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Effect of Sequential Application of Pre and Post-emergence Herbicides on Yield and Soil Microbial Activity in *kharif* greengram

G. K. Shruthi, S. R. Salakinkop and R, Basavarajappa

ABSTRACT: *A field experiment was conducted during kharif season of 2013 to study the effect of sequential application of pre and post-emergence herbicides on yield and soil microbial activity in greengram growing field. Among the sequential treatments, pre-emergence application of pendimethalin 1.0 liter ha^{-1} followed by imazethapyr 75 g ha^{-1} as post emergence at 20 days after sowing (DAS) significantly reduced weed growth and as a consequence gave significantly higher seed yield (1110 kg ha^{-1}). At the recommended dose all the tested pre emergence herbicides viz., oxyfluorfen, pendimethalin and alachlor reduced the microbial population initially, but at later stages they recovered as evidenced by higher concentration of dehydrogenases and phoaphatases resulted in degradation and disappearance of herbicides in soil. Among the herbicides, oxyflourfen reduced the soil microbial population significantly as compared to other herbicides at initial crop growth stage.*

Key words: *Herbicides, Greengram, Soil microbes, dehydrogenase and phosphatase.*

INTRODUCTION

The sustainable agriculture involves optimizing agricultural resources and at the same time maintaining the quality of environment and sustaining natural resources for future generation. In achieving this optimization, the soil microbial community composition is of great importance, because they play a crucial role in carbon flow, nutrient cycling and residue decomposition, which in turn affect soil fertility and plant growth, and hence occupy a unique position in biological cycles in terrestrial habitat. The soil microbial biomass is considered as active nutrient pool to plants and plays an important role in nutrient cycling and decomposition in ecosystem. A healthy population of soil microorganisms can stabilize the ecological system in soil due to their ability to regenerate nutrients to support plant growth. Any change in their population and activity may affect nutrient cycling as well as availability of nutrients, which indirectly affect productivity and other soil functions.

Natural and anthropogenic factors may affect the soil enzyme activities directly or indirectly. Among anthropogenic factors, pesticides are of primary importance due to their continuous entry into the soil environment. Herbicides are one of the major groups of pesticides, used to kill or suppress the growth of unwanted plants and vegetation in order to minimize the cultivation cost as well as to sustain high yield. A number of herbicides have not only been introduced as pre- or post-emergence weed killer but also leave unwanted residues in soil, which are ecologically harmful. Preferred herbicides should not only have good efficacy, but also poses minimum adverse effects to crop, ecology and environment.

Herbicides not only affect the target organisms, but also microbial communities in soil. These non-target effects may reduce the performance of important soil functions. Herbicides are extraneous to soil component pools, and are expected to affect the catalytic efficiency, behavior of soil enzymes, which contribute to the total biological activity of the soil-plant environment under different states. The interaction between herbicides and soil microorganisms may be of practical significance because of possible inhibition in microbial activities contributing to soil fertility. By keeping above information in view a field experiment was undertaken to study the influence of sequential application of pre and post-emergence herbicides on green gram yield, soil micro flora and dehydrogenase and phosphatase activity.

* Department of Agronomy, University of Agricultural Sciences, Dharwad – 580 005, India, *E-mail: shruthi.gk7@gmail.com*

