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Inhibitive Activity of Cow Urine and Cow Dung against *Sclerotinia sclerotiorum* of Cucumber

A. B. Basak*, Min Woong Lee[1] and Tae Soo Lee[2]

Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh
[1]Department of Biology, Dongguk University, Seoul 100-715, Korea
[2]Department of Biology, University of Inchon, Inchon 402-749, Korea

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A study on comparative efficacy and *in vitro* activity of fresh cow urine and cow dung for controlling Sclerotinia rot caused by *Sclerotinia sclerotiorum* of cucumber was carried out following mycelial growth inhibition test, treated and untreated sclerotia with these organic matters at different days of incubation. Results showed that cow urine suppressed more effectively the mycelial growth even after 5 days of incubation in comparison to cow dung. The highest inhibition 75.9% of mycelial growth was recorded in cow dung potato dextrose agar (CUPDA) after 3 days of incubation and least 22.7% was in cow dung potato dextrose agar (CUPDA) after same days of incubation. Mycelial growth from sclerotia of *S. sclerotiorum* was also influenced by PDA medium mixed with cow urine and cow dung. After 6 days of incubation in CUPDA mycelial growth was only 12.9 mm whereas in CDPDA and PDA the corresponding growth at the same time were 65.8 mm and 80.0 mm. Treated sclerotia of the selected fungus with cow urine had a very effective role on suppression of mycelial growth than that of untreated one. No mycelial growth was observed up to 4 days in treated sclerotia with cow urine. After 5 days only 0.9 mm mycelial growth was measured in treated sclerotia, while in case of untreated sclerotia the growth was 42.6 mm. Application of cow urine and cow dung on growing plants inoculated with the pathogen at different concentrations also proved their inhibitive effects.

KEYWORDS: Cow urine and cow dung, Cucumber, Inhibitive activity, *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum (Lib.) de Bary a most common fungal pathogen causing Sclerotinia rot of many vegetable crops including cucumber, has been found to cause severe infection every year in the crop fields and glass houses of Korea and other countries of the world (Jagger, 1920; Kohn, 1979a, b; Gamundi and Spinedi, 1987; Graf and Schumacher, 1995; KSPP, 1998; Cho *et al.*, 1997; Kim *et al.*, 1999). Due to relatively low temperature and high relative humidity in most of the glass houses, the occurrence of the fungal pathogen is very common and which can survive or overwinter in the soil as sclerotia for a long period (Agrios, 1988). Symptoms produced by Sclerotinia rot results in not only sudden death of plants but also appears in stem and fruit. Many researchers recorded the disease as stem rot, stem blight, fruit rot, timber rot and cottony rot depending on host (Farr *et al.*, 1989). In an intensive survey on six cucurbitaceous crops namelynetted melon, cucumber, pumpkin, summer squash, water melon and oriental melon, Kim *et al.* (1999) recorded as high as 30~70% disease incidences. Many researchers also identified and illustrated the causal organism of the Sclerotinia rot with characteristic typical symptoms (Williams and Spooner, 1991; Farr *et al.*, 1989; Purdy, 1979; Cho *et al.*, 1997). Though it is a serious pathogen on many vegetable crops, very little study on cultural and chemical control of the pathogen *S. sclerotiorum* has been carried out from different parts of the world (Walker, 1952; Nyvall, 1989; Agrios, 1988). Recently Basak and Lee (2001a, b) reported that fresh cow urine and cow dung has positive response in suppression of mycelial growth of *Fusarium solani* f. sp. *cucurbitae* and *F. oxysporum* f. sp. *cucumerinum*, and protective effect on Fusarium root rot and wilt of cucumber by applying these bio matters under pot conditions.

This research carried out to determine the efficacy of cow urine and dung on *Sclerotia sclerotiorum* under *in vivo* and *in vitro* conditions.

Material and Methods

Selection of fungus. *Sclerotinia sclerotiorum* was isolated from the infected samples collected from the different selected glass houses located at Gimpo city and Dongguk university farm at Go-yaung city, Korea.

