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EFFECT OF SOME BOTANICAL EXTRACTS AND COW'S URINE ON *Sclerotium rolfsii* CAUSAL AGENT OF FOOT AND ROOT ROT OF BETEL VINE

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----------ABSTRACT----------
To evaluate the effect of different plants extracts and namely rhizome of turmeric, rhizome ginger, neem leaf, tobacco leaf, tobacco leaf extract in water, tobacco leaf extract in cow's urine, and cow's urine at different concentrations (70%, 60%, 50%, 40% and 30%) on the growth and sclerotia formation of Sclerotium rolfsii, causal agent of foot and root rot disease of betel vine. Radial growth and formation of sclerotia were recorded. Radial growth inhibition percentage was calculated thereafter. In all the cases, the growth inhibition was found to be increased with the increase of concentration No growth of the tested fungi was observed in all concentrations of tobacco leaf extract in cow's urine, 70%, 60%, 50%, 40% concentration of cow's urine alone, and 70% and 60% concentration of tobacco leaf extracts in water and consequently no sclerotia were formed. In other concentration of tobacco leaf extracts in water (50%, 40% and 30%) growth inhibitions were 92.7%, 69.87% and 47.28% respectively. Considerable growth inhibitions were observed in all concentrations of turmeric rhizome which were 47.16%, 50.04%, 55.97%, 56.43% and 57.13% at 30%, 40%, 50%, 60% and 70% concentrations respectively. Few sclerotia were formed in the plates treated with tobacco leaf extract in water at 50% and 40% (14 and 53 respectively). Less than 50% sclerotia were formed at all concentration of turmeric rhizome extract.

KEY WORD: *Botanical extract, Cow's urine, Sclerotium rolfsii*

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I. INTRODUCTION

Betel vine, (*Piper betel* L.) is an important cash crop of Bangladesh. It is a perennial dioecious creeper belonging to the family Piperaceae. It is locally known as "Pan" and thought to have originated in Malaysia, Sumatra and possibly Java. It is a climbing plant with shiny, green, heart-shaped leaves. The stem is climbing by many short adventitious roots (Hassan and Shahadat, 2005). Leaves of betel vine are chewed along with areca nut as a masticator. Usually the people of South Asia, Southeast Asia, Gulf States and Pacific islands chew betel leaves. All classes of people in Bangladesh chew betel vine not only as a habit but also as an item of rituals, etiquette and manners.

Bangladesh is the second largest grower of betel vine on about 14000 hectares. Total annual production of the crop in Bangladesh is about 72,500 tons. The average yield is 2.27 tons per acre (Anonymous, 2006). But the acreage of betel vine is decreasing fast because of some physical and socioeconomic barriers like unavailability of credit facilities, uncontrolled marketing system and infestation of diseases and pest (Islam 2005). Disease damage to the crop is one of several known limiting factors. The betel vine is highly susceptible to diseases, pests and some natural calamities (Sayeeduzzaman, 1988). Humid and moist shaded conditions are favorable for betel vine growth and also favor a variety of root and foliage disease development (Goswami *et al*, 2002). The recurrence of disease leads to complete destruction and crop failure after a few year. Among the diseases of betel vine, foot and root rot caused by *Sclerotium rolfsii* is the most overwhelming disease which decreases the production of betel leaf to a great extent. Farmers growing *Piper betel* in three upazilas of Rajshahi incurred a huge loss as foot rot disease damaged about 60% of the cultivation in the year of 2004. (Islam, 2005).*Sclerotium rolfsii* Sacc. is a serious soil borne pathogenic fungus and harmful to many crops which are economically valuable in most of the tropical and subtropical region of the world (Aycock, 1966). It has a wide host range and it has been referred as an almost omnipathogenic organism (Talukder, 1974). The fungus *S. rolfsii* is a facultative parasite and can maintain continuity of generation under adverse situation by the formation of sclerotia (Ahmed, 1980). As the fungus *S. rolfsii* is soil borne and omnipathogenic,