MATERIAL AND METHODS

The experiment was conducted during *kharif* season of 2013, at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka state. The soil of experimental site was clay loams comprising maximum clay content (70.1%) with bulk density and particle density of 1.15 g cc^{-1} and 2.65 g cc^{-1}, respectively. The soil pH was 6.5 (neutral in reaction). It was low in available nitrogen and available phosphorus and high in available potassium. Fifteen treatments *viz.,* oxyfluorfen 0.10 kg ha^{-1} (PRE) fb 1 HW and 1 IC at 30 DAS (T_1) or fb imazethapyr 75 g ha^{-1} (POST) at 25 DAS (T_2) or fb fenoxyprop-p-ethyl 75 g ha^{-1} (POST) at 25 DAS (T_3); alachlor 1.5 liter ha^{-1} (PRE) fb 1 HW and 1 IC at 30 DAS (T_4) or fb imazethapyr 75 g ha^{-1} (POST) at 25 DAS (T_5) or fb fenoxyprop-p-ethyl 75 g ha^{-1} (POST) at 25 DAS (T_6); pendimethalin 1.0 liter ha^{-1} (PRE) fb 1 HW and 1 IC at 30 DAS (T_7) or fb imazethapyr 75 g ha^{-1} (POST) at 25 DAS (T_8) or fb fenoxyprop-p-ethyl 75 g ha^{-1} (POST) at 25 DAS (T_9); tank mix application of alachlor 1.0 liter ha^{-1} + pendimethalin 0.5 liter ha^{-1} (PRE) fb 1 HW and 1 IC at 30 DAS (T_{10}) and post-emergence alone application of imazethapyr 75 g ha^{-1} at 20 DAS (T_{11}) or fenoxyprop-p-ethyl 75 g ha^{-1} at 20 DAS (T_{12}). These weed control treatments were compared with farmers' practice (1 HW and 1 IC at 20-25 and 1 IC at 40 DAS- T_{13}), weedy (T_{14}) and weed free check (T_{15}). These fifteen treatments were laid out in complete randomized block design with three replications. Herbicides were sprayed with a knap sack sprayer fitted with a flat fan nozzle using 500 liters of water per hectare. Pre-emergence herbicides were applied either one day after sowing, whereas post-emergence herbicides were sprayed 25 days after sowing.

The greengram variety DGGV-2 was sown in June 2013 with a spacing of 30 cm X 10 cm using a seed rate of 15 kg ha^{-1}. Enumeration of bacteria, fungi, actinomycetes and phosphorus solubalizing micro-organisms was done by serial dilution and plate count method. Nutrient agar medium, Martin's rose Bengal medium and Katznelson and Bose medium as described by Subba Rao [15] were used for enumeration of total bacteria, total fungi and phosphate solubilizing fungi (PSM), respectively. Dehydrogenase activity was measured following reduction of 2,3,5-triphenylotetrazolium chloride (TTC) to red-coloured triphenyl formazon (TPF), which were determined spectrophotometrically [11]. Phospatase activity was measured following p-nitrophenyl phosphate (pNPP) is hydrolysed to p-nitrophenol (pNP), which is usually determined spectrophotometrically at 400 nm, under alkaline conditions.

RESULTS AND DISCUSSION

Effect on Crop

All the herbicide treatments produced significantly higher seed yield (714-1110 kg ha^{-1}) compared to weedy check (500 kg ha^{-1}). Unweeded check registered 57.4 per cent reduction in seed yield compared to weed free check owing to sever competition offered by uncontrolled weeds for nutrients, soil moisture, space and light. Among the weed control treatments, significantly higher seed yield (1175 kg ha^{-1}) was obtained with season long weed free check (T_{15}) as compared to weedy check (T_{14}) and treatments consisted of only post-emergence herbicides (T_{11} and T_{12}) (Table 1). However, it was on par with all herbicide treatments involving sequential and pre-emergence herbicides application fb cultural practices *viz.,* pendimethalin fb post-emergence application of either imazethapyr (T_8-1110 kg ha^{-1}) or fenoxyprop-p-ethyl (T_9-1060 kg ha^{-1}) or 1 HW and 1 IC (T_7-1103 kg ha^{-1}), pre-emergence application of alachlor fb post-emergence application of either imazethapyr (T_5- 1026 kg ha^{-1}) or 1 HW and 1 IC (T_4-1012 kg ha^{-1}), tank mix application of alachlor + pendimethalin as pre-emergence fb 1 HW and 1 IC (T_{10}- 1019 kg ha^{-1}) and farmers' practice (T_{13}-1084 kg ha^{-1}). The extent of yield increase in T_{15}, T_8, T_9, T_7, T_5, T_4, T_{10} and T_{13} was to the tune of 135, 122, 112, 121, 106, 102, 104 and 117 per cent, respectively over weedy check. The superior performance of these treatments was mainly due to effective control of weeds since from the sowing to maximum vegetative stage which created conditions similar to weed free environment due to sequential application of herbicides and pre-mergence application of herbicides fb cultural practices. The results are akin to those reported by Vijayalaxmi [17], Dwivedi [7] and Younesabadi [18]..

