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Original Research Article

DYNAMICS OF SOIL MICROBIAL POPULATION AND ENZYMES ACTIVITIES UNDER DISTILLERY SPENTWASH IRRIGATION

ABSTRACT

7 Spentwash is a rich source of organic matter and nutrients like nitrogen, phosphorus, potassium,
8 calcium and sulphur. The effect of different levels and methods of spentwash application on soil enzymatic
9 activity was examined through a field experiment. The field experiment was conducted using Sesame VRI
10 (Sv) 2 as a test crop at Research and Development Farm, The Sakthi Sugars pvt. Ltd., Appakkudal,
11 Erode District. The experiment was formulated with six treatments with four replications, laid out in
12 Randomised Block design. As per the treatment schedule the calculated quantity of biomethanated
13 distillery spentwash for pre-sown application was uniformly applied to the plots before sowing viz., 25, 50,
14 75,100% along with recommended dose of NP for four treatments viz., 100%, 75%, 50%, 25% and
15 Recommended dose NPK was treated as one treatment (control).The soil samples were collected at 30
16 days intervals and analysed for the changes in soil microbial population and enzyme activities. The
17 results of the study showed that the microbial population and enzymatic activities of the soil were
18 substantially increased throughout the crop growth period due to biomethanated distillery spentwash
19 application.

20 **Keywords:** Post methanated distillery spentwash, microbial population, enzyme activities

1.INTRODUCTION

22 The spentwash is acidic (pH 3.94 - 4.30), dark brown liquid with high BOD (45,000 – 1,00,000 mg
23 l⁻¹) and COD (90,000 – 2,10,000 mg l⁻¹), and produce obnoxious odour. Although it does not contain toxic
24 substances, its discharge without any treatment brings about immediate discolouration and depletion of
25 dissolved oxygen in the receiving water streams, in turn posing serious threat to the aquatic flora and
26 fauna (Mane *et al.*, 2006). Distillery waste is rich in organic matter and nutrients especially nitrogen and
27 potassium and also can be utilized as a source of irrigation water in water scarcity areas. However, they
28 are also characterized by high soluble salts coupled with high BOD and COD. Hence while aiming for
29 better crop production; their utilization has to be optimized for sustaining the environment. On an average,
30 distillery effluent release 80 million kg of nitrogen and 520 million kg of potassium annually. Thus the
31 availability of nutrients in distillery effluents and the possibility of substituting these for inorganic fertilizer
32 in agriculture have a great promise (Joshi and Singh, 2010).The addition of organic matter through the
33 BDS may be favorable for microorganisms and enzymes in soils. Batch *et al.* (1993) observed that the
34 spentwash at 250 m³ ha⁻¹ rate stimulated the soil microorganisms and increased the dehydrogenase
35 activity in soil. The spentwash addition increased the phosphatase, dehydrogenase and urease enzymes
36 in dry land black and red soils especially at levels of 125 m³ ha⁻¹ (Murugaragavan, 2002).

37 2.MATERIALS AND METHODS

38 A field experiment was conducted at Research and Development Farm, The Sakthi Sugars pvt.
 39 Ltd., Appakkudal, Erode District, Tamil Nadu in randomized block design with three replications using
 40 sesame (*Sesamum indicum*) var .VRI (Sv)2 as a test crop. The experimental field was laid out and the
 41 calculated quantity of BDS (Table 1) was uniformly applied in each plot as per the treatment details given
 42 below. Then, the soil was ploughed at 10 days interval for providing better soil aeration and consequent
 43 reduction of BOD level in the soil-water system.

44 Treatment Details:

45	T₁	:	Absolute control.
46	T₂	:	Control – 100% recommended dose of NPK.
47	T₃	:	25 % N through DSW and 75 % N through inorganic 48 source based on crop requirement.
49	T₄	:	50 % N through DSW and 50 % N through inorganic 50 source based on crop requirement.
51	T₅	:	75 % N through DSW and 25 % N through inorganic 52 source based on crop requirement.
53	T₆	:	100 % N through DSW.

54 While applying P, the available P in DSW and inorganic P will be taken together to meet the P
 55 requirement of crop. Potassium will be skipped in DSW applied treatments.