It is very difficult to control even by the use of chemical fungicide. Moreover, most of the chemicals are costly so the farmers have to spend a large amount of money to buy these chemicals. Now-a-days use of chemical for management of crop disease is being discouraged due to health hazards and environmental pollution. So, control of the pathogen through botanical pesticides or plant extracts and natural biocides might be a good alternative. Botanical extracts are biodegradable (Devlin and Zettel, 1999) and their use in crop protection is a practical sustainable alternative. It reduces environmental contamination and health hazards (Grange and Ahmed, 1988). Research on the active ingredients, fungicide preparation, application rates and environmental impact of botanical fungicides is a prerequisite (Buss and Park, 2002) for sustainable agriculture. Botanical fungicides are unique because they can be produced easily by the farmers and small industries (Roy *et al.*, 2005). Few works have been done by using tobacco, neem, garlic and some other plant extracts to control some other fungi. Different natural biocides also used separately or in combination with plant extracts to control some other fungi by the farmers. Antifungal activities of garlic, neem, allamanda have been reported by many researchers (Islam, 2005; Rahman *et al.*, 1999; Arun *et al.*, 1995 and Mohanty *et al.*, 1995).It is thus dire necessity to work extensively to examine the effect of different concentration of tobacco, neem, garlic and other indigenous plant extract and natural biocides like ginger, turmeric, cow's urine etc. in controlling disease which are easily available. These botanical pesticides are affordable by low income farmers and they have the potentiality for use in agriculture, especially with the dramatic increase towards the consumption of organically produced plants and ensure the sound ecology and friendly environment without any pollution. So, the present experiment was undertaken to study the effect of some botanical extracts in water and cow's urine and cow's urine alone on the growth and sclerotia formation of *S. rolfsii in vitro*.

II. MATERIALS AND METHOD

The experiment was conducted in the plant protection laboratory of Agrotechnology Discipline, Khulna University, Khulna to evaluate the effect of different botanical extracts namely rhizome of turmeric, rhizome of ginger, neem leaf, tobacco leaf in water (soaked tobacco leaf in water for 24 hours), tobacco leaf in cow's urine (soaked tobacco leaf in cows urine for24 hours), and cows urine at different concentrations (30%, 40%, 50%, 60%, and 70%) on the growth and sclerotia formation of *S. rolfsii*, causal agent foot and root rot disease of betel vine. In this experiment the crude extracts of the above indigenous plants and cows urine along with the crude extract of the selected indigenous plants extract were mixed with Potato Dextrose Agar (PDA) medium at different concentrations and the fungal mycelia were inoculated here to grow. The p^H of the medium was adjusted to 6.5 by using p^H meter with the help of 1N HCl or 1N NaOH. Data on the radial growth and number of sclerotia were recorded from there. The fungus *S. rolfsii* was collected from fungal isolates preserved in the plant protection laboratory of Agrotechnology Discipline, Khulna University. Botanical extracts were prepared by using a newly adapted method following the standard procedure (Vijayalakshmi *et al.*, 1999). Following materials were required for the preparation of botanical extracts- tobacco leaves, neem leaves, rhizome of turmeric, rhizome of ginger, and cows urine, conical flask, beaker, soap, detergent, water, glass beaker, measuring cylinder, blender, grinding stone and hand sprayer.The basic PDA medium was modified by using the crude extracts and natural biocides of turmeric, ginger, neem, tobacco leaves in water, tobacco leaves in cow's urine and cow's urine at specified concentrations. The p^H of the medium was adjusted to 6.5. The plates were inoculated 5mm discs of 36 hours old PDA culture and the plates were incubated at 26 ± 2^0C. The radial growths of mycelium and colony characters in each plate were recorded after 60 hours of incubation. After 60 hours of incubation, radial growth (cm) of *S. rolfsii* in petridishes was recorded. The radial growth (cm) of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony and then these plates were kept for 30 days for sclerotia formation.

Percentage inhibition of growth was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{x-y}{X} \times 100$$

Where, x = Average growth (cm) of *S. rolfsii* in control petridishes.

y= Average growth (cm) of *S. rolfsii* in each plant extract and biocides treated petridishes.

