Botany Research International 2 (4): 267-272, 2009
ISSN 2221-3635

Effect of Biomethanated Spent Wash on Soil Enzymatic Activities

P. Kalaiselvi and S. Mahimairaja

Department of Environmental Sciences,
Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Abstract: The biomethanated distillery spentwash is a nutrient rich liquid organic waste obtained from molasses based distillery industries after biomethanation process. The spentwash, being loaded with organic compounds could bring remarkable changes on the biological properties of soils and thus influences the fertility of soil significantly. The spentwash application appeared to have promoted the enzyme activities in soil. The effect of different levels and methods of spentwash application on soil enzymatic activity was examined through a field experiment. The field experiment was conducted using Groundnut (*Arachis hypogea* L.) as a test crop at Research and Development Cane Farm, The Salem Co-operative Sugar Mills Ltd., Mohanur, Namakkal District. The highest enzyme activities were resulted due to the application of spentwash at the rate of 120 m^3 ha^{-1} plus NP fertilizers. The continuous application of split doses was found better than one time application of spentwash in promoting the enzyme activities throughout the crop growth, mainly by providing steady supply of nutrients and organic matter. The positive significant correlations observed between enzyme activities and nutrients mineralization clearly demonstrate the important role of these enzymes on the nutrients dynamics and the availability in soil.

Key words: Spentwash · Organic waste · Enzyme activities · Nutrient dynamics

INTRODUCTION

The distillery spentwash is rich in nutrients and organic components with high BOD. Therefore, upon field application, it increases the soil organic matter content, nutrient content and mineral content. Also the high concentration of soluble carbon added from the spentwash application might be responsible for the enhanced enzyme activities. This condition may be favourable for number of enzymes in soils. [1] reported that the soil enzyme activities were maintained with 50 times diluted spentwash irrigation, but the phosphatase enzyme activity was not suppressed by the undiluted spentwash irrigation which might be due to the presence of copious quantity of phosphate in the spentwash. [2] reported that the application of distillery wastewater to a field soil at rates equivalent to 40, 80 and 160 m^3 ha^{-1} of irrigation increased soil microbial biomass and dehydrogenase activity. The spentwash addition increased the activity of phosphatase, dehydrogenase and urease enzymes in dry land black and red soils especially at levels of 125 m^3 ha^{-1} [3]. The current study was, therefore, aimed at examining the enzymatic activities in soil under spentwash application.

MATERIALS AND METHODS

Collection and Characterization of distillery spentwash: The biomethanated distillery spentwash was characterized for its nutritive value and pollution potential and used in all the experiments (Table 1). The biomethanated distillery spentwash sample was collected from the Salem Co-operative Sugar Mills Ltd, Mohanur, Namakkal District, Tamil Nadu, India.

Field Experiment: A field experiment was conducted using Groundnut (*Arachis hypogea* L.) as a test crop to examine the effect of spentwash on nutrient dynamics at Research and Development Cane Farm, The Salem Co-operative Sugar Mills Ltd., Mohanur, Namakkal, District Tamil Nadu, India during the rabi season (August 31, 2007 to December 11, 2007).

Soil Characteristics: The experimental soil was sandy loam in texture; taxonomically the soil belongs to the family *Typic Rhodustalfs*. A representative soil sample, at 0-15 cm depth, was collected from the experimental plot to determine the initial properties of the soil.

Corresponding Author: P. Kalaiselvi, Department of Environmental Sciences,
Tamil Nadu Agricultural University, Coimbatore - 641 003, Tamil Nadu, India

Table 1: Characteristics of biomethanated distillery spentwash

S. No	Characters	Biomethanated spentwash*
1.	Colour	Dark brown
2.	Odour	Unpleasant burnt sugar
3.	pH	7.1
4.	EC (dS m^{-1})	38
5.	Total dissolved solids	50000
6.	Total suspended solids	3300
7.	Total solids	53300
8.	Biological oxygen demand	12800
9.	Chemical oxygen demand	35000
10.	Carbon (g L^{-1})	24
11.	Nitrogen	420
12.	Phosphorus	40
13.	Potassium	9097
14.	Sodium	357
15.	Calcium	4600
16.	Magnesium	1752
17.	Chloride	13471
18.	Bicarbonates	195
19.	Sulphate	947
20.	Oil and grease	19.6
21.	Total sugars (%)	3.49
22.	Reducing sugars (%)	1.77
23.	Total phenols	84
24.	Zinc	7.20
25.	Iron	78
26.	Manganese	5.3
27.	Copper	5.5
29.	Bacteria (x 10^6 CFU ml^{-1} of effluent)	12
30.	Fungi (x 10^4 CFU ml^{-1} of effluent)	19
31.	Actinomycetes (x 10^3 CFU ml^{-1} of effluent)	Nil