Effect on Root Nodules

In the present investigation application of pre-emergence herbicides *viz.*, Oxyfluorfen 0.10 kg ha^{-1} reduced the number of effective nodules per plant both in sequential application of herbicides (14.23 and 15.59 at 30 DAS and 44.14 and 44.17 at 60 DAS) as well as pre-emergence herbicide fb I HW and IC (14.78 and 44.72 at 30 and 60 DAS, respectively) as compared to weed free check (25.92 and 64.29, respectively at 30 and 60 DAS). The similar reduction in the number of effective nodules per plant due to application of

Table 1
Soil microbial population in greengram as influenced by sequential application of pre and post-emergence herbicides

Treatment	*Seed yield ($kg\ ha^{-1}$)*	*Root nodules*		*Soil microbial population (days after pre emergence herbicide application)*								
				Bacteria (cfu X 10^5)			*Fungi (cfu X 10^3)*			*Actinomycetes (cfu X 10^3)*		
		30 DAS	*60 DAS*	*7 DAS*	*14 DAS*	*21 DAS*	*7 DAS*	*14 DAS*	*21 DAS*	*7 DAS*	*14 DAS*	*21 DAS*
T_1	897	14.78	44.72	43.00	42.24	43.17	11.82	12.08	13.52	7.65	8.31	8.94
T_2	874	14.23	44.14	31.00	37.40	40.45	9.23	11.44	12.66	6.59	7.98	8.15
T_3	849	15.59	44.17	32.56	37.45	41.04	9.27	11.63	12.93	6.35	7.71	8.17
T_4	1012	21.93	59.65	42.15	42.17	43.15	11.08	12.33	13.77	7.22	8.53	8.83
T_5	1028	21.69	58.85	37.15	37.54	41.35	9.70	11.67	12.25	6.06	7.78	8.18
T_6	988	22.81	59.00	35.98	37.86	40.56	9.25	11.73	12.78	6.91	7.90	8.13
T_7	1103	23.94	59.77	43.87	42.55	43.95	11.96	12.67	13.38	7.78	8.10	8.89
T_8	1110	24.89	59.18	38.01	37.47	42.66	9.63	11.11	12.77	6.65	7.74	8.18
T_9	1060	24.33	59.00	39.00	37.38	41.67	9.48	11.66	13.26	6.37	7.69	8.20
T_{10}	1019	21.67	58.41	42.87	42.07	43.37	10.48	12.26	13.79	7.16	8.27	8.85
T_{11}	794	23.49	59.67	34.99	35.56	41.51	9.33	11.18	12.99	6.24	7.64	8.19
T_{12}	714	22.26	58.27	36.21	39.55	41.14	9.15	11.40	12.82	6.04	7.69	8.20
T_{13}	1084	25.33	63.22	44.00	42.66	43.51	12.00	12.44	13.89	8.24	8.48	8.85
T_{14}	500	20.89	54.95	44.89	44.61	44.96	12.70	13.59	14.04	8.19	8.40	9.00
T_{15}	1175	25.92	64.29	44.21	43.73	44.28	12.26	13.29	13.95	7.97	8.30	8.97
S.Em.±	55	1.65	2.98	1.37	2.30	1.62	0.59	0.77	0.72	0.42	0.23	0.31
CD (P=0.05)	160	4.79	8.65	3.97	NS	NS	1.69	NS	NS	1.22	NS	NS

