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Influence of herbicides on soybean yield, soil microflora and urease enzyme activity

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ABSTRACT

An experiment was done during *Kharif* 2008 and 2009 at BCKV, Kalyani to study the weed control efficiency of herbicide as well as its effect on soil microorganisms including urease enzyme in Soybean crop field. Dominent weeds were: *Echinochloa colona, Eleusine indica, Dactyloctenium aegyptium, Digitaria sanguinalis, Cyperus rotundus, Euphorbia hirta, Digera arvensis, Physalis minima, Phyllanthus niruri, Alternanthera philoxeroides* and *Amaranthus viridis*. The treatment UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha was found best for efficient weed control as well as safe use for soil microflora including urease enzyme activity among all the treatments even in comparison to hand weeding treatment.

Key words: Seed yield, Soil microflora, Soybean, Urease enzyme activity

Soybean contains 43.2% protein, 20% fat, 31.3% carbohydrate and 432 Calories per 100 g (Kundu *et al.* 2011). Soybean oil can be used as edible oil as well as vegetable oil. Soybean forage and protein also provide excellent nutritive feed for livestock and poultry. Being a leguminous crop, it restores the fertility of soil also. Soybean is grown mostly in *Kharif* season as rainfed crop. In this season, The problem of weed is much more than other season crop causing reduction in yield. To overcome this problem, an effective method which is less costly and environmentally safe in comparison to costly hand weeding method was attempted.

MATERIALS AND METHODS

An experiment was done during *Kharif* 2008 and 2009 at Farm (latitude: 22°57'E, longitude: 88°20'N and altitude: 9.75 m) of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India to study the weed control efficiency of herbicide as well as its effect on soil microorganisms including urease enzyme in Soybean crop field. The experimental soil was well drained, alluvial in nature and sandy loam in texture (sand 63%, silt 21%, clay 16%), having pH 6.86, organic carbon 0.58%, available nitrogen 236.3 kg/ha, available phosphorus 20.0 kg/ha and available potassium 178.6 kg/ha.

The experiment consisted twelve treatments and replicated thrice in RBD was conducted during *Kharif* sea son of 2008 and 2009 in Soybean crop with variety PK-327. The treatments were: T_1- UPH-203 60 g/ha, T_2- UPH-203 80 g/ha, T_3- UPH-203 100 g/ha, T_4-UPH-203 60 g/ha + Na-acifluorfen 10% SL 123.7 g/ha, T_5-UPH-203 80 g/ha + Na-acifluorfen 10% SL 165 g/ha, T_6- UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha, T_7- Na-acifluorfen 10% SL 123.7 g/ha, T_8-Na-acifluorfen 10% SL 165 g/ha, T_9- Na-acifluorfen 10%SL 206.2 g/ha, T_{10}- imazethapyr 10% SL 1000 g/ha, T_{11}- hand weeding twice (15 and 30 DAS) and T_{12}- untreated control. First two herbicides were sprayed at 23 days after sowing whereas imazethapyr was spreyed at 10 DAS.

To count the weed population/m^2 in different plots, quadrate of 0.5 x 0.5 m was thrown at four random places in each plot at 30 DAS, 60 DAS and at harvest.

The enumeration of the microbial population was done on agar plates containing appropriate media following serial dilution technique and pour plate method (Pramer and Schmidt 1965). Plates were incubated at 30^{0}C. The counts were taken at 5th day of incubation. The results were recorded as number of cells per gram of soil. For counting total number of viable bacteria, Thornton's agar medium was used. Jensen's agar medium was used for counting aerobic non-symbiotic nitrogen fixing bacteria. Total number of phosphate solubilizing microorganisms was estimated in Pikovskaia's agar medium. Martin's rose Bengal streptomycin agar medium of the following composition was used for counting total fungi. Soil samples were collected from the rhizosphere of soybean before spraying, 15 days after application of herbicide and at harvest.

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The method was based on determination of ammonia released after the incubation of soil samples with urea solution for 2 hours at 37^0C (Tabatabai and Bremner 1972).

