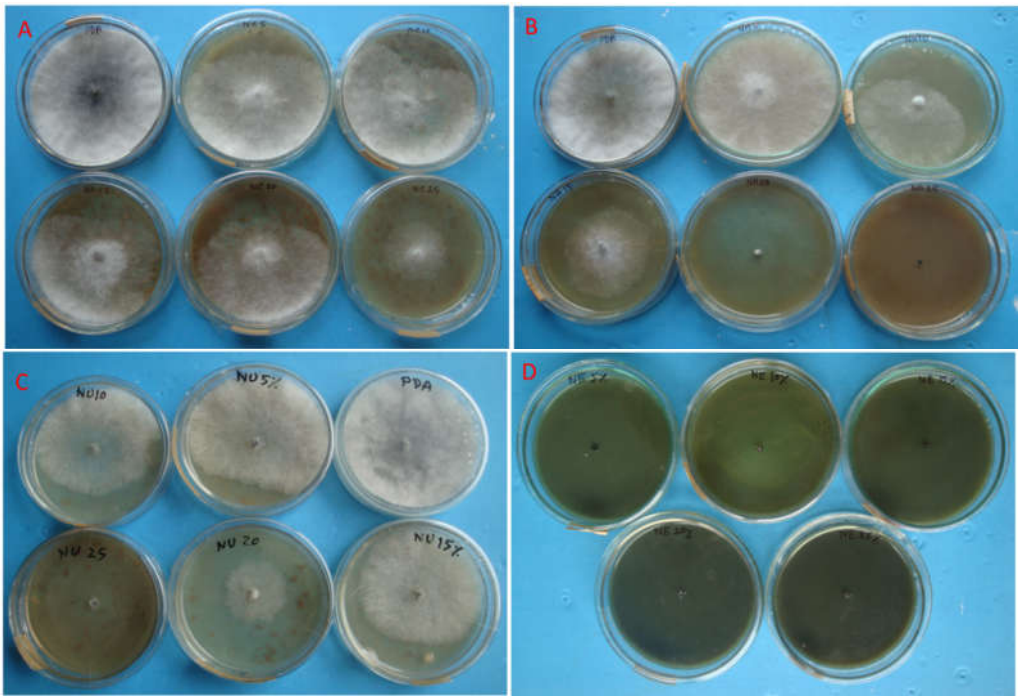

Fig. 2. Mycelial growth of *Macrophomina phaseolina* in Control (PDA) and *Azadirechta indica* plant extract with (A) cold water, (B) hot water, (C) cow urine and (D) ethanol extract

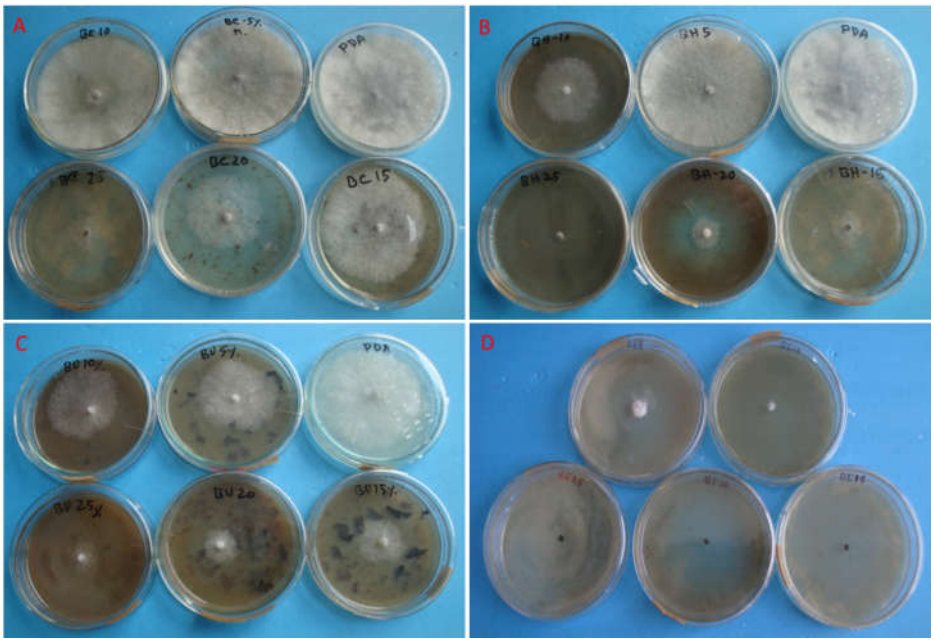

Fig. 3. Mycelial growth of *Macrophomina phaseolina* in Control (PDA) and *Adhatoda vasica* plant extract with (A) cold water, (B) hot water, (C) cow urine and (D) ethanol extract

