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In vitro Inhibition Effect of Plant Extracts, Urine, Fertilizers and Fungicides on Stem Rot Pathogen of *Sclerotium rolfsii*

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Twenty plant extracts were tested against mycelial growth, sclerotium formatiom and dry weight of mycelium with sclerotia of *Sclerotium rolfsii* Sacc. The highest (90 mm) mycelial growth was measured in *Adhatoda vasica, Tegetes erecta, Allium cepa,* and *Curcuma longa.* The lowest (25 mm) was in *Azadirachta indica.* No mycelial growth was found in any concentration of cow, buffalo, and goat urine. The highest (90 mm) and the lowest (15 mm) mycelial growth were measured in Biomil and Urea, respectively. No mycelial growth was observed in Zinc. The highest (60 mm) and the lowest (2 mm) mycelial growth were recorded in Macuprex (Dodine; 65% WP) and Boron (100% Boric acid and 17% Boron) respectively. Mycelial growth was totally inhibited in Rovral (Iprodione; 50% WP).

KEYWORDS: Fertilizers, Fungicides, Plant Extracts, *Sclerotium rolfsii*, Urine

Betelvine (*Piper betle*) is an important crop in terms of consumption and intercropping with medicinal plants. Its cultivation is considered economically inefficient (Bhalerao, 1990). As a masticatory the betelvine leaf is credited with being aromatic, digestive, stimulant and carminative. Some pathogenic fungi and bacteria cause destructive diseases of betelvine that pose a major threat to the betelvine industry and make it a risky proposition (Balasubrahmanyam *et al.*, 1994). Stem rot of betelvine caused by *S. rolfsii* is an important and devastating disease in Bangladesh. Due to the disease, the farmers are faced a great loss every year. Among pesticides used to protect crops, fungicides were perceived until recently as relatively safe (Wilson *et al.*, 1997). A 1986 National Academy of Sciences (NAS) report (Research Council, Board of Agriculture, 1987) of pesticide residues on food indicated that fungicides pose more of a carcinogenic risk than insecticides and herbicides together. Therefore, synthetic fungicides are suspect in our food chain, and pressure is increasing to find safer alternatives. Additionally, resistance by pathogens to fungicides has rendered certain fungicides ineffective, creating a need for new ones with alternative modes of action (Wilson *et al.*, 1997). Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides (Shekhawat and Prasad, 1971; Appleton and Tansey, 1975; Egawa *et al.*, 1977; Misra and Dixit, 1976; Singh *et al.*, 1986; Dubey, 1991). Plant extracts show antifungal activity against a wide range of fungi (Davidson and Parish, 1989; Grange and Ahmed, 1988). Ark and Thompson (1959) showed that garlic extracts contain a potent fungicidal compound. They are able to effectively protect peaches against brown rot (*Monilinia fruticola)* with deodorized garlic extract preparations. Cow urine and cow dung are capable of suppressing conidial germination and mycelial growth of fungi (Basak and Lee, 2001). The present study was undertaken to find out the growth inhibitory effect against stem rot pathogen caused by *Sclerotium rolfsii* using plant extracts, chemical fungicides, fertilizer, and urine *in vitro.*

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Materials and Methods

Fungus used. *Sclerotium rolfsii* Sacc. was isolated from stem rot of *Piper betle* on potato dextrose agar (PDA) and the culture was maintained at 28°C.

Mycelial growth in plant extract medium. The experiment of mycelial growth and sclerotia formation inhibition was carried out following Miah *et al.* (1990). Twenty plant extracts (*Rauvolfia serpentina, Adhatoda vasica, Tegetes erecta, Catharenthus roseus, Ocimum sanctum, Capsicum frutescens* (ripe), *Allium cepa, Zingiber officinale, Capsicum frutescens* (green), *Eucaliptus camadulensis, Cymbopogon citratus, Cajanus cajan, Paederia foetida, Cinnamomum tamala, Datura metel* (bark), *Datura metel* (green fruit), *Datura metel* (ripe fruit), *Datura metel* (leaf) and *Azadirachta indica*) were mixed with potato agar (PA) medium to have 5~25% concentra-

tion of each crude extracts. After autoclaving at 121°C and 15 lbs/inch^2 pressures, these were poured in sterilized Petri dishes. Extracts of plant species were tested following the methods of Basher and Rai, 1991. Single sclerotium of old culture media was taken and placed in the center of each Petri dish. The experiment was replicated three times and radial growth of mycelium was measured by following Brown methods (1923).

