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Influence of cow urine and butter milk on the activity of lytic enzymes secreted by three soil-borne pathogens of soybean

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Soybean crop suffers badly with soil-borne pathogens (*Rhizoctonia bataticola, Sclerotium rolfsii, Fusarium solani* f. sp. *glycine*. The inherent hazardous effects involved in conventional chemical management coupled with the inclination of farmer towards organic farming, use of FYM, green manuring and animal by- products such as cow urine, buttermilk as described in Vedas, Arthshastra, Aginpuran, Surapala's etc for plant protection measures is gaining importance.Various preparation of cow urine viz., Panchagavya, Devversa, Gotech, Goratana, are available in the market for the management of insect-pest and diseases. Antifungal properties have been reported by various workers but very few scientific literature is available on the effect on enzymatic activity. This study indicatyed that ability of the three pathogen to secrete macerating, pectin methyl esterase and carboxy methyl cellulase enzyme were slightly retarded by cow urine and butter milk.

INTRODUCTION

Enzymes play key role in pathogenesis. *Rhizoctonia bataticola, Sclerotium roflsii* and *Fursairum solani* f.sp. *glycine,* causing charcoal rot, collar rot and root rot of soybean, are capable of secreting macerating enzyme (ME), pectin methyl esterase (PME), and cellulase (Cx) during pathogenesis. For the management of pest and diseases, use of bio-control agents and use of indigenous technologies i.e. cow urine and butter milk are being preferably used over chemicals. Cow urine and buttermilk are not only used by Indian farmers but also in other countries like Sri Lanka, Brazil, Korea, Zimbabwe for plant protection measures. Lack of sufficient literature available on these aspects, the present investigation aimed to study the role of cow urine and butter milk on the activity of these enzymes.

MATERIALS AND METHODS

The information about the animal products used in the present investigation are given with their source.

Cow urine

Cow urine collected from dairy farm, JNKVV, Jabalpur, were filtered and sterilized by fltering it through Seitz filter. The filtrate was collected in sterilized stoppered glass bottle and stored at room temperature for further studies.

Butter milk

Butter milk was collected from Sanchi Dugdhashangh Maryadit, Jabalpur. It was filtered and sterilized by filtering it through Seitz filter. The filtrate was kept in a glass stoppered bottle and stored at room temperature.

Determination of enzymatic activities of Rhizoctonia bataticola, Sclerotium roflsii and Fursairum solani f. sp. glycine

The macerating enzyme (ME), pectin methyl esterase (PME), and cellulase (Cx) were determined. Filtrates of 15 days culture grown on Richards, medium were used for the study.

Preparation of culture filtrate

Richards' solution 100 ml was dispensed to each of the 500 ml Erlenmeyer flasks and was sterilized in an autoclave. Ten mm (diameter) mycelial disc of 7 days old culture of *Rhizoctonia bataticola, Sclerotium roflsii* and *Fursairum solani f.* sp. *glycine* was transferred in each flask and incubated at 25°C for 15 days. The medium was filtered through a double layered muslin cloth to separate mycelial mat. This filtrate was then passed through a sterilized Seitz filter unit. The filtrate thus obtained was collected in sterilized conical flask and used for assaying enzymatic activities.

Preparation of buffers

Two buffers with pH 4.0, and 9.2 were prepared. Adjustment of pH was done by Elico pH meter using sodium citrate buffer. To obtain pH 4.0, 30.7 ml of 0.1 m citric acid (19.21 g in 100 ml) was added to 19.3 ml of 0.2 M dibasic sodium phosphate (71.1 g $Na_2NHPO_4.12H_2O$ in 1000 ml) and finally diluted to 100 ml. To obtained pH 9.2, 4.3 ml of 0.2 M monobasic sodium phosphate (27.8 g in 1000 ml) was added to 95.7 ml of 0.2 M dibasic sodium phosphate and finally diluted to 200 ml. The prepared buffers were rechecked and adjusted by pH meter. These were stored in glass stopped corning bottles (250 ml) at 8°C.