RESULTS AND DISCUSSION

Weed flora

Grasses: *Echinochloa colona Digitaria sanguinalis, Dactyloctenium aegyptium,Eleusine indica Cynodon dactylon, Leersia hexandra, Echinochloa crus-galli etc.* Sedges: *Cyperus rotundus, Cyperus difformis, Fimbristylis littoralis*; and broad-leaved weeds: *Digera arvensis, Physalis minima, Phylanthus niruri, Alternanthera philoxeroides, Amaranthus viridis, Euphorbia hirta, Cleome viscosa, Stellaria media* and *Spilanthus paniculata.* Similar findings were reported by Norsworthy (2008).

Hand weeding twice at 15 and 30 DAS recorded significantly lowest weed density (27.3) than all other treatments (Table 1). The highest weed density (120) was recorded in weedy check. UPH-203 100 g/ha + Na-acifluorfen10% SL 206.2 g/ha recorded significantly lowest weed population at all the stages than the sole application of UPH-203 and Na-acifluorfen 10% SL. Again imazethapyr 10% SL 1000 g/ha treatment was statistically at par with UPH-203 60 g/ha+ Na-acifluorfen 10% SL 123.7 g/ha, UPH-203 80 g/ha + Na-acifluorfen 10% SL 165 g/ha and UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha in this respect.

Soybean yield

Highest grain yield (2.35 t/ha) was recorded (Table 1) in the treatment of hand weeding twice, which gave significantly higher seed yield of soybean over all other treatments. Again the treatments UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha (2.16 t/ha) and imazethapyr 10% SL 1000 g/ha (2.04 t/ha) were statistically at par among themselves and showed their best performance in this respect among the chemical treatments. Herbicide UPH-203 as single chemical gave good results and recorded significantly higher seed yield over unweeded control but lower than hand weeding treatment. On the other hand, Na-acifluorfen 10% SL at different doses though at par among themselves with respect to seed yield but gave significantly lower yields when they were compared with combined chemical treatments of UPH-203 and Na-acifluorfen10% SL. Among all the treatments, unweeded control resulted lowest seed yield (1.12 t/ha). Similar results were recorded by Bhattacharya *et al.* 1(998) and Pandey *et al.* (2007).

In respect of net present value (NPV), the highest value was obtained with the treatment T_6 (UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha) (1.15) which was closely followed by the treatment T_{11} (twice hand weeding at 15 and 30 DAS) (T_{11}:1.10) and T_{10} (imazethapyr 10% SL 1000.0 g/ha) (0.99). Hand weeding treatment (T_{11}) showed lower NPV value in comparison to T_6 due to higher expenditure on labour wages. On the contrary, lowest value of cost: benefit ratio was obtained in unweeded control (0.18).

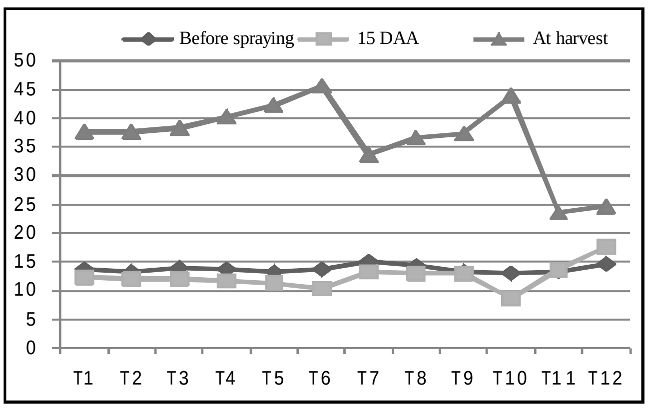
Non-symbiotic N_2-fixing bacteria

Before spraying, population of aerobic non-symbiotic nitrogen fixing bacteria did not differ signifi-

Table 1. Effect of treatments on total weed density and soybean yield (pooled data)

