International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 1016-1023

Received: 01-09-2017
Accepted: 04-10-2017

Raghavendra KS
Department of Agricultural Microbiology, PhD Scholar, UAS, Dharwad, Karnataka, India

Gundappagol RC
Department of Agricultural Microbiology, PhD Scholar, UAS, Dharwad, Karnataka, India

Santosh GP
Department of Agricultural Microbiology, PhD Scholar, UAS, Dharwad, Karnataka, India

Correspondence
Raghavendra KS
Department of Agricultural Microbiology, PhD Scholar, UAS, Dharwad, Karnataka, India

Impact of post emergence herbicides on soil microorganisms, nodulation and yield of chickpea

Raghavendra KS, Gundappagol RC and Santosh GP

Abstract
A field experiment was conducted to study the effect of post emergent herbicides [Quizalofop Ethyl, Phenaxoprop Ethyl, Propaquizafop Ethyl, Oxyfluorfen and Imazethapyr] on soil microflora, nodulation, growth and yield of chickpea under rainfed condition during the rabi season of 2012-13. In the investigation, observations recorded at initial soil sampling showed non-significant difference in treatment plots, microbial population *viz*., bacteria, fungi, actinomycetes, *Azotobacter*, *Rhizobium* and PSM. The populations of micro flora were differed due to different weed control treatments. The increased population of bacteria, fungi, actinomycetes, *Azotobacter*, *Rhizobium* and PSM found with increase in the age of the host plant up to 60 DAS. Whereas, at 90 DAS microbial population recorded was lesser than that of 30 and 60 DAS samples. Seed yield differed significantly due to different herbicide treatments. The growth parameter *viz.*, nodule count, nodule dry weight, shoot and root dry weight, total dry weight, plant height, number of branches, number of pods and dry weight of pods of chickpea increased with increase in the age of the host. Significantly higher seed yield (997 kg/ha) was recorded in weed free check. Among the herbicidal treatments, significantly yield (848 kg/ha) was recorded in phenaxprop ethyl.

Keywords: Microorganism, *Rhizobium*, *Azotobacter*, phosphorous solublising microorganisms (PSM), nodulation, herbicides

Introduction
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 and persists on the top soil (Ayansina*et al*., 2003) [3]. These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plant, wild life and man (Amakiri, 1982) [2]. 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) [12]. Herbicides may affect biological nitrogen fixation either by affecting plant growth or by directly affecting nitrogen-fixing *Rhizobia*. There are complexes of processes which are affected by herbicides. The more important could be photosynthesis, respiration and protein synthesis. The overall effect or herbicides is reflected in dry matter production. Either above-ground plant growth or root growth or both can be affected by the herbicides.

The use of pesticide is an integral and essential part of modern agricultural production. Soil represents a major environmental compartment on which most of applied pesticides are finally deposited and most synthetic pesticides are accumulating in the soil and ground water where they threaten the health of entire ecosystem. Microorganisms are an integral part of biogeochemical cycles of different elements in the ecosystem. If there is an imbalance in the population of these floras, then the cycling of different elements in the ecosystem is adversely affected. 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 toxicity of different herbicides on the growth and multiplication of agriculturally important microorganisms, which in turn could affect the crop growth and yield.

The field experiment was conducted to study the effect of herbicides on soil microflora, nodulation, growth and yield of chickpea under rainfed condition during the rabi season of 2012-13 at Agricultural Research Station, Klaburagi. The materials used and the methods followed are presented as below.

Material and Methods

The soil samples were collected from the field (Before sowing, 30, 60 daysafter sowing (DAS) and at harvest (90 DAS) by the standard method described by Jackson (1967) [14]. The collected samples were brought in the polythene bags and in refrigerator to maintain their chemical and biological properties for further study.

Observations

The observations on soil properties (pH and EC), general microflora (Bacteria, fungi and actinomycetes) and beneficial microflora (*Rhizobium*, *Azotobacter*, Phosphorus Solubilizing Microorganism (PSM)) were recorded at different stages (Before sowing, 30, 60 DAS and at harvest).

Enumeration of general microflora.

Soil samples collected from different treatment plots were used for enumeration of general soil microorganisms *viz.*, bacteria, fungi and actinomycetes at different stages of crop growth.

Bacteria

Each soil sample was sieved through the 1000micromesh 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 hat 28 ºC. Colonies that appeared on the media were enumerated and expressed in terms of colony forming units per gram of soil on dry weight basis (Bunt and Rovira, 1955) [4].

Fungi

Each soil sample was sieved through the 1000 micromesh to remove the bigger particles and debris and were used for enumeration of fungi using Martin's rose bengal agar medium (MRBA) by standard plate count method. The plates were incubated for 4 days at 28ºC. Colonies that appeared on MRBA media were enumerated and expressed in terms of CFU per gram of soil on dry weight basis (Martin, 1950) [17].

Actinomycetes

Each soil sample was sieved through the 1000 micromesh to remove the bigger particles and debris, and were used for enumeration of actinomycetes using Kuster's agar medium by standard plate count method. The plates were incubated for 6 days at 28 ºC. Colonies that appeared on Kuster's agar media were enumerated and expressed in terms of CFU per gram of soil on dry weight basis.

