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Impact of Herbicides on Soil Microorganisms, Nodulation and Yield of Chickpea

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ABSTRACT

Background: Chickpea yield losses due to weed competition have been estimated to range between 40 and 87% depending on weed species and density. As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil. These herbicides could be leached down, then accumulate to toxic levels in the soil and become harmful to microorganisms, plant, wild life and human being. Hence, there is a need to determine the effect of different herbicides on the growth and multiplication of agriculturally important microorganisms, which in turn could affect the crop growth and yield. Thus there is a need to test pre and post-emergent herbicides for their effect on beneficial soil microflora, as they could be both beneficial and harmful depending on the herbicide used. In this context, a field experiment was conducted to study the effect of herbicides on soil microflora, root nodulation, growth and yield of chickpea under rainfed condition during the rabi season of 2017-18 and 2018-19 at Zonal Research Station, Chianki, Palamau, (Birsa Agricultural University, Ranchi), Jharkhand (India).

Methods: In this experiment conducted during rabi season 2017-18 and 2018-19 at Zonal Research Station, Chianki, Birsa Agricultural University, Ranchi, Jharkhand, India. The eight treatments were taken under RBD in three replications. Observations and analysis were carried out by following the standard procedures.

Result: The present investigation clearly brought out that two hand weeding followed by pendimethalin was the best sought out option on controlling weed population, for higher growth and yield of chickpea. Based on the results obtained, it could be inferred that two hand weeding are costly as compared to pendimethalin and it had least adverse effect on microbial population and nodulation. Therefore, pendimethalin can be used to get the higher plant growth and yield of chickpea.

Key words: Chickpea, Herbicides, Nodulation, Rhizobium, Soil microorganism.

INTRODUCTION

Chickpea (*Cicer arietinum*) is one of the important grain legumes of the world, which is grown in 44 countries across five continents. It is cultivated in about 8.7 million hectares (m ha) worldwide. Of this area, 24% is found in the West Asia and North Africa (WANA) region. It accounts for 14% of the total world area sown to pulses (Anonymous, 2016). India is the largest producer of chickpea accounting to 75% of the world production (Anonymous, 2016). The major chickpea growing states in India are Maharashtra andhra Pradesh, Bihar, Karnataka, Madhya Pradesh (Singh and Singh, 2011). Chickpea is a good source of carbohydrates and protein, which together constitute about 80% of the total seed dry weight (Singh and Singh, 2011).

Two main types of chickpea are recognized i.e. *desi* type with small and brown seed accounts for nearly 90 % and *kabuli* type with bold and cream coloured seed is grown in around 10 % area (Khan *et al*., 2006). About 80.1% of chickpea cultivation is followed under rain fed conditions (Khan *et al*., 2006). In India it is cultivated in 29.46 m ha with 22.95 million tones (m t) production and average productivity of 779 kg/ha (Anonymous, 2012). India's demand of pulses by 2030 is expected to be as high as 32 m t (Singh and Joshi, 2011). Chickpea yield losses due to weed competition have been estimated to range between 40 and 87% depending on weed species and density (Punia *et al*. 2011). High yield losses occur because chickpea has a slow initial growth rate and limited leaf area development at early stages of crop growth of 30-45 DAS. Weed flora is dependent on climate, crop rotation and time of sowing. The weed flora in the experimental field consisted of grasses like *Echinochloa glabrescens*, *Bracharia sp.*; sedges like *Cyperus rotundus* and broad-leaved weeds like *Parthenium hysterophorus*, *Physalis minima*, *Digeria arvensis*, *Euphorbia hirta, Convolvulus arvensis, Phyllanthus niruri, Portulaca oleracea, Abutilon indicum and Cyanotis axillaris* etc.

In general, chickpea is more sensitive to herbicides than cereals (Kumar *et al*., 1989). As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil. But the fate of these compounds in the soil is becoming increasingly important since they could be leached in which case groundwater is contaminated, or immobile

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and persists on the top soil (Ayansina *et al*., 2003). These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plant, wild life and human being (Amakiri, 1982). There is an increasing concern that herbicides not only affect the target organisms (weeds) but also the microbial communities present in soils and these non-target effects may reduce the performance of important soil functions. These critical soil functions include organic matter degradation, the nitrogen cycle and methane oxidation (Hutsch, 2001). Herbicides may affect biological nitrogen fixation either by affecting plant growth or by directly affecting nitrogen-fixing *Rhizobia*. If the herbicides used have adverse effect on soil microflora then it will affect the availability of nutrients to the plants, which in turn affects the crop yield. Hence, there is a need to determine the effect of different herbicides on the growth and multiplication of agriculturally important microorganisms, which in turn could affect the crop growth and yield. Thus there is a need to test pre and post-emergent herbicides for their effect on beneficial soil microflora, as they could be both beneficial and harmful depending on the herbicide used. In this context, a field experiment was conducted to study the effect of herbicides on soil microflora, root nodulation, growth and yield of chickpea under rainfed condition during the *rabi* season of 2017-18 and 2018-19 at Zonal Research Station, Chianki, Palamau, (Birsa Agricultural University, Ranchi), Jharkhand (India).