DAS= Days after spraying, PRE=Pre-emergence, POST=Post-emergence, fb= followed by, IC= Intercultivation, HW= Hand weeding, PSM=Phosphorus solubalizing micro-organisms, NS= Non Significant

T_1- Oxyfluorfen 0.10 kg ha^{-1} (PRE) fb 1 HW and 1 IC, T_2- Oxyfluorfen 0.10 kg ha^{-1} (PRE) fb Imazethapyr 75 g ha^{-1}(POST), T_3- Oxyfluorfen 0.10 kg ha^{-1} (PRE) fb Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_4- Alachlor 1.5 l ha^{-1} (PRE) fb 1 HW and 1 IC, T_5- Alachlor 1.5 l ha^{-1} (PRE) fb Imazethapyr 75 g ha^{-1}(POST), T_6- Alachlor 1.5 l ha^{-1} (PRE) fb Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_7- Pendimethalin 1.0 l ha^{-1} (PRE) fb 1 HW and 1 IC, T_8- Pendimethalin 1.0 l ha^{-1} (PRE) fb Imazethapyr 75 g ha^{-1}(POST), T_9- Pendimethalin 1.0 l ha^{-1} (PRE) fb Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_{10}- Alachlor 1.0 l ha^{-1} (PRE) + Pendimethalin 0.5 l ha^{-1} (PRE) 1 HW and 1 IC, T_{11}- Imazethapyr 75 g ha^{-1}(POST), T_{12}- Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_{13}- Farmer 's practice(1 HW and 2 IC), T_{14}- Weedy check, T_{15}- Weed free

Table 2
Soil microbial population in greengram as influenced by sequential application of pre and post-emergence herbicides

Treatment	*Soil microbial population and enzymes activity (days after application of pre-emergence herbicides)*								
	PSM (cfu X 10^4)			*Dehydrogenase activity (?g TPF formed g^{-1} soil h^{-1})*			*Phosphatase activity (?g PNP formed g^{-1} soil d^{-1})*		
	7 DAS	*14 DAS*	*21 DAS*	*7 DAS*	*14 DAS*	*21 DAS*	*7 DAS*	*14 DAS*	*21 DAS*
T_1	6.45	7.01	8.13	1.84	4.95	10.21	18.19	26.76	36.89
T_2	5.81	6.97	8.07	1.80	4.92	10.40	18.01	28.02	36.89
T_3	5.40	6.75	8.07	1.90	5.01	10.67	18.39	28.18	36.51
T_4	6.77	7.35	8.13	2.30	5.61	10.00	20.56	39.33	38.61
T_5	5.46	6.68	8.04	2.31	5.40	10.20	20.38	39.75	38.46
T_6	5.30	6.87	8.04	2.33	5.24	10.15	20.34	39.30	38.84
T_7	6.70	7.33	8.11	2.39	5.36	10.16	21.01	39.29	37.27
T_8	5.98	6.72	8.01	2.26	5.48	10.27	20.55	39.70	38.43
T_9	5.18	6.98	8.06	2.33	5.24	10.07	21.05	39.36	39.46
T_{10}	6.48	7.31	8.15	2.05	5.06	10.14	19.11	31.99	39.47
T_{11}	5.36	6.63	8.08	5.52	6.96	10.53	24.39	41.37	38.52
T_{12}	5.15	6.81	8.09	5.25	6.85	11.92	23.93	41.74	39.63
T_{13}	7.88	7.45	8.12	5.52	6.96	11.04	22.66	41.52	39.75
T_{14}	8.35	8.11	8.14	5.25	6.85	11.12	23.63	42.02	39.83
T_{15}	7.74	7.80	8.18	5.47	7.14	11.32	23.63	41.22	40.27
S.Em.±CD	0.55	0.31	0.06	0.34	0.30	0.44	0.75	1.62	1.53
(P=0.05)	1.61	NS	NS	0.97	0.88	NS	2.17	4.70	NS