Enumeration of beneficial microflora

Soil samples collected from study area were used for enumeration of beneficial soil microorganisms like *Rhizobium*, *Azotobacter*, Phosphorus Solubilizing Microorganism (PSM).

Rhizobium

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 28ºC. Colonies that appear on the YEMA medium were enumerated and expressed in terms of CFU per gram of soil on dry weight basis.

Azotobacter

Enumeration of *Azotobacter* population in different soil samples at different stages was carried out by pour plate method using Waksman No. 77 medium. The plates were incubated for 48 hrs at 28 ºC. Colonies that appeared on the Waksman No. 77 medium were enumerated and expressed in terms of CFU per gram of soil on dry weight basis.

PSM

The phosphate solubilizing microorganisms were enumerated from the soil samples by serial dilution pour plate method using Pikovskaya's agar medium (Pikovskaya's, 1948) containing tricalcium phosphate (TCP). The plates were be incubated at 28 ± 2 ºC for two to seven days. Phosphate solubilizers producing clear hallo zones around the microbial colonies in media were enumerated.

Nodule number and dry weight of nodules per plant

The number of root nodules per plant at 30, 60 and 90 days (at harvest) 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. To determine the nodule dry weight, root nodules collected from five plants were dried in an oven at 70 ºC to constant weight and the average weight was expressed as milligrams (mg) per plant.

Chemical analysis of plant sample

The chickpea plant samples collected at 30, 60 DAS and at harvest from individual treatment were dried in an oven at 70ºC till constant weight was observed and further ground to fine powder in Willey mill with stainless steel blades. The powdered samples were used for the estimation of nitrogen and phosphorus contents.

The total nitrogen content in powdered samples was estimated by modified Micro Kjeldahl method as outlined by Jackson (1967) [14]. The total phosphorous in the plant samples was estimated by Vanadomolybdate yellow colour method as out lined by Jackson (1973) [15].

N content

Nitrogen content was estimated by modified Micro kjeldahl method (Jackson, 1967) [14] at 30, 60 DAS and at harvest. Nitrogen uptake was expressed as percentage at different growth stages. The total N uptake was calculated for each treatment separately using the following formula.

$$\text{Nitrogen (\%)} = \frac{\text{Titration value xN of } H_2SO_4 \text{x Dilution factor}}{\text{Weight of plantsample(g)}} \text{ x } 100$$

P content

The oven dried samples were ground to fine powder separately in a willey mill and used for estimation of phosphorous content at 30, 60 and harvest.

Estimation of phosphorus

Five grams of plant sample was taken in a 250 ml capacity conical flask and was added with 2.5 ml of concentrated HNO_3. The flasks were swirled to moisten the entire sample and then placed on a hot sand bath for 30 minutes and then on an electric hot plate at 180ºC to 200ºC. The suspension was boiled until taken nearly to dryness.

Wet oxidation

Five ml of triacid mixture (Conc. HNO_3, Conc. H_2SO_4 and 60% $HClO_4$ in the ratio of 10:1:4) was added to pre-digested

sample and further digestion was carried out at 180ºC to 200ºC on a digestion mantle until the content in the flask became clear white. The contents of the flasks were cooled and 10-15 ml of 6 N HCl was added and stirred well. The acid digest was transferred to 50 ml volumetric flask and the volume was made up to 50 ml with the distilled water. From this wet oxidized digested sample, P was estimated by Vanadomolybdate- phosphoric yellow colour method (Jackson, 1967) [14].

Ten ml of wet oxidized digested sample was taken in a 50 ml volumetric flask and 10 ml Vanadomolybdate reagent was added. The volume was made up to 50 ml with the distilled water and allowed to react for 30 minutes. The intensity of yellow color developed was read at 490 nm using a spectrophotometer. The P content was obtained by the standard curve prepared using KH_2PO_4.

Grain Yield and yield parameters

Number of pods per plant

At harvest (90 DAS) pods were separated from the five randomly selected plants and counted. The average weight of pods of five plants was then expressed as pod weight in grams per plant.

Dry weight of pods per plant

Pods of five plants were dried and dry weight of the pods was recorded in each treatment. The average weight of the pod of five plants was then expressed as pod weight in grams per plant.

Grain yield per hectare

On the basis of grain yield per net plot, the seed yield per hectare was calculated and expressed in quintals per hectare.

$$\text{Grain yield (kgha}^{-1}) = \frac{\text{Grain yield (kg) per plot}}{\text{Area harvested (m}^2)} \text{ x } 10000$$

Statistical analysis of the data

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.

Experimental Results

The present study emphasized evaluation of different herbicides on the population of total microorganisms and beneficial microflora at different growth stages of chickpea, apart from determining nodulation and yield parameters under field condition at ARS Gulbarga, University of Agricultural Sciences, Raichur, Karnataka during the year 2012-2013.The results of the field investigation conducted are furnished in the tables.

Effect of herbicides on general and beneficial microflora at different growth stages in chickpea rhizosphere

The bacterial, fungal and actinomycetes populations of soil samples varied at different stages of crop growth. General microbial population of soil samples of different treatments, were maximum at 60 DAS compared to other stages of plant growth and is presented in Table 1.