56 **2.1 Collection and analysis of soil samples:** Soil samples were collected at 30 DAS (R1), 60 DAS
 57 (R2), 90 DAS (R3), and 120 DAS (R4), and the number of fungi ,bacteria and actinomycetes colonies
 58 were assessed by plating dilution techniques (Waksman and Fred ,1922). The activities of urease,
 59 phosphatase and dehydrogenase enzymes were assayed as per the standard procedures (Tabatabai and
 60 Bremner, 1972).

61 **Table 1.Characteristics of Post methanated distillery spentwash (PMDSW)**

Characters	Unit	Values
Physical properties		
Colour	-	Greenish brown
Odour	-	Unpleasant
Moisture	%	82
Total suspended solids	mg L ⁻¹	6850
Total dissolved solids	mg L ⁻¹	45,120
Total solids	mg L ⁻¹	51,970
Specific gravity	g cc ⁻¹	1.12
Physico-chemical properties		

pH	-	7.75
EC	dS m ⁻¹	37.8
Biological oxygen demand	mg L ⁻¹	8,740
Chemical oxygen demand	mg L ⁻¹	37,476
Organic carbon	mg L ⁻¹	26,110
Total Nitrogen	mg L ⁻¹	1,700
Total Phosphorus	mg L ⁻¹	450
Total Potassium	mg L ⁻¹	11,550
Total Sodium	mg L ⁻¹	845
Total Calcium	mg L ⁻¹	2,272
Total Magnesium	mg L ⁻¹	1,580
<i>Water soluble cations</i>		
Calcium	m.e. L ⁻¹	52.89
Magnesium	m.e. L ⁻¹	61.25
Sodium	m.e. L ⁻¹	32.87
Potassium	m.e. L ⁻¹	227.35
<i>Water soluble anions</i>		
Carbonate	m.e. L ⁻¹	Absent
Bicarbonate	m.e. L ⁻¹	54.12
Chloride	m.e. L ⁻¹	240.82
Sulphate	meq L ⁻¹	75.70
SAR		4.56
RSC	meq L ⁻¹	-61.20
SSP	Per cent	9.87
Potential salinity	meq L ⁻¹	258.24
<i>Biological properties</i>		
Bacteria	× 10 ⁶ CFU ml ⁻¹	23.6
Fungi	× 10 ⁴ CFU ml ⁻¹	11.2
Actinomycetes	× 10 ² CFU ml ⁻¹	7.2

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63 3.RESULTS AND DISCUSSION

64 **3.1 Soil microflora:** In field experiment conducted with sesame under rainfed condition, the highest
65 population of bacteria, fungi and actinomycetes (36.70×10^6 CFU g⁻¹, 15.27×10^4 CFU g⁻¹ and 6.87×10^2 CFU g⁻¹
66 of soil) were observed in the treatment that received 100 per cent N through DSW at rainfed condition

67 respectively (Table.2). This is in line with Mattiazo and Ada Gloria (1985) who reported the increase in soil
68 microbial activity due to oxidation of organic matter in treated spentwash applied soil. Devarajan *et al.* (1993)
69 studied the population dynamics of bacteria, fungi and actinomycetes in field soil grown with turmeric, paddy,
70 gingelly, cotton, and groundnut and the populations were increased with spentwash irrigations at different doses.
71 Patil *et al.* (1982) stated that the spentwash contained 42.7 per cent polysaccharides which served as a source of
72 carbon and sulphate for microbial proliferation. Such effect of spentwash on the population of bacteria, fungi and
73 actinomycetes in the soil was also reported by Tauk *et al.* (1990); Goyal *et al.* (1995); Rajukkannu *et al.* (1996);
74 Valliappan (1998). Similar results were reported in spentwash applied soils by Gopal *et al.* (2001) and Latha
75 (2008). The above findings add strength to the present investigation.

