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Effect of Nanoencapsulated Pre-emergence Sulfentrazone Herbicide on Soil Microbiome and Nodulation of Irrigated Blackgram (*Vigna mungo* L.)

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A B S T R A C T

Field experiments were conducted in the wetland farms of Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore during *Rabi* and *Summer* 2019-2020. Both the experimental trials consists of nine treatments of randomized block design which were replicated thrice. The treatments comprise of sulfentrazone with and without encapsulation @ 0.30 kg ha^{-1} applied at 1 DBS and 2 DAS followed by general recommended herbicides and weed management methods for blackgram. Sulfentrazone herbicide was encapsulated by using solvent evaporation method for season long weed management and to reduce the leachability. These treatments were tested to know their effect on soil bacterial, fungal and actinomycetes population and also the nodulation ability of blackgram crop. All the herbicide applied treatments were showed reduction in bacterial, fungal and actinomycetes population at 25 DAS compared to initial population, but slight increase in the population of T_7 (Two hand weedings at 15 and 30 DAS), T_8 (Weed free check)and T_9 (Absolute control)treatments in both the trials. At 50 DAS there was great increase in microbial population compared to 25 DAS in all herbicide applied treatments. There was no significant difference among all the treatments at 50 DAS in microbial population. Higher nodule count and nodule dryweight were noticed at 30 DAS in T_9 (Absolute control), T_8 (Weed free check) and T_7 (Two hand weedings at 15 and 30 DAS) which is followed by T_1 (Encapsulated sulfentrazone @ 0.30 kg a.i. ha^{-1}applied at 1 DBSand T_6 (Pendimethalin @ 1kg a.i. ha^{-1}applied at 2 DAS *fb* hand weeding at 20 DAS). But at 60 DAS there was no significant difference among the treatments except with unweeded control.

Introduction

Soil is the restless harbour for plant growth and is the mother land for most of the microbes. Use of pesticides and fertilizers in crop protection and production affects soil in many ways. Their concentration, threshold level, half-life, movement and also type of crop that harbours the particular soil influence soil biology and ecology. Blackgram is a nutritious edible seed of leguminous crop, have become an essential part of the human diet. It is an important pulse crop cultivated in tropical and subtropical regions of the world.

These leguminous plants are symbiotically connected with rhizobia and their interaction plays a vital role in crop growth (Vijay *et al.*, 2018). Symbiotic nitrogen fixing ability of this crop helps in enrichment the soil, with this reason blackgram became an important crop for crop rotation. N-fixing bacteria and fungi are accountable up to 80% of nitrogen and up to 75% of phosphorus, that is assimilated by plants annually (Nongmaithem and Pal, 2013). Microorganisms are influenced by several factors including the application of herbicides (Pampulha *et al.*, 2007). Among the different soil microbes, more sensitive microbes to herbicides are bacteria (Ghinea *et al*., 1998). Sulfentrazone herbicide belongs to the family of phenyl triazolinone, has mean partition coefficient K_{oc} = 43 and sorption coefficient K_d< 1 and also has high horizontal and vertical leaching potential (Martinez *et al.*, 2008). It has high Groundwater Ubiquity Score (GUS) of 6.75 which is far more than broad spectrum herbicides like pendimethalin and glyphosate which are having GUS of 0.66 and 0.42 respectively (Gustafson, 1989). This is the prime reason for encapsulation of sulfentrazone using solvent evaporation method. This study is mainly aimed to know the effect of sulfentrazone with and without encapsulation and other different treatments on soil microbial population changes with time and also to know nodulation ability of blackgram.

