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Effect of biomethanated distillery spentwash on physiological and biochemical aspects of maize *(Zea mays)*

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The demand for wastewater reuse has increased significantly worldwide. In particular, the arid and semiarid areas of the world can easily augment 15-20 % of their water supply through reuse of wastewater. Such reuse accomplishes several purposes such as minimizing the cost of wastewater treatment and disposal, providing much needed plant nutrients to the soil when used for agriculture. Spentwash, a liquid waste, rich in organic load and nutrients is being discharged from the molasses based distillery industry to the tune of 14 - 15 litres for every litre of alcohol production. In India, about 40 billion litres of spentwash is being discharged annually from 319 distilleries (Kanimozhi and Vasudevan 2010). The raw spentwash contains high Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and solids, which is again subjected to biomethanation process in order to get the effluent with 80 per cent BOD reduction namely the Biomethanated Distillery Spentwash (BDS). Unlike other industrial wastes, this liquid does not contain any hazardous materials detrimental to soil health and plant growth. Being originated from a plant source, it contains large amounts of organic carbon, macro, micro nutrients and plant growth promoters and it is substitute for market purchased inorganic fertilizer has a great promise (Joshi and Singh 2010)

Maize is considered as a promising option for diversifying agriculture in upland areas and ranks as the third most important foodgrain crop in India. The area has slowly expanded over the past few years to about 6.2 million ha. Since opportunities are limited for further expansion of maize area, future increase in maize supply will be achieved through the intensification of current maize production systems. Though the growth and yield parameters for many crops have been standardized, the information on the influence of BDS on physiological and biochemical aspects of maize is lacking. Only a few research reports regarding the effect of distillery spentwash on crop physiology are available (Bharagava *et al.* 2008). Keeping this in view, the present study was made to assess the impact of biomethanated distillery spentwash on physiological and biochemical parameters and to optimise the dosage of spentwash and inorganic fertilizers for maize crop.

The field experiment was conducted during August to December, 2009 at Aalathu kombai (11° 27' to 11° 47'N latitude and 76° 51' to 77° 28' E longitude; 600 m above mean sea level), Erode, Tamil Nadu where a distillery with spentwash generation capacity of 60 kilo liters/day from molasses is operating since 1996. The location comes under the soil series of Irugur with the subgroup of *Typic Ustorthent* under the soil order Entisol. The annual precipitation is around 700 mm and minimum temperature ranged from 18 to 25°C and the maximum from 28°C to 36°C. The soil was sandy loam, neutral pH (7.04), low in organic carbon (0.38%) and available N (104 kg/ha), medium in available P (19 kg/ha) and K (224 kg/ha). Different doses of BDS along with inorganic fertilizers using Maize hybrid COH (M) 5 as test crop have been tried. The experiment was laid out in Randomized Block Design with three replications; seed rate adopted was 18 kg/ha with the spacing of 60 cm × 25 cm. The treatment consisted of T_1 – RD (Recommended Dose) NPK, T_2 - BDS @ 50 kilo liters/ha + RD-NP, T_3 - BDS @ 50 kilo liters/ha + RD-NPK, T_4 - BDS @ 100 kilo liters/ha + RD-NP, T_5 - BDS @ 100 kilo liters/ha + RD-NPK, T_6 - BDS @ 150 kilo liters/ha + RD-NP, T_7 - BDS @ 150 kilo liters/ha + RD-NPK. Spentwash was applied as per the treatment and incorporated into the soil at 30 days before sowing in order

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Table 1 Influence of BDS on physiological parameters of maize

Treatment	Chlorophyll Stability Index (%)			Leaf Water Potential (-Mpa)			Relative Water Content (%)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	50.67	60.43	33.12	0.42	1.05	1.17	59.55	62.10	48.76
T_2	51.45	65.61	39.31	0.39	0.95	1.14	67.72	70.30	56.23
T_3	53.83	71.25	48.46	0.37	0.89	1.02	69.45	74.52	59.74
T_4	65.83	82.25	60.46	0.31	0.65	0.80	76.69	79.30	65.84
T_5	73.34	86.25	69.34	0.24	0.58	0.67	80.48	84.84	68.24
T_6	55.67	75.34	54.73	0.34	0.82	0.94	71.52	74.80	60.34
T_7	57.34	78.34	58.71	0.33	0.75	0.87	73.52	76.80	62.27
SEm±	0.48	0.62	0.52	0.01	0.01	0.01	0.74	0.75	0.69
CD (P=0.05)	1.48	1.89	1.61	0.01	0.03	0.02	2.28	2.31	2.11

to reduce the BOD and COD. The experimental plots were irrigated immediately after sowing. Recommended dose of nitrogen @ 150 kg/ha as urea, phosphorus @ 75 kg/ha as single super phosphate and potassium @75 kg/ha as muriate of potash was applied as per the treatment.

The plant samples were collected from individual treatments at 30 days interval and dried in an oven at 80°C. Chlorophyll Stability Index (CSI) was determined by the method suggested by Murty and Majumdar (1962). Leaf Water Potential (LWP) was measured by using pressure pump apparatus suggested by Scholander *et al.* (1965). Relative Water Content (RWC) was estimated as per the method given by Barrs *et al.* (1962). Chlorophyll content was estimated by the method suggested by Yoshida *et al*. (1976). Soluble protein content of leaf was estimated as per the procedure described by Lowry *et al.* (1951). Nitrate reductase activity was determined by adopting the method of Nicholas *et al.* (1976).