76 The treatment of distillery spentwash with addition of inorganic fertilizer significantly influenced
77 the soil bacterial population at different stages of crop growth. Among the treatments, T₆ (100 per cent N
78 through distillery spentwash) recorded the highest soil bacterial population of 36.70×10^6 CFU g⁻¹ of soil,
79 followed by T₅ (75 per cent N through distillery spentwash + 25 per cent N through inorganic source) of
80 34.07×10^6 CFU g⁻¹ of soil while the lowest bacterial population of 18.55×10^6 CFU g⁻¹ of soil was
81 recorded in T₁ (Control). The interaction effect on stages and different doses of DSW was positive only at S
82 I (30DAS) and S II (60 DAS) stages. With respect to the stages of sampling, the highest soil bacterial
83 population of 29.28×10^6 CFU g⁻¹ of soil was recorded at S₁ (vegetative stage) and the lowest soil
84 bacterial population of 26.25×10^6 CFU g⁻¹ of soil was recorded at S₄ (at harvest stage). The interaction
85 effects of treatments and various stages were significant.

86 **3.2 Fungal population**

87 The different treatments significantly influenced the soil fungal population at four different stages
88 of observation. Among the treatments, T₆ recorded the highest soil fungal population of 15.27×10^3 CFU
89 g⁻¹ of soil, which was on par with T₅ of 14.23×10^3 CFU g⁻¹ of soil, while the lowest soil fungal population
90 of 8.15×10^3 CFU g⁻¹ of soil and 9.35×10^3 CFU g⁻¹ of soil was recorded in T₁ (control) and T₂ (100 per
91 cent RD of NPK) and were on par with each other. With respect to the stages of sampling, the highest soil
92 fungal population of 13.15×10^3 CFU g⁻¹ of soil was recorded at S₁ (vegetative stage) and the lowest
93 soil fungal population of 10.45×10^3 CFU g⁻¹ of soil was recorded at S₄ (at harvest stage). The interaction
94 effects of treatments and various stages were non- significant.

95 **3.3 Actinomycetes population**

96 Application of distillery spentwash significantly influenced the soil actinomycetes population at
97 different stages of observation. Among the treatments, T₆ (100 per cent distillery spentwash) recorded the
98 highest soil actinomycetes population of 6.87×10^2 CFU g⁻¹ of soil, followed by T₅ (75 per cent N through
99 distillery spentwash + 25 per cent N through inorganic source) of 6.15×10^2 CFU g⁻¹ of soil, while the lowest
100 soil actinomycetes population of 4.77×10^2 CFU g⁻¹ of soil was recorded in T₁ (Control). With respect to
101 the stages of sampling, the highest soil actinomycetes population of 6.11×10^2 CFU g⁻¹ of soil was

102 recorded at (vegetative stage) and the lowest value of 4.91×10^2 CFU g^{-1} of soil was recorded at S_4 (at
103 harvest stage). The interaction effects of treatments and various stages were significant.

104 **3.4 Dehydrogenase activity:** The DSW application increased the activities of dehydrogenase,
105 phosphatase and urease with different doses of DSW viz., 100 per cent N through DSW in the field
106 experiments with sesame at rainfed condition, respectively (Fig.1, Fig.2 & Fig.3).The dehydrogenase
107 activity of the soil was also influenced by other doses of DSW application. Significantly higher
108 dehydrogenase activity of 31.62 and 28.15 μg of TPF g^{-1} of soil was recorded in T_6 and T_5 , which were on par
109 with each other. The lowest enzyme activity of 12.42 μg of TPF g^{-1} of soil was recorded in T_1 (Control). The soil
110 dehydrogenase activity significantly differed at all stages of crop growth. The dehydrogenase enzyme activity
111 was lowest at S_4 (at harvest stage) of 19.72 μg of TPF g^{-1} of soil and highest at S_1 (vegetative stage) of 23.40
112 μg of TPF g^{-1} of soil.