Test of different urine effect. In this test, urine of different sources (Cow, Buffalo and Goat) was added with PA in different (5, 10, 15, 20 and 25%) concentrations. The media were inoculated and mycelial growth, dry weight of mycelium, sclerotium formation and number of total slcerotium was recorded as described above.

Mycelial growth inhibition test in different fertilizers. Fixed concentration (2%) of six fertilizers namely Zinc, Urea, Sulpher, M.P (Murate of potash), Zipsum and Biomil were added with PA. The culture media were inoculated with a single sclerotium of the fungus and mycelial growth, dry weight of mycelium, sclerotium formation day and number of total slcerotium were recorded as described above.

Test of different fungicidal effect. A fixed concentration (0.35 g/100 *ml*) of nine fungicides namely Bavistin (Carbendazim; 50% WP), Cupravit (Copper oxychloride; WP), Microthial (90% Sulphur WP), Thiovit (Wettable sulphur; 80% WP), Dithane M-45 (Coordinated product of zinc ion and maneb; 80% WP), Rovral (Iprodione; 50% WP), Boron (100% boric acid and 17% boron), Macuprex (Dodine; 65% WP) and Cumulus (80% maneb and 20% inerts including Zinc; 80% WP) were added with PA and the media were inoculated with the fungus. Mycelial growth, dry weight of mycelium, sclerotium formation and total number of slcerotium were recorded as described above.

Results and Discussion

Twenty plant extracts were considered as fungal growth inhibitor and their effects on mycelial growth, dry weights of mycelium and sclerotium formation of *S. rolfsii* are presented in Table 1. The highest (90 mm) mycelial growth was found in *Adhatoda vasica*, *Tegetes erecta*, *Allium cepa* and *Curcuma longa*, and the lowest (25 mm) was found in *Azadirachta indica*. The mycelial growth in other tested plant extracts were intermediate. The highest (300 mg) and the lowest (30 mg) dry weight of mycelium were measured in *Datura metel* (ripe fruit) and in *Azadirachta indica* extracts respectively, in rests of the cases it was intermediary. The first sclerotia were formed on 3rd days of incubation in *Allium cepa*, *Datura metel* (green fruit) and *Datura metel* (ripe fruit), but most (10) of the cases it was formed on 6th days of incubation. The high-

Table 1. Effect of different plant extracts on mycelial growth and sclerotia formation of *Sclerotium rolfsii* on 8 days of incubation at 28°C

Name of plant	Radial growth of mycelium[a] (mm) in different incubation period (day)								Sclerotium formation day	Total number of Sclerotia on 6th day	Dry weight of mycelium on 6th day (mg)
	1	2	3	4	5	6	7	8			
Rauvolfia serpentina	2	11	26	41	56	65	76	84	5	150	140
Adhatoda vasica	4	15	32	46	59	70	81	90	6	50	80
Tegetes erecta	4	14	31	44	57	68	80	90	5	200	100
Catharenthus roseus	2	10	22	32	41	50	59	66	6	70	40
Ocimum sanctum	0	1	5	8	12	17	23	34	6	16	60
Capsicum frutescens (ripe)	4	12	27	43	58	68	77	86	5	140	120
Allium cepa	3	15	31	46	58	71	82	90	3	440	90
Zingiber officinale	3	14	30	44	55	68	78	85	4	240	90
Curcuma longa	3	14	32	47	59	72	81	90	5	130	60
Capsicum frutescens (green)	0	2	10	23	37	48	57	65	5	180	50
Eucaliptus camaldulensis	4	16	33	47	60	71	80	89	6	96	130
Cymbopogon citratus	3	14	28	41	54	65	75	86	4	220	220
Cajanus cajan	2	10	21	31	40	49	58	69	6	88	110
Paederia foetida	2	9	18	28	37	46	55	63	6	60	80
Cinnamomum tamala	3	13	26	39	52	63	73	82	6	24	90
Datura metel (bark)	2	10	23	35	46	57	67	76	6	80	140
Datura metel (green fruit)	4	15	32	47	57	68	78	89	3	340	250
Datura metel (ripe fruit)	0	2	9	21	34	42	50	58	3	380	300
Datura metel (leaf)	0	0	2	7	13	19	26	33	6	50	50
Azadirachta indica	0	0	2	6	11	16	21	25	6	20	30

[a]Mean of three replications.