After 30 days the sclerotia of each petridish were separated by using camel hair brush and number of sclerotia of each petridish was counted manually. Here a petridish was maintained as control to compare it with others.

III. RESULTS AND DISCUSSION

Effect of different plant extracts and cow's urine on mycelial growth of *S. rolfsii* at different concentration

The different plant extracts and cow's urine in different concentration inhibited the mycelial growth of *S. rolfsii* significantly ($p<0.01$).The mycelial growth inhibition in different concentration of ginger rhizome extracts was significantly increased with the increase of concentration (Table 1). The highest percent inhibition (33.83%) was observed at 70% concentration and the lowest (14.13%) was at 30% concentration which was statistically similar (14.60% and 17.38%) to 40% and 50% concentration respectively. Regression equation (Fig. 1) between concentration of ginger rhizome extract and mycelial growth inhibition percentage revealed that almost 88% of the variation in increased inhibition percentage could be explained by the increase of concentration. The mycelial growth inhibition in different concentration of turmeric rhizome extracts was significantly increased with the increase of concentration (Table 1). The highest percent inhibition (57.13%) was observed at 70% concentration which was statistically similar (56.43%, 55.97% and 55.04%) to 60%, 50%, and 40% concentration respectively and the lowest (47.16%) was at 30% concentration and differed significantly from other concentration. Regression equation (Fig. 1) between concentration of turmeric rhizome extracts and mycelial growth inhibition percentage revealed that 68% of the variation in increased inhibition percentage could be explained by the increase of concentration.

The mycelial growth inhibition in different concentration of neem leaf extracts was significantly increased with the increase of concentration (Table 1). The highest percent inhibition (13.67%) was observed at 70% concentration which was statistically similar (13.44%, 11.01% and 10.17%) to 60%, 50% and 40% concentration respectively and the lowest percentage inhibition (6.491%) was at 30% concentration which was statistically similar (10.17%) to 40% concentration. Regression equation (Fig. 1) between concentration of neem leaf extract and mycelial growth inhibition percentage revealed that more than 91% of the variation in increased inhibition percentage could be explained by the increase of concentration. The mycelial growth inhibition in different concentration of water and tobacco leaf extracts was significantly increased with the increase of concentration (Table 1). The highest percentage inhibition (100%) was observed at 70% concentration and the lowest percent of inhibition of mycelial growth (47.28%) was at 30% concentration. Effect of all concentrations differed significantly among each other. Regression equation (Fig. 1) between concentration of tobacco leaf in water and mycelial inhibition percentage revealed that more than 87% of the variation in increased inhibition percentage could be explained by the increase of concentration. No mycelia growth of *S. rolfsii* was found at any concentration of tobacco extract in cow's urine (Table 2, Plate 1 and Fig. 7) i.e percentage inhibition was (100%) at all the concentration used. The mycelial growth inhibition in different concentration of cow's urine was significantly increased with the increase of concentration (Table 1). The highest percent inhibition (100%) was observed at all concentrations (70%, 60%, 50%, and 40%) except 30% where inhibition was only 7.77% which is not consistent to other concentrations.

Effect of different plant extracts and cows urine on sclerotia formation of *S. rolfsii* at different concentration

The different plant extracts and cow's urine at different concentration influence significantly ($p<0.01$) on sclerotia formation of *S. rolfsii* over control. The number of sclerotia produced at different concentration of ginger rhizome extracts was significantly decreased with the increase of concentration (Table 2). The highest number of sclerotia (543) was produced at control condition and the lowest number of sclerotia (255) at 70% concentration. The number of sclerotia (489) produced at 30% concentration was statistically similar (479) to 40% concentration. Numbers of sclerotia produced at other concentration were significantly different among each other. Regression equation (Fig. 2) between concentration and number of sclerotia formation revealed that almost 86% of the variation in decreased number of sclerotia formation could be explained by the increase of concentration. The number of sclerotia produced at different concentration of turmeric rhizome extracts was significantly decreased with the increase of concentration (Table 2). The lowest number of sclerotia (255) at 70% concentration which was statistically similar (277, 269, 267 and 260) to all other concentration (30%, 40%, 50%, 60%, and 70%) of this extract. Regression equation (Fig. 2) between concentration and number of sclerotia formation revealed that more than 74% of the variation in decreased number of spore formation could be explained by the increase of concentration. The number of sclerotia produced at different concentration of neem leaf extracts was significantly decreased with the increase of concentration (Table 2). Number of sclerotia at 70% concentration (402) was statistically similar (404, 407, 427, and 435) to 60%, 50%, 40%, and 30% concentration respectively but differed from control (543). Regression equation (Fig. 2) between concentration and number of sclerotia formation revealed that more than 86% of the variation in decreased number of sclerotia formation could be explained by the increase of concentration

