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Performance of Cumbu Napier Hybrid Grass as influenced by Distillery Industrial Byproducts under Irrigated Condition

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A field experiment was conducted during 2009 to 2011 at Research and Development farm, M/s. Bannari Amman Sugars Distillery Division Ltd., Ealur, Erode to assess the performance of cumbu napier hybrid grass by utilizing distillery industry byproducts *viz.,* distillery spentwash, biocompost and spentwash ash. Treatments involved are distillery spentwash @ 37.5 and 50 kilo l per ha at full and split dose, biocompost @ 5.0 tonnes per ha and spentwash ash @ 400 kg per ha with recommended dose of fertilizers and the parameters were assessed at 12th, 26th, 39th and 52nd weeks after planting (1st, 3rd, 5th and 7th cuttings). *Results of the field experiment revealed that the* **application of distillery spentwash @ 50 kilo l per ha at full dose with recommend dose of nitrogen and phosphorus increased the physiological parameters, green fodder yield and soil biological properties and decreased the biochemical parameters over recommended dose of fertilizers.**

Key words: Distillery spentwash, Biocompost, Spentwash ash, Soluble protein, Bacterial Count.
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Distilleries, one of the most important agro-based industries in India, produce alcohol from molasses. They generate large volume of foul smelling coloured wastewater known as spentwash. For production of each litre of alcohol, 12- 15 l of spentwash is produced. Approximately 40 billion litres of wastewater is generated per annum from 319 distilleries in the country (Kanimozhi and Vasudevan, 2010). The raw spentwash is acidic in nature (pH < 4.0) and is generally characterized by high levels of Biochemical Oxygen Demand (BOD=40000-50000 mg L^{-1}) and Chemical Oxygen Demand (COD=90000-100000 mg L^{-1}). Alternatively, the raw spentwash is subjected to biomethanation treatment to decrease the BOD and COD levels and the effluent obtained is known as Biomethanated Distillery Spentwash (BDS). It contains nutrients, organic matter and plant growth promoting chemicals viz., gibberellic acid and indole acetic acid (Selvakumar, 2006). Therefore, upon field application, it increases the soil organic matter content and available nutrients. Also the high concentration of soluble carbon added from the spentwash might be responsible for the enhanced microbial and enzyme activities.

Biocompost is being prepared by using pressmud and distillery spentwash at the ratio of 1:2.5. It is being used as a source of nutrients with a potential of increasing crop production on sustainable manner (Selvaseelan *et al.*, 2000). The distillery spentwash contains about 1.2 per cent of potassium but this concentration is increased to the range between 10-18 per cent, when it is ignited into ash (Jadhav *et al*., 2010).

In India, the projected demand for fodder in 2015 is estimated to be 589 million tonnes of dry fodder and 1061 million tonnes of green fodder. Hence, there is a need to increase fodder production without much investment. Forage crops that require high amount of N and K are the better choices in assessing the nutrient potential of spentwash since it is rich in K and N (Galavi *et al.,* 2009). Only a few research reports regarding the effect of distillery spentwash on forage crops (Banulekha, 2007; Latha, 2008). Keeping this in view, the present study was made to assess the performance of distillery industry byproducts on physiological, biochemical parameters and soil biological properties on Cumbu Napier hybrid grass.