Inhibitive activity on mycelial growth. (1) Cow urine: Inhibition activity of cow urine on mycelial growth of *S. sclerotiorum* was surveyed on cow urine potato dextrose agar (CUPDA) medium. The ingredients and their quantities are as follows: peeled and sliced potato-20 grams, dextrose-20 grams, cow urine- 500 m*l* and distilled water-500 m*l* (pH 7.4). Mycelial blocks of 5 mm diameter of

*Corresponding author <E-mail: abbasak@ctgu.edu>

S. sclerotiorum taken from 10 days old culture were used. Sclerotia collected directly from full grown PDA culture medium were also used in the experiment. In each Petri dish a disc of mycelial block (5 mm) and a sclerotia of *S. sclerotiorum* was placed at the center. Every after 24 hours mycelial growth from the disc or sclerotia was measured and it was continued for 5 days. Percentage of inhibition of mycelial growth was calculated (Ashrafuzzaman, 1976)

(2) Cow dung: For this test, cow dung potato dextrose agar (CDPDA: cow dung- 20 grams, peeled and sliced potato- 200 grams, dextrose 20 grams, agar- 20 grams and distilled water-1000 m*l*) was used. The same method was followed as described under cow urine.

Effect of cow urine on activity of sclerotia. Scleotia were collected from full grown culture medium and dipped in cow urine for 24 hour. The treated sclerotia were placed on PDA and mycelial growth from the sclerotia was daily measured for 5 days.

Inhibitive activity of cow urine and dung on *S. sclerotiorum* inoculated plants grown in pots. Inhibitive activity of cow urine and dung was also tested on *S. sclerotiorum* inoculated cucumber plants grown in pots. *S. sclerotiorum* was grown in corn dextrose agar (CDA) medium at 25°C for 10 days. In all 90 cucumber plants in pots were selected for this experiment. Inocula of *S. sclerotiorum* from 10 days old culture were placed at the surrounding soils of the collar parts of 2 months old plants. Among the plants, 40 plants 10 each were applied with cow urine and the other 40 plants 10 each with cow dung at concentrations of un-dilution, 1 : 1, 1 : 2, and 1 : 4. The remaining 10 plants were not treated with either cow urine or dung. After application of these bio matters observation was taken if any kinds of wilt symptoms or death of plants occurred every after 24 hours and this observation was continued for 10 days.

Results and Discussion

Effects of cow urine and cow dung on mycelial growth of *S. sclerotiorum* varied widely (Table 1, Fig. 1A and 1B). Results showed that cow urine significantly decreased the mycelial growth even after 5 days of incubation in comparison to cow dung. After an hour of incubation, fungal growth of CUPDA and CDPDA were 5.6 and 6.2 mm, whereas in PDA the mycelial growth was 13.6 mm. After 5 days of incubation, in presence of cow urine in medium, the mycelial growth was recorded as 17.2 mm whereas in PDA the growth was 53.8 mm. In case of percentage inhibition of mycelial growth the highest inhibition (75.9%) was recorded in CUPDA after 3 days of incubation and the least (22.7%) in CDPDA after same days of incubation (Table 1).

Table 1. Effects of cow urine and cow dung on the mycelial growth from a mycelial of *Sclerotinia sclerotiorum* after different days of incubation

Days of incubation	Mycelial growth (mm) in			% inhibition of mycelial growth	
	CUPDA[a]	CDPDA[b]	PDA	CUPDA	CDPDA
1	05.6	06.2	13.6	58.6	54.7
2	07.0	08.4	22.0	68.2	61.7
3	09.3	29.7	38.4	75.9	22.7
4	14.9	34.7	45.9	67.5	24.5
5	17.2	40.8	53.8	68.0	24.1

[a]CUPDA : Cow urine potato dextrose agar.
[b]CDPDA : Cow dung potato dextrose agar

Effect of cow urine and cow dung medium on direct mycelial growth from sclerotia of *S. sclerotiorum* also differed greatly (Table 2, Fig. 1B and 1E.). No mycelial growth in CUPDA was recorded within 4 days of incubation; while in CDPDA and PDA some growth of mycelium was started. After 6 days of incubation, mycelial growth was only 12.9 mm in CUPDA, whereas in CDPDA and PDA the corresponding growth was 65.8 mm and 80.0 mm respectively. In case of percentage inhibition of mycelial growth the highest values (100%) were recorded after second, third and fourth days of incubation and the lowest (17.8%) after 6 days of incubation in CDPDA.