MATERIALS AND METHODS

A Field experiment was conducted during *rabi* season 2017-18 and 2018-19 at Zonal Research Station, Chianki, Birsa Agricultural University, Ranchi, Jharkhand, India. The soil was clay loam in texture, near to neutral in reaction (pH 6.5), low in organic carbon (0.14%), whereas medium in available nitrogen (255 kg/ha), phosphorus (18.7 kg P_2O_5/ ha) and available potassium (131 kg K_2O/ha). The experiment was laid out in randomized block design (RBD) comprising eight treatment combinations *viz*., W_1- Weedy Check, W_2- Two hand weeding (at 25 DAS and 45 DAS), W_3- Pendimethalin @ 1.0 litre a.i./ha (PE), W_{4-} Quizalofop Ethyl @ 0.60 litre a.i./ha at 25 DAS,W_5- Imazethapyr @ 0.60 litrea.i./ha at 25 DAS, W_6- Pendimethalin @ 1.0 litre a.i./ha (PE) followed by Imazethapyr @ 0.60 litrea.i./ha at 25 DAS,W_7- Pendimethalin @ 1.0 litre a.i./ha (PE) followed by Quizalofop Ethyl @ 0.60 litre a.i./ha at 25 DAS, W_8 - One hand weeding at 25 DAS. Pendimethalin was applied as pre-mergence (After 1 DAS), while Imazethapyr was applied as post emergence (25 DAS).

The soil samples were collected from the field (before sowing and at harvest by the standard method described by Jackson (1967). The collected samples were brought in the polythene bags and kept in refrigerator to maintain their biological properties for further study. The observations on general microflora (Bacteria, fungi and actinomycetes) and beneficial microflora (*Rhizobium*) were recorded before sowing and at harvest. Each soil sample was sieved through the 1000 micromesh to remove the bigger particles and debris and was used for enumeration of bacteria using soil extract agar medium by standard plate count method. The plates were incubated for 48 h at 28 ºC. Colonies that appeared on the media were enumerated and expressed in terms of colony forming units (CFU) per gram of soil (Bunt and Rovira, 1955). For enumeration of fungi, Martin's Rose Bengal agar medium (MRBA) by standard plate count method was used. The plates were incubated for 4 days at 28°C. Colonies that appeared on MRBA media were enumerated and expressed in terms of CFU per gram of soil (Martin, 1950). For enumeration of actinomycetes using Kuster's agar medium by standard plate count method was followed. The plates were incubated for 6 days at 28°C. Colonies that appeared on Kuster's agar media were enumerated and expressed in terms of CFU per gram of soil. Enumeration of *Rhizobium* was carried out by plate technique using Yeast extract mannitol agar (YEMA) medium with congo red. The plates were incubated for 7 days at 28°C. Colonies that appear on the YEMA medium were enumerated and expressed in terms of CFU per gram of soil. Initial bacteria, fungi and actinomycetes population varied from 6.85-8.37 x 10^6CFU /g soil, 3.56-3.93 10^3CFU / g soil and 2.9-4.02 x 10^4CFU /g soil. To assess the effect of herbicides on nitrogen fixing bacteria, the number of root nodules per plant at 30, 60 and 90 days of crop growth were recorded by carefully uprooting five plants from each plot, followed by dipping in water to remove soil clods without losing the nodules. The number of root nodules on each of the five randomly selected plants was counted and the average number was expressed as number of the nodules per plant. The Data recorded on various growth and yield parameters were subjected to Fisher's method of analysis of variance and interpretation of data as given by Gomez and Gomez (1984) The level of significance used in 'F' test and't' test was P = 0.05.

The materials used and the methods followed are presented as below:

Herbicides	Mode/ method of application	Dosage
Pendimethalin	Pre emergence	2.5 ml/l
Quizalofop Ethyl	Post emergence	1.5 ml/1
Imazathyper	Post emergence	1.5 ml/1
Weedy check	Control	Control
Hand weeding twice	Herbicides were not imposed	

RESULTS AND DISCUSSION

Effect on weed density

Critical perusal on pooled data taken at 25 DAS indicated that density of weeds was significantly lowest in pendimethalin. At 45 DAS, however, lowest density (Table 1) of all weed species was recorded after two hands weeding (at 25 and 45 DAS). The weeds that emerged at the later stage were not serious as crop canopy smothered them and limited the harmful effect on crop under the pre-emergence

herbicide treatment and treatments having pre-emergence followed by post emergence herbicide. Similar results were reported by Singh and Joshi (2011). They found that weed density was lowest in the treatment having pendimethalin.