Estimation of macerating enzyme activity

The method described by Singh and Wood (1956) was used for estimation of macerating enzyme activity in the culture filtrate as given below :

10 ml of the culture filtrate (pH 4.0, and 9.2) was poured into 9 mm sterilized petriplates. Five potato discs (10 × 0.5 mm) of the c.v. Chandramukhi were placed in each petriplates at equal distance. The discs were cut with the help of a hand microtome. The macerating activity was judged by the length of time taken for complete maceration of potato discs. Maceration was observed after 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 hr intervals till complete maceration. If the discs were easily teased to needles it indicated complete maceration. Culture filtrate heated at 100°C for 10 min. eliminate enzymes served as control. The experiment was carried out at room temperature (17 to 25°C).

Estimation of Pectin methyl esterase activity

Pectin methyl esterase activity was determined by measuring loss in viscosity of 1.2 per cent pectin solution (Muse *et al.*, 1972). Viscometric measurements were made with Ostwald's viscometer at 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 h intervals. The reaction mixtures were prepared as under : (a) 5 ml of 1.2 per cent pectin solution (pH 9.2). (b) 2 ml of sodium citrate buffer (pH 9.2). (c) 2 ml of enzyme preparation.

Determination of Cellulase (Cx) enzymes activity

Cellulase activity was determined by measuring the loss in viscosity of 0.5 per cent carboxymethyl cellulase (CMC) solution (Muse *et al.*, 1972). Viscometric measurements were made at 0, 1, 2, 3, 4, 5, 6, 9, 12 and 24 h intervals. The reaction mixtures (pH 4.0) were prepared as described earlier.

The loss in viscosity for PME and CMC was calculated by the formula :

$$\text{Per cent loss in viscosity} = \frac{T_0 - T_1}{T_0 - T_w} \times 100$$

Where,

T_0 = Flow time of reaction mixture at 0 min., T_1 = Flow time of reaction mixture at a particular time interval, T_w = Flow time of distilled water.

RESULTS AND DISCUSSION

Effect of cow urine and butter milk on the enzymatic activity of three pathogens

The data presented in Table 1 indicated that potato discs were teasable with pressure after 3 h in *Fursairum solani* and *Sclerotium rolfsii* and 2 h in *Rhizotonia bataticola* at 9.2 pH and easily teasable after 9 h in *F. solani* and *R. bataicola* after 12 h in *S. rolfsii* with control filtrate. In case of cow urine amended media teasable with pressure was recorded after 9 h in *S. rolfsii* and after 6 h in *Rhizoctonia bataticola* whereas in buttermilk it was 12 h in *F. solani* and *S. rolfsii* and after 6 hrs in *R. bataticola.*

Table 1 : Influence of culture filtrate of *Rhizoctonia bataticola, Sclerotium rolfsii and Fusarium solani f.* sp. *glycine* grown on Richard's medium amended with cow urine (CU) and buttermilk (BM) on the activity of macerating enzyme activity

Time interval (h)	Per cent loss of viscosity in								
	Rhizoctonia bataticola			*Sclerotium rolfsii*			*F. solani f.* sp. *glycine*		
	C.U.	B.M.	Control	C.U.	B.M.	Control	C.U.	B.M.	Control
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	-	-	+	-	-	-	-	-	-
3	-	-	+	-	-	+	-	-	+
6	+	+	+	-	-	+	-	-	+
9	+	+	+ +	+	-	+	+	-	+ +
12	+	+ +	+ +	+	+	++	+	+	+ +
24	++	+ +	+ +	+	+	+ +	+ +	+ +	+ +