Before sowing the observations recorded on the general population of soil samples collected before implementation of the treatments and at 30 DAS indicated that there was no significant variation in the general microbial population (Table 3). At 60 DAS, among the soil samples of different treatments, more number of bacterial population of 8.77 x 10^6cfu per gram of soil, fungal 4.27 x 10^4cfu per gram of soil,andactinomycetes4.14 x 10^4cfu per gram of soil, was noticed in plots where herbicides were not imposed with any herbicides, Whereas in herbicides treated plots, treatment received Phenaxoprop Ethyl recorded highest bacterial population of 7.37x 10^6 cfu per gram of soil, fungal 3.30x 10^4cfu per gram of soil and actinomycetes 3.82x 10^4 cfu per gram of soil and oxyflorfen recorded lowest and the same trend was followed at 90 DAS and the beneficial microflora population also recorded to be same trend as flowed in general microflora in Table 3.

Effect of herbicides on nodule number and nodule dry weight of chickpea

Observations recorded on the nodule number and nodule dry weight of chickpea at different growth stages (30, 60 DAS and at harvest) are presented in Table 4 and Fig1. respectively.

Nodule number and nodule dry weight

Observations recorded on the nodule number of chickpea, generally found to vary at different stages (30, 60 DAS and at harvest) of the crop growth. Among the treatments, more number of nodules was noticed in the plots where herbicides were not imposed in plots (weed free and weedy check plots) when compared with different pre and post emergence herbicides imposed plots. The nodule number per plant was recorded and found highest at 60 DAS.

At 30 DAS, observations of nodule number at 30 days after sowing ranged from 17 to 37 per plant and noticed highest in weed free (37 per plant). Whereas, lowest nodules per plant was noticed in weedy check. Among herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (24 per plant) and less number of nodules (20 per plant) were noticed in oxyfluorfen treatment.).

At 60 DAS, observations of nodule number at 60 days after sowing ranged from 21 to 41 nodules per plant and the highest was noticed in weed free check (41 per plant). Whereas, weedy check recorded lowest count of nodules per plant. Whereas, among herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (28 per plant) and less number of (24 per plant) nodules per plant were noticed in oxyfluorfen treatment. (figure. 1).

At harvest (90 DAS) Number of nodules per plant was lowest at 90 days after sowing when compared to 30 days after sowing and at harvest. Observation of nodule number at 60 days after sowing ranged from 18 to 40 nodules per plant and noticed highest in weed free (40 per plant) and lowest nodule was recorded in weedy check (15 per plant). Whereas, among herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (21 per plant) and less number of nodules were noticed in oxyfluorfen (19 per plant) treatment and Dry weight of chickpea nodules at different growth stages (30, 60 DAS and at harvest) were recorded. The nodule dry weight per plant was noticed and found highest at 60 DAS, when compared with 30 DAS and harvest. Among the different treatments, highest nodule dry weight was noticed in herbicides free plots (weed free and weedy check plots) when compared with different herbicides treated plots at different growth stages the nodule dry weight depicts the same trend as nodule number presented in Table 4.

Effect of herbicides on Nitrogen content at different growth stages of chickpea

The data pertaining to Nitrogen content, recorded at different growth stages of chickpea at 30, 60 DAS and at harvest as influenced by different herbicide treatments are presented in [Table 5]. However, the N content in the different growth stages of chickpea, finds highest at 90 DAS.

Observations at 30 days after sowing recorded on nitrogen content in the chickpea plant ranged from 2.04-2.60% per plant. The highest N content was noticed in weed free (2.60%) and lowest N content in plant was recorded in treatment weedy check (2.04%). Among post emergence herbicides, highest N content (2.40%) was recorded in phenaxoprop ethyl and lowest N content (2.23%) was observed in oxyfluorfen. At 60 days after sowing, the N content was found to be highest (3.10%) in weed free and lowest N content (2.22%) was recorded in treatment weedy check. Among herbicides, highest N (2.55%) content was recorded in phenaxoprop ethyl and lowest N. Observations recorded at 90 days after sowing showed that among all the treatments, weed free check was found to be highest (3.29%) and lowest N content (2.32%) was recorded in treatment weedy check. Among herbicides, highest N (2.68%) content was recorded in phenaxoprop ethyl and lowest N content (2.52%) was observed in oxyfluorfen.

Effect of herbicides on Phosphorus content at different growth stages of chickpea

The data depicting the Phosphorus content, recorded at different growth stages of chickpea at 30, 60 DAS and at harvest as influenced by different weed control treatments are presented in table 5. However, the P content in the different growth stages of chickpea was found to be highest at harvest.

At 30 days after sowing, phosphorus content in the chickpea plant ranged from 0.20 to 0.41% per plant. The highest P content (0.41%) was noticed in weed free and lowest P content (0.20%) in weedy check. Among herbicides treated highest P content (0.25%) was recorded in phenaxoprop ethyl and lowest P content (0.21%) was observed in oxyfluorfen.

At 60 days after sowing, the P content was found to be the highest (0.52%) in weed free and the lowest P content (0.35%) was recorded in weedy check treatment, but results were non-significant. However, among herbicides, numerically highest P content (0.44%) was recorded in phenaxoprop ethyl and lowest P content (0.36%) was observed in oxyfluorfen.