Effect of cow urine treated and untreated sclerotia of *S. sclerotiorum* on average diameter of mycelial growth in PDA medium after different days of incubation varied widely with respect to days on incubation and nature of treatment (Table 3, Fig. 1C). Results showed that treated sclerotia with cow urine had a very effective role on suppression of mycelial growth than that of untreated one. No mycelial growth was recorded up to 4 days in treated sclerotia with cow urine. After 5 days only 0.9 mm mycelial growth was measured in treated sclerotia while in case of untreated scleroitia the growth was 42.6 mm.

Results of inhibitive of cow urine and cow dung on inoculated plants with *S. sclerotiorum* at different concentrations are presented in Table 4, Fig. 1G and 1H. It was revealed from the data that cow dung had better inhibitive effect on pathogen inoculated plants in comparison to plants treated with cow urine. No plant infection was recorded at un-dilution, 1 : 1 and 1 : 2 concentrations of cow dung solution when applied to the pathogens inoculated plants. However direct application of fresh cow dung at un-diluted condition caused the death of all inoculated plants. Percentage of infected plants gradually increased with the decrease of concentration rates of cow urine.

In the present study, cow urine and dung had different inhibitive activities both *in vitro* and *in vivo* conditions. With the increase of concentration of these bio matters the

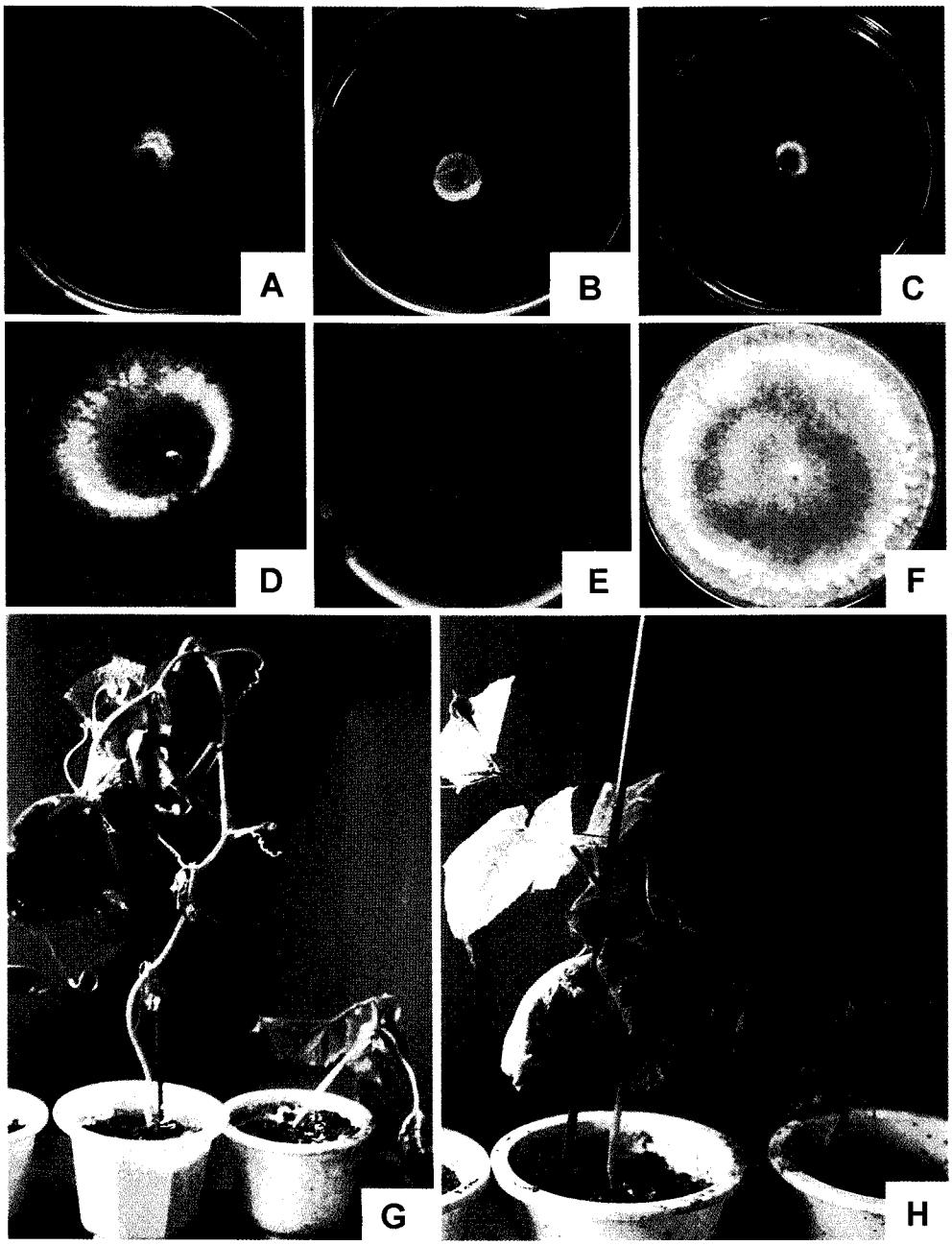

Fig. 1. Effect of cow urine (A-C) and dung (D-E) on mycelial growth of *Sclerotinia sclerotiorum* and control. Mycelial growth of the fungus from a mycelial block (A), and from sclerotium (B) in cow urine PDA medium and from treated sclerotium (C) in PDA medium. Radial growth of the fungus from a mycelial block (D), untreated sclerotium (E) in cow dung PDA medium and fungal growth in PDA medium (F) as control. Effect of Cow urine (G) and dung (H) on inoculated plants with *S. sclerotiorum*. In both cases, one healthy plant with the bio matter treatment (left) and another infected plant (right) without treatment are growing in pots.

percentage of plant infection decreased. So it is indicated clearly that toxicity of these bio matters against the pathogens depends on their concentrations. Moreover direct application of cow dung to the pathgen-inoculated plants caused the death of plant. Therefore, cow dung at undiluted concentrations possessed high toxicity.