Materials and Methods

The BDS was collected from the distillery unit of M/s. Bannari Amman Sugars Ltd., Periyapuliyur, Erode district, Tamil Nadu and analyzed for its physico-chemical properties by following standard procedures (APHA, 1989). Biocompost is being prepared and marketed by M/s. Bannari Amman Sugars Ltd., Ealur, by utilizing the pressmud as a raw material and the composting process is being carried out by mechanized open windrow system with the use of bioinoculants *viz., Pseudomonas* sp, *Trichoderma* sp, *Bacillus* sp, *Cellulomonas* sp for about 70-80 days. The product is then sun dried, ground and sieved by mechanical separator and mixed with super phosphate before marketing and the sample was analyzed for its physico - chemical properties. Spentwash ash is being produced by M/s. Bannari Amman Sugars Ltd., Distillery division, Alakangi village near Nanjangud in Karnataka State. The raw spentwash contains only about 15 per cent of solids and can be increased upto 60 per cent by neutralization of spentwash with lime followed by filtration and evaporation. The concentrated spentwash is burnt in fluidized bed boiler (840°C to 950°C) and converted into ash and collected in collection chamber and analyzed for its physico - chemical properties. BDS was dark brown colour and a neutral pH (7.42) with high EC (32.5 dS m^{-1}), BOD (6,545 mg L^{-1}) and COD (34,476 mg L^{-1}). It contains highest K (8,376 mg L^{-1}) followed by N (2,116 mg L^{-1}), Ca (2,072 mg L^{-1}), Mg (1,284 mg L^{-1}) and very low content of P (52.8 mg L^{-1}). The biocompost showed a neutral pH (7.26) and 1.74 dS m^{-1} EC with 15.42 per cent organic carbon content. Among the nutrients, the K content was highest (4.08 %), followed by Ca (3.72 %), Mg (2.46 %), P (2.06 %), Na (1.54 %) and N (1.24 %). The spentwash ash was alkaline nature (pH 8.96) with high EC (17.8 dS m^{-1}) and no organic carbon and N content. Among the nutrients, the K content was the highest (10.25 %), followed by Ca, Mg and Na (3.16, 2.54 and 0.65 %), respectively.

The field experiment was conducted during 2009 to 2011, at research and development farm, M/S. Bannari Amman Sugars Distillery Division Ltd., Ealur (11° 29' to 11° 48' N latitude and 76° 50' to 77° 27' E longitude; 600 m above mean sea level), Erode, Tamil Nadu. 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 to 36°C. The soil texture of the experimental site was sandy loam, neutral pH (7.24), non - saline (0.28 dS m^{-1}) and rich in organic carbon (3.56 g kg^{-1}). With regard to nutrient status, the soil was low in N (118.5 kg ha^{-1}), medium in P (19.2 kg ha^{-1}) and K (248 kg ha^{-1}). Considerable population of microorganisms (bacteria, fungi and actinomycetes) and appreciable amount of enzyme (dehydrogenase, phosphatase and urease) activities were also assayed in the soil.

The effect of different doses of BDS, biocompost and spentwash ash along with inorganic fertilizers using Cumbu Napier hybrid grass (CO (CN) 4) as test crop have been tried. The experiment was laid out in randomized block design with three replications; 40,000 two budded stem cuttings ha^{-1} was used with the spacing of 50 x 50 cm. The treatment consisted of T_1 - Recommended dose of NPK (RD), T_2 - Biocompost @ 2.5 t ha^{-1} + RD of NP, T_3 - Spentwash ash @ 400 kg ha^{-1} + RD of NP, T_4 - BDS @ 37.5 kilo l ha^{-1} at full dose + RD of NP, T_5 - BDS @ 37.5 kilo l ha^{-1} at split dose (Basal 40 % and 10 % after each cutting) + RD of NP, T_6 - BDS @ 50 kilo l ha^{-1} at full dose + RD of NP, T_7 - BDS @ 50 kilo l ha^{-1} at split dose + RD of NP. Spentwash was applied as per the treatment and incorporated into the soil at 30 days before planting in order to reduce the BOD and COD. Biocompost and spentwash ash were applied as basal. Recommended dose of nitrogen at 150 kg ha^{-1} as urea, phosphorus at 50 kg ha^{-1} as single super phosphate and potassium at 40 kg ha^{-1} as muriate of potash was applied as per the treatment. The first harvest was scheduled on 90th days after planting, the crop was allowed for ratooning at 45 days intervals by supplementing N as top dress at the rate of 75 kg ha^{-1}.

