The Pharma Innovation Journal 2022; 11(7): 1863-1866

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 1863-1866

www.thepharmajournal.com
Received: 05-05-2022
Accepted: 09-06-2022

Effect of pre and post emergence herbicides alone and mixtures on soil dehydrogenase, micro flora and yield of soybean [*Glycine max* (L.) Merrill] in vertisols

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Abstract
Herbicide application for controlling weeds in soybean crop is an important part of plant protection in agriculture. In this study, among the various application of herbicides, maximum leaf area index, chlorophyll content, nodules/plant, dry weight/plant were recorded by fomesafen + fluazifop-p-butyl 220 G A.I./ha, propaquizafop + imazethapyr 125 G A.I./ha, and sodium acifluorfen + Clodinafop-propargyl 245 G A.I./ha as compared to other applied herbicides. Pre-emergence application of pendimethalin + imazethapyr 960 G A.I/ha and pendimethalin 1.0kg A.I/ha significantly reduced dehydrogenase enzyme activity compared to control. However, maximum phosphatase enzyme activity (30.17µg PNP/g soil/hr) was observed under two hand weeding followed by fomesafen + fluazifop-p-butyl 220g A.I./ha. All herbicidal weed control measures applied as pre-emergence and post- emergence did not influence total bacterial, fungi, actinomycetes populations. Herbicides application had not significant effect on the soil microbial population and soil enzymes.

Keywords: Pre and post emergence, herbicides, dehydrogenase, microflora, yield, soybean

Introduction
Soybean [*Glycine max* (L) Merrill] has emerged as a potential protein as well as oilseed crop globally. It has attracted the Indian farmers especially Rajasthan, Maharashtra and Madhya Pradesh due to its wider adaptability and high yield potential as compared to any other oilseed and pulse crop in the *kharif* season. In India, soybean occupied an area of 11.84 m ha with the production of 10.45mt during 2020 (Anonymous, 2021) [1]. Rajasthan occupied an area of 11.29 lakh ha and production of 10.94 lakh tones having hovering productivity of 969 kg/ha (Anonymous, 2020-21) [2]. The productivity of soybean in the state of Rajasthan is deplorable low as compared to Madhya Pradesh (1231 kg/ha), Maharashtra (1132 kg/ha), India (1192 kg/ha) and the world (2491 kg/ha. World market and trade USDA, 2022).
Soybean being a rainy season crop, suffers severely due to competitive stress of weeds. Weeds predominantly associated with soybean were *Echinochloa crusgalli*, *Echinochloa colona*, *Commelina benghalensis*, *Panicum dichotomiflorum*, *Polygonum spp*., *Aeschynomene indica* and *Digitaria sanguinalis*, however, *Eleusine aegyptium*and *Cyperus* spp. Weeds make a complete seizure of the land in the early stage of soybean crop growth. Yield reduction in soybean due to weeds may vary from 30-80 per cent (Gupta *et al*., 2006) [7]. Weed management in soybean had really been a challenging factor mainly in *kharif* season due to unpredictability of rains, entailing to non-workable conditions of soil in rainy days and timely non-availability of labour. Works on various herbicides have been done so far but for the dynamic evaluation of recent available new herbicides bioefficacy microbial activity and enzymes are very meager in the soybean growing regions particularly in the Rajasthan state which is a great concern to the safe cropping system and sustainability. Soil enzymes also play a vital role in maintaining the physical and chemical properties and thus, conserve soil ecology as well as soil health (Paul 2007) [13]. Kepler *et al*. (2020) [9] found that glyphosate did not affect the overall microbial community composition in maize or soybean grown soil. Studying the effects of herbicides on the microorganisms and enzyme activity in vertisol soil is helpful to explicate the mechanisms of herbicides affecting both plant growth and soil environment.