The number of sclerotia produced at different concentration of tobacco leaf in water was significantly decreased with the increase of concentration (Table 2). The number of sclerotia produced at different concentration varied significantly. No sclerotia were formed at 70% and 60% concentration. Only 14 sclerotia were formed at 50% concentration followed by 40% (53) and 30% (147) and they differed significantly ($p<0.01$) among each other. Regression equation (Fig. 2) between concentration and number of sclerotia formation revealed that more than 85% of the variation in decreased number of sclerotia formation could be explained by the increase of concentration.

No sclerotia formation was observed at any concentration of tobacco leaf extract in cow's urine at any concentration (Table 2). No sclerotia were formed in the plates with cow's urine at 70%, 60%, 50%, and 40% concentration. Only sclerotia (379) were formed at a concentration of 30%. Number of sclerotia formed at a concentration of 30% seems to be inconsistent (Table 2).

Effect of different plant extracts and cow's urine on mycelial growth of *S. rolfsii* at specific concentration

The different plant extracts and cow's urine at specific concentration significantly ($p<0.01$) inhibit the mycelial growth of *S. rolfsii* over control. The mycelial growth inhibition was significantly differing due to the addition of different plant extracts and cows urine at a specific concentration (30%) (Table 1). The highest percent inhibition (100%) was observed in tobacco leaf in cow's urine and the lowest percentage inhibition (6.491%) was in neem leaf extract. The mycelial growth inhibition in turmeric rhizome extracts (47.16%) was statistically similar (47.28%) to extract from tobacco in water and growth inhibition (7.77%) in cow's urine was statistically similar (6.49%) to neem leaf extract. The mycelial growth inhibition was significantly differing due to the addition of different plant extracts and cows urine at a specific concentration (40%) (Table 1). The highest percent inhibition (100%) was observed in tobacco leaf in cow's urine which was statistically similar (100%) to tobacco leaf in water and the lowest percent inhibition was (10.17%) in neem leaf extract. The mycelial growth inhibition was significantly differing due to the addition of different plant extracts and cow's urine at a specific concentration (50%) (Table 1). The highest percentage inhibition (100%) was observed in tobacco leaf in cow's urine which was statistically similar (100%) to tobacco leaf in water and the lowest percent inhibition (11.01%) was in neem leaf in extract. The mycelial growth inhibition was significantly differing due to the addition different plant extracts and cow's urine at a specific concentration (60%) (Table 1). The highest percent inhibition (100%) was observed in tobacco leaf in cow's urine which was statistically similar (100%) to tobacco leaf in water and (100%) to cow's urine and the lowest percent inhibition (13.44%) was in neem leaf in extract. The mycelial growth inhibition was significantly differing due to the addition different plant extracts and cow's urine at a specific concentration (70%) (Table 2). The highest percentage inhibition (100%) was observed in tobacco leaf in cow's urine, cow's urine and tobacco leaf in water and the lowest percentage inhibition (13.67%) was in neem leaf in extract.