* Mean of triplicate samples; (Values are in mg L^{-1} unless otherwise stated)

Experimental Details: The experiment was laid out in a split plot design with two main plots and eight sub plot treatments with three replications consisting of different levels of spentwash with and without NP fertilizers were allotted to plots of 13.5 m^2 size (6 m x 2.25 m) leaving 1 m between each replication for irrigation purpose following random principles.

Treatment Details

Main Plots:

M_1 - One time application

M_2 - Continuous split doses of application

Sub Plots:

T_1 - Control

T_2 - RD of NP

T_3 - Spentwash @ 40 m^3 ha^{-1}

T_4 - Spentwash @ 40 m^3 ha^{-1}+ RD of NP

T_5 - Spentwash @ 80 m^3 ha^{-1}

T_6 - Spentwash @ 80 m^3 ha^{-1}+ RD of NP

T_7 - Spentwash @ 120 m^3 ha^{-1}

T_8 - Spentwash @ 120 m^3 ha^{-1}+ RD of NP

(RD – Recommended Dose)

The different levels of spentwash was applied to the field uniformly by spraying manually to each plot 15 days before sowing for first main plot treatment (M_1). In the second main plot treatment (M_2), the spentwash was applied in three equal splits along with irrigation water. The first split dose of spentwash was applied 15 days after sowing. The crop was supplied with N and P fertilizers, as per the treatments at the recommended dose of 17 and 34 kg ha^{-1}, respectively. The fertilizers were applied in the form of urea and single super phosphate. The K was entirely supplied through the spentwash. Sowing was done with groundnut seeds of TMV 7 by adopting a seed rate of 125 kg ha^{-1} and a spacing of 30 cm x 10 cm. All other routine cultural operations until the harvest of the crop were followed as per the recommendations of crop production guide of Tamil Nadu Agricultural University.

Enzymatic Activity

Urease: A 10 g of soil was taken in a 100 ml volumetric flask. To this 1.5 ml of toluene was added, mixed well and incubated for 15 min. Then 10 ml of 10 % urea solution and 20 ml of citrate buffer were added, mixed thoroughly, stoppered and incubated for 3 hrs at 37°C in an incubator. Then the volume was made up to 100 ml with distilled water, mixed by shaking immediately. The contents were filtered through Whatman No.1 filter paper and 1ml of filtrate was pippetted out into 50 ml volumetric flak. To this 9 ml of distilled water, 4 ml of phenate and 3 ml of NaOCl were added, mixed well and allowed to stand for 20 min. The volume was made up to 50 ml and mixed well. The bluish green colour developed was read at 630 nm. Simultaneously a blank was also prepared (without urea solution). The concentration of urease in the sample was obtained from the standard graph using diammonium sulphate [4].

Phosphatase: A 5 g of soil sample was taken in a boiling tube. To this 10 ml of distilled water, 0.25 ml of toluene and 1ml of 10 mM p- nitrophenyl phosphate (PNPP) were added and incubated at room temperature for 1hr. Then 5 ml of 0.5 M $CaCl_2$ and 20 ml of 0.5 M NaOH were added. The content was filtered using Whatman No.42 and volume made up to 50 ml with distilled water. The colour intensity was read at 420 nm. The concentration of phosphatase was obtained from a standard graph [5].

Dehydrogenase: A 5 g of soil sample was taken in a boiling tube. To this 1 ml of 3 % 2, 3, 5-Tri phenyl tetrazolium chloride was added. Then 1 ml of 1 % glucose and 2.5 ml of distilled water was added and incubated for 24 hrs. After that 10 ml of methanol were added and incubated for another 5 hrs. The content was filtered through Whatman No.1 filter paper. The samples were washed thoroughly with methanol. The red colour developed was read at 485 nm. The concentration of dehydrogenase in the sample was obtained from the standard graph using triphenyl farmazane [6].