Materials and Methods
A field experiment was carried out in soybean [*Glycine max* (L.) Merrill] crop during *kharif*2021 at Agricultural Research Station, Ummedganj, Kota (Agriculture University, Kota)

which is situated at 25°13' N latitude and 75°25' E longitude at an altitude of 258 m above mean sea level. This region comes under Agro-climatic zone V *i.e.* Humid South Eastern Plain of Rajasthan. The experiment was laid out in randomized block design (RBD) with sixteen treatments and three repetitions. Soybean crop was sown with the cultivar of JS 20-34 and seed was treated with carbendazim @ 1g/kg. A uniform recommended fertilizer dose of nitrogen, phosphorus and potash (20:40:40 kg/ha) was drilled in furrow at a depth of 8-12 cm at the time of sowing. Nitrogen, phosphorus and potash were applied through urea, single super phosphate and muriatic of potash, respectively. Sowing was done by tractor drawn seed drill kept in row space 30 cm apart a depth of 2-3 cm using a seed rate is 80 kg/ha. Weed management practices of hand weeding and herbicides application has been adopted as per experimental treatment in each earmarked plots as per schedule. Pre-emergence herbicides *i.e.* pendimethalin 1.0 kg A.I./ha and pendimethalin + imazethapyr 960g A.I./ha were applied just after sowing of soybean. Whereas, all the post emergence herbicides i.e. imazethapyr 100g a.i./ha, fluthiacet-methyl 12.5g a.i./ha, clodinafop propargyl 60g a.i./ha, fomesafen 250g a.i./ha, fluazifop-p-butyl 250g a.i./ha, propaquizafop 50g a.i./ha, propaquizafop + imazethapyr 93.75g a.i./ha, propaquizafop + imazethapyr 125g a.i./ha, sodium acifluorfen + clodinafop propargyl 183.7g a.i./ha, sodium acifluorfen + clodinafop propargyl 245g a.i./ha, fomesafen + fluazifop-p-butyl 165 g a.i./ha and fomesafen + fluazifop-p-butyl 220 g a.i./ha were applied with use of 0.1 per cent non-ionic surfactant at 16 DAS. All the herbicides were sprayed through knapsack sprayer using flat fan nozzle using 500litre water/ha as per treatments. Leaf area index (LAI) was computed by Sestale *et al*., 1971.

$$\text{Leaf area index (LAI)} = \frac{\text{Leaf area per plant (cm}^2\text{)}}{\text{Ground area occupied by each plant (cm}^2\text{)}}$$

The Chlorophyll content of leaves was determined by extracting using dimethyl sulphoxide DMSO extraction method (Hiscox and Israelstam, 1979) [8] by suspending the leaves samples in 5 ml of dimethyl sulphoxide (DMSO) and incubating it at 60°C for about one hour in a pre-heated hot air oven. Absorbance of each extract was measured using spectrophotometer at 645 and 663 nm wavelength. The total chlorophyll content was measured using the following equations (Arnon, 1949) [3]:

$$\text{Total chlorophyll content (mg/g)} = \frac{(20.2 \times A_{645}) + (8.03 \times A_{663})}{1000 \times \text{weight of leaf sample (g)}}$$

The nodules were counted per plant were dugout from wet soil condition and then roots were washed gently and thoroughly with water in sieve. Soil samples collected were analyzed for dehydrogenase enzyme activity (Klein *et al.*, 1971) [9]. The enumeration of microbial population (bacteria, fungi and actinomycetes) was done on agar plates containing appropriate media following serial dilution techniques and pour plate method (Collins and Lyne, 1985) [6] and population was recorded as number of CFU per gram of soil.

Result and Discussion

Leaf area index

Leaf area index was recorded at 50 per cent flowering stage and found significant due to various weed control treatments. Significantly higher LAI (5.08) was recorded under two hand weeding over rest of the herbicidal treatments and weedy check. The next best treatments were fomesafen + fluazifop-p-butyl 220g a.i./ha, propaquizafop + imazethapyr 125g a.i./ha and sodium acifluorfen + clodinafop-propargyl 245g a.i./ha. It was found that herbicide mixtures of higher as well as lower dose recorded significantly higher LAI over alone application of herbicides. Weed control through various herbicides registered higher LAI (2.23-3.97) at 50 per cent flowering compared to weedy crop (1.14). A greater variation in leaf area index of soybean were observed with different weed control treatments. Leaf area index seems to be the function of reduction in weed spread due to these treatments. This could have provided more space for the plants to extend the foliage and branches, thereby providing for more leaves per unit area of land. Similar sort of findings has been reported by Wadafale *et al.* (2011) [17] and Kumar *et al.* (2018) [11].