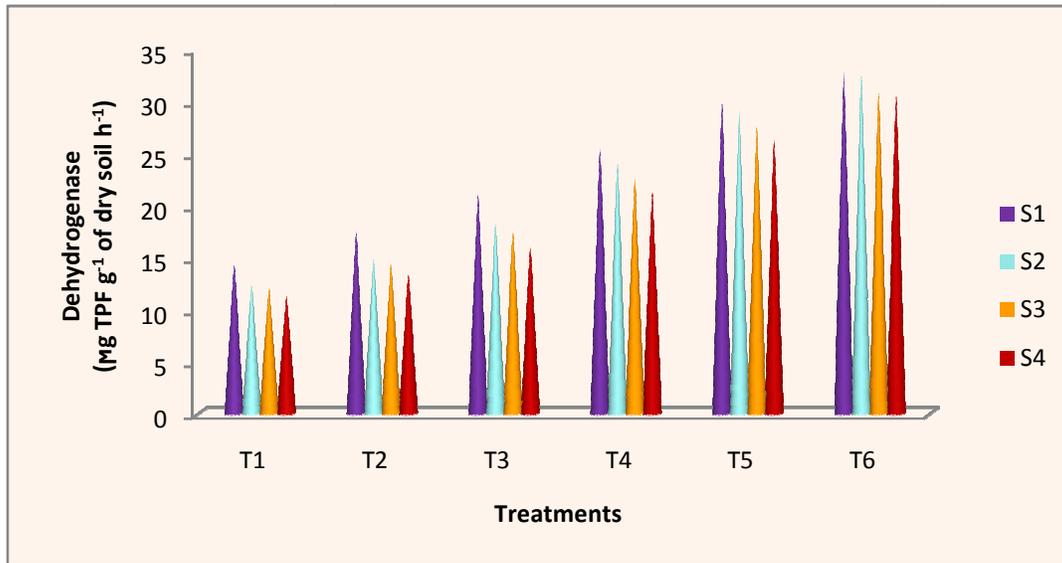
113 **3.5 Phosphatase activity:** The phosphatase activity of the soil was highly influenced by different doses
114 of DSW application. Significantly higher phosphatase activity of 16.32 μg of PNPP g^{-1} of soil was recorded
115 in T_6 (100 per cent N through distillery spentwash) followed by the treatments T_5 , T_4 and T_3 . The lowest
116 enzyme activity of 9.85 μg of PNPP g^{-1} of soil was recorded in T_1 (Control). The soil phosphatase activity
117 significantly differed at all stages of sesame crop growth. The enzyme activity was lowest at S_4 (harvest
118 stage) of 11.60 μg of PNPP g^{-1} of soil and the highest at S_1 (Vegetative stage) of 14.21 μg of PNPP g^{-1} of
119 soil.

120 **3.6 Urease activity** Urease activity of the soil was measured in distillery spentwash applied field.
121 Significantly higher urease activity of 15.25 μg of ammonia released g^{-1} of soil h^{-1} was recorded in T_6 (100 per
122 cent N through distillery spentwash), which was on par with T_5 (75 per cent N through distillery spentwash + 25
123 per cent N through inorganic source) of 14.00 μg of ammonia released g^{-1} of soil h^{-1} . The lowest enzyme
124 activity of 8.87 μg of ammonia released g^{-1} of soil h^{-1} was recorded with T_1 (Control). The soil urease activity
125 significantly differed at all stages of crop growth. The enzyme activity was the lowest at S_4 (at harvest
126 stage) of 10.41 μg of ammonia released g^{-1} of soil h^{-1} and the highest at S_1 (vegetative stage) of 13.66 μg
127 of ammonia released g^{-1} of soil h^{-1} . This might be due to tremendous increase in the microbial population,
128 availability of most of the essential nutrients and organic carbon content of the soil applied with different
129 levels of DSW. This is in close agreement with the findings of Kamalakumari and Singaram (1995) who
130 observed a strong positive relationship among the available NPK and organic carbon for enzyme
131 activities of the soil. The work of Goyal *et al.* (1995) and Rajannan *et al.* (1998), Murugaragavan (2002) lend
132 support for the increased activities of soil enzymes owing to the addition of spentwash. Similar results
133 were obtained by Sivashankari (2009) and Nandha Kumar (2009).