DAS= Days after spraying, PRE=Pre-emergence, POST=Post-emergence, fb= followed by, IC= Intercultivation, HW= Hand weeding, PSM=Phosphorus solubalizing micro-organisms, NS= Non Significant

T_1- Oxyfluorfen 0.10 kg ha^{-1} (PRE) fb 1 HW and 1 IC, T_2- Oxyfluorfen 0.10 kg ha^{-1} (PRE) fb Imazethapyr 75 g ha^{-1}(POST), T_3- Oxyfluorfen 0.10 kg ha^{-1} (PRE) fb Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_4- Alachlor 1.5 l ha^{-1} (PRE) fb 1 HW and 1 IC, T_5- Alachlor 1.5 l ha^{-1} (PRE) fb Imazethapyr 75 g ha^{-1}(POST), T_6- Alachlor 1.5 l ha^{-1} (PRE) fb Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_7- Pendimethalin 1.0 l ha^{-1} (PRE) fb 1 HW and 1 IC, T_8- Pendimethalin 1.0 l ha^{-1} (PRE) fb Imazethapyr 75 g ha^{-1}(POST), T_9- Pendimethalin 1.0 l ha^{-1} (PRE) fb Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_{10}- Alachlor 1.0 l ha^{-1} (PRE) + Pendimethalin 0.5 l ha^{-1} (PRE) 1 HW and 1 IC, T_{11}- Imazethapyr 75 g ha^{-1}(POST), T_{12}- Fenoxyprop-p-ethyl 75 g ha^{-1}(POST), T_{13}- Farmer 's practice(1 HW and 2 IC), T_{14}- Weedy check, T_{15}- Weed free

oxyfluorfen was reported by - Choudhary [6]. Among the weed control treatments, weed free check recorded significantly higher number of effective nodules per plant (25.92 and 64.29, respectively at 30 and 60 DAS) as compared to pre-emergence application of oxyfluorfen 0.10 kg ha^{-1} fb imazethapyr 75 g ha^{-1} or fb fenoxyprop-p-ethyl 75 g ha^{-1} or fb 1 HW and 1 IC. This increase in number of effective nodules per plant was mainly attributed to complete removal of weed competition in terms of allelopathy due to effective control of weeds. While, weedy check recorded significantly lower number of effective nodules per plant (20.89 and 54.95, respectively at 30 and 60 DAS) due to allelopathic effect caused by weeds and also due to reduced supply of energy from crop for fixation of atmospheric nitrogen as a result of reduced crop growth due to sever weed competition.

Effect on oil microbial load, dehydrogenase activity and phosphatase activity

Similar to root nodules, soil microbial activities were also get affected due to application of pre-emergence herbicides initially (Table 1). At 7 DAS, all the herbicides treatments recorded significantly lower activities of microbes in soil *viz.*, Bacteria (31.0 to 39.00 cfu X 10^5), Fungi (9.19 to 11.82 cfu X 10^3), Actinomycetes (6.35 to 7.37 cfu X 10^3) and PSM (5.06 to 7.48 cfu X 10^4) as compared to non-herbicides treatments viz., farmers practice, weedy and weed free check. However, at 14 DAS, all weed control treatments recorded on par microbial activities (bacteria 41.53-43.54 cfu X 10^5, fungi 12.33-14.46 cfu X 10^3, Actinomycetes 8.08-8.35 cfu X $10^3$and PSM 6.37-7.48 cfu X 10^4) as compared to non-herbicides treatments, except pre-emergence application of oxyfluorfen @ 0.10 kg ha^{-1} (bacteria 37.40-37.91 cfu X 10^5, fungi 10.96-11.44 cfu X 10^3, Actinomycetes 7.98-8.31 cfu X 10^3 and PSM 6.97-7.08 cfu X 10^4). This clearly indicated that among the herbicides tested, oxyfluorfen was found toxic to microorganisms initially and significantly reduced the microbial count as compared to other herbicide treatments. But, at 21 DAS the soil microflora gets recovered and hence, all the herbicides treatments were recorded statistically on par microbial activities as that of non-herbicide treatments. These results are in line with the Latha and Gopal [8]. They reported that in pre-emergence herbicides practices, high proportion of herbicide reaches the soil and accumulates in the microbiologically active top layer of 0 to 15 cm of soil. The detracting effect of herbicides towards all bacteria, fungi and enzyme population decreased with time. This is because of the recovery of microbial population and enzyme population after initial inhibition due to microbial adaptation to these chemicals or due to their degradation. It can also be due to microbial multiplication on increased supply of nutrients available in form of microorganisms killed by herbicides.