est (440) and the lowest (16) number of sclerotia were counted in *Allium cepa* and *Ocimum sanctum* respectively. Statistical analysis revealed that there are highly significant relationship between incubation period and radial mycelial growth of *S. rolfsii* in different tested plant extracts. The analysis also indicates that the role of tested plant extracts on mycelial growth of *S. rolfsii* in different incubation period is significantly different. Poswal *et al.* (1993) examined *in vitro* fungicidal properties of aqueous extracts from different parts of 10 species against mycelial growth of *Macrophomina phaseolina*, *Alternaria zinnae* and *Sclerotium rolfsii*. The inhibitory extracts were from *Dichapetalum cymosum* stems, *Melia azedarach* seeds and bark, *Solanum nigrum* berries and *Pavetta harborri* stems. Basak and Paul (1999) has already observed that plant extracts of *Azadirachta indica*, *Polygonum hydropiper*, *Lantana camara*, *Cassia tora* and *Moringa olifera* had successful effect on the inhibition of mycelial gowth of six major fruit rot fungal pathogen of chilli.

In case of different concentrations (5, 10, 15, 20 and 25%) of *Allium cepa*, *Zingiber officinale*, *Capsicum frutescens* (green), *Ocimum sanctum*, *Detura metal* (leaf) and *Azadirachta indica* extracts, the highest (90, 90, 90, 89, 87 and 82 mm) and the lowest (59, 50, 64, 35, 42 and 36 mm) mycelial growth was measured at 5 and 25% concentrations respectively (Table 2). Rests of the tested concentrations of these extracts, were intermediate. The highest (290, 190, 230, 220 and 200 mg) and the lowest (110, 80, 80, 80 and 150 mg) dry weights of mycelium were measured in *Allium cepa*, *Capsicum frutescens* (green), *Ocimum sanctum*, *Datura metel* (leaf) and *Azadirachta indica* at the concentration of 5 (for highest) and 25% (for lowest), respectively. But in case of *Zingiber officinales*, the highest (250 mg) and the lowest (90 mg) dry weight of mycelium was found at 5 and 20% concentrations, respectively. The first sclerotium was formed on 3rd days of incubation at 5 and 25%, 4th days at 10 and 20% and 5th days at 15% concentrations of the extract of

Table 2. Effect of different concentrations of selected plant extract on mycelial growth and sclerotia formation of *Sclerotium rolfsii* on 8 day of incubation at 28°C.

Source of extracts	Concentrations (%)	Radial growth of mycelium[a] (mm) in different incubation period (day)								Sclerotium formation day	Total number of Sclerotia on 6th day	Dry weight of mycelium (mg) on 6th day
		1	2	3	4	5	6	7	8			
Allium cepa	5	5	17	31	44	56	67	79	90	5	96	290
	10	4	15	28	41	52	62	73	82	4	250	220
	15	3	14	26	38	48	57	67	75	4	200	210
	20	2	12	23	35	44	52	61	66	5	100	190
	25	1	9	19	31	39	44	54	59	4	250	110
Zingiber officinale	5	4	17	31	45	59	72	82	90	3	165	250
	10	3	17	25	35	53	65	75	84	4	120	150
	15	2	13	30	38	45	56	67	77	5	90	170
	20	2	10	24	33	40	47	56	65	4	148	90
	25	0	3	10	19	28	35	42	50	3	180	150
Capsicum frutescens (Green)	5	10	22	38	49	58	70	81	90	4	42	190
	10	8	19	34	45	52	65	76	85	5	70	140
	15	6	17	25	35	55	62	68	74	4	122	100
	20	4	13	22	33	48	56	63	70	4	65	120
	25	2	10	20	31	41	50	58	64	4	53	80
Ocimum sanctum	5	2	10	21	38	54	71	80	89	4	19	230
	10	0	4	14	28	41	53	65	76	4	14	180
	15	0	3	12	22	33	44	53	62	4	11	180
	20	0	2	9	17	25	33	41	49	5	10	150
	25	0	0	2	8	15	22	29	35	5	4	80
Datura metel (Leaf)	5	3	16	30	44	57	68	78	87	4	75	220
	10	2	14	27	40	51	60	68	76	4	79	200
	15	1	11	23	34	44	52	59	66	4	60	170
	20	0	2	11	20	28	37	46	54	5	18	80
	25	0	0	1	9	18	26	34	42	5	17	80
Azadirachta indica	5	3	15	27	39	50	61	71	82	7	15	200
	10	3	13	24	35	44	54	63	71	0	0	180
	15	2	10	17	24	30	37	44	52	0	0	170
	20	0	2	9	15	22	29	37	44	0	0	170
	25	0	0	2	8	15	23	30	36	0	0	150

[a]Mean of three replications.