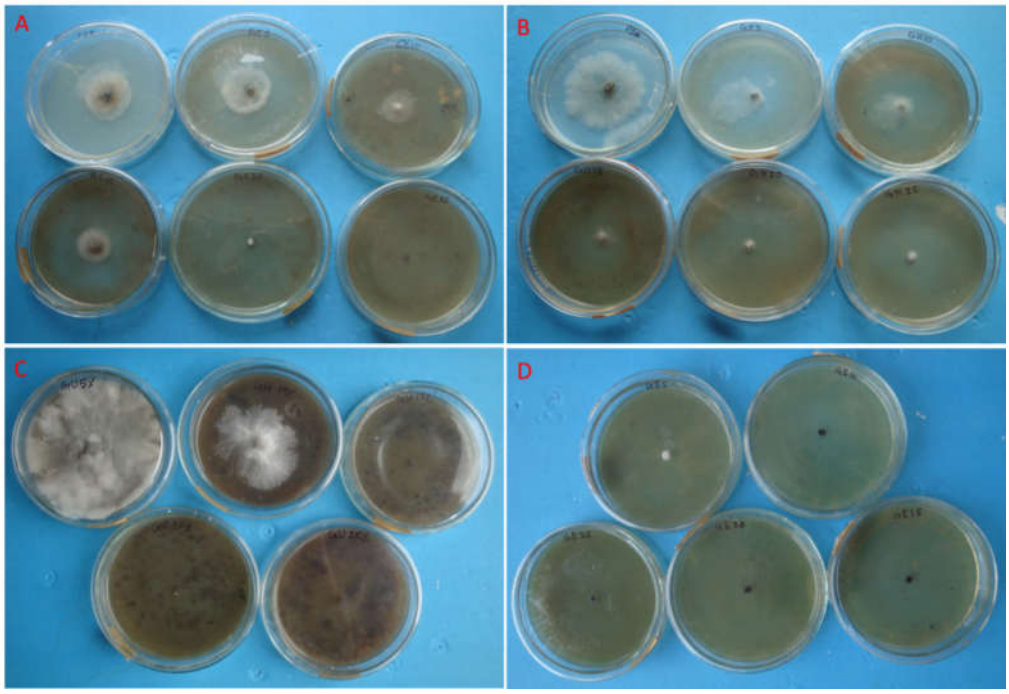

Fig. 4. Mycelial growth of *Macrophomina phaseolina* in Control (PDA) and *Tagetes patula* plant extract with (A) cold water, (B) hot water, (C) cow urine and (D) ethanol extract