Materials and Methods

Field experiments were conducted in the wetland farms of Department of Agronomy, TNAU, Coimbatore during *Rabi* and *Summer* 2019-2020. Both the experimental trials consists of nine treatments of randomized block design which were replicated thrice. The treatments are T_1-Encapsulated(e^+) Sulfentrazone @ 0.3 kg a.i. ha^{-1} at 1 DBS, T_2-Non-encapsulated(e^-) Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^- at 1 DBS, T_3-Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^+ at 2 DAS, T_4-Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^- at 2 DAS, T_5-Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS fb Quizalofop-ethyl @ 50 g a.i. ha^{-1} and Imazethapyr @ 50 g a.i. ha^{-1} at 20 DAS, T_6-Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS fb 1 HW at 20 DAS, T_7-HW twice at 15 and 30 DAS, T_8-Weed free check and T_9-Absolute control.

The soil type of the field trials is clay loam in texture, slightly basic pH (8.4), low EC (0.43 dSm^{-1}), medium in organic carbon (0.70 per cent), low in available N (263.5 kg ha^{-1}), medium in available P_2O_5 (15.2 kg ha^{-1}) and high in available K (891.7 kg ha^{-1}). Proper need based crop management practices and plant protection measures were followed in all the treatments as per the crop production guide, TNAU, 2019. Microbial population dynamics in various treatments was studied from the experimental soil before sowing, at 25 and 50 DAS by serial dilution plate count technique. Weighed and transferred 1 gram of soil in to 10 ml sterile distilled water and shaked rigorously. This gives 10^{-1} dilution, from this 1 ml of suspension was transferred to 9 ml of sterile distilled water using a sterile pippete to get 10^{-2} dilution. Consequent 10^{-3}, 10^{-4} 10^{-5} and 10^{-6} dilutions were made similarly. The appropriate media viz., nutrient agar, rose bengalagar and kenknightagar for bacteria, fungi and actinomycetes respectively were melted, cooled and poured in to sterile petri plates by pour plate method carrying respective dilution. Petri plates were incubated at 30^{o}C, 2 days, 4 days and 7 days for bacteria, fungi and actinomycetes respectively. After incubation time, emerged colonies were counted and expressed as CFU per gram of soil. For nodule count and dryweight five plants were selected and pulled out after giving irrigation then counted No. of nodules per plant. After that nodules were collected, shade dried and taken dry weight per plant in mg $plant^{-1}$at 30 and 60 DAS.

Results and Discussion

Effect on soil microbiome

All the herbicide applied treatments were showed reduction in bacterial, fungal and actinomycetes population at 25 DAS compared to initial population, but slight increase in the population of T_7, T_8 and T_9treatments in both the trials. At 50 DAS there was great increase in microbial population compared to 25 DAS in all herbicide applied treatments. There was no significant difference among all the treatments at 50 DAS in microbial population. This might be due to carbon released from degraded herbicide leads to an increase of the soil microflora population (Bera and Ghosh, 2013). In sulfentrazone applied plots initially at 25 DAS there was less bacterial, fungal and actinomycetes population compared to control. But at 50 DAS there was gradual increase in population of microbes (Table 1 and Table 2). This was supported by Sulfentrazone applied to sugarcane crop at lower doses of 720 and 840 g a.i. ha^{-1} did not affect the microflora but in case of higher doses of 1320 and 2400 g a.i. ha^{-1} initial reduction of microflora was observed and recovered 30 days after application (Kalaiyarasi, 2012).