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137 **Table 2. Effect of Distillery spentwash on soil microbial population at various stages of sesame**
 138 **under rainfed condition**

Treatment / stages	Bacteria (x 10 ⁶ CFU g ⁻¹ of soil)					Fungi (x 10 ³ CFU g ⁻¹ of soil)					Actinomycetes (x 10 ² CFU g ⁻¹ of soil)				
	S ₁	S ₂	S ₃	S ₄	Mean	S ₁	S ₂	S ₃	S ₄	Mean	S ₁	S ₂	S ₃	S ₄	Mean
T ₁	19.80	19.00	18.40	17.00	18.55	8.70	7.70	8.00	8.20	8.15	5.60	5.20	4.60	3.70	4.77
T ₂	23.00	22.30	21.50	20.50	21.82	10.20	8.40	9.20	9.60	9.35	5.40	5.00	4.40	4.10	4.75
T ₃	26.70	24.20	23.00	22.40	24.07	10.70	9.40	8.50	8.40	9.25	5.60	5.30	5.10	5.00	5.25
T ₄	31.00	30.40	29.60	28.80	29.95	14.80	12.20	10.70	10.10	11.94	5.80	5.50	5.20	4.60	5.27
T ₅	35.80	34.20	33.70	32.60	34.07	16.70	14.30	13.10	12.90	14.23	6.90	6.30	5.60	5.80	6.15
T ₆	39.40	35.50	35.10	36.80	36.70	17.90	15.60	14.00	13.60	15.27	7.40	7.00	6.80	6.30	6.87
Mean	29.28	27.60	26.88	26.35	27.52	13.15	11.27	10.58	10.45	11.36	6.11	5.71	5.28	4.91	5.50
	SEd		CD (0.05)			SEd		CD (0.05)			SEd		CD (0.05)		
T	1.03		2.06			0.56		1.12			0.15		0.31		
S	0.84		1.68			0.46		0.92			0.12		0.25		
TxS	2.06		4.12			1.13		2.26			0.31		0.62		
Treatments:															
T ₁ - Absolute control ,T ₂ -Control – 100% recommended dose of NPK ,T ₃ - 25 % N through DSW and 75 % N through inorganic source ,T ₄ -50 % N through DSW and 50 % N through inorganic source, T ₅ - 75 % N through DSW and 25 % N through inorganic source ,T ₆ - 100 % N through DSW															
Sampling periods: S ₁ : 30 DAS, S ₂ : 60 DAS, S ₃ : 90 DAS, S ₄ : At the time of harvest															

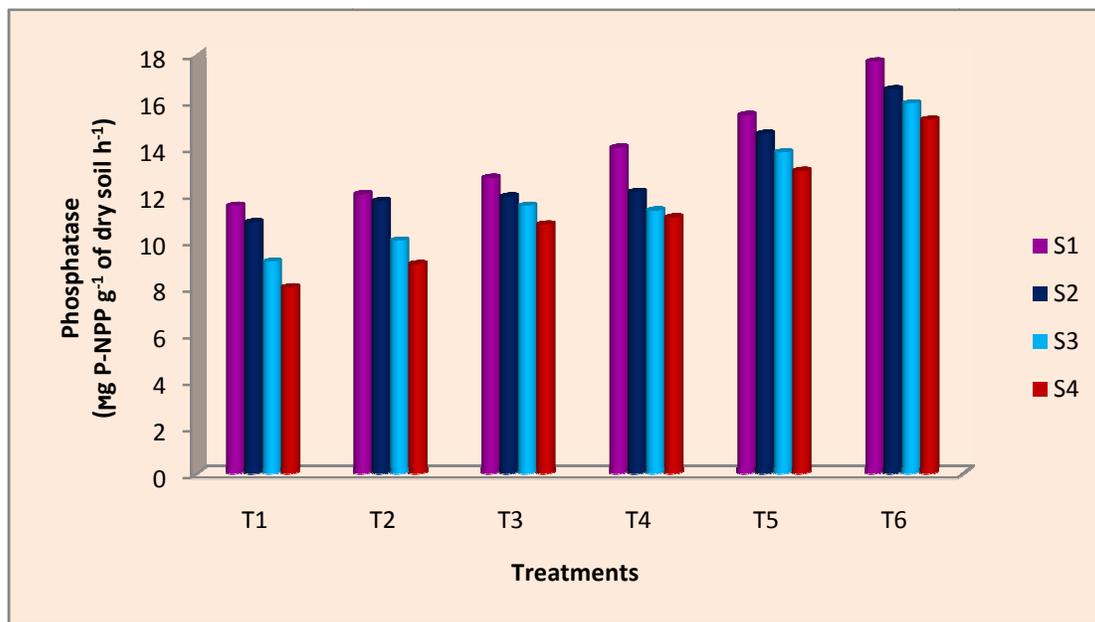


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Fig.1. Effect of distillery spentwash application on soil dehydrogenase activity in rainfed sesame crop