Observations recorded at 90 days after sowing showed that among all the treatments, highest P content (0.59%) was observed in weed free check and lowest P content was recorded in treatment weedy check (0.40%). Among herbicides traeated highest P content (0.52%) was recorded in phenaxoprop ethyl and lowest P content (0.42%) was observed in oxyfluorfen.

Effect of herbicides on number, dry weight of pods per plant and yield

Observation recorded on the number of pods were generally found to be varied at different herbicide treatments and are presented in Table 5. Observations of chickpea pods ranged from 16 to 31 per plant and noticed highest in weed free check (31 per plant). Treatment receiving weedy check recorded lowest number of pods (16 per plant). Among herbicides treatments highest number of pods (23 per plant) was recorded in phenaxoprop ethyl and lowest (19 per plant) in oxyfluorfen. Dry weight of pods, ranged from 4.28 to 6.79 g per plant and noticed highest in weed free check (6.79 g per plant). Treatment receiving weedy check recorded lowest number of pods (4.28 g per plant). Among herbicides treated, highest pods (5.95 g per plant) were recorded in phenaxoprop ethyl and lowest (5.11 g per plant) in oxyfluorfen.

Grain yield (g/plant)

The grain yield differed significantly due to different herbicides treatments. Grain yield per plant ranged from 4.08 to 6.59 g per plant. Significantly highest grain yield (6.59 g per plant) was recorded in weed free check and lowest grain yield (4.08 g per plant) was recorded in the weedy check. Among post emergence herbicides, highest grain yield per plant was noticed in phenaxoprop ethyl (5.75 g per plant) and lowest in oxyfluorfen (4.91 g per plant).

Grain yield (kg/ha)

Grain yield differed significantly due to different weed control treatments. Grain yield per plant ranged from 565 to 997 kg/ha. Significantly highest grain yield (997 kg/ha) was recorded in weed free and lowest grain yield (564 kg/ha) was recorded in the weedy check as presented in the Table 5. Among herbicides treated highest grain yield per plant (848 kg/ha) was noticed in phenaxoprop ethyl and lowest (628 kg/ha) in oxyfluorfen.

Table 1: Effect of herbicides on bacterial, fungal and actinomycetes population at different growth stages of chickpea

Treatments	Bacteria x 10^6Cfu /g of soil				fungal x 10^3Cfu /g of soil				Actinomycetes x 10^4Cfu /g of soil			
	Before sowing	30 DAS	60 DAS	At harvest	Before sowing	30 DAS	60 DAS	At harvest	Before sowing	30 DAS	60 DAS	At harvest
T_1: Quizalofop Ethyl (POE)	3.23	8.21	7.33	5.24	2.13	3.90	3.31	2.95	2.13	3.98	3.84	3.16
T_2: Phenaxoprop Ethyl (POE)	3.37	8.32	7.37	5.27	2.27	3.89	3.32	2.97	2.17	4.02	3.88	3.20
T_3: Propaquizafop Ethyl (POE)	3.23	8.19	7.23	5.14	2.13	3.87	3.30	2.95	1.83	3.97	3.82	3.15
T_4: Oxyfluorfen (POE)	3.27	8.10	6.30	4.21	2.17	3.91	3.13	2.75	2.17	3.66	3.57	2.70
T_5:Imazethapyr (POE)	3.37	8.13	6.54	4.24	2.27	3.85	3.23	2.89	1.97	3.72	3.68	3.09
T_6: Weedy check (WC)	3.37	8.12	8.40	6.02	2.20	3.90	4.19	3.40	2.10	3.90	3.98	3.84
T_7: Weed free check (WF)	3.27	8.37	8.77	6.16	2.17	3.93	4.27	3.42	2.07	3.93	4.14	4.04
S.Em±	0.20	0.14	0.13	0.12	0.18	0.18	0.19	0.12	0.18	0.19	0.20	0.18
C.D at 0.05%	NS	0.40	0.38	0.34	NS	0.51	0.46	0.35	NS	0.54	0.58	0.52

DAS = Days after sowing, NS = Non significant, POE = post-emergence herbicide

Table 2: Effect of herbicides on Azotobacter, *Rhizobium* and phosphorous solublising Microorganism PSM population at different growth stages of chickpea

Treatments	*Azotobacter*x 10^3Cfu /g of soil				*Rhizobium*x 10^4Cfu /g of soil				Phosphorus solubilising microorganism x 10^3Cfu /g of soil			
	Before sowing	30 DAS	60 DAS	At harvest	Before sowing	30 DAS	60 DAS	At harvest	Before sowing	30 DAS	60 DAS	At harvest
T_1: Quizalofop Ethyl (POE)	2.30	4.83	4.70	3.31	2.10	4.10	4.06	3.10	2.12	2.40	2.33	2.24
T_2: Phenaxoprop Ethyl (POE)	2.20	4.79	4.71	3.60	2.33	4.12	4.08	3.30	2.15	2.43	2.37	2.30
T_3: Propaquizafop Ethyl (POE)	2.10	4.81	4.60	3.41	2.08	4.09	4.05	3.06	2.17	2.39	2.29	2.27
T_4: Oxyfluorfen (POE)	2.10	4.87	4.34	3.26	2.15	4.13	3.16	2.15	2.13	2.36	2.26	2.12
T_5:Imazethapyr (POE)	2.35	4.96	4.43	3.34	2.25	4.10	3.18	2.67	1.79	2.38	2.28	2.15
T_6: Weedy check (WC)	2.20	4.81	5.81	4.00	2.12	4.02	6.33	4.31	1.74	2.40	2.72	2.56
T_7: Weed free check (WF)	2.33	5.02	5.86	4.04	2.35	4.13	6.54	4.53	1.52	2.45	2.84	2.62
S.Em±	0.15	0.59	0.40	0.41	0.31	0.33	0.43	0.47	0.41	0.08	0.07	0.07
C.D at 0.05%	NS	1.69	1.19	1.20	NS	0.96	1.26	1.37	NS	0.24	0.21	0.22