Results also showed that cow dung showed better inhibitive activity against the selected fungal pathogen than that of cow urine. As cow dung is composed of many digested and undigested plant materials so it is very easy for cow dung to protect plants creating toxic barriers against the inoculated pathogen. Moreover, chemicals or gases present in cow urine may be evaporated after application to the plants. So inhibitive activity for cow urine may be lowered in comparison to cow dung.

Recently, Basak and Lee (2001) reported that cow urine

Table 2. Effects of cow urine and cow dung on mycelial growth from Sclerotia of *Sclerotinia sclerotiorum* after different days of incubation

Days of Incubation	Mycelial growth (mm) in			% inhibition of mycelial growth	
	CUPDA[a]	CDPDA[b]	PDA	CUPDA	CDPDA
1	–	–	–	–	–
2	–	02.6	07.2	100.0	63.5
3	–	13.2	25.0	100.0	47.1
4	–	42.2	58.0	100.0	29.8
5	07.4	50.3	75.6	90.2	33.5
6	12.9	65.8	80.0	84.0	17.8

[a]CUPDA : Cow urine potato dextrose agar.
[b]CDPDA : Cow dung potato dextrose agar.

Table 3. Effect of cow urine treated and untreated sclerotia of *Sclerotinia sclerotiorum* on average diameter of mycelial growth in PDA medium after different days of incubation

Days of incubation	Radial growth growth (mm) of mycelium on PDA medium of	
	Treated sclerotia	Untreated sclerotia
1	–	–
2	–	02.7
3	–	10.6
4	–	25.5
5	0.9	42.6

Table 4. Effect of cow urine and cow dung on inoculated plants with *Sclerotinia sclerotium* at different concentrations

Bio matters Concentration	5 infected plants in		
	CDA[a]	CDA + Cow urine	CDA + Cow dung
Un-dilution	–	30.0	0.0
1 : 1	–	40.0	0.0
1 : 2	–	60.0	0.0
1 : 4	–	70.0	40.0
Control	100.0	–	–

[a]CDA : Corn dextrose agar.

and cow dung has an effective role on mycelial growth of *F. oxysporum* f. sp. *cucumerinum* and *F. solani* f. sp. *cucurbitae* causing fusarium wilt and root rot of cucumber, respectively. Both cow urine and cow dung have been used in Indo -Bangla sub continent for curing some human diseases. It is pertinent to mention here that the role of cow urine and cow dung in controlling plant pathogens never have been studied. So results of the present experiment will provide a new technology for controlling plant pathogens instead of using different chemical fungicides that are very hazardous to both environment and soil structure. Complete suppression of mycelial growth of *S. sclerotiorum* may be possible if different herbal plant extracts are added with fresh cow urine and cow dung before application. In order to establish the above results for the commercial use a series of different trials should be initiated.

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