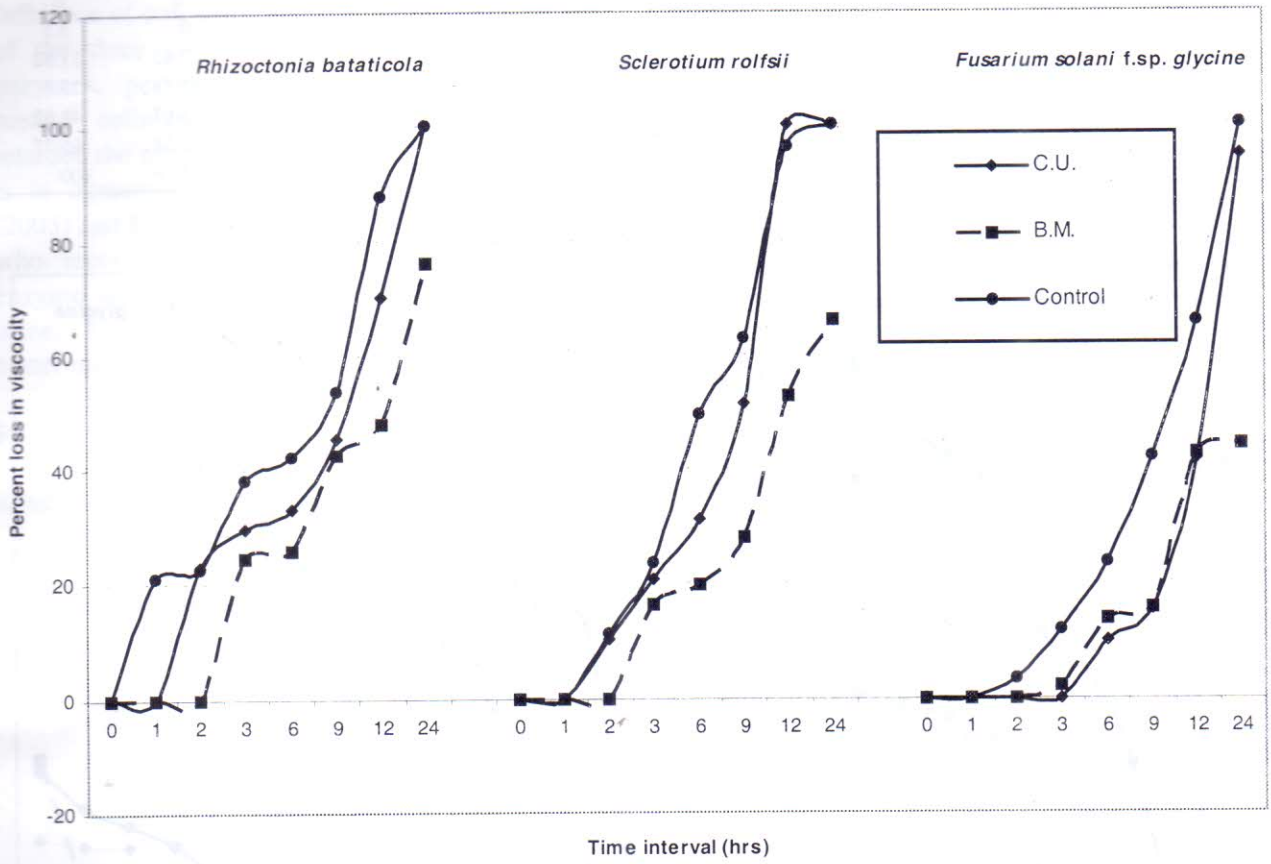

Fig. 1 : Showing per cent loss of viscocity of PME as influenced by cow urine and butter milk

The data presented in Table 2 and Fig. 1 clearly indicated that the per cent loss in viscosity varied with fungi and treatment used. Per cent loss of viscosity (in control) of 21.15 was recorded in *R. bataticola* at 1 h followed by 11.34 and 3.50 per cent at 2 h in *S. rolfsii* and *F. solani* respectively. In case of cow urine amended media 23.0 and 10.21 per cent loss was observed at 2 h in *R. bataticola* and *S. rolfsii* whereas it was 10.09 per cent in *F. solani*. Activity in butter milk amended medium was noted at 3 h in *R. bataticola* (24.42 per cent). and *S. rolfsii* (16.44 per cent). Cent per cent loss of viscosity (100%) were recorded at 24 h in control and cow urine amended media but not in butter milk amended media.