The plant samples were collected from the field at 12th, 26th, 39th and 52nd weeks after planting (WAP) (1st, 3rd, 5th and 7th cuttings) and dried in hot air oven at 65^{o}C to determine the moisture percentage. The total chlorophyll and carotenoid content were estimated by adopting the procedure of Yoshida *et al*. (1971) and soluble protein content was determined by the procedure described by Lowry *et al*. (1951). The cellulose content was estimated by adopting the method of Updegraph (1969), hemicellulose content was estimated by the method described by Goering and Vansoest (1975) and lignin content was estimated by following the method of Chesson (1978). Each harvesting was made at above the ground level in each plot and the total green biomass was weighed and expressed in t ha $^{-1}$. The four cutting values of physiological and biochemical parameters were cumulated and the mean value was presented in Table 1 and 2. The collected soil samples were dried under shade, powdered with wooden mallet and sieved through 2 mm sieve and the number of bacteria, fungi and actinomycetes colonies were assessed by plating dilution technique by adopting the analytical methods outlined by Waksman and Fred (1922). Dehydrogenase activity was determined by triphenly farmazane method (Casida *et al.,* 1965), phosphatase activity was determined by adopting p-nitrophenyl phosphate (PNPP) method outlined by Tabatabai and Bremmer (1969), Urease activity was determined by NH_4-N Distillation method (Bremner and Keeney, 1966). The data were analyzed statistically and the treatment means were compared using LSD at 5 % probability (Panse and Sukhatme, 1985).

Results and Discussion

Physiological and biochemical parameters

Application of BDS and biocompost on CN hybrid grass had significant influence on the physiological parameters *viz.,* total chlorophyll, carotenoid and soluble protein compared to spentwash ash and RD (Table 1). Among the treatments, BDS @ 50 kilo l ha^{-1} at full dose + RD of NP registered the highest total chlorophyll (3.10 mg g^{-1}), carotenoid (0.53 mg g^{-1}) and soluble protein (27.2 mg g^{-1}) which was on par with BDS @ 37.5 kilo l ha^{-1} at full doses + RD

Table 1. Effect of BDS, biocompost and spentwash ash on physiological parameters of CN hybrid grass

Treatment	Total chlorophyll (mg/g)	Carotenoid (mg/g)	Reducing sugar (%)	Soluble protein (mg/g)
T_1- RD	2.64	0.41	0.45	24.8
T_2 - Biocompost @ 2.5 t ha^{-1} + RD of NP	2.95	0.50	0.53	26.2
T_3 - Spentwash ash @ 400 kg ha^{-1} + RD of NP	2.77	0.44	0.47	25.2
T_4 - BDS @ 37.5 kilo l ha^{-1} at full dose + RD of NP	3.01	0.51	0.54	26.7
T_5 - BDS @ 37.5 kilo l ha^{-1} at split dose + RD of NP	2.82	0.45	0.49	25.9
T_6 - BDS @ 50 kilo l ha^{-1} at full dose + RD of NP	3.10	0.53	0.56	27.2
T_7 - BDS @ 50 kilo l ha^{-1} at split dose + RD of NP	2.85	0.47	0.50	26.1
CD (0.05)	0.10	0.03	0.02	0.52

RD - Recommended Dose of NPK ; BDS- Biomethanated Distillery Spentwash

of NP. The lowest total chlorophyll (2.64 mg g^{-1}), carotenoid (0.41 mg g^{-1}) and soluble protein (24.8 mg g^{-1}) was recorded by RD which was on par with spentwash ash @ 400 kg ha^{-1}. The productivity of crop depends on photosynthesis and partitioning of assimilates to the economically important parts. An increased content of total chlorophyll and carotenoid was due to the application of BDS. This reflected the high manurial potential of the distillery effluents (Sivasankari, 2009). Plant cells might have retained higher water potential with the application of BDS which might have prevented protein degradation metabolism and enhanced the soluble protein synthesis by activating enzyme activity (Koach and Mengel, 1977) which was coincided with the present study.