Application of spentwash of maize had significant influence on the physiological and biochemical parameters (Table 1). The highest CSI was recorded with BDS @ 100 kilo liters/ha + RD-NPK (73.34, 86.25 and 69.34 % at 30, 60 and 90 DAS, respectively), LWP (0.24, 0.58 and 0.67 -Mpa at 30, 60 and 90 DAS respectively) and RWC (80.48, 84.84 and 68.24 % at 30, 60 and 90 DAS, respectively) and the lowest was recorded in RDF. The enhanced physiological and biochemical parameters might be due to the addition of increased plant nutrients to the maize crop through the distillery spentwash. In the present investigation, the LWP and RWC of maize were significantly influenced by the application of graded doses of BDS. The reason for this phenomenon is the accumulation of K in maize at high levels through the addition of potassium rich BDS (12 650 mg/l). Potassium acts as osmoticum helped the plant for having better stomatal regulation and further application of BDS also increased root growth. The present study is in close agreement with previous findings of Sivasankari (2009).

Similar to physiological parameters, biochemical parameters, viz. total chlorophyll content (2.62, 3.13 and 2.52 mg/g at 30, 60 and 90 DAS, respectively), soluble protein (2.78, 8.38 and 5.95 mg/g at 30, 60 and 90 DAS, respectively) and nitrate reductase activity (67.45, 124.34 and 98.46 µmol of NO_2/g/hr at 30, 60 and 90 DAS, respectively) recorded in BDS @ 100 kilo liters/ ha + RD-NPK and the lowest in RDF. An increase in chlorophyll content was recorded in leaves of maize at all the BDS concentration on 30 and 60 DAS and subsequently decreased due to the onset of senescence (Table 2). This reflects the high manurial potential of the distillery effluent up to 60 DAS. A positive correlation between carbon dioxide exchange rate and chlorophyll content was also reported in maize (Girardin *et al.* 1985). The increased soluble protein might be due to cells retained higher water potential with the application of spentwash. This might have prevented protein degradation metabolism and enhanced soluble protein synthesis by activating enzyme activity. Nitrate reductase activity is the key enzyme for nitrogen assimilation as nitrate is the major N source for most of higher plants and is substrate inducible. The increased nitrate reductase activity was observed up to flowering stage and decreased thereafter in all the treatments. The higher activity of the enzyme in the plants treated with distillery effluent might possibly be due to the high nitrogen content present in the effluent (1 200 mg/l). Distillery effluents significantly increased the grain yield of maize compared to RDF (Table 2). This might be due to the favorable effect of organic matter and nutrients in distillery wastes which improved the soil fertility status and physical environment and might have promoted better germination, root proliferation, nutrient and water uptake by the crops (Hati *et al*. 2007). The physiological and biochemical parameters have contributed to obtain the higher grain yield of maize. The higher chlorophyll content would have increased the photosynthetic rate reflected on higher water and nutrients uptake, thus enhanced yield. Soluble protein prevents the protein degradation mechanism, thus facilitated more protein synthesis and enhanced the grain yield. Similar to chlorophyll, more nitrate reductase activity increased the nitrogen content, increased more leaf surface area, enhanced photosynthetic activity and increased yield of maize crop. Among the treatments BDS @ 100 kilo liters/ ha + RD-NPK

Table 2 Impact of BDS on biochemical parameters and grain yield of maize

Treatment	Total chlorophyll (mg/g)			Soluble protein (mg/g)			Nitrate reductase (µmol of NO_2/g/hr)			Grain yield
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	(kg/ha)
T_1	2.22	2.67	2.02	1.45	6.82	4.22	37.00	71.25	41.25	3917
T_2	2.33	2.79	2.13	1.87	7.24	4.85	41.03	96.00	74.34	4265
T_3	2.35	2.82	2.17	2.06	7.53	5.17	48.12	98.54	75.16	4493
T_4	2.51	3.01	2.41	2.54	8.14	5.74	56.25	107.18	84.62	5759
T_5	2.62	3.13	2.52	2.78	8.38	5.95	67.45	124.34	98.46	5815
T_6	2.4	2.88	2.22	2.25	7.72	5.36	44.52	110.21	90.33	5543
T_7	2.46	2.99	2.34	2.34	7.86	5.68	49.07	108.05	82.34	5589
SEm±	0.03	0.03	0.03	0.03	0.08	0.06	0.67	1.53	1.07	65.8
CD (P=0.05)	0.09	2.67	0.09	0.08	0.22	4.22	2.01	4.68	3.28	192

T_1, RD-NPK; T_2, BDS @ 50 kilo liters/ha + RD-NP; T_3, BDS @ 50 kilo liters/ha + RD-NPK; T_4, BDS @ 100 kilo liters/ha + RD-NP; T_5, BDS @ 100 liters/ha + RD-NPK; T_6, BDS @ 150 liters/ha + RD-NP; T_7, BDS @ 150 liters/ha + RD-NPK

recorded 33 % higher grain yield (5 815 kg/ha) which was on par with BDS @ 100 kilo liters/ha + RD-NP (5 759 kg/ha) over RDF (3 917 kg/ha).

SUMMARY

The results obtained from the experiments revealed that the application of biomethanated distillery spentwash @ 100 kilo liters/ha + Recommended Dose of NPK followed by BDS @ 100 kilo liters/ha+ Recommended Dose of NP significantly increased the physiological (chlorophyll stability index, leaf water potential relative water content) and biochemical (total chlorophyll, soluble protein and nitrate reductase activity) parameters total chlorophyll, soluble protein and nitrate reductase activity and these are highly correlated with yield of maize crop. Hence, any of these two methods could be adopted for the utilization of BDS for cultivation of crops. Distillery spentwash from sugar mills hitherto considered as factory waste could be used as a source of nutrients to maize.

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