Zingiber officinale. But it was first formed on 4th days of incubation at 10, 15 and 25% concentrations of *Allium cepa*, 5, 15, 20 and 25% of *Capsicum frutescens* (green), 5, 10 and 15% of *Ocimum sanctum*, 5, 10 and 15% of *Datura metel* (leaf). With rests of the concentrations of these plant extracts [*Allium cepa*, *Capsicum frutescens* (green), *Ocimum sanctum*, and *Datura metel* (leaf)], the first sclerotia were formed on 5th days of incubation. On 7th days of incubation, the first sclerotia were formed at 5% concentration of *Azadirachta indica*. In other concentrations of *Azadirachta indica*, the sclerotia were not formed. The highest number of slcerotia (260, 180, 122, 19, 79 and 15) were counted at the concentration of 25% of *Allium cepa*, 25% of *Zingiber officinale*, 15% of *Capsicum frutescens* (green), 5% of *Ocimum sanctum*, 10% of *Datura metel* (leaf) and 5% of *Azadirachta indica*, respectively. The lowest number of slcerotia (96, 90, 42, 4, and 17) were counted at the concentration of 5% of *Allium cepa*, 15% of *Zingiber officinale*, 5% of *Capsicum frutescens* (green), 25% of *Ocimum sanctum* and 10% of *Datura metel* (leaf) respectively. But no sclerotia were formed at the concentrations of 10~25% of *Azadirachta indica*. Statistical analysis indicates that there is highly significant relationship between incubation periods and mycelial radial growth of the fungus. The role of different concentrations of tested plant extracts on mycelial growth in different incubation periods is significantly different. Alam *et al.* (2002) reported that inhibition of conidial germination of four fungi viz., *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 inhibited 100% spore germination of *Bipolaris sorokiniana* and *Rhizopus artocarpi* when it was immersed from 5~30 minutes at 5 : 1.25 (w/v) concentration. *A. indica* (leaf, root and seed) extracts showed good (100%) inhibition results on *R. artocarpi*. Alam *et al.* (2002) reported that ten plant-extracts considered as fungicides were tested, out of which *Tagetes erecta* leaf and *Azadirachta indica* bark extracts were the most effective in inhibit of germination of conidia of *Colletotrichum gloeosporioides* after 5~30 minutes of immersion and at 5 : 1.25 (w/v) concentration. Alam *et al.* (1999) reported that seed, leaf and root extracts of *Azadirachta indica* were proved to be very effective on the inhibition of growth of *A. tenuis* (79, 69 and 53%) respectively after immersion for 30 minutes and *Vinca rosea* leaf and root extracts inhibited 45 and 41% growth of the fungus, respectively.

During the study of mycelial growth and sclerotia formation of *S. rolfsii* on different concentrations (5, 10, 15, 20 and 25%) of Cow, Buffalo and Goat urine, no mycelial growth and sclerotia were formed after 8 days of incubation at 28°C. The inhibitory effect of above mentioned urine against *S. rolfsii* in *in vitro* condition showed good antifungal activities. This result also indicates that with the application of cow, buffalo and goat urine, the growth of the fungus may inhibit in field condition. Basak *et al.* (2002) studied on comparative efficacy and *in vitro* activity of cow dung and cow urine against *Fusarium solani* f. sp. *cucurbitae*. The germination of conidia and percentage inhibition of mycelial growth of the fungus decreased or suppressed and varied greatly with respect to different hours and days of incubation and kind of bio matters. In between two bio matters cow urine was more effective than that of cow dung in conidial germination.

In case of different fertilizers, after 7 days of incubation, the highest (90 mm) and the lowest (15 mm) mycelial growth was measured in Biomil and Urea, respectively. No mycelial growth was observed in Zinc. The mycelial growth in other tested fertilizer was intermediary (Table 3). The highest (550 mg) and the lowest (100 mg) dry weight of mycelium were measured in Biomil and Urea, respectively. In rests of the fertilizer (except Zinc), it was intermediary. The first sclerotia had formed on 4th day of incubation in Biomil and in case of Sulpher, M.P and Zipsum it was occurred on 5th day of incubation. No sclerotium was formed in Zinc and Urea. The highest (40) and the lowest (15) number of sclerotia were counted in Sul-