Table 2. Effect of BDS, biocompost and spentwash ash on biochemical parameters of CN hybrid grass

Treatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Oxalic acid (%)
T_1- RD	37.5	20.2	13.6	2.43
T_2 - Biocompost @ 2.5 t ha^{-1} + RD of NP	34.9	18.4	12.4	2.33
T_3 - Spentwash ash @ 400 kg ha^{-1} + RD of NP	37.1	19.9	13.3	2.40
T_4 - BDS @ 37.5 kilo l ha^{-1} at full dose + RD of NP	34.2	18.1	12.2	2.29
T_5 - BDS @ 37.5 kilo l ha^{-1} at split dose + RD of NP	36.3	19.3	13.0	2.35
T_6 - BDS @ 50 kilo l ha^{-1} at full dose + RD of NP	33.3	17.5	11.5	2.26
T_7 - BDS @ 50 kilo l ha^{-1} at split dose + RD of NP	35.1	18.8	12.6	2.37
CD (0.05)	0.65	0.44	0.36	0.09

RD - Recommended Dose of NPK ; BDS- Biomethanated Distillery Spentwash

Application of BDS and biocompost had significantly influenced the biochemical parameters of CN hybrid grass (Table 2). Compared with treatments, BDS @ 50 kilo l ha^{-1} at full dose + RD of NP registered the lowest cellulose (33.3 %), hemicellulose (17.5 %) and lignin (11.5 %) which was on par with BDS @ 37.5 kilo l ha^{-1} at full dose. The highest parameter of 37.5, 20.2 and 13.6 % was recorded by RD which was on par with spentwash ash @ 400 kg ha^{-1} + RD of NP. High nitrogen and other nutrients present in BDS increased in leaf stem ratio and forage protein enlargement and decreased in stem fibre. This could be because of the water content and solution carbohydrate enlargement that have eventually resulted in decrease of cellulose, hemicelluloses and lignin in forage crops (Mohammad Rusan *et al.,* 2007). This was confirmed the present study. High percentage of N and K in spentwash increased the N uptake and might have better effect on protein structure resulting in decreased the cellulose, hemicelluloses and lignin content (Tas, 2005).

Green fodder yield

The application of BDS and biocompost had significant influence in increasing the total green fodder yield when compared to RD and spentwash ash (Fig. 1). The BDS @ 50 kilo l ha^{-1} at full dose +

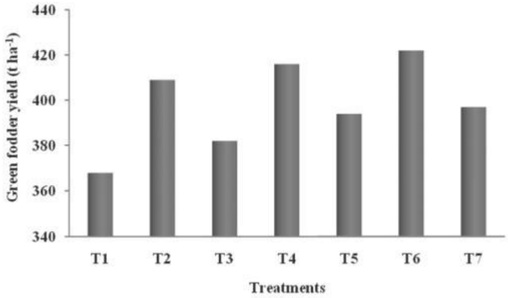

Fig. 1. Effect of BDS, biocompost and spentwash ash on total green fodder yield (t ha^{-1}) of CN hybrid grass. T_1-T_7 treatments as detailed in materials and methods.

RD of NP (422 t ha^{-1}) was recorded the highest yield which was on par with BDS @ 37.5 kilo l ha^{-1} at full dose + RD of NP (416 t ha^{-1}). All the cutting, RD showed the lowest yield (416 t ha^{-1}) which was on par with spentwash ash @ 400 kg ha^{-1} + RD of NP. The reason might be due to the favourable 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). Similar result was also obtained from the present study. Basal application of spentwash registered higher dry matter yield compared to split dose.

Microbial population

The effect of addition of BDS, biocompost and spentwash ash on soil microbial population is presented in Table 3. The mean bacterial, fungal and actinomycetes population ranged from 20.2 to 30.5, 8.7 to 16.0 and 4.3 to 6.6 x 10^{6} CFU g^{-1} of soil respectively. Among the treatments, BDS @ 50 kilo l ha^{-1} at full dose + RD of NP significantly recorded the highest soil bacterial population (30.5 x 10^{6} CFU g^{-1} of soil), fungal population (16.0 x 10^{4} CFU g^{-1} of soil) and actinomycetes population (6.6 x 10^{2} CFU g^{-1} of soil) followed by BDS @ 37.5 kilo l ha^{-1} at full dose + RD of NP. The lowest bacterial, fungal and actinomycetes population count was recorded by RD which was on par with spentwash ash @ 400 kg ha^{-1} + RD of NP. The microbial population decreased significantly as the cuttings advanced. The interaction effects of treatments with various stages of cuttings were found to be non significant for bacterial population but it was significant for fungal and actinomycetes population count.