Treatment	Weed density (no./m^2)			Seed yield (t/ha)	NPV
	30 DAS	60 DAS	At harvest		
T_1- UPH-203 60 g/ha	75.3	114.3	135.0	1.68	0.74
T_2- UPH-203 80 g/ha	74.6	105.0	139.7	1.69	0.74
T_3- UPH-203 100 g/ha	71.0	101.7	136.3	1.73	0.78
T_4- UPH-203 60 g/ha + Na-acifluorfen 123.7 g/ha	63.7	94.00	129.3	1.78	0.81
T_5- UPH-203 80 g/ha + Na-acifluorfen 165 g/ha	56.0	87.7	120.0	1.84	0.85
T_6- UPH-203 100 g/ha + Na-acifluorfen 206.2 g/ha	40.0	74.7	90.0	2.16	1.15
T_7- Na-acifluorfen 123.7 g/ha	91.3	129.3	159.3	1.52	0.56
T_8- Na-acifluorfen 165.0 g/ha	82.0	117.3	151.7	1.61	0.64
T_9- Na-acifluorfen 206.2 g/ha	77.7	110.3	139.3	1.65	0.67
T_{10}- Imazethapyr 1000.0 g/ha	49.3	91.0	101.7	2.04	0.99
T_{11}- Twice hand weeding at 15 and 30 DAS	27.3	56.7	80.0	2.35	1.10
T_{12}- Weedy check (untreated)	120.0	162.3	186.7	1.12	0.18
LSD (P=0.05)	16.7	24.2	30.4	0.14	

cantly among the treatments (Fig. 1). At 15 DAA, unweeded control plot exerted a significant increase in the population of aerobic non-symbiotic nitrogen fixing bacteria in soil over all the treatments. On the other hand, all the treatments except hand weeding twice showed a significant reduction in number of the micro flora at 15 DAA as compared to unweeded control. Unweeded control treatment recorded highest population which was followed by Na-acifluorfen 10% SL 123.7 g/ha. At harvest, population of aerobic non-symbiotic nitrogen fixing bacteria was remarkably increased in all the chemical treatments due to release of carbon from degraded chemicals. However, UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha recorded significantly higher value over the other treatments and statistically at par with imazethapyr 10% SL 1000 g/ha. These herbicides might nourish bacteria with nutrient and energy sources for higher proliferation.



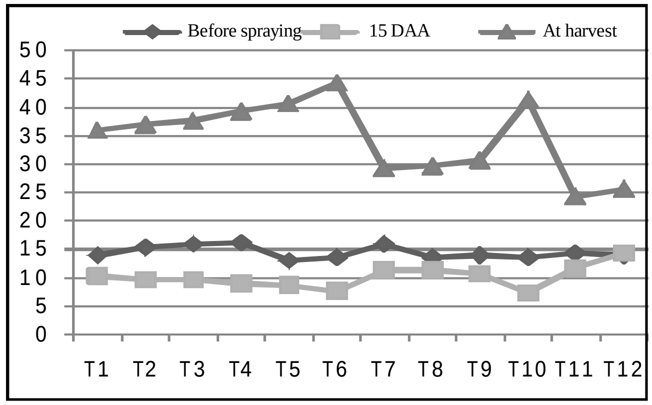
Treatment details are given in Table 1

Fig. 1. Influence of treatments on the population of aerobic non-symbiotic N_2- fixing bacteria (CFU x10^6/g of soil)

The population of aerobic non-symbiotic nitrogen fixing bacteria decreased on the 15 DAA as compared to before spraying and then increased at harvest. The decrease in the bacterial population at initial stage after application of herbicides was due to competitive influence and the toxic effect of chemicals in soil. On the contarary, the population was seen to increased by the commensalic or protocooperative influence of various micro-organisms on non-symbiotic nitrogen fixing bacteria in the rhizosphere soil of soybean crop after the degradation of the applied herbicides in soil within a considerable time.

Phosphate-solubilising bacteria

The population of phosphate solubilising bacteria did not differ significantly with the treatments before spraying of herbicides (Fig. 2). Similar findings were recorded in case of aerobic non-symbiotic nitrogen fixing bacteria at 15 DAA. At harvest, treatments recorded a significant increase in the population of phosphate solubilising bacteria except the treatment hand weeding twice. UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha was followed by imazethapyr 10% SL 1000 g/ha in this respect.