Data (Table 3) revealed that with the increase in time interval there was increase in per cent loss of viscosity and ranged from 19.26 to 60.01 (*R. bataticola*), 5.66 to 45.63 (*S. rolfsii*) and 2.55 to 19.18 (*F. solani*) in control filtrate. In cow urine per cent loss ranged from 10 to 48.20 (*R. bataticola*), 0.87 to 9.06 (*S. rolfsii*) and 1.70 to 12.25 (*F. solani*). The activity in buttermilk was noted 1.0 at 1 h in *R. bataticola*, 1.51 at 2 h in *S. rolfsii* and 1.66 at 3 h in *F. solani*. Maximum loss in viscosity 60.01 was recorded in *R. bataticola* (control).

Table 2 : Influence of culture filtrate of *Rhizoctonia bataticola, Sclerotium rolfsii and Fusarium solani f.* sp. *glycine* grown on Richard's medium amended with cow urine and butter milk on the activity of pectin methyl esterases

Time interval (h)	Per cent loss of viscosity in								
	Rhizoctonia bataticola			*Sclerotium rolfsii*			*F. solani f.* sp. *glycine*		
	C.U.	B.M.	Control	C.U.	B.M.	Control	C.U.	B.M.	Control
0	0	0	0	0	0	0	0	0	0
1	0	0	21.15	0	0	0	0	0	0
2	23.0	0	22.69	10.21	0	11.34	0	0	3.5
3	29.61	24.42	38.26	20.75	16.44	23.47	0	2.02	11.82
6	33.07	25.76	42.30	31.06	19.67	49.67	10.09	13.78	23.5
9	45.46	42.69	53.64	51.37	27.89	62.81	15.31	15.67	41.82
12	69.96	48.07	87.67	100	52.53	96.28	41.64	42.58	65.77
24	100	76.14	100	100	66.0	100	94.6	44.16	100