Table 1. Effect of different botanical extracts and cows urine at different concentration on mycelial growth inhibition of *S. rolfsii*

Plant extract and cow's urine	Inhibition percentage at different concentration				
	30%	40%	50%	60%	70%
Ginger rhizome	14.13 hij	14.60 hi	17.38 h	29.55 g	33.83 f
Turmeric rhizome	47.16 e	55.04 d	55.97 d	56.43 d	57.13 d
Neem leaf	6.491l	10.17 jkl	11.01 ijk	13.44 hij	13.67 hij
Tobacco leaf in water	47.28 e	69.87 c	92.7 b	100 a	100 a
Tobacco leaf in cow's urine	100 a	100 a	100 a	100 a	100.0 a
Cow's urine	7.77 kl	100 a	100 a	100 a	100.0 a
CV (%)	3.99				

Means followed by a different letters are significantly different at 1% level

Effect of different plant extracts and cow's urine on sclerotia formation of *S. rolfsii* at specific concentration

The different plant extracts and cow's urine at specific concentration significantly ($p<0.01$) influence the formation of sclerotia of *S. rolfsii* over control.The number of sclerotia (543) production at control condition (0% concentration) was similar for all the treatments (plant extracts and cow`s urine). The number of sclerotia formation was significantly differing on different plant extracts and cow's urine at a specific concentration

(30%) (Table 2). The highest number of sclerotia (489) was formed in ginger rhizome extracts and the lowest number of sclerotia (147) in tobacco leaf in water but no sclerotia formed in tobacco leaf in cow's urine.
The number of sclerotia formation was significantly differing on different plant extracts and cow's urine at a specific concentration (40%) (Table 2). The highest number of sclerotia (479) was formed in ginger rhizome extracts. The lowest number of sclerotia (53) in tobacco leaf in water. There were no sclerotia formed in cow's urine and tobacco leaf in cow's urine. The number of sclerotia formation was significantly differing on different plant extracts and cow's urine at a specific concentration (50%) (Table 2). The highest number of sclerotia (407) was formed in neem leaf extracts which was statistically similar (402) to ginger rhizome extract and the lowest number of sclerotia (14) in tobacco leaf in water. No sclerotia were formed in cow's urine and tobacco leaf in cow's urine. The number of sclerotia formation was significantly differing on different plant extracts and cow's urine at a specific concentration (60%) (Table 2). The highest number of sclerotia (404) was formed in neem leaf extracts and lowest number of sclerotia (260) in turmeric rhizome extract. There were no sclerotia formed in tobacco leaf in water, cow's urine and tobacco leaf in cow's urine. The number of sclerotia formation was significantly differing on different plant extracts and cows urine at a specific concentration (70%) (Table 2). The highest number of sclerotia (402) was formed in neem leaf extract and the lowest numbers of sclerotia (255) in ginger rhizome extract which was statistically similar to turmeric rhizome extract (255). There were no sclerotia formed in tobacco leaf in water, cow's urine and tobacco leaf in cow's urine.

Table 2. Effect of different botanical extracts and cows urine at different concentration on sclerotia formation of *S. rolfsii*

Plant extract and cow's urine	Number of sclerotia at different concentration					
	30%	40%	50%	60%	70%	Control
Ginger rhizome	489 b	479 b	402 cd	324 e	255 f	543 a
Turmeric rhizome	277 f	269 f	267 f	260 f	255 f	
Neem leaf	435 c	427 c	407 cd	404 cd	402 cd	
Tobacco leaf in water	147 g	53 h	14 i	00 i	00 i	
Tobacco leaf in cow's urine	00 i	00 i	00 i	00 i	00 i	
Cow's urine	379 d	00 i	00 i	00 i	00 i	
CV (%)	8.34					