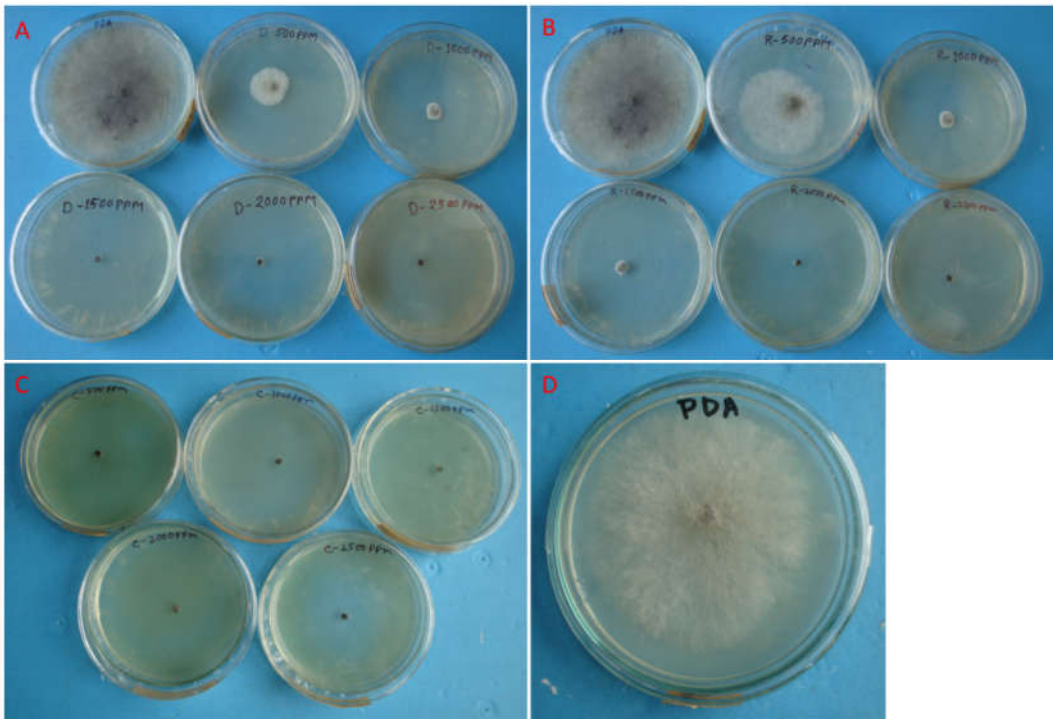

Fig. 5. Mycelial growth of *Macrophomina phaseolina* in (A) Dithan M 45, (B) Redomil, (C) Cupravit and Control

Table 8. Effect of Redomil on the radial mycelial growth of *M. phaseolina* after different incubation period

Concentration (ppm)	Radial growth of mycelium (mm)[a] in different incubation period (days)[b]				% of inhibition of mycelia growth in different incubation period (days)[b]				Dry wt. (mg)
	1	2	3	4	1	2	3	4	
500	00	15	22	35	100	62.50	66.15	61.11	20
1000	00	00	8	11	100	100	87.69	87.77	15
1500	00	00	07	09	100	100	89.23	90	10
2000	00	00	00	00	100	100	100	100	00
2500	00	00	00	00	100	100	100	100	00
PDA (Control)	20	40	65	90					32
F value ($LSD_{p\leq0.05}$)	12.54(11.70)				7.65 (9.31)				171.19 (2.31)

[a]Radial growth of mycelium (mm) [b]Incubation period (days)

In case of hot water extract of *A. vasica*, the maximum growth inhibition of the fungus was found 91.11% at 25% concentration and the minimum was 5.56% at 5% concentration. Cow urine extract of *A. vasica* showed, the highest 57.78% growth inhibition in 25% concentration and the lowest was 34.44 in 5% concentration. There was no growth occurred at 15, 20 and 25% concentrations of ethanol extract of *A. vasica* (Fig. 3). The lowest growth inhibition was 77.78% in 5% concentration (Table 4). *T. patula* extracts with cold water, the highest growth inhibition was found 88.89 at 25% concentration and the lowest was 50% at 5% concentration. In case of hot water extract of *T. patula*, the maximum mycelial growth inhibition was found 100% at 25% concentration and the minimum was 54.44% at 5% concentration. The mycelial growth of *M. phaseolina* was not occurred at 15, 20 and 25% concentrations of cow urine extracts of *T. patula* (Fig. 4). In case of ethanol extract of *T. patula*, the highest growth inhibition was 100% at 15, 20 and 25% concentrations and the lowest was 87.22% in 5% concentration (Table 6).