Table 3. Effect of distillery industry wastes on soil microbial population at various cuttings of CN hybrid grass

Treatments/Cuttings	Bacterial Count (x 10^{6} CFU g^{-1})				Fungal Count (x 10^{4} CFU g^{-1})				Actinomycetes Count (x 10^{2} CFU g^{-1})			
	1st Cut	3rd Cut	5th Cut	7th Cut	1st Cut	3rd Cut	5th Cut	7th Cut	1st Cut	3rd Cut	5th Cut	7th Cut
T_1- RD	21.9	20.7	19.4	18.7	9.60	9.10	8.5	7.40	4.7	4.4	4.1	3.9
T_2 - Biocompost @ 2.5 t ha^{-1} + RD of NP	28.6	27.6	26.1	24.3	14.8	13.6	12.2	10.7	6.8	5.8	5.4	5.1
T_3 - Spentwash ash @ 400 kg ha^{-1} + RD of NP	22.8	21.1	20.1	19.4	9.80	9.30	9.10	7.80	4.9	4.6	4.3	4.1
T_4 - BDS @ 37.5 kilo l ha^{-1} at full doses + RD of NP	32.1	29.7	28.8	27.1	16.5	15.2	14.3	13.8	7.2	6.1	5.8	5.4
T_5 - BDS @ 37.5 kilo l ha^{-1} at split doses + RD of NP	23.8	22.8	20.1	19.8	11.8	11.4	10.3	9.2	5.7	5.2	4.8	4.7
T_6 - BDS @ 50 kilo l ha^{-1} at full doses + RD of NP	33.4	30.4	29.6	28.7	17.9	16.3	15.7	14.2	7.9	6.4	6.1	5.9
T_7 - BDS @ 50 kilo l ha^{-1} at split doses + RD of NP	24.4	23.4	22.5	21.3	12.3	11.8	10.6	9.6	6.3	5.5	5.2	4.9
	CD (0.05)				CD (0.05)				CD (0.05)			
T	0.76				0.37				0.17			
C	0.58				0.28				0.13			
T X C	NS				0.74				0.33			

RD - Recommended Dose of NPK ; BDS- Biomethanated Distillery Spentwash

Being rich in nutrients and organic matter, particularly easily oxidizable and soluble organic carbon, the spentwash might have favoured the proliferation of microbial population in soil. This supports the earlier findings of Murugaragavan (2002). Split dose of spentwash application recorded the lowest population and the reason for such reduction of microbial population might be due to faster depletion of oxygen in the soil because of high BOD of effluent and the resulting anaerobic soil environment prevailed immediately after its application (Saliha *et al*., 2005). The spentwash contained sufficient amount of organic matter which might have served as a source of food for microbial proliferation (Patil and Shinde, 1995). The reduction in the microbial activities during the advancement of the crop growth, particularly at end of the crop growth was probably due to the exhaustation of nutrients and organic matter as a result of intense microbial activity and crop uptake of nutrients during the crop growth (Goyal *et al*., 1995).