RESULTS AND DISCUSSION

Effect of Spentwash on Enzyme Activities in Soil

Urease Activity: The urease activity was markedly affected due to different levels and methods of spentwash application (Table 2). Before sowing, the urease activity in soil ranged from 5.47 to 10.47 µg NH_4-N g^{-1} of soil hr^{-1}under one time application (M_1) and from 5.03 to 5.70 µg NH_4-N g^{-1} of soil hr^{-1}under continuous application of spentwash (M_2). Increase in the rate of application recorded considerable increase in the urease activity and such increase was more with NP fertilizers. Irrespective of methods of application the urease activity was found increased significantly at pod formation stage, but decreased at post harvest stage. At all times, irrespective of methods of application, the urease activity was the lowest in soil with no spentwash application (T_1) and the highest with the application of spentwash at a rate of 120 m^3 ha^{-1} plus NP fertilizers (T_8). The interaction effect of methods of application and different levels of spentwash was significant.

Phosphatase Activity: The varied levels and methods of spentwash application markedly influenced the phosphatase activity in soil (Table 3). Initially, before sowing, the phosphatase activity in soil was between 8.8 and 18.8 µg p-nitrophenol g^{-1} of soil hr^{-1} with one time application (M_1) and from 8.5 to 9.2 µg p-nitrophenol g^{-1} of soil hr^{-1} with continuous split application (M_2) of spentwash. Increases in the rate of application progressively increased the phosphatase activities. The highest value was recorded in the soil which received the spentwash at a rate equivalent to 120 m^3 with NP fertilizers (T_8) and the lowest activity was in the control (T_1). In general, irrespective of the treatments, the phosphatase activity was found increased up to pod formation stage and decreased at harvest stage. Both at pod formation and at harvest stages split application (continuous application) of spentwash appeared to have relatively higher values than one time application of spentwash. The interaction effect of methods of application and different levels of spentwash was significant.

Dehydrogenase Activity: The activity of dehydrogenase enzyme was significantly influenced by the application of different levels and methods of spentwash application (Table 4). Before sowing dehydrogenase activity was only 2.20 µg TPF g^{-1} of soil hr^{-1} in the control (T_1); whereas, it ranged from 2.20 to 6.80 µg TPF g^{-1} of soil hr^{-1} under one time application of spentwash (M_1). Increasing levels of spentwash significantly enhanced the dehydrogenase

Table 2: Effect of different levels of spentwash application on urease activity (µg NH_4-N g^{-1} of soil hr^{-1}) in soil

	Stage I			Stage II			Stage III		
Treatments	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
T_1- Control	5.47	5.47	5.47	7.83	7.40	7.62	3.80	4.63	4.22
T_2 - NP alone	5.47	5.23	5.35	10.47	11.43	10.95	7.50	8.27	7.88
T_3 - Spentwash @ 40 m^3 ha^{-1}	6.60	5.03	5.82	9.67	14.60	12.13	6.97	9.67	8.32
T_4- Spentwash @ 40 m^3 ha^{-1}+ NP	8.97	4.83	6.90	15.70	18.47	17.08	11.43	13.97	12.70
T_5- Spentwash @ 80 m^3 ha^{-1}	6.97	5.23	6.10	13.97	15.70	14.83	9.67	10.47	10.07
T_6- Spentwash @ 80 m^3 ha^{-1} + NP	9.67	5.03	7.35	18.47	20.93	19.70	11.87	14.60	13.23
T_7- Spentwash @ 120 m^3 ha^{-1}	7.83	5.23	6.53	14.60	17.70	16.15	10.47	11.87	11.17
T_8- Spentwash @ 120 m^3 ha^{-1}+ NP	10.47	5.70	8.08	20.93	22.83	21.88	14.60	16.53	15.57
Mean	7.68	5.22	6.45	13.95	16.13	15.04	9.54	11.25	10.39
	Sed		CD (0.05)	Sed		CD (0.05)	Sed		CD(0.05)
T	0.19		0.40	0.46		0.99	0.34		0.72
M	0.09		0.20	0.22		0.47	0.15		0.32
T x M	0.27		0.57	0.64		1.37	0.45		0.96
M x T	0.27		0.57	0.63		1.34	0.42		0.90

