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In Vitro Inhibitory Activity of Cow Urine and Dung to *Fusarium solani* f. sp. *cucurbitae*

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This paper deals with the study on comparative efficacy and *in vitro* activity of cow urine and cow dung for controlling root rot disease of cucumber caused by *Fusarium solani* f. sp. *cucurbitae* Snyder & Hansen following slide germination and mycelial growth inhibition tests. Results showed that both germination of conidia and the percentage inhibition of mycelial growth decreased or suppressed and varied greatly with respect to different hour and days of incubation and kind of bio-matters. In between two bio-matters cow urine was found more effective than that of cow dung in conidial germination. No germination of conidia was recorded after one hour of incubation in any medium whereas in cow urine germination of conidia was not also observed even after 2 hours of incubation. After 7 hours of incubation out of 200 conidia of *F. solani* f. sp. *cucurbitae*, 28 in cow urine and 64 in cow dung were germinated while in control a total germinated conidia was 185. In case of percentage inhibition of conidial germination the highest percentage (100%) was recorded in cow urine after 2 hours of incubation followed by 3 hours (96.0%), 4 hours (91.0%) and 6 hours (89.4%). During the test on inhibition of mycelial growth, the highest percentage (62.8%) was recorded in cow urine potato dextrose agar (CUPDA) medium tested after 4 days of incubation, followed by 3 days (60.5%), 5 days (56.5%) and 2 days (55.0%). In this test cow dung potato dextrose agar (CDPDA) had less efficacy in suppression of the percentage inhibition of mycelial growth.

KEYWORDS: Control, Cow dung, Cow urine, Cucumber, *Fusarium solani* f. sp. *cucurbitae*, Inhibitory activity, Root rot disease

Cucumber (*Cucumis sativus* L.), the important vegetable crop of Korea suffers from more than 13 fungal diseases (Holliday, 1970; Cho *et al.*, 1997; Anonymous, 1998; Kim *et al.*, 1999). Among them, root rot disease caused by *Fusarium solani* f. sp. *cucurbitae* Snyder & Hansen is one of the most important pathogen which cause sudden death of growing plants and cause heavy yield loss to the crop (Booth and Waterson, 1964; Walker, 1952). Many other crops are also infected with *Fusarium* spp. In fact, this pathogen can survive or over winter in the soil of glass houses and fields for long time (Agrios, 1988). So it is very difficult to eliminate this pathogen from the soil by chemical fungicides. Moreover chemical fungicides are very hazardous to environment causing air pollution and changes in soil properties. Literature review shows that some research works had already been carried out for controlling this fungal disease by cultural practice and biological control (Bakker *et al.*, 1990; Datnoff *et al.*, 1995; Leeman *et al.*, 1996; Boer *et al.*, 1999; Lee *et al.*, 2001). To eliminate this pathogen from the crop field and glass house is very much needed for growing healthy plants. Basak and Lee (2001a) reported that cow dung is very effective for controlling Fusarium wilt of cucumber. In another study, Basak and Lee (2001b) observed that cow urine and cow dung are capable of suppressing conidial germination and mycelial growth of *F. oxysporum* f. sp. *cucumerinum* Owen causing Fusarium wilt of the crop.

So in the present study an attempt was made to study efficacy and *in vitro* activities of cow urine and cow dung against *F. solani* f. sp. *cucurbitae*, the causal agent of root rot disease of cucumber.

Materials and Methods

Selection of fungi. In this study, *F. solani* f. sp. *cucurbitae* Snyder & Hansen responsible for causing wilt diseases of cucumber was used. This fungal pathogen was isolated from the diseased samples collected from cucumber grown in some glass houses of New Sali, Kimpo city, Korea.

Slide germination test. (a) For cow urine. Five-milliliter spore suspension from 10 days old culture of *F. solani* f. sp. *cucurbitae* was made and adjust the concentration of spore to approximately 20~30 per optical field (400×). From this stock solution 10-μl suspensions was taken on a slide as a drop and equal amount of fresh cow urine was mixed together in a small watch glass. This spore suspension was kept on a slide. A moist chamber was prepared by adding sterile water to Whatman filter paper on the bottom of petri dishes. Several replications of slide with the mixture of spore suspension and cow urine was kept

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at the bottom of petri dishes and incubated in the moist chamber up to 7 hours at room temperature. After every hour germination of spores in slide were counted. Two hundred conidia per treatment for germination after a definite period of incubation were counted under compound microscope. Before counting spore germination, a drop of cotton blue or lactophenol solution on a slide was taken and cover slip was kept on spore suspension to check further germination. Percentage inhibition of spore germination and percentage of inhibition of mycelial growth were calculated (Ashrafuzzaman, 1976):

(b) For cow dung. Five grams of fresh cow dung and five milliliter of sterile water were mixed. As the cow dung was full of different undigested plant tissues so spore germination test were somewhat difficult to observe under microscope. To avoid this problem, cow dung mixture was filtered through Whatman filter paper No. 1001 110. From this filtrate 10 m*l* cow dung solution was taken and kept on slide where 10 m*l* spore suspension of *F. solani.* sp. *cucurbitae* was taken earlier. The same methodology for spore germination in cow dung solution was followed as described under cow urine test.