Effect on nodulation of blackgram

Higher nodule count and nodule dryweight were noticed at 30 DAS in T_9 (Absolute control), T_8 (Weed free check) and T_7 (Two hand weedings at 15 and 30 DAS) which is followed by T_1 (Encapsulated sulfentrazone @ 0.30 kg a.i. ha^{-1}applied at 1 DBSand T_6 (Pendimethalin @ 1kg a.i. ha^{-1}applied at 2 DAS *fb* hand weeding at 20 DAS). But at 60 DAS there was no significant difference among the treatments except with unweeded control. At 60 DAS higher nodule count and nodule dry weight were noticed in T_8 and T_7 followed by T_6, T_5 (Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS *fb*Quizalofop-ethyl @ 50 g a.i. ha^{-1} and Imazethapyr @ 50 g a.i. ha^{-1} at 20 DAS) and T_1 (Encapsulated sulfentrazone @ 0.3 kg a.i. ha^{-1} at 1 DBS).According to Raman and Krishnamoorthy (2005) nodulation in black gram was not affected significantly due to the application of chemical herbicides. With this experiment it was found that sulfentrazone with and without encapsulation @ 0.30 kg a.i. ha^{-1}applied at 1 DBS and 2 DAS did not differ significantly with others except absolute controlin both nodule number and dryweight (Table 3). Pendimethalin, imazethapyr and quizalofop-ethyl also did not affect the nodule number and dryweight. Similar observations were recorded by Mishra and Chandra Bhanu (2006).

Hence concluded, in both the field experiments conducted during *Rabi* and *Summer* 2019-2020, it was observed that all the herbicide applied treatments were showed reduction in microbial count at 25 DAS compared to initial population. There was no significant difference among all the treatments at 50 DAS in microbial population. Higher nodule count and nodule dryweight were noticed at 30 DAS in Absolute control, Weed free check and Two hand weedings at 15 and 30 DAS which are followed by Encapsulated sulfentrazone @ 0.30 kg a.i. ha^{-1}applied at 1 DBS and Pendimethalin @ 1kg a.i. ha^{-1}applied at 2 DAS *fb* hand weeding at 20 DAS.But at 60 DAS there was no significant difference among the treatments except with unweeded control. In this experiment it is concluded that in sulfentrazone @ 0.30 kg a.i. $ha^{-1}$with and without encapsulation and also in other herbicidal treatments even though there was slight decrease in microbial population, nodule count and nodule dryweight at initial stages of blackgram, later due to herbicidal degradation by microbes there was gradual increase in soil microbiome and nodulation ability.

Table.1 Effect of weed management treatments on microbial population (CFU) of soil in trial I

T. No.	Treatments	25 DAS			50 DAS		
		Bacteria (x 10^6 CFU)	Fungi (x 10^4 CFU)	Actinomycetes (x 10^3 CFU)	Bacteria (x 10^6 CFU)	Fungi (x 10^4 CFU)	Actinomycetes (x 10^3 CFU)
T_1	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^+ at 1 DBS	30.96	8.33	18.18	45.99	14.72	24.98
T_2	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^- at 1 DBS	30.31	8.17	17.86	45.25	14.70	24.46
T_3	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^+ at 2 DAS	30.95	7.58	17.58	45.35	14.59	24.27
T_4	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^- at 2 DAS	30.92	7.53	17.51	45.24	14.51	24.29
T_5	Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS *fb*Quizalofop-ethyl @ 50 g a.i. ha^{-1} and Imazethapyr @ 50 g a.i. ha^{-1} at 20 DAS	29.75	7.79	17.05	47.51	14.86	25.47
T_6	Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS *fb* 1 HW at 20 DAS	33.85	9.25	18.37	48.41	15.39	26.47
T_7	HW twice at 15 and 30 DAS	43.55	12.25	22.67	48.50	15.79	27.20
T_8	Weed free check	44.51	12.08	22.74	48.45	16.39	26.90
T_9	Absolute control	46.90	11.67	23.48	48.34	15.49	27.30
	SEd	2.31	0.84	0.83	2.74	0.79	1.51
	CD(P= 0.05)	4.89	1.79	1.76	NS	NS	NS

e^+ - with encapsulation e^- - without encapsulation DBS – Day before sowing DAS – Days after sowing HW - Hand weeding

*Initial microbial population (Before ploughing): Bacteria – 41.55 x 10^6 CFU Fungi – 8.50 x 10^4 CFU Actinomycetes – 15.37 x 10^3 CFU

Table.2 Effect of weed management treatments on microbial population (CFU) of soil in trial II