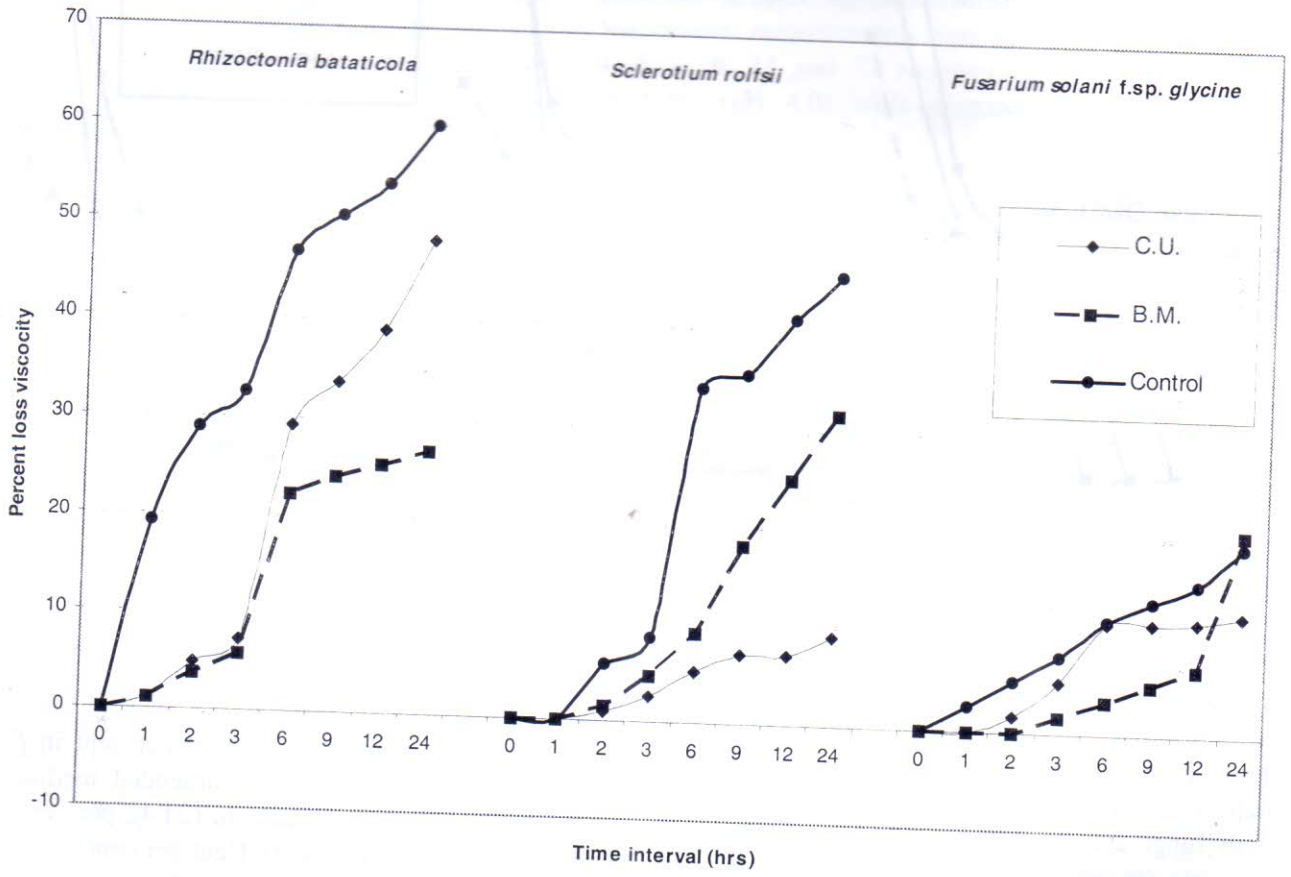

Fig. 2 : Showing per cent loss of viscocity of CMC as influenced by cow urine and butter milk

Table 3 : Influence of culture filtrate of *Rhizoctonia bataticola, Sclerotium rolfsii and Fusarium solani* f.sp. *glycine* grown on Richard's medium amended with cow urine and buttermilk on the activity of carboxy methyl cellulases

Time interval (h)	Per cent loss of viscosity in								
	Rhizoctonia bataticola			*Sclerotium rolfsii*			*F. solani f.* sp. *glycine*		
	C.U.	B.M.	Control	C.U.	B.M.	Control	C.U.	B.M.	Control
0	0	0	0	0	0	0	0	0	0
1	1.0	1.0	19.26	0	0	0	0	0	2.55
2	4.84	3.75	28.89	0.87	1.51	5.66	1.70	0	5.11
3	7.27	5.62	32.44	2.61	4.53	8.5	5.15	1.66	7.66
6	29.09	22.15	46.85	5.22	9.06	33.98	11.18	3.32	11.50
9	33.63	24.0	50.56	7.0	18.12	35.44	11.18	4.98	13.49
12	39.0	25.25	53.92	7.0	25.0	41.10	11.46	6.64	15.33
24	48.20	26.78	60.01	9.06	31.63	45.63	12.25	20.46	19.18

Influence of cow urine and buttermilk on the ability of the three pathogens to produced macerating enzymes, pectin methyl esterase and carboxy methyl cellulase indicated that both of them retarded the enzymatic activity. The present finding is in agreement with the finding of Kurucheve (2003) and Raja and Kurucheve (1997, 1998, 1999) who reported retarded production of hydrolytic enzyme in *Macrophomina phaseolina* by buffalo urine. Literature pertaining to the study on buttermilk is lacking.

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