Treatment details are given in Table 1

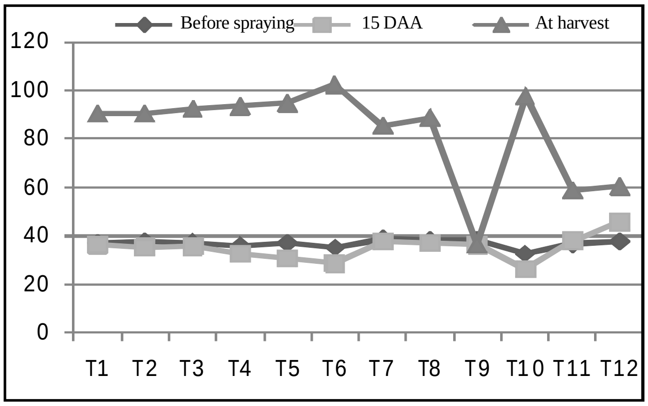
Fig. 2. Influence of treatments on the population of phosphate-solubilising bacteria (CFU x 10^6/g of soil)

The population of phosphate solubilising bacteria decreased at 15 DAA as compared to the observation before spraying and then again it increased at harvest. The increase may be due to the commensalic or protocooperative influence of various micro-organisms on phosphate solubilising bacteria in the rhizosphere soil of soybean crop after degradation of applied herbicides in soil.

Total bacteria

Similar trends in case of total bacteria population have been found (Fig. 3). Before spraying, population of total bacteria did not differ significantly among the treatments. At 15 DAA and at harvest similar trends were recorded regarding total bacterial population. UPH-203 100 g/ha Na-acifluorfen 10% SL 206.2 g/ha recorded highest population count followed by imazethapyr 10% SL1000 g/ha.

Here also, the population of total bacteria decreased on the 15th day of application as compared to that of before spraying and then increased at harvest. The decrease in the bacterial population was due to competitive influence and the toxic effect of chemicals in soil. On the other hand, the increase might be due to the commensalic or protocooperative influence of various micro-organisms on total bacteria in the rhizosphere soil of soybean crop (Ghosh *et al.* 2012).



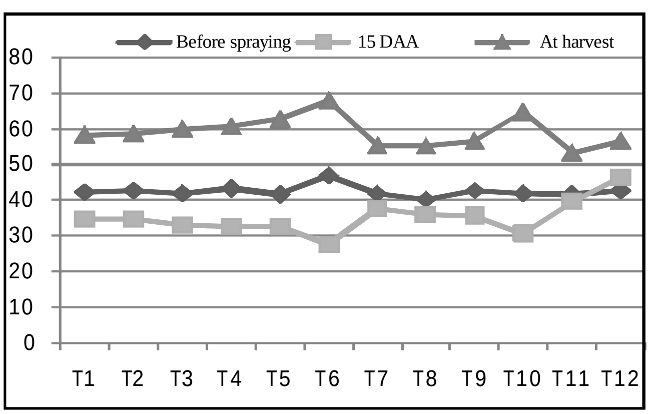
Treatment details are given in Table 1

Fig. 3. Influence of treatments on the population of total bacteria (CFU x 10^6/g of soil)

Actinomycetes

Before spraying, population of actinomycetes did not differ significantly among the treatments (Fig. 4). The actinomycetes population decreased at 15 DAA as compared to before spraying. This might be due to the competitive influence of various micro-organisms on the population of actinomycetes in the rhizosphere soil of soybean as well as toxic effect of the chemicals applied. Highest actinomycetes population was recorded in unweeded control plot which was statistically different than other treatments.

Before spraying, at 15 DAA and at harvest, similar trends were recorded regarding actinomycetes like other microorganisms. At harvest, UPH-203 80 g/ha + Na-acifluorfen 10% SL 165 g/ha also recorded promising result in population which was significant over all other treatments including control plot. In general, the population



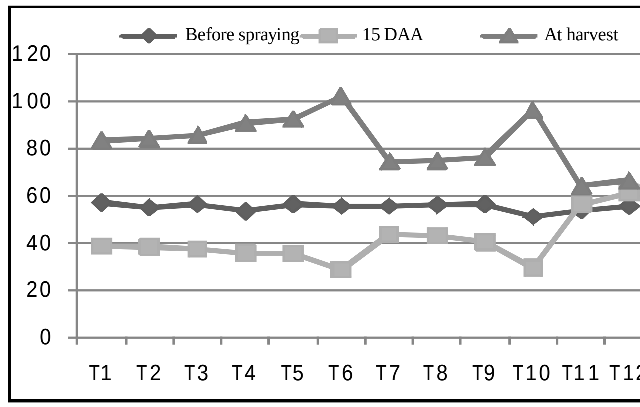
Treatment details are given in Table 1

Fig. 4. Influence of treatments on the population of tactinomycetes (CFU x 10^5/g of soil)

count for actinomycetes increased at harvest in all the treatments in comparison to 15 DAA because at that time chemicals were degraded and the availability of carbon was increased in the soil which ultimately helped in increasing the population in soil. Sapundjieva *et al.* (2008) reported similar findings.