Effect on number of pods per plant and yield

In the present study, it was noticed that among the treatments, two hand weeding recorded highest number of pods per plant (49.1) followed by pendimethalin (45.6), which was statistically at par. Weed check recorded lesser number of pods (20.4) due to the presence of more number of weeds associated with the crop, which exhibited severe competition throughout the crop growth for nutrients, light and moisture.

Two hand weeding gave significantly highest yield (14.3 q/ha) followed by pendimethalin (13.9 q/ha) that remained statistically at par. Yield was adversely affected in weedy check, where weeds population was noticed significantly higher; hence the grain yield was recorded lowest when compared to all other treatments. These results are in close conformation with the findings of Channappagoudar and Biradar (2007). This shows that the reduction in yield was apparently due to reduction in growth and yield components caused by weed infestation.

Effect on nodules

Observations recorded on the nodule number of chickpea, generally found to vary at different stages (30, 60 and 90 DAS) of the crop growth. Among the treatments, more number of nodules was noticed in the plots where herbicides were not imposed in plots (hand weeding) compared to pre and post emergence herbicides treated plots. At 30 DAS, nodule number was varied from 8.2 to 21.1 per plant and noticed highest in two hand weeding (21.1 per plant), whereas, lowest nodules per plant were noticed in weedy check (8.2). Among herbicides, more number of nodules was observed in pendimethalin treated plot (19.3 per plant). At 60 DAS, nodule number ranged from 12.3 to 25.4 per plant and the highest was recorded in two hand weeding (25.4 per plant) plots. Among herbicides, more number of nodules were observed in pendimethalin treated plot (23.4 per plant). Number of nodules per plant was recorded lowest at 90 DAS when compared to 30 DAS in all treatments. Therefore, it is possible that an herbicide, which induces reduction in nodules formed per plant may be due to the restricting root growth and hence the number of root sites available for infection (Khan *et al*., 2006). Higher nodules in two hand weeding may be due to weed suppression for longer period, better soil aeration and soil structural manipulation. Pendimelthalin was the next best performing treatment after pure manual weed control methods. Ahemad and Khan (2010) also reported that herbicides decreased the symbiotic activity in lentil. *Rhizobium leguminosarum,* bacteria responsible for nodulation in lentil and its symbiotic association with the leguminous plant was affected by the herbicides, decreasing the nitrogenase activity and biological nitrogen fixation by the crop. Punia *et al.* (2011) also reported 20-30% injury to legumes (clusterbean) and its nodule formation with Chlorimuron ethyl application.

Herbicides whose mechanism of action is thought the inhibition of amino acids biosynthesis have different effects on *Rhizobium* (Drew *et al*., 2007). These results suggest that herbicides may be affected the some functional aspects of the rhizobial cells that subsequently reduced its ability to nodulate the chickpea roots.

Microbial population

Before sowing, the general population of microflora in soil samples indicated that there was no significant variation. The bacterial population was ranged between 6.85 to 8.37 $x10^6$ CFU / g soil Table 3. There was least effect on bacterial population when pendimethalin was sprayed. The bacterial population was more reduced with post emergence spray of Imazethapyr (2.59 $x10^6$ CFU / g) soil. Fungal population was ranged between 3.56 to 3.90 x 10^3 CFU / g soil before sowing. Pendimethalin had less effect on fungal population. The actinomycetes population was ranged from 2.90 x 4.02 10^4 CFU / g soil. Lower effect of pendimethalin was observed on actinomyces activity in soil. Similar findings were observed by Ahmad and Khan (2010).

Table 1: Effect of herbicides on yield, growth parameters and yield of chickpea.

Treatment	Plant height	Weed density (/m^2)		No. of pods	Seed index	Grain yield
	(cm)	25 DAS	45 DAS	/ plant	(gm)	(q/ha)
Quizalofop Ethyl (POE)	37.0	103	62	31.8	23.4	11.2
Imazethapyr (POE)	36.9	105	29	31.5	23.1	10.5
Weedy check (WC)	32.6	116	204	20.4	22.8	6.07
Pendimethalin	38.6	40	38	42.6	25.5	13.9
HW at 25 and 45 DAS	38.4	98	10	49.1	26.2	14.3
Pendimethalin fb Quizalofop Ethyl	37.6	45	28	36.5	23.5	12.2
Pendimethalin fb Imazethapyr	38.1	42	23	37.2	24.4	12.9
One HW at 25DAS	36.2	110	31	29.1	23.5	8.8
S. Em $_{(\pm)}$	1.52	0.98	0.79	1.41	0.95	0.59
C.D (P= 0.05)	NS	2.86	2.42	4.07	NS	1.74

HW-Hand weeding; fb- followed by; DAS-Date after sowing.