DAS = Days after sowing, NS = Non significant, POE = post-emergence herbicide

Table 3: Effect of post emergence herbicides on nodule number and nodule weight at different growth stages of chickpea

Treatments	Nodule number/plant			Nodule dry weight (mg/plant)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1: Quizalofop Ethyl (POE)	23	27	20	25.85	28.92	20.76
T_2: Phenaxoprop Ethyl (POE)	24	28	21	26.67	29.74	20.79
T_3: Propaquizafop Ethyl (POE)	23	27	21	25.80	28.87	20.68
T_4: Oxyfluorfen (POE)	20	24	19	23.08	26.15	17.48
T_5:Imazethapyr (POE)	21	25	20	24.66	27.73	18.59
T_6: Weedy check (WC)	17	21	15	20.36	23.43	14.18
T_7: Weed free check (WF)	37	41	40	40.66	43.73	42.23
S.Em±	0.68	0.71	0.45	0.39	0.55	0.42
C.D at 0.05%	2.04	2.10	1.30	1.17	1.60	1.22

DAS = Days after sowing, NS = Non significant, POE = post-emergence herbicide

Table 4: Effect of post emergence herbicides on plant N content at different growth stages of chickpea

Treatments	N content in Plant (%)			P content in Plant (%)		
	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest
T_1: Quizalofop Ethyl (POE)	2.39	2.55	2.67	0.24	0.43	0.49
T_2: Phenaxoprop Ethyl (POE)	2.40	2.55	2.68	0.25	0.44	0.52
T_3: Propaquizafop Ethyl (POE)	2.38	2.55	2.67	0.24	0.43	0.48
T_4: Oxyfluorfen (POE)	2.23	2.39	2.52	0.21	0.36	0.42
T_5:Imazethapyr (POE)	2.38	2.54	2.66	0.23	0.38	0.44
T_6: Weedy check (WC)	2.04	2.22	2.32	0.20	0.35	0.40
T_7: Weed free check (WF)	2.60	3.10	3.29	0.35	0.52	0.59
S.Em±	0.05	0.16	0.21	0.06	0.08	0.03
C.D at 0.05%	0.15	0.48	0.68	0.17	NS	0.10

DAS = Days after sowing, NS = Non significant, POE = post-emergence herbicide

Table 5: Effect of pre and post emergence herbicides on number, dry weight of pods, grain yield kg^{-1} ha.

Treatments	No. of pods /plant	Dry weight of pods (g/plant)	Grain yield (g /plot)	Grain yield (kg/ha)
T_1: Quizalofop Ethyl (POE)	23	5.79	672.33	800
T_2: Phenaxoprop Ethyl (POE)	23	5.95	712.33	848
T_3: Propaquizafop Ethyl (POE)	22	5.55	660.33	786
T_4: Oxyfluorfen (POE)	19	5.11	527.67	628
T_5:Imazethapyr (POE)	20	5.41	609.67	726
T_6: Weedy check (WC)	16	4.28	474.67	565
T_7: Weed free check (WF)	30	6.79	837.67	997
S.Em±	1.41	0.53	89.98	61
C.D at 0.05%	4.07	1.50	258.60	172