Fungi

Before spraying, population of fungi did not differ significantly among the treatments. Hand weeding twice exerted a significant enhancement in the population of fungi in soil at different stages. At 15 DAA, population of fungi decreased in the chemical treated plots. However, at harvest, all the treatments in comparison to 15 DAA showed a significant increase in the population of fungi in soil (Fig. 5).



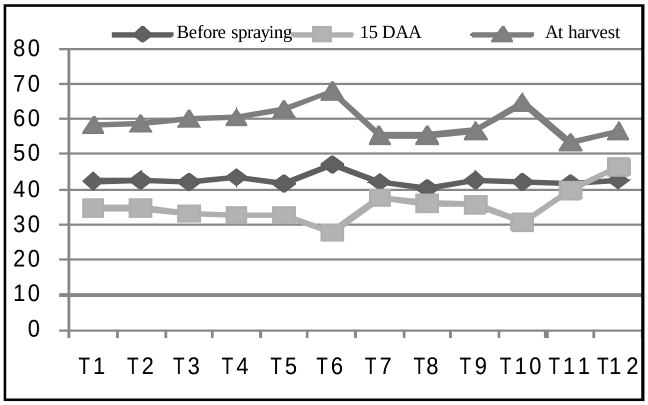
Treatment details are given in Table 1

Fig. 5. Influence of treatments on the population of fungi (CFU x 10^4/g of soil)

The decrease in fungal population at 15 DAA might be due to the toxic effect or ammensalic or competitive influence of various micro-organisms on the population of fungi in the rhizosphere soil of soybean. At harvest the population was again significantly increased in all treatments because chemicals were degraded at that time and no toxic effect in the soil remained afterward. Similar findings were recorded by Sokolova and Gulidova (2010).

Urease enzyme activity in rhizosphere

Among all herbicidal treatments, UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha showed best performance in urease enzyme activity in all the three observations. At 15 DAA, UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha treatment was statistically at par with UPH-203 80 g/ha + Na-acifluorfen 10% SL 165 g/ha in this respect (Fig. 6). At 30 DAA and 45 DAA, similar trends were recorded. Imazethapyr 10% SL 1000 g/ha recorded the lowest urease activity at 15 DAA and 30 DAA which was significantly lower than other treatments. At 45 DAA, similar trends were recorded. Application of UPH-203 as sole or in combination with Na-acifluorfen 10% SL recorded significantly better urease activity than that of imazethapyr 10% SL treatment and untreated control.



Treatment details are given in Table 1

Fig. 6. Influence of treatments on the population of urease enzyme activity (µg NH_4-N/g of soil 2/h at 37^0C)

It is very clear from the data presented regarding soil microflora populations and urease enzyme activity that all kinds of soil microflora (total bacteria, non-symbiotic nitrogen fixing bacteria, phosphate solubilising bacteria, actinomycetes and fungi) has a positive relationship with the activity of urease enzyme. Microflora and urease activity were highly positively correlated. But it was not enough to effect on yield negatively. Byrnes and Freney (1995) also reported that high biological activity at the soil surface promote soil enzyme urease which was similar to the present findings.

It may be concluded that considering the seed yield of soybean as well as soil health (soil beneficial microflora population and urease enzyme activity), combined chemical methods can replace hand weeding twice. Amongst the different methods used in this experiment, UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha gave higher economic yield over other methods (except hand weeding twice). It can further be concluded that in spite of 8.60% less yield in this treatment UPH-203 100 g/ha + Na-acifluorfen 10% SL 206.2 g/ha was also superior over the hand weeding twice as it gave higher benefit: cost ratio (1.15) whereas hand weeding twice is laborious, time consuming, costly (benefit: cost ratio 1.10) and problematic as labourers were not available at the critical period of crop weed competition.

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