Nodules/plant

All the weed control treatments increased the number of nodules/plant significantly over weedy check. Highest nodules/plant (57.33) was observed with two hand weeding at 20 & 40 DAS which was significantly superior over rest of the treatments. The per cent increase in nodules/plant to the tune of 84.1 over weedy check. The next best effective treatments were application of fomesafen + fluazifop-p-butyl 220g a.i./ha (52.1), propaquizafop 50g/ha + imazethapyr 125g a.i./ha (51.8) and sodium acifluorfen + clodinafop-propargyl 245g a.i./ha (51.2) in comparison to rest of the herbicidal treatments and weedy check. Sole application of herbicides *viz.* fomesafen 250g a.i./ha, imazethapyr 100g a.i./ha, fluthiacet-methyl 12.5g a.i./ha, clodinafop-propargyl 60g a.i./ha, fluazifop-p-butyl 250g a.i./ha, propaquizafop 50g a.i./ha and pendimethalin 1.0 kg a.i./ha (PE) were found at par with each other but significantly superior over weedy check. Nodules/plant recorded under two hand weeding, fomesafen + fluazifop-p-butyl 220g a.i./ha, sodium acifluorfen + clodinafop-propargyl 245g a.i./ha, propaquizafop + imazethapyr 125g a.i./ha were 17.6, 151.2, 143.1 and146.2 per cent higher than those observed under weedy check, respectively. (Table 1).

Table 1: Effect of weed control measures on leaf area index, chlorophyll content, nodules/plant, dehydrogenase activity and soil microflora (after harvest) in soybean

Treatments	Leaf Area Index	Nodules/ Plant (Nos.)	Dehydrogenase (µgTPF/g soil/d)	Total Bacteria (10^4 x cfu/g)	Total Fungi (10^4 x cfu/g)	Total Actinomycetes (10^4 x cfu/g)	Seed yield (kg/ha)
T1: Weedy check	1.14	31.14	0.331	22.33	13.67	12.33	626
T2: 2 HW 20 & 40 DAS	5.08	57.33	0.329	21.67	13.00	12.00	1970
T3: Pendimethalin 1.0kg PE	2.30	39.00	0.308	18.33	10.67	11.00	970
T4: Imazethapyr 100g/ha PoE	2.73	44.00	0.312	19.67	11.67	10.67	1145

T5: Fluthiacet methyl 12.5g/ha PoE	2.50	40.00	0.311	20.33	11.67	11.67	1113
T6: Clodinafop-propargyl 60g/ha PoE	2.25	39.80	0.312	19.67	12.00	12.00	1072
T7: Fomesafen 250g/ha PoE	3.37	40.40	0.313	20.67	11.00	10.67	1103
T8: Fluazifop-p-butyl 250g/ha PoE	3.00	41.00	0.311	20.67	12.00	11.67	1014
T9: Propaquizafop 50g/ha PoE	2.23	39.20	0.315	20.00	12.00	11.33	1053
T10: Pendi. + Imaz. 960g/ha PE	2.73	44.40	0.305	17.67	10.00	10.33	1127
T11: Propaqf. + Imaz. 93.7g/ha PoE	2.87	42.40	0.313	21.00	11.33	11.67	1407
T12: Propaqf. + Imaz.125g/ha PoE	3.95	51.80	0.321	20.33	12.33	11.67	1730
T13: Sod Acif. + Clodina.F.183.7g/ha PoE	2.60	44.40	0.315	20.00	11.67	10.33	1340
T14: Sod. Acif. + Clodina.F.245g/ha PoE	3.82	51.20	0.323	20.33	12.00	11.00	1628
T15: Fomsaf. + Fluazi.FB165g/ha PoE	2.85	43.80	0.311	21.00	12.33	11.33	1413
T16: Fomsf. + Fluazi.FB 220g/ha PoE	3.97	52.10	0.323	20.33	11.67	11.00	1760
SEm ±	0.20	1.78	0.004	0.81	0.61	0.43	63
CD (P=0.05)	0.59	5.15	0.013	NS	NS	NS	181