M_1 - One time application; Stage I - Before sowing;
M_2 - Continuous application Stage II - Pod formation; Stage III - Post harvest

Table 3: Effect of different levels of spentwash application on phosphatase activity (µg p-nitrophenol g^{-1} of soil hr^{-1}) in soil

Treatments	Stage I			Stage II			Stage III		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
T_1- Control	8.8	8.9	8.9	9.1	9.2	9.2	5.9	6.1	6.0
T_2 - NP alone	8.9	8.7	8.8	9.2	9.4	9.3	6.2	6.5	6.4
T_3 - Spentwash @ 40 m^3 ha^{-1}	11.8	8.5	10.2	12.5	13.6	13.1	10.3	11.7	11.0
T_4- Spentwash @ 40 m^3 ha^{-1}+ NP	13.8	8.6	11.2	14.4	15.2	14.8	11.6	12.3	12.0
T_5- Spentwash @ 80 m^3 ha^{-1}	14.6	8.9	11.8	15.2	16.3	15.8	12.1	13.2	12.7
T_6- Spentwash @ 80 m^3 ha^{-1} + NP	15.2	9.0	12.1	16.3	17.6	17.0	13.8	14.5	14.2
T_7- Spentwash @ 120 m^3 ha^{-1}	16.6	9.2	12.9	17.2	18.4	17.8	15.0	15.9	15.5
T_8- Spentwash @ 120 m^3 ha^{-1}+ NP	18.8	8.9	13.9	19.1	20.3	19.7	16.5	18.3	17.4
Mean	13.6	8.8	11.2	14.1	15.0	14.6	11.4	12.3	11.9
	Sed		CD (0.05)	Sed		CD (0.05)	Sed		CD(0.05)
T	0.31		0.67	0.29		0.59	0.23		0.48
M	0.17		0.37	0.09		0.19	0.08		0.15
T x M	0.47		0.99	0.34		0.70	0.28		0.57
M x T	0.49		1.04	0.27		0.54	0.21		0.43

M_1 - One time application Stage I - Before sowing

M_2 - Continuous application Stage II - Pod formation Stage III - Post harvest

Table 4: Effect of different levels of spentwash application on dehydrogenase activity (µg TPF g^{-1} of soil hr^{-1}) in soil

Treatments	Stage I			Stage II			Stage III		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
T_1- Control	2.20	2.20	2.20	2.60	2.63	2.62	2.30	2.33	2.32
T_2 - NP alone	2.20	2.20	2.20	4.53	4.60	4.57	3.37	3.60	3.48
T_3 - Spentwash @ 40 m^3 ha^{-1}	3.23	2.23	2.73	4.13	4.40	4.27	3.53	3.90	3.72
T_4- Spentwash @ 40 m^3 ha^{-1}+ NP	4.37	2.20	3.28	5.70	6.67	6.18	4.83	5.60	5.22
T_5- Spentwash @ 80 m^3 ha^{-1}	3.47	2.20	2.83	4.50	4.93	4.72	3.90	4.30	4.10
T_6- Spentwash @ 80 m^3 ha^{-1} + NP	5.50	2.20	3.85	6.33	7.23	6.78	5.33	6.53	5.93
T_7- Spentwash @ 120 m^3 ha^{-1}	4.03	2.20	3.12	4.60	5.23	4.92	4.03	4.73	4.38
T_8- Spentwash @ 120 m^3 ha^{-1} + NP	6.80	2.23	4.52	8.23	10.63	9.43	6.67	8.10	7.38
Mean	3.98	2.21	3.09	5.08	5.79	5.44	4.25	4.89	4.57
	Sed		CD (0.05)	Sed		CD (0.05)	Sed		CD(0.05)
T	0.10		0.21	0.19		0.41	0.15		0.33
M	0.05		0.11	0.07		0.16	0.07		0.14
T x M	0.14		0.30	0.24		0.52	0.20		0.44
M x T	0.14		0.30	0.21		0.45	0.19		0.40

M_1 - One time application; Stage I - Before sowing;

M_2 - Continuous application Stage II - Pod formation; Stage III - Post harvest

activity and such increase was more due to NP fertilizers. Soil that received 120 m^3 of spentwash plus NP fertilizers (T_8) had the highest dehydrogenase activities.