On the other hand the soil enzyme activities *viz.*, Dehydrogenase and phaphatase were increased significantly due to application of pre-emergence herbicides as compared to non-herbicide treatments. The dehydrogenase activity significantly higher in all the herbicide treated plots at 7 DAS (4.30 – 5.23 ?g TPF formed g^{-1} soil h^{-1}), 14 DAS (7.24 – 8.83?g TPF formed g^{-1} soil h^{-1}) and 21 DAS ((9.67 – 11.67?g TPF formed g^{-1} soil h^{-1}) as compared to non herbicide treatments viz. farmers practice, weedy check and weed free check (2.25-2.52?g TPF formed g^{-1} soil h^{-1} at 7 DAS, 3.85 – 4.14 ?g TPF formed g^{-1} soil h^{-1} at 14 DAS and 7.04-7.32 ?g TPF formed g^{-1} soil h^{-1} at 21 DAS). Among the herbicide treatments, oxyfluorfen treated plots recorded significantly higher dehydrogenase activity as compared to other herbicides at all the stages of observations.

The soil phosphatase activity at pH 6.5 also followed similar trend as that of dehydrogenase activity. Wherein all the herbicide treatments recorded significantly higher phosphatase activity at pH 6.5 at 7 DAS (23.83-28.39 ?g TPF formed g^{-1} soil h^{-1}), 14 DAS (33.29-38.18 ?g TPF formed g^{-1} soil h^{-1}) and 21 DAS ((37.27-42.51?g TPF formed g^{-1} soil h^{-1}) as compared to non herbicide treatments viz. farmers practice, weedy check and weed free check (17.66 18.63?g TPF formed g^{-1} soil h^{-1} at 7 DAS, 29.19-30.52 ?g TPF formed g^{-1} soil h^{-1} at 14 DAS and 36.52-37.49 ?g TPF formed g^{-1} soil h^{-1} at 21 DAS). Among the herbicide treatments, oxyfluorfen treated plots recorded significantly higher dehydrogenase activity as compared to other herbicides at all the stages of observations.

The differences in both the enzyme activity in various herbicide treated soil could be due to the herbicide induced changes in starch degrading enzyme [1], and the unavailability of nutrients thus inducing stress [2]. Herbicides are decomposed by enzymes produced by the soil microorganisms, which subsequently use the metabolite as a source of biogenous elements [5]. Besides, certain groups of microorganisms start to decompose herbicides a few days after the application [10].

CONCLUSION

Pre-emergence herbicides significantly decreased the microbial load including root nodules in the soil at initial stages of application. But at later stages they recovered due to rapid degradation of herbicides due to production of soil enzymes. Among the herbicides tested, oxyfluorfen was found toxic to soil microbes at initial stage of its application. Among the weed control measures, sequential application of pendimethalin @ 1.0 kg ha^{-1} as pre-emergence fb imazethapyr @ 75 g ha^{-1} as post-emergence at 25 DAS was found economically viable and it can be used as substitute to farmers' practice for effective weed management in greengram grown in northern transitional zone. Results indicated that sequential application pre-emergence followed by post emergence herbicides treatments were found superior over individual applications in reducing the weed population and improving greengram yield.

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