DAS = Days after sowing, NS = Non significant, POE = post-emergence herbicide

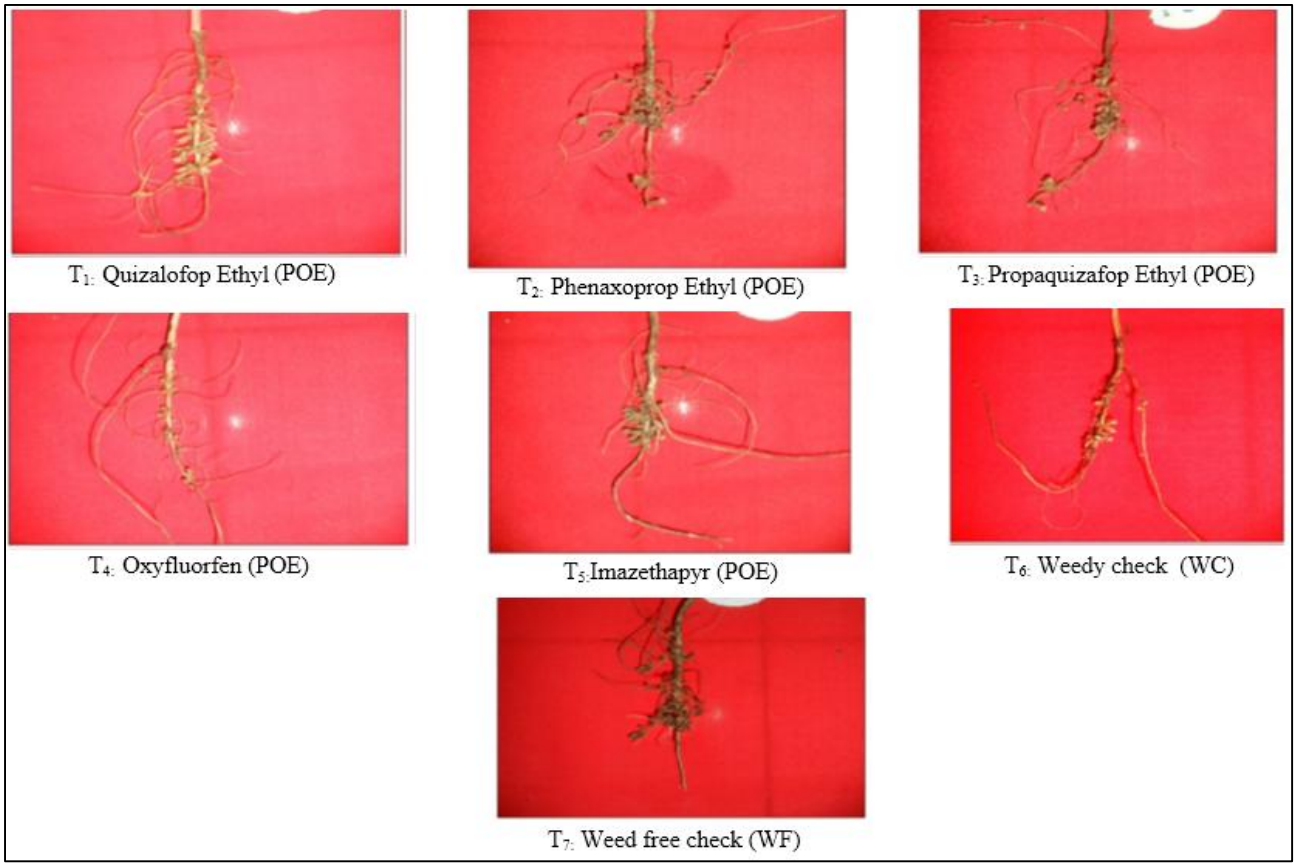

Fig 1: Effect of herbicides on nodule count at 60 DAS

Discussion

In the present study, an attempt was made to find out the effect of different pre and post emergence herbicides on soil microflora, nodulation and yield parameters of chickpea. The results obtained during the experimentation are discussed herewith.

Effect of herbicides on general microfloral (bacteria, fungi and actinomycetes) population in chickpea

In the present investigation the soil samples were collected at different growth stages (Before sowing, 30, 60 and 90 DAS (at harvest) of chickpea and investigated the population of general microflora (Bacteria, fungi and actinomycetes) and beneficial microorganism (*viz*., *Azotobacter, Rhizobium* and Phosphorus Solubilizing Microorganism (PSM)). At initial soil sampling, the general microbial population was on par with each other and found to be non-significant.

The population of general micro flora was found to be increased with increasing in the age of the host up to 60 DAS whereas; at harvest, the population was lesser than that of 60 DAS. Among the treatments, weed free and weedy check (control) recorded maximum number of microbial population when compared to different pre and post emergence herbicides applied plots. Herbicides significantly affect microbial growth and multiplication. However, herbicides also affect the microbes physiologically: a) by changing their biosynthetic mechanism (change in the level of protein biosynthesis is reflected on the ratio of extracellular and intracellular enzymes); b) by affecting protein biosynthesis (induction or repression of synthesis of certain enzymes); c) by affecting the cellular membranes (changes in transport and excretion processes); d) by affecting plant growth regulators (transport of indoleacetic acid, gibberellin synthesis and ethylene level); e) applied in high doses, they may kill microorganisms (Cook and Hutter, 1981) [7].

At 60 DAS the general microbial population was reduced where post emergence herbicides were imposed. Similarly, the effect of herbicides on soil microbial population (*viz*., Bacteria, fungi and actinomycetes) decreased upon treatment with herbicides when compared to the control. Sebiomo*et al*. (2011) [19] determined the effects of herbicides might affect the microbial population and microbial community structure in agricultural soils (Changpeng *et al*., 2010) [6]. Similarly, the same findings were reported by Ismail and Shamsuddin (2005) [13] they investigated the impact of two acetanilide herbicides *viz.*, Alachlor and Metolachlor on bacterial and fungal population and biomass. The effect of the two herbicides was monitored for 70 days under ambient condition. Metolachlor caused greater reduction in bacterial count than on fungal population. There was approximately 75% reduction in bacterial count in 14 days after treatment with 2µg/g metolachlor. Alachlor caused a reduction in bacterial counts at 7 and 14 days after treatment with 2µg/g or above. Fungal population decreased significantly in the presence of 20 µg/g alachlor at 7 days after treatment with respect to control. At 90 DAS, the population among all the treatments, combined application of pre followed by post recorded lowest population.