Means followed by a different letters are significantly different at 1% level

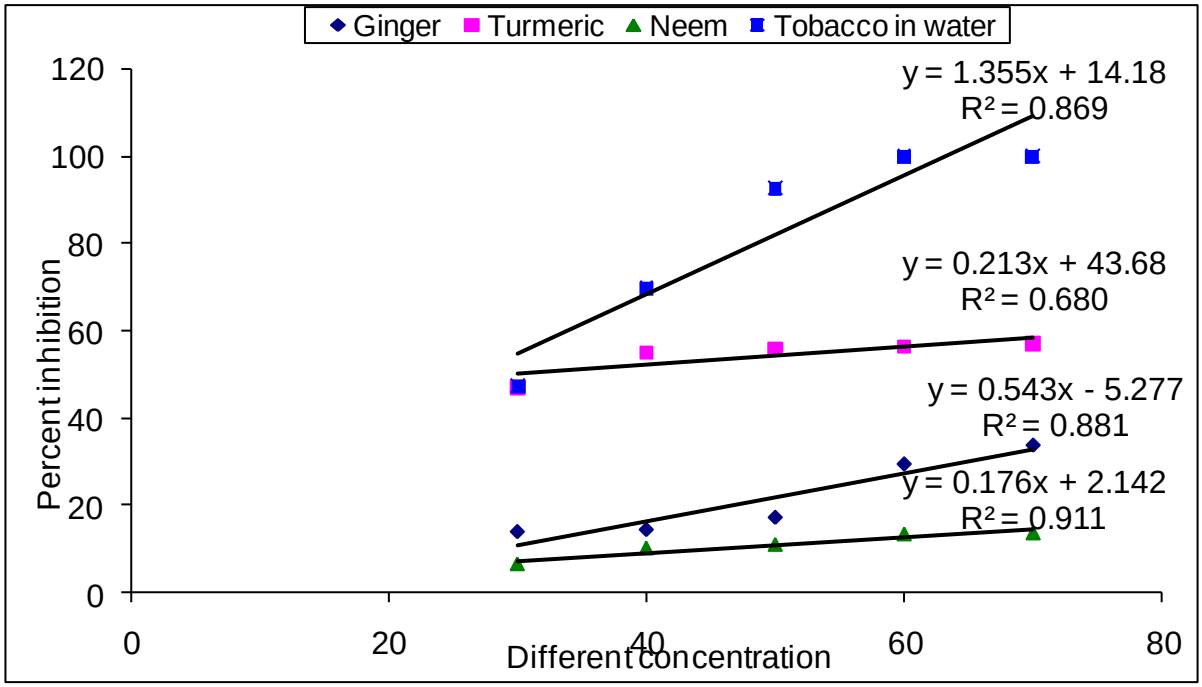

Fig 1: Functional relationship between concentrations and mycelial growth inhibition percentage