Table 2: Effect of herbicides on nodules per plant and rhizobium population at different growth stages of chickpea.

Treatment	Nodules/plant at DAS			Rhizobium population at DAS		
	30	60	90	30	60	90
Quizalofop Ethyl (POE)	17.0	20.4	14.4	2.75	3.75	2.12
Imazethapyr (POE)	18.0	19.6	14.2	2.69	3.11	1.51
Weedy check (WC)	8.2	12.3	6.1	2.73	3.14	1.54
Pendimethalin	19.3	23.4	18.3	3.51	5.20	2.91
HW at 25 and 45 DAS	21.1	25.4	19.1	4.13	6.54	3.33
Pendimethalin fb Quizalofop Ethyl	19.0	22.2	16.2	3.47	4.12	2.10
Pendimethalin fb Imazethapyr	19.0	20.1	16.0	3.43	3.96	1.46
One HW at 25DAS	14.0	16.7	10.0	2.85	3.75	2.12
S. Em $_{(\pm)}$	0.68	0.71	0.65	0.47	0.48	0.16
C.D (P=0.05)	2.04	2.10	1.92	NS	1.43	0.47

HW-Hand weeding; fb- followed by; DAS-Date after sowing.

Table 3: Effect of herbicides on bacterial, fungal and actinomycetes population at different growth stages of chickpea.

Treatment	Bacteria x 10^6CFU /g soil		Fungi x 10^3CFU /g soil		Actinomycetes x 10^4CFU /g soil	
	Before sowing	At harvest	Before sowing	At harvest	Before sowing	At harvest
Quizalofop Ethyl	8.21	5.24	3.90	3.18	3.98	3.16
Imazethapyr	8.13	2.59	3.89	2.89	4.02	3.01
Pendimethalin fb Quizalofop	6.91	4.24	3.71	2.95	3.99	2.91
Pendimethalin fb Quizalofop	6.85	3.68	3.82	2.99	3.98	3.10
Weedy check (WC)	8.12	5.94	3.90	3.10	3.90	3.19
Two hand weeding	8.37	6.16	3.93	3.42	3.93	4.04
One HW	8.26	5.90	3.56	3.10	3.16	3.26
Pendimethalin	7.43	6.02	3.62	3.35	2.90	3.84
S. Em $_{(\pm)}$	0.08	0.06	0.06	0.03	0.19	0.07
C.D (P=0.05)	NS	0.18	NS	0.08	NS	0.21

HW-Hand weeding, fb- followed by.

Rhizobium population

The *Rhizobium* populations in soil samples varied at different stages of growth in chickpea. In general, it was in different treatments was maximum at 60 DAS as compared to other stages of plant growth (Table 2). At 30 DAS, highest *Rhizobium* population of 4.13 x 10^4 CFU/g soil was noticed in the treatment of two hand weeding. However, among herbicides, highest population of *Rhizobium* was observed in the pendimethalin treated plots (3.51 x 10^4 CFU/ g soil). The lowest population of *Rhizobium* was observed in the imazethapyr treated plots (2.69 x 10^4 CFU/ g soil). At 60 DAS, more number of Rhizobium was noticed in two hand weeding treatment (6.54 x 10^4 CFU/g of soil). Among herbicides, significantly higher population of *Rhizobium* was observed in pendimethalin applied treatment (5.20 x 10^4 CFU/ g soil) and significantly lowest population of *Rhizobium* was found in imazethapyr applied treatment (3.11 x 10^4 CFU/ g soil). At 90 DAS, among the different herbicide treatments, the *Rhizobium* population was more in two hand weeding treatment compared to herbicides treated plots. Among herbicides, significantly highest *Rhizobium* population was observed in pendimethalin and significantly lowest population of *Rhizobium* was noticed in imazethapyr applied treatment (1.51 x 10^4 CFU/g of soil). Similar findings were observed by Ahmad and Khan (2010).

CONCLUSION

The present investigation clearly brought out that two hand weeding followed by pendimethalin was the best sought out option on controlling weed population, for higher growth and yield of chickpea. Based on the results obtained, it could be inferred that two hand weeding are costly as compared to pendimethalin and it had least adverse effect on microbial population and nodulation. Therefore, pendimethalin can be used to get the higher plant growth and yield of chickpea.

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