Effect of herbicides on beneficial microorganism (*viz*., *Azotobacter*, *Rhizobium* and Phosphorus Solubilizing Microorganism (PSM)) in chickpea

Soil samples were collected at different growth stages (Before sowing, 30, 60 and 90 DAS (at harvest)) of chickpea and investigated the population of beneficial microflora (*viz*., *Azotobacter*, *Rhizobium* and Phosphorus Solublising Microorganism (PSM)) by adopting standard enumeration techniques. At initial soil sample, the beneficial micro floral population was on par with each other and found to be non-significant.

In the present investigation, the population of beneficial microflora (*viz*., *Azotobacter*, *Rhizobium* and Phosphorous Solublising Microorganism (PSM)) were lesser in the herbicides applied treatments. Whereas, weed free recorded highest population since, herbicides were not applied throughout the crop growth period which enabled zero effect on beneficial microorganism population. Similarly, Eberbach and Douglas (1989) [8] reported that the population of *R. trifoli.* and *Mesorhizobium ciceri* reduced, when herbicides applied to field. Adeleye*et al.* (2004) [1] reported that the herbicides were more toxic to *Azotobacter vinelandii* and *Rhizobium phaseoli*. Hence, the percentage survival decreased with increased concentration of herbicides. Similar results reported by Singh and Wright (2002) [20] that the adverse effect of herbicides on nodulation and nitrogen fixation in legumes by affecting the nitrogen-fixing *Rhizobia* (*Rhizobium leguminosarum* population). Whereas, Felipe *et al.* (1987) [9-10] suggested that, the direct effects of herbicides on the plant, decreased the number of nitrogen-fixing bacteroides.

Observations recorded on the nodule number and nodule dry weight of chickpea at different growth stages (45, 60 DAS and at harvest). Nodule number varied at different stages of the crop growth. Observations, at 45 DAS recorded highest in weed free (41 per plant) and nodule dry weight (43.73 mg/plant). At 60 DAS similar trend was followed. Gupta *et al.,* (2002)[11] by his experimentation, led to concluded that, the herbicides application can result in substantial loss of nodules from the roots, likely due to the herbicide-induced stress on the plant *Rhizobium* symbiosis. Similarly Khan *et al.,* (2006)[16] studied the biotoxic effect of herbicide on growth, nodulation, nitrogenase activity and seed production in chickpea, and they found that the effects of pre-emergent (PRE) application of methabenzthiazuron (MBT), terbutryn, and linuron on Nodulation and nodule count per plant decreased consistently with increased herbicide rates. In the present investigation, the lowest nodule number and nodule dry weight were recorded pendimethalin with Imazethapyr applied treatments.

Effect of herbicides on chickpea pods

In the present study it was noticed that, among the treatments, weed free check recorded highest number of pods per plant (30 pods per plant) and pod dry weight of (6.79 g per plant). Weed check recorded lesser number of pods 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. In the present study oxyfluorfen recorded lowest pods per plant when compared to weed free treatments. Due to the effect of herbicide on beneficial microflora (*Rhizobium* and PSM) plant growth, nutrient uptake was inhibited and also delayed flowering and maturity. Similar observation were pointed by Taran *et al.* (2013)[21] showed that, pre-emergence application of low-rate imazethapyr cause minor injury to the plants and slightly increased ascochyta blight severity, it had only minor effects on plant development and yield compared with sulfentrazone. In contrast, post-emergence applications of imazethapyr, imazamox and metribuzin increased ascochyta blight severity significantly and delayed flowering maturity, and pod yields. Similar results was reported by Munees (2012) [18] wherein with the recommended field rate of quizalofop-p-ethyl decreased root, shoot biomass, symbiotic attributes (numbers, dry weight and leghaemoglobin content in nodules), nutrient-uptake (nitrogen and phosphorus), seed protein and yields of the tested legumes varied significantly in the presence of quizalofop -p-ethyl.

Effect of different post emergence herbicides on chickpea yield

The various yield components were significantly influenced by different weed control treatments. Weed free recorded maximum number of pods per plant and recorded highest number of yield per plot. The higher yield components in weed free was mainly due to the complete elimination of weeds throughout the crop growth, which enabled the greater population of general and beneficial micro flora (*Rhizobia* and PSM), plant growth along with more nodules, branches and pods which resulted in higher yield attributing parameters. Whereas, these yield components were adversely affected in weedy check, where in the microbial population was not affected but the weeds population were noticed significantly highest in the treatment hence the grain yield was recorded lowest when compared to all the treatments. These results are in close conformation with the findings of Channappagoudar and Biradar (2007) [5] and Vyas *et al.* (2003) [22]. While, weedy check recorded lower yield due to heavy weed infestation and more crop weed competition throughout the crop growth resulting in low nutrient uptake by crop, while weeds removed more quantity of nutrients throughout the crop growth period. This shows that the reduction in yield was apparently due to reduction in growth and yield components caused by weed infestation.