Inhibitory effect of fungicides against radial mycelial growth of *Macrophomina phaseolina:* The effect of different concentrations of three fungicides namely, Dithane M 45, Redomil and Cupravit were used to inhibit the effect of mycelia growth of *M. phaseolina*. In case of Dithane M 45, the highest inhibition percentage of mycelial growth was recorded 100% in 1500, 2000 and 2500 ppm concentrations and the lowest was 75.55% at 500 ppm concentration of Dithane M 45. After 4 days of incubation the maximum mycelial dry weight was found 16 mg at 500 ppm concentration and the minimum was 10 mg at 1000 ppm concentration (Table 7). There was no growth occurred at 2000 and 2500 ppm concentrations of Redomil. The highest inhibition percentage of mycelial growth was recorded 100% in 2000 and 2500 ppm concentrations and the lowest was 61.11% at 500 ppm concentration of Redomil (Table 8). Results showed that, Cupravit have a very effective role on suppression of mycelial growth than the other fungicides. Cupravit significantly ($P \leq 0.05$) decreased the mycelial growth of *M. phaseolina* after 4 days of incubation in comparison to control (PDA). There was no growth occurred at all concentrations of Cupravit (Table 9) (Fig. 5).

DISCUSSION

Diseases are major limiting factor for jute production. Stem rot is a devastating disease of jute plant and *Macrophomina phaseolina* is one of the causal agents of this disease. Control measures were done by using cow urine, cow dung, medicinal plant extracts and fungicides. The main reason to use the fungicides along with other treatment to know the comparison between antifungal biological compounds. The highest inhibition percentage of mycelial growth was recorded 100% in 2 and 2.5% concentrations and the lowest was 11.11% at 0.5% of CDPDA medium. On the other hand, the highest mycelial growth of inhibition 75.5% was recorded at 50% concentration and the lowest inhibition was 5.45% at 20% concentration of CUPDA. In a research study Basak et al. (2002a, 2002b) proved that cow urine and cow dung had some effectiveness in suppression of conidial germination and mycelial growth of *Sclerotinia sclerotiorum* and *F. oxysporum* f. sp. *cucumerinum* causing disease of cucumber plants. *A. vasica* extracts with cold water, hot water and cow urine showed 75.56%, 91.11% and 57.78% highest growth inhibition of the fungus respectively at 25% concentration (Akhter et al., 2006).

In case of *A. indica* extracts with cold water, hot water and cow urine the highest growth inhibition of the fungus was recorded 55.55%, 43.33% and 54.44% respectively at 25% concentration. A similar finding have had been reported recently by Javaid and Saddique (2011) and Dubey et al. (2009) who found mycelial growth inhibition of *M. phaseolina* isolated from soil-borne and diseased roots of soybean treated with *D. metel* and *A. indica*. On the other hand, *T. patula* extracts with cold water, hot water and cow urine showed highest 88.99%, 100% and 100% growth inhibition of the fungus was recorded respectively at 25% concentration. In similar study Tiwari and Das (2011) observed *in vitro* and in vivo inhibitory efficacy of cow urine extracts of some medicinal plants against *Rhizoctonia solani*, causal agent of sheath blight of rice. Hassanein et al. (2008) reported that neem and chinaberry extracts derived by ethanol and ethyl acetate solvents were more effective against *Fusarium oxysporum* and *Alternaria solani* than their aqueous extracts.

On the contrary, Oluma and Elaigwe (2006) observed that extracts of *A. indica* had no inbibitory effect against *Macrophomina phaseolina.* But in our study, all the concentrations of ethanol extract of *A. vasica, A. indica* and *T. patula* showed 100% inhibition of the fungus. The present findings correlates with the findings of Farooq (2002), who evaluated the effects of different concentrations of plant extract of *Achillea millefoliumon* linear growth of *Macrophomina phaseolina*. His results also legitimated our findings that the growth rate of pathogen decreases by increasing the concentration of plant extract. Alam *et al*. (1999) reported that, the growth inhibition (*in vitro*) of chilli fruit rot pathogen *Alternaria tenuis* and found that Redomil, Dithone M-45, Cupravit, Bavistin and Rovral proved to be the most effective against *A. tenuis* when immerged for 5 to 30 minute at 500 to 2500 ppm concentrations. But in our study, out of three fungicides Dithane M45, Redomil and Cupravit; Cupravit was the most effective against this pathogen in all concentrations at 4 days of incubation. The present study suggest that, as the ethanol extract of all plants showed 100% growth inhibition of *M. phaseolina*. So it can be utilized as phytofungicide to control this pathogen in compare to the other biological control.

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