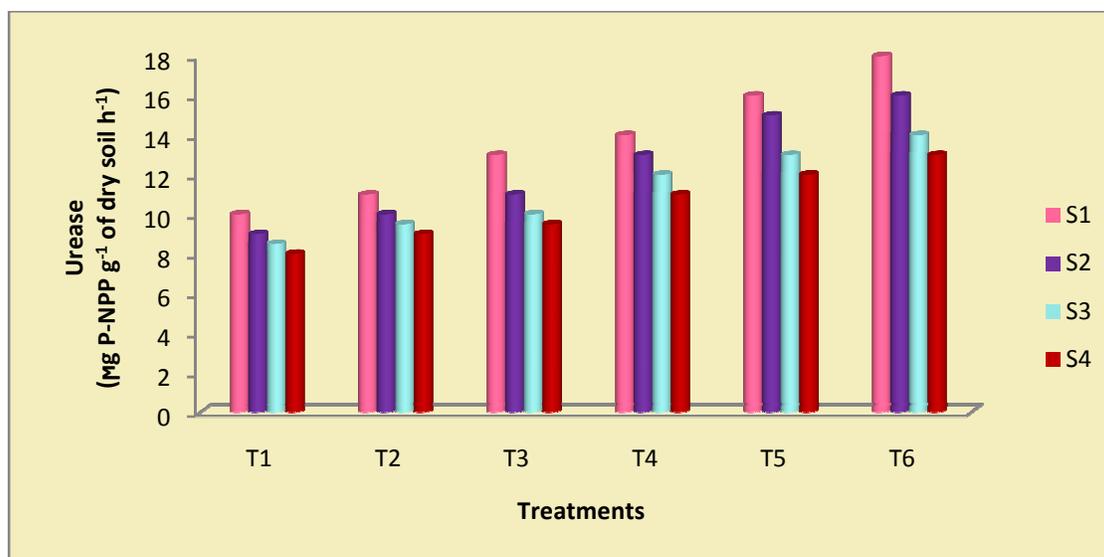
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Fig.2. Effect of distillery spentwash application on soil phosphatase activity in rainfed sesame crop



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148 **Fig.3. Effect of distillery spentwash application on soil urease activity in rainfed sesame crop**

T₁- Absolute control ,T₂-Control – 100% recommended dose of NPK ,T₃ - 25 % N through DSW and 75 % N through inorganic source ,T₄ -50 % N through DSW and 50 % N through inorganic source, T₅ - 75 % N through DSW and 25 % N through inorganic source ,T₆ - 100 % N through DSW

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150 **CONCLUSION**

151 Results of the present study indicated that application of post methanated distillery spentwash
152 increased the microflora and enzyme activities of the soil throughout the crop growth period of sesame.
153 Application of distillery spentwash in treatment T₆ (100 per cent N through distillery spentwash) recorded the
154 highest bacterial, fungal and actinomycetes population at all four stages of crop growth compared to other
155 treatments in rainfed condition. The increase in soil microbial activity due to oxidation of organic matter in
156 treated spentwash applied soil .The enzyme activities viz., phosphatase, dehydrogenase and urease
157 recorded the highest value of 16.32 µg p-nitrophenol g⁻¹ soil h⁻¹, 31.62 µg TPF g⁻¹ soil h⁻¹ and 15.25 µg
158 NH₄-N g⁻¹ soil h⁻¹ respectively, in the treatment T₆ (100 per cent N through distillery spentwash)
159 respectively under rainfed condition. Enhancement of the activities of phosphatase, dehydrogenase and
160 urease enzyme was observed in the soil that received different doses of Post methanated Distillery
161 SpentWash and maintained stable microbial population and enzyme activities till the harvest stage of the
162 crop.

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