Thus herbicide affected the nitrogen-fixing bacteroides, plant growth, nutrient uptake and also delayed flowering and maturity. Similar observations were observed by Taran*et al.* (2013) [21] which showed that, application of low-rate imazethapyr cause minor injury to the plants and slightly increased ascochyta blight severity, in contrast, post-emergence applications of imazethapyr, imazamox and metribuzin increased drying and delay in flowering, maturity and decreased yield. Meanwhile, the nodulation and nutrients uptake also noticed lowest when compared to individual application of herbicides and herbicide free treatments. Felipe *et al.* (1987) [9-10] suggested that, the direct effects of herbicides on the plant, which decreased number of nitrogen-fixing bacteroids. Similar findings observed in the study, that the herbicide imposed treatments recorded less when compared to herbicide free treatments.

Conclusion

The present investigation clearly brought out that weed free followed by Phenaxoprop Ethyl was the best sought out option on controlling weed population, improving microbial population (nitrogen-fixing bacteria) and their activity in soil, nodulation, nutrient (N and P) uptake, growth and yield of chickpea. In general, the population of the general microflora, beneficial microflora and nodules noticed highest in 60 DAS when compared to 30 and 90 DAS. Based on the result obtained, it could be inferred that weed free had no effect on microbial population, nodulation, plant growth and yield parameters of chickpea.

Reference

1. Adeleye IA, Okorodudu E, Lawal O. Effect of some herbicides used in Nigeria on *Rhizobium phaseoli*, *Azotobacter vinelandii* and *Bacillus subtilis*. J Environ. Biol. 2004; 25:151-156.
2. Amakiri MA. Microbial degradation of soil applied herbicides. Nigeria J. Microbiol. 1982; 2:17-21.

3. Ayansina ADV, Ogunshe AAO, Fagade OE. Environment impact Assessment and microbiologist: An overview. Proc. of 11thAnnl. Natl. Conf. of Environment and Behavior Assocn. of Nigeria, 2003, 26-27.
4. Bunt JS, Rovira AD. Microbiological studies of some subantarctic soils. J.Soil Sci. 1955; 6:119-128.
5. Chanappagoudar BB, Biradar NR. Physiological approaches for weed management in soybean and redgram (4:2rp) intercropping system. Karnataka J Agric. Sci.2007; 20:241-244.
6. Changpeng Zhang, Xingang Liu, Fengshou Dong, Jun Xu, YongquanZheng Jing Li. Soil microbial communities response to herbicide 2, 4-dichlorophenoxyacetic acid butyl ester. European J. Soil Biol.2010; 46:175-180.
7. Cook AM, Hutter R. s-Triazines as nitrogen sources for bacteria. J Agril. Food Chem.1981; 29:1135-1143.
8. Eberbach P, Douglas LA. Herbicide effects on the growth and nodulation potential of *Rhizobium trifolii* with *Trifolium subterraneum* L. Plant and Soil.1989; 119:15-23.
9. FelipeMR, Fernández-Pascual M, Pozuelo JM. Effects of the herbicides Lindex and Sitnazine on chloroplast and nodule development, nodule activity, and grain yield in Lupinusalbus L. Plant and Soil.1987; 101:99-105.
10. FelipeMR, Fernández-Pascual M, Pozuelo JM. Effects of the herbicides Lindex and Sitnazine on chloroplast and nodule development, nodule activity, and grain yield in *Lupinus albus* L. Plant and Soil.1987; 101:99-105.
11. Gupta V, Roget D, Davoren B. Nitrogen fixation by grain legumes in the low rainfall Mallee soils - Potential effects of herbicide application. Grain Research and Development Corporation, 2002.
12. Hutsch BW. Methane oxidation in non-flooded soils as affected by crop production. European J. Agron.2001; 14:237-260.
    ication in Soil Science and Plant Analysis. 37:1783-1793.
13. Ismail BS, Shamsuddin N. Effect of Alachlor and metolachlor on microbial population in the soil. Malaysian J Microbiol. 2005; 1:36-41.
14. Jackson ML. Soil Chemical Analysis. Prentice Hall of India, Pvt. Ltd., New Delhi.1967; 1:111-203.
15. Jackson ML. Soil Chemical Analysis. Prentice Hall of India, Pvt. Ltd., New Delhi, 1973, 38-82.
16. Khan MS, Zaidi A, Rizvi PQ. Biotoxic effects of herbicides on growth, nodulation, nitrogenase activity and seed production in chickpea. Commun, 2006.
17. Martin UP. Use of acid, rose Bengal and streptomycin in plate method for estimating soil fungi. Soil Sci. 1950; 20:19-25.
18. Munees A. A Comparative Analysis of the Growth Parameters of Different Legume Crops Grown In quizalofop-p-ethyl Applied Soils. Insight Biotec. 2012; 2:1-6.
19. Sebiomo A, Ogundero VW, Bankole SA. Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. African J Biotechnol. 2011; 10:770-778.
20. Singh G, Wright D. *In vitro* studies one effect of herbicides on the growth of Rhizobia. Lett. Appl. Microbiol. 2002; 35:12-16.
21. Taran B, Holm F, Banniza S. Response of chickpea cultivars to pre and post emergence herbicide applications. Canada. J Plant Sci. 2013; 93:279-286.
22. Vyas MD, Jain RC, Dubey S. Productivity and weed control efficiency of integrated weed management practices in pigeonpea + soybean intercropping system under rainfed condition. Indian J Weed Sci.2003; 35:87-89.