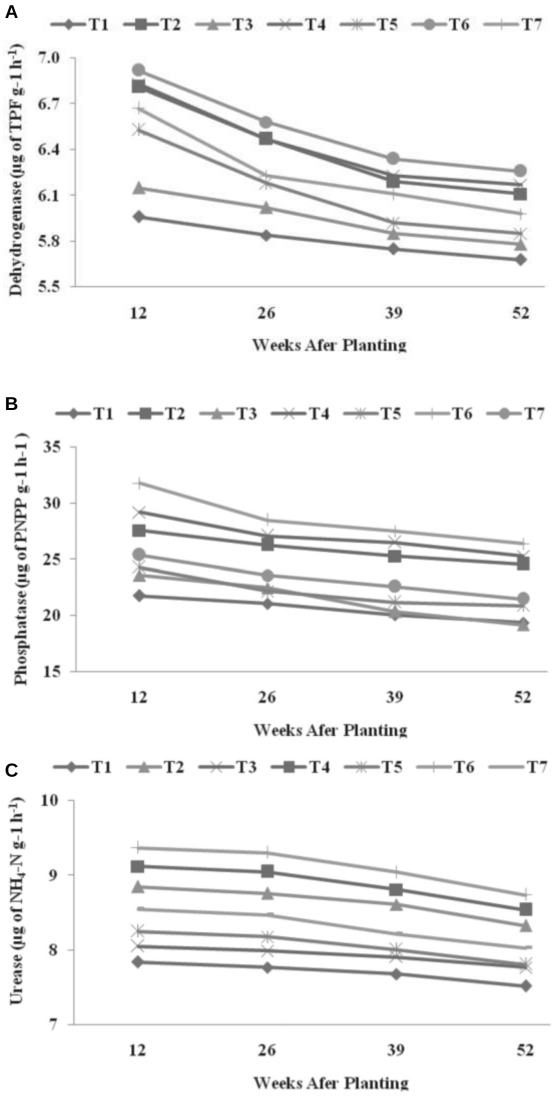

Fig. 2. Impact of distillery spentwash, biocompost and spentwash ash with inorganic fertilizers on changes in dehydrogenase (A), phosphatase (B) and urease activity (C) of soil grown with CN hybrid grass. T_1-T_7 treatments as detailed in materials and methods.

Enzyme activities

Enzyme activity of the soil was significantly influenced by the addition of BDS, biocompost and spentwash ash (Fig 2). The mean dehydrogenase, phosphatase and urease activity for the treatments ranged from 5.81 to 6.53 µg of TPF g^{-1} of soil h^{-1}, 20.6 to 28.6 µg of PNPP g^{-1} of soil h^{-1} and 7.70 to 9.11mg NH_4-N g^{-1} of soil h^{-1} respectively. Among the treatments, BDS @ 50 kilo l ha^{-1} at full dose + RD of NP recorded the highest dehydrogenase (6.53 µg of TPF g^{-1} of soil), phosphatase (28.6 µg of PNPP g^{-1} of soil) and urease activity (9.11mg NH_4-N g^{-1} of soil h^{-1}) which was on par with BDS @ 37.5 kilo l ha^{-1} at full dose + RD of NP and biocompost @ 2.5 t ha^{-1} + RD of NP. The lowest enzyme activity was recorded in RD which was on par with spentwash ash @ 400 kg ha^{-1} + RD of NP. The enzyme activity decreased significantly as the cuttings advanced. The interaction effects of treatments with various stages of cuttings were found to be non significant for all the three enzymes.

There was an increase in the activities of urease, phosphatase and dehydrogenase due to spentwash application which supplemented the organic matter and nutrients to the soil which in turn subsequently enhanced the microbial biomass. It is implied that organic and inorganic nutrients provided a nutrient rich environment, which is essential for the synthesis of enzymes. This is in accordance with the findings of Ramana *et al.* (2002). Generally, organic manure addition was found to enhance the microbial activities which in turn favour the synthesis of various enzymes in soil (Dinesh *et al.,* 2000). The mineralization rate of organic P is relevant to both P nutrition of crops and phosphatase activity in soil. Higher enzyme activities in soil indicated the greater mineralization of N and P due to the application of spentwash (Rajannan *et al*., 1998).

Conclusion

From the present investigation, it could be concluded that the application of BDS @ 50 kilo l ha^{-1} at full dose + RD of NP increased the physiological parameters, green fodder yield and soil biological properties and decreased the biochemical parameters. Thus application of spentwash to the agricultural field, as an amendment, might be a viable option for the safe disposal of this industrial waste with concomitant enhancement of soil biological properties. However, the level of application should be within the prescribed limit to avoid development of soil salinity in the long run and not to affect the ground water quality also.

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