Table 3. Effect of different fertilizers (2%) on mycelial growth and sclerotia formation of *Sclerotium rolfsii* on 7 days of incubation at 28°C

Name of fertilizer (2%)	Redial growth of mycelium[a] (mm) in different incubation period (day)							Sclerotia formation day	Total number of sclerotia on 7th days	Dry weight of mycelium (mg) on 7th days
	1	2	3	4	5	6	7			
Zinc	0	0	0	0	0	0	0	0	0	0
Urea	0	0	0	2	6	10	15	0	0	100
Sulpher	0	0	3	7	12	19	27	5	40	150
M.P (Murate of potash)	2	10	19	29	41	54	65	5	15	390
Zipsum	2	11	20	31	43	57	68	5	16	420
Biomil	3	18	34	49	64	78	90	4	20	550

[a]Mean of three replications.

Table 4. Effect of fixed amount of different Fungicides on mycelial growth of *Sclerotium rolfsii* on 9 days of incubation at 28°C

Name of fungicides (0.35 gm/100 *ml*)	Radial growth of mycelium[a] (mm) in different incubation period (day)									Sclerotia formation day	Total number of Sclerotia on 9th day	Dry weight of mycelium (mg) on 9th day
	1	2	3	4	5	6	7	8	9			
Bavistin (Carbendazim; 50% WP)	0	0	3	9	15	21	28	35	42	7	03	200
Cupravit (Copper oxychloride; WP)	0	0	0	1	6	12	20	29	35	8	02	170
Microthial (90% Sulphur WP)	0	0	0	0	0	1	3	9	13	-	-	80
Thiovit (Wettable sulphur; 80% WP)	0	0	0	0	1	4	9	12	15	-	-	90
Dithen M-45 (Coordinated product of zinc ion and maneb; 80% WP)	0	0	2	6	12	19	25	32	38	8	01	170
Rovral (Iprodione; 50% WP)	0	0	0	0	0	0	0	0	0	-	-	-
Boron (100% boric acid and 17% boron)	0	0	0	0	0	0	0	1	2	-	-	20
Macuprex (Dodine; 65% WP)	0	4	11	18	25	32	41	51	60	8	04	340
Cumulus (80% maneb and 20% inerts including Zinc; 80% WP)	0	0	0	0	0	0	1	4	8	-	-	80

[a]Mean of three replications.

pher and M.P (Murate of potash) respectively. In rests of the cases it was intermediary. Statistical analysis indicate that there is highly significant relationship between incubation period and redial mycelial growth of *S. rolfsii* in different fertilizer and the role of different tested fungicides on mycelial growth of *S. rolfsii* in different incubation period is significantly different.

The effects of fixed concentration (350 mg/100 *ml*) of tested fungicides on mycelial growth, dry weight and sclerotia formation of *S. rolfsii* are shown in (Table 4). After 9 days of incubation, the highest (60 mm) and the lowest (2 mm) mycelial growth was measured in Macuprex (Dodine; 65% WP) and Boron (100% boric acid and 17% boron) respectively. No mycelial growth was observed in Rovral (Iprodione; 50% WP). The mycelial growth in other tested fungicides was intermediary. The highest (340 mg) and the lowest (20 mg) dry weight of mycelium were measured in Macuprex and Boron respectively. In rests of the fungicides (except Rovral), it was intermediary. The first sclerotia had formed on 7th days of incubation in Bavistin and on 8th days of incubation in Cupravit, Dithane M-45 and Macuprex. No sclerotia were formed in other tested fungicides. The highest (4) and the lowest (1) number of sclerotia were counted in Macuprex and Dithane M-45 respectively. Statistical analysis indicates that there is highly significant relationship between incubation period and mycelial growth of *S. rolfsii* in different fungicides and the role of different fungicides on mycelial growth in different incubation period is significantly different. Hossain *et al.* (2001) reported the efficacy of different fungicides in controlling purple blotch of onion seed-crop and observed that combined application of rovral 50wp 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 50wp 0.2% and score 250EC 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 and tilt were the most effective fungicides. They stated that at 500 to 2500 ppm and 1/10 to 1/1000 *ml* concentrations were most effective after 5 to 30 minutes immersion. Alam *et al.* (1999) reported the growth inhibition (*in vitro*) of chilli fruit rot pathogen *Alternaria tenuis* and found that redomil, dithane M-45, cupravit, bavistin and rovral proved to be the most effective against *A. tenuis* when immersed for 5 to 30 minutes at 500 to 2500 ppm concentrations.

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