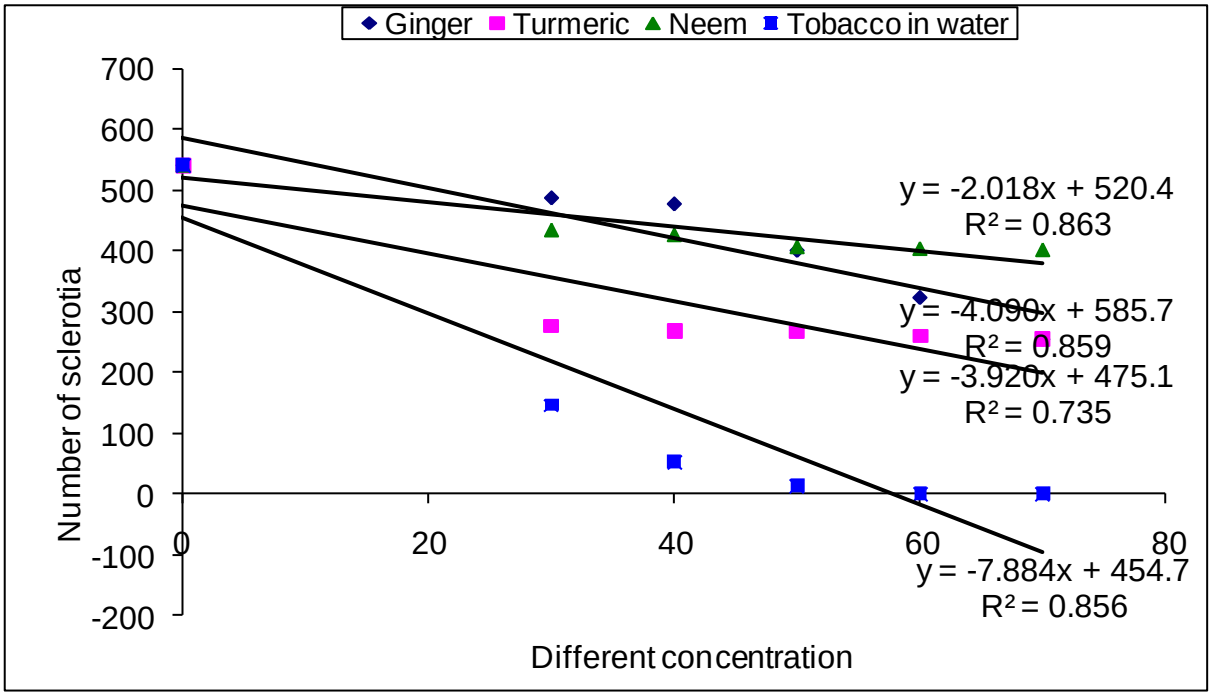

Fig 2: Functional relationship between concentrations and formation of sclerotia

IV. CONCLUSION

For all the plant extracts and cow's urine, mycelial growth inhibition was increased and sclerotia formation was decreased with the increase of concentration. In the specific plant extracts and natural biocides tobacco leaf extract in cow's urine was more effective in both of mycelial growth inhibition and sclerotia formation.

V. REFERENCES

[1] Ahmed, F. 1980. Control of foot and root rot disease of wheat. A M.Sc. thesis submitted to Plant Pathology Department,Bangladesh Agricultural University (BAU), Mymensingh.
[2] Anonymous, 2006.Asiatic society of Bangladesh.Arun, A. C., Tekha and A. Chitra.1995. Effects of alicin of garlic extract and Bigonia on two fungi. Indian J. Mycol. Plant
[3] Path. 2593): 316-318.
[4] Aycock, R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. North Carolina Agricultural experiment Station
[5] Technical Biulletin, 2: 174-202.
[6] Buss, E. A. and Park, S. G. 2002. Journal of Natural Products for Insect Pest Management. 10(4): 311-318.
[7] Delvin, J. F. and Zettel, T. 1999. Eco-agriculture: Initiatives in Eastern and Southern Africa. 6(2): 150-152.
[8] Goswami, B. K., K. A. Kader, S. K. Adhikary, M. R. Islam, K. G. Quddus and P. K. Malaker. 2002. Severity of leaf rot of
[9] betel vine (*Piper betel* L.) through the year. Bangladesh J. of Agril. Res. 27(3): 497-501.
[10] Grange, N. and Ahmed, S. 1988. Handbook of Plants with Pest Cpntrol Properties. 7(5): 75-78.
[11] Hassan, S. A. and Shahat , S. 2005. Diseases affecting betelvine. 3(2):4-5.
[12] Islam, M. 2005. Country news, Holiday Publication Limited, 8: 3-4.
[13] Mohanty, A. K., A. K. Kar and P. N. Setti. 1995. Efficacy of crude plant extracts of some selected plants in controlling
[14] brinjal blight and fruit rot pathogen, *Phomopsis vexans*. Crop Research Hisar., 9(3): 447-448.
[15] Rahman, G. M. M., M. R. Islam and M. A. Wadud. 1999. Seed treatment with plant extracts and hot water: a potential
[16] biophysical method of controlling seed borne infection of wheat. Bangladesh J. Training Develop. 12(1-2): 185-190.
[17] Roy, B., Amin, R., Uddin, M. N., Islam A. T. M. S., Islam, M. J. and Halder, B. C. 2005. Leaf extracts of Shiyalmutra
[18] (*Blumea lacera*) as botanical pesticides against lesser grain borer and rice weevile. Journal of Biological Sciences, 5(2): 201-204.
[19] Sayeduzzaman, M. 1988. An economic geographical study of betel leaf cultivation in Bangladesh. A M.Sc. thesis submitted
[20] to Department of Geography, University of Dhaka.
[21] Talukder, M. 1974. Plant diseases in Bangladesh. Bangladesh J. of. Agril. Res.1(1): 64-68.
[22] Vijayalakshmi, K., Subhashini, B., Koul, S. 1999. plants in pest Control, pongam, tulasi and aloe. Centre for Indian
[23] Knowledge Systems, Chennai, India.