Mycelial growth inhibition test. (a) For cow urine. In this test, cow urine potato dextrose agar (CUPDA-Peeled

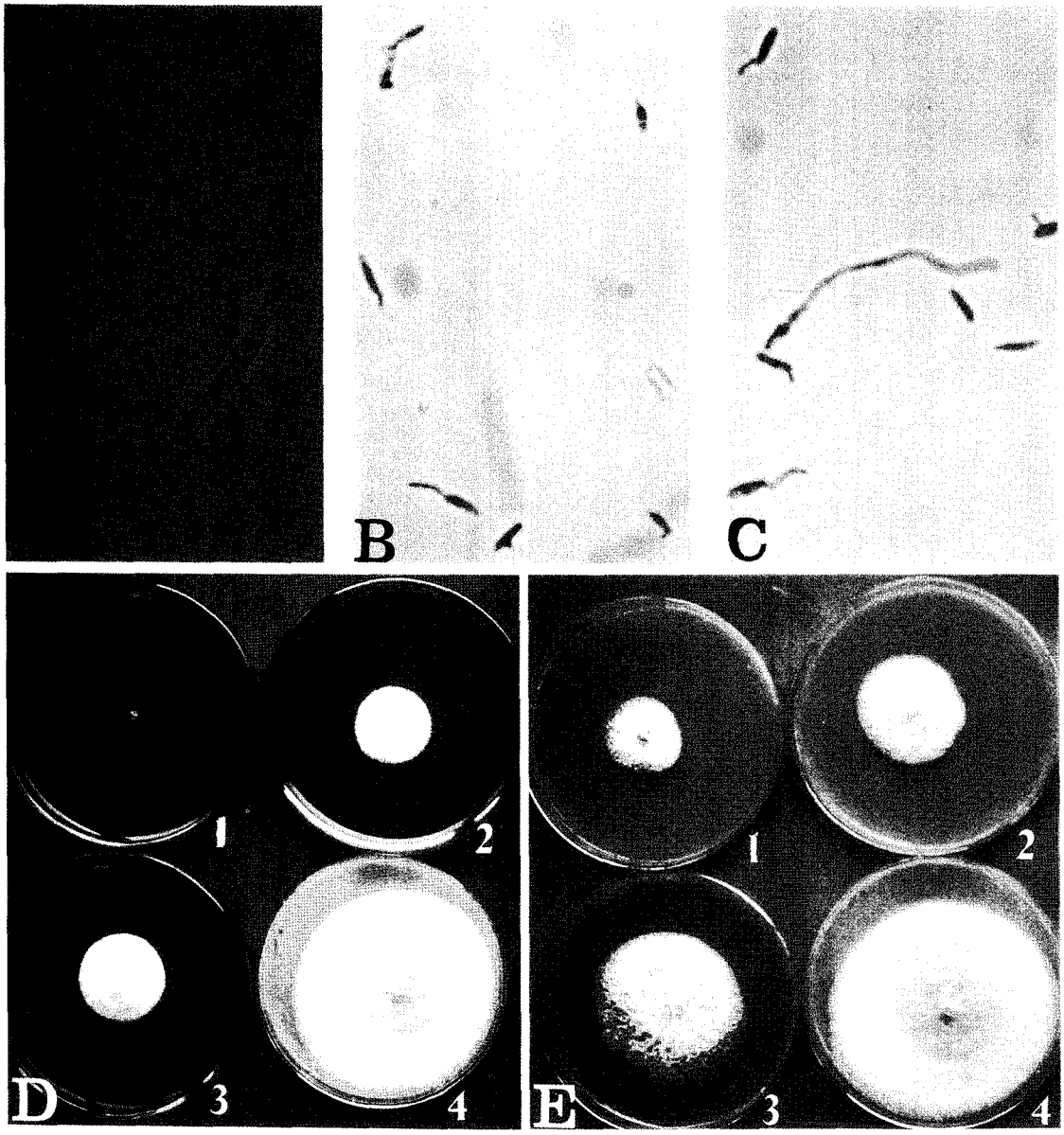

Fig. 1. Effects of cow urine (A) and cow dung (B) on conidial germination and mycelial growth (D & E) of*Fusarium solani* f. sp. *cucubitae* at different incubation period. A. Germination of conidia in cow urine solution, B. germination of conidia in cow dung solution, and C. germination of conidia in sterile water after 7 hours of incubation. D1, D2 & D3. Mycelial growth in cow urine PDA medium; E1, E2 & E3. Mycelial growth in cow dung PDA medium and D4 & E4. Mycelial growth in PDA medium after 7 days of incubation.

and sliced potato-20 grams, Dextrose-20 grams, Cow urine-500 m*l*. and Distilled water-500 m*l*, pH 7.4) medium was used. Mycelial blocks of 5-mm. diameter of *F. solani*. sp. *cucurbitae* taken from 10 days old culture were used. Every after 24 hours mycelial growth from the disc was measured and it was continued till 7 days. Percentage of inhibition of mycelial growth was calculated (Ashrafuzzaman, 1976).