Similar to urease and phosphatase, dehydrogenase activities were also found increased up to pod formation stage and decreased at harvest stage. At pod formation stage, the dehydrogenase activity ranged between 2.60 and 8.23 µg TPF g^{-1} of soil hr^{-1} due to one time application of spentwash (M_1); whereas, it was between 2.63 and 10.63 µg TPF g^{-1} of soil hr^{-1} under continuous application of split doses of spentwash (M_2). At all stages, irrespective of methods of application, the dehydrogenase activity was the lowest in soil with no spentwash application (T_1); the highest was with the application of spentwash at a rate of 120 m^3 ha^{-1} plus NP fertilizers (T_8). The interaction effect of methods of application and different levels of spentwash was significant.

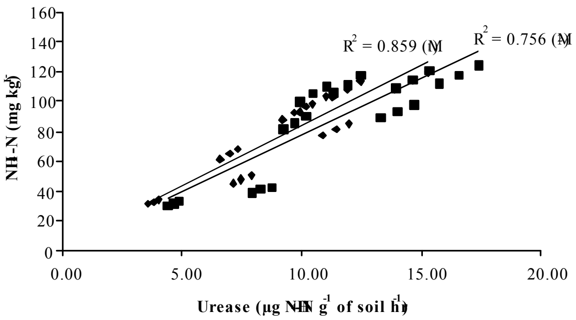

Fig. 1: Relationship between urease and NH_4-N in soil under spentwash application

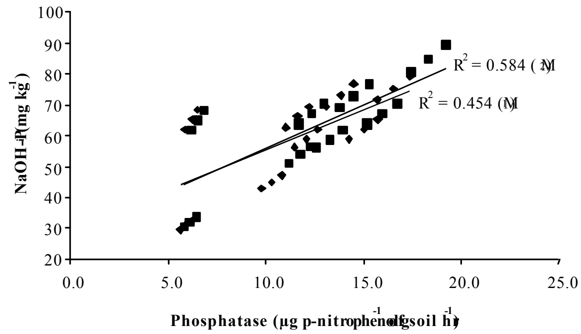

Fig. 3: Relationship between phosphatase and NaOH-Pi in soil under spentwash application

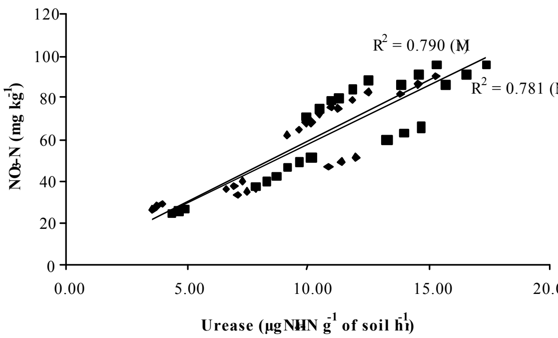

Fig. 2: Relationship between urease and NO_3-N in soil under spentwash application

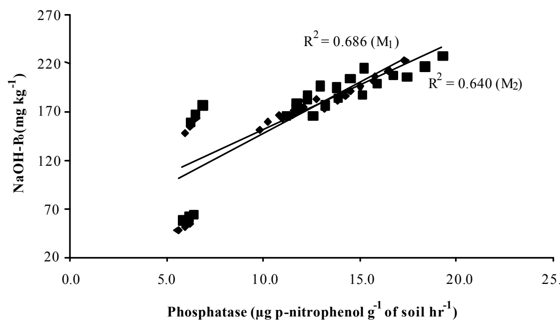

Fig. 4: Relationship between phosphatase and NaOH-Po in soil under spentwash

Enzyme activity in soil is an indirect indication on the microbial activity, which is directly correlated with soil microbial population. In the present investigation, greater activities of dehydrogenase, urease and phosphatase were associated with the spentwash application. Increase in the rate of spentwash application resulted in a marked increase in the enzyme activities. The treatment that received the spentwash @ 120 m^3 ha^{-1} + NP was found to have greater enzyme activities over the control followed by NP alone (T_2). The spentwash being a liquid organic manure, increases the organic matter and nutrients content of the soil and subsequently enhanced the microbial biomass. The high dose of spentwash along with the recommended dose of NP recorded the highest value. It implies that organic and inorganic nutrients provide a nutrient rich environment, which is essential for the synthesis of enzymes [7].