Dehydrogenase activity

Two hand weeding at 20 & 40 DAS was recorded maximum dehydrogenase enzyme activity (0.329 µgTPF/g soil/d) closely followed by application of herbicides mixture and alone herbicide while pre-emergence application of pendimethalin + imazethapyr 960g a.i./ha and pendimethalin 1.0kg a.i./ha significantly reduced dehydrogenase enzyme activity compared to control. However, the value of dehydrogenase enzyme activity was also higher in weedy check. Generally, soil enzymatic activities were greater in the untreated control which can be ascribed to the greater contribution of weeds and crop stand stimulating production of soil enzymes through microbial and plant origin. Application of post emergence herbicides in soybean did not affect soil dehydrogenase but stimulated urease activity and enhanced yield. This corroborates with the findings of Ramesh *et al*. (2000) [14] and Yogesh (2000) [18].

Soil microflora

All herbicidal weed control measures applied as reemergence and post emergence did not influence total bacterial populations. Though, weedy check recorded maximum bacterial population (22.33 x 10^4cfu/g) followed by two hand weeding at 20 & 40 DAS. All herbicidal weed control measures could not bring significant variation in total fungi population and found non-significant. However, maximum total fungi population was recorded under weedy check (13.67 x 10^4cfu/g) and two hand weeding at 20 & 40 DAS (13.00 x 10^4cfu/g) and least under pendimethalin + imazethapyr 960 g a.i./ha (10.00 x 10^4cfu/g). All herbicidal weed control treatments did not bring significant variation in total actinomycetes population and found non-significant. While, maximum total actinomycetes population was recorded under weedy check (12.33xCFU/g) closely followed by two hand weeding at 20 & 40 DAS (12.00 x 10^4cfu/g). Since post-emergence herbicides were applied on the foliage of weeds, the amount of herbicide molecules that comes in contact with soil particles were lesser thus their application might have not influence microbial proliferation and activity (Auspurg *et al*., 1989) [4] and Bhimwal *et al.* (2018) [5].

Soybean Yield

Two hand weeding and ready mixture of fomesafen + fluazifop-p-butyl 220g a.i./ha gave significantly higher values of growth characters as well as yield attributes over alone herbicides and weedy check. Two hand weeding and ready mix of fomesafen + fluazifop-p-butyl 220g a.i./ha, sodium acifluorfen + clodinafop-propargyl 245g a.i./ha and propaquizafop + imazethapyr 125g a.i./ha, were found significantly superior over rest of the treatments in enhancing seed, straw and biological yield. Seed yield increased significantly by all herbicidal treatments and two hand weeding as compared to weedy check. Two hand weeding recorded significantly higher seed (1970kg/ha), straw (2636kg/ha) and biological yield (4606kg/ha) followed by ready mix of fomesafen + fluazifop-p-butyl 220 g a.i./ha (1760, 2364 & 4124 kg/ha), propaquizafop + imazethapyr 125g a.i./ha (1730, 2323 & 4053kg/ha) and sodium acifluorfen + clodinafop-propargyl 245g a.i./ha (1628, 2204 & 3832 kg/ha) over weedy check. Which were increased seed yield to the tune of 214.7, 181.2, 176.4 & 160.0 per cent over weedy check, respectively.

Conclusion

Weed control treatments were found statistically superior in improving leaf area index, nodule number over weedy check. Higher LAI, nodule numbers and their dry weight were registered by controlling weeds with two hand weeding followed by ready mix of fomesafen 11.1% + fluazifop-p-butyl 11.1% SL @ 220g a.i./ha, propaquizafop 2.5% + imazethapyr 3.75% @125g a.i./ha and sodium acifluorfen 16.5% EC + clodinafop-propargyl 8% EC @ 245 g a.i./ha (RM) in comparison to other treatments. Two hand weeding at 20 & 40 DAS was recorded maximum dehydrogenase enzyme activity (0.329 µgTPF/g soil/d) closely followed by herbicides mixture and alone while pre-emergence application of pendimethalin + imazethapyr 960g a.i./ha and pendimethalin 1.0kg a.i./ha significantly reduced dehydrogenase enzyme activity compared to control. The treatment proceeding soybean crop did not influence the basic microbial population.

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