(b) For cow dung. For this test, cow dung potato dextrose agar (CDPDA-Cow dung-20grams, Peeled and sliced potato-200 grams, Dextrose-20 grams, Agar-20 grams and Distilled water-1000 m*l*) was used. The same methodology was followed for preparing CDPDA as mentioned earlier under slide germination test. In this test 20 grams cow dung was used instead of 500 m*l* of cow urine.

Results and Discussion

Effects of cow urine and cow dung on the percentage germination of conidia and their inhibition of *Fusarium solani* f. sp. *cucurbitae* at different hours of incubation are presented in Table 1 (Figs. 1A and B). It was revealed from the data that both the bio-matters had an effective role in controlling germination and its inhibition of conidia. In between two bio matters cow urine was found to be more effective than that of cow dung in conidial germination. No germination of conidia was recorded after 1 hour of incubation in any media whereas in cow urine germination of conidia was not also found in 2nd hour of incubation. After 3 hours of incubation only two conidia were germinated in cow urine treatment. In case of percentage inhibition of conidial germination the highest percentage (100%) was recorded in cow urine after 2 hours of incubation followed by 3 hours (96.0%), 4 hours (91.1%) and 6 hours (89.4%) period of incubation.

The efficacy of cow urine and cow dung on suppression of mycelial growth and their percentage inhibition

Table 1. Effects of cow urine and cow dung on the germination and percentage inhibition of conidia of *Fusarium solani* f. sp. *cucurbitae* at different hours of incubation

Hours of incubation	Total no. of conidial germination[a]			% inhibition of conidial germination	
	Cow urine	Cow dung	Control	Cow urine	Cow dung
1	–[b]	–	–	–	–
2	–	8	16	100.0	50.0
3	2	12	50	96.0	76.0
4	8	30	90	91.0	66.7
5	12	44	108	88.9	59.3
6	18	56	170	89.4	67.1
7	28	64	185	84.9	65.4

[a]Based on 200 conidia.
[b]No germination.

Table 2. Effects of cow urine and cow dung on mycelial growth (mm) of *Fusarium solani* f. sp. *cucurbitae* at different days of incubation

Days of incubation	Radial growth (mm) in		
	CUPDA[a]	CDPDA[b]	PDA
1	05.0	05.0	05.0
2	06.7	08.4	14.9
3	11.8	14.8	29.9
4	14.6	18.6	39.2
5	19.5	24.9	44.8
6	23.1	29.9	52.8
7	27.3	34.4	59.0

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

Table 3. Effects of cow urine and cow dung on inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum* at different days of incubation

Days of incubation	% Inhibition of mycelial growth in	
	CUPDA[a]	CDPDA[b]
1	–	–
2	55.0	43.6
3	60.5	50.5
4	62.8	51.8
5	56.5	44.4
6	56.3	43.4
7	53.8	41.7

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

are shown in Table 2 and Table 3 (Figs. 1D and E). Both cow urine and cow dung was more effective in suppressing mycelial growth. Cow urine had comparatively better efficacy than that of cow dung in suppressing mycelial growth. After 7 days of incubation the highest suppression of mycelial growth 27.3 mm was recorded in CUPDA medium whereas in CDPDA and PDA the corresponding values were 34.4 mm and 59.0 mm respectively. In case of inhibition of mycelial growth the highest percentage ((62.8%) was recorded in CUPDA medium tested after 4 days of incubation followed by 3 days (60.5%), 5 days (56.5%) and 2 days (55.0%) of incubation. In this test CDPDA had less efficacy in suppression of the percentage inhibition of mycelial growth.

Cow urine and cow dung are the two important bio matters that are extensively used by the growers as manure for production of different crops. It is also believed that cow urine and cow dung have some medicinal properties that are employed in ayurvediic or unani (herbal) medicine for curing many human diseases. In India, a cow research center, Ramteck, Nagpur, Mahasashtra has already been established and manufactured some medicine from cow urine and cow dung for treating human diseases. Moreover, cow dung solution is sprayed around the house pre-

mises of hindu family in every early morning. But there was no scientific proof in favor of positive response of cow urine and cow dung for controlling plant pathogens. In a research study Basak and Lee (2001b) proved that cow urine and cow dung had some effectiveness in suppression of conidial germination and mycelial growth of *F. oxysporum* f. sp. *cucumerinum* causing Fusarium wilt of cucumber plants. So the results obtained in the present experiment are more or less coherent with the findings of Basak and Lee (2001b). It is experimentally proved that extracts of different medicinal plants had the suppression effect on mycelial growth of some pathogenic fungi. In an experimental report, Basak and Paul (1999) has already observed that plant extracts of *Azadiracta idica*, *Polygonum hydropiper*, *Lantana camera*, *Cassia tora* and *Moringa oleifera* had suppressive effect on mycelial growth of six major fruit rot fungal pathogens of chilli. So more experiment might be initiated after mixing plant extracts with cow urine and cow dung for complete control of this root rot pathogen. This type of control measure will not create any adverse effect on environment pollution as well as changes in soil properties. Moreover every grower will be able to use this biological control measure with out expending much money.

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