T. No.	Treatments	25 DAS			50 DAS		
		Bacteria (x 10^6 CFU)	Fungi (x 10^4 CFU)	Actinomycetes (x 10^3 CFU)	Bacteria (x 10^6 CFU)	Fungi (x 10^4 CFU)	Actinomycetes (x 10^3 CFU)
T_1	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^+ at 1 DBS	27.57	7.33	15.40	42.17	14.42	21.42
T_2	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^- at 1 DBS	26.89	7.33	15.04	41.48	14.10	21.02
T_3	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^+ at 2 DAS	27.62	6.33	14.58	41.58	14.36	21.05
T_4	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^- at 2 DAS	27.54	6.67	14.06	41.19	14.08	20.55
T_5	Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS *fb* Quizalofop-ethyl @ 50 g a.i. ha^{-1} and Imazethapyr @ 50 g a.i. ha^{-1} at 20 DAS	26.56	6.00	14.15	43.50	14.28	21.84
T_6	Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS *fb* 1 HW at 20 DAS	31.35	7.33	15.60	43.77	14.92	22.66
T_7	HW twice at 15 and 30 DAS	38.79	10.33	20.70	44.08	15.33	23.28
T_8	Weed free check	39.63	11.33	20.47	44.03	16.17	22.92
T_9	Absolute control	41.84	9.33	21.29	44.35	15.00	23.43
	SEd	1.94	0.60	0.86	2.35	0.72	1.23
	CD(P= 0.05)	4.11	1.27	1.83	NS	NS	NS

e^+ - with encapsulation e^- - without encapsulation DBS – Day before sowing DAS – Days after sowing HW - Hand weeding

*Initial microbial population (Before ploughing): Bacteria – 35.50 x 10^6 CFU Fungi – 7.33 x 10^4 CFU Actinomycetes – 15.55 x 10^3 CFU

Table.3 Effect of weed management treatments on nodule count (No.plant^{-1}) and nodule dryweight (mg plant^{-1}) of blackgram in trial I and II

T. No.	Treatments	Trial I				Trial II			
		30 DAS		60 DAS		30 DAS		60 DAS	
		Count	Dry weight	Count	Dry weight	Count	Dry weight	Count	Dry weight
T_1	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^{+} at 1 DBS	26.33	84.88	40.67	179.29	28.85	95.35	43.86	194.80
T_2	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^{-} at 1 DBS	18.67	77.31	39.67	179.07	20.57	84.11	42.40	194.59
T_3	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^{+} at 2 DAS	22.33	80.47	39.00	178.39	24.68	89.64	42.02	193.78
T_4	Sulfentrazone @ 0.3 kg a.i. ha^{-1} e^{-} at 2 DAS	17.33	74.53	38.33	177.98	19.03	80.70	41.29	193.43
T_5	Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS *fb*Quizalofop-ethyl @ 50 g a.i. ha^{-1} and Imazethapyr @ 50 g a.i. ha^{-1} at 20 DAS	16.67	76.42	41.67	180.16	18.33	84.40	43.78	195.75
T_6	Pendimethalin @ 1.0 kg a.i. ha^{-1} at 2 DAS *fb* 1 HW at 20 DAS	27.67	82.28	41.67	179.94	30.28	92.11	44.32	195.66
T_7	HW twice at 15 and 30 DAS	33.33	98.08	42.33	180.81	39.02	103.15	45.70	202.55
T_8	Weed free check	34.33	99.21	42.33	182.13	37.75	108.26	45.95	202.04
T_9	Absolute control	41.33	84.89	36.67	101.54	45.43	94.43	37.25	114.50
	SEd	2.22	7.42	2.16	17.97	2.52	7.74	2.27	17.91
	CD(P= 0.05)	4.70	15.74	4.58	38.10	5.34	16.40	4.82	37.97

e^{+} - with encapsulation e^{-} - without encapsulation DBS – Day before sowing DAS – Days after sowing HW - Hand weeding

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