[8] found a positive correlation between the organic residues and dehydrogenase, â-glucosidase, urease and protease activities of the soil. [9] also reported that the enzyme activities were increased due to the application of distillery effluent. Generally, organic manure addition was found to enhance the microbial activities which in turn favour the synthesis of various enzymes in soil [10].

[11] also reported that the spentwash contains about 42.7 % polysaccharides which might have served as a source of carbon substrate for enzyme activities. These three enzymes play a significant role in the bio-transformation of nutrients in soil and thus influence the nutrients availability in soil. The positive correlation between urease and N mineralization (NH_4/ NO_3), as well as phosphatase and P mineralization (P_i and P_o) in soil, as depicted in Fig. 1 to 4.

Clearly demonstrate the role of these enzyme activities in the nutrient dynamics in soil. The phosphatases hydrolyze organic P to inorganic P, catalyze the rate limiting steps of P nutrient cycling and therefore, phosphatase activity plays a significant role in P availability to plants from native organic P compounds. The phosphatase activity, thus, can be a good indicator of the organic P mineralization potential and biological activity of soils [12]. The mineralization rate of organic P is relevant to both P nutrition of crops and phosphatase activity in soil. Therefore, higher enzyme activities in soil suggested that the mineralization of N and P was greater due to the application of spentwash. Similar result was reported by [13]. Thus the spentwash application even at its high concentration appeared to have promoted the enzyme activities in soil.

ACKNOWLEDGEMENT

The authors thanks the Salem Cooperative Sugar mills Ltd., Mohanur in Namakkal District, Tamil Nadu, India. for their financial assistance.

REFERENCES

1. Deverajan, L., G. Rajannan, G. Ramanathan and G. Oblisami, 1993. Sugarcane cultivation with distillery effluent. SISSTA Sugar J., 20(3): 23-25.
2. Goyal, S., K. Chander and K.K. Kapoor, 1995. Effect of distillery wastewater application on soil microbial properties and plant growth. Environ. Ecol., 13(1): 89-93.
3. Murugaragavan, R., 2002. Distillery spentwash on crop production in dryland soils. M.Sc. Thesis, Tamil Nadu Agric. Univ., Coimbatore.
4. Tabatabai, M.A. and J.M. Bremner, 1972. Assay of urease activity in soil. J. Soil. Biol. Biochem., 4: 479-487.
5. Halstead, R.L., 1964. Phosphatase activity of soils as influenced by lime and other treatments. Can. J. Soil Sci., 44: 137-144.
6. Casida, L.E.Jr., D.A. Klein and T. Santoro, 1964. Soil dehydrogenase activity. Soil Sci., 98: 371-376.
7. Kamalakumari, K. and P. Singaram, 1995. Relationship among soil chemical biochemical properties and enzyme activities. Madras Agric. J., 82: 69-70.
8. Engracia Madejon, S., P. Burgos, R. Lopez and F. Cabrera, 2003. Agricultural use of three organic residues: effect on orange production and on properties of a soil of Spain. Nutr. Cycl. Agroecosyst., 65: 281-288.
9. Ramana, S., A.K. Biswas and A.B. Singh, 2002. Effect of distillery effluents on some physiological aspects in maize. Bioresour. Technol., 84: 295-297.
10. Dinesh, R., R.P. Dubey, A.N. Ganeshamurthy and G. Shyam Prasad, 2000. Organic manuring in rice-based cropping system: Effects on soil microbial biomass and selected enzyme activities. Curr. Sci., 79: 1716-1720.
11. Patil, J.D., S.V. Arbatti and D.G. Hapase, 1982. Humification of spentwash in soil. J. Maharashtra Agric. Univ., 7(1): 80-83.
12. Dick, W.A. and M.A. Tabatabai, 1993. Significance and potential uses of soil enzymes. In: F.B. Meeting, (Ed.) Soil microbial ecology: Application in agricultural and environmental management. Marcel Dekker, New York, pp: 95-125.
13. Rajannan, G., L. Devarajan and G. Oblisami, 1998. Impact of distillery effluent irrigation on growth of banana crop. In: Proceedings of national seminar on application of treated effluents for irrigation, held at REC, Triuchirapalli, Mar., 23: 56.