Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 1649-1655
Received: 28-07-2017
Accepted: 29-08-2017

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Effect of herbicides on soil microcosm, nodulation and yield in chickpea (*Cicer arietinum* L.)

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Abstract
Herbicides are commonly used in kalaburagi to control weeds in chickpea. In addition to their impact on weeds, these herbicides are also affecting soil microorganisms which are responsible for numerous biological processes essential for crop production. In the present study, we assessed the impact of five commonly used herbicides three are pre emergence and two are post emergence [Pendimethalin (PRE), Chlormuron ethyl (PRE), Oxyfluorfen (PRE), Quizalofop ethyl (POE) and Phenaxoprop ethyl (POE)] on soil microbial populations in chickpea cultivation experimental plots. Our study showed that the herbicide treatments sinhibited the development of microbial populations in the soil, and the degree of inhibition closely related to the mode application as pre emergence or post emergence and varied with the types of herbicide. Weed free check recorded the highest number of microorganisms at all the different growth stages and differed in herbicides treated plots. Among the pre emergence pendimethalin recorded highest and in post emergence herbicides phenaxoprop ethyl resulted more in different stages of chickpea sampling. The study suggests that the herbicide application to soil of chickpea cultivation cause transient impacts on microbial population growth, when applied at recommended field application rate.

Keywords: Herbicides, soil microcosm, *Rhizobium*, Nodulation, chickpea.

Introduction
Weeds emerge fast and grow rapidly competing with the crop severally for growth resources viz., nutrients, moisture, sunlight and space during entire vegetative and early reproductive stages of chilli (Isik *et al.,* 2009). Presence of weeds reduces the photosynthetic efficiency, dry matter production and its distribution to conomical parts and there by reduces sink capacity of crop resulting in poor yield. To eliminate weed we go for the application of herbicide we know that, Soil is an important component of the ecosystem, serves as a medium for plant growth through the activity of microbial communities. This soil microbial communities (like bacteria, fungi and actinomycetes) play critical role in litter decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth (Singh *et al*., 1999; Chauhan *et al*., 2006; Tripathi *et al.,* 2006; Pandey *et al*., 2007) [24, 6-18, 27, 21]. However, soil micro-organisms are greatly influenced by factors including the application of herbicides (Pampulha *et al*., 2007) [20], which are applied in modern agricultural practices to attain optimum crop yields (Zabaloy *et al.,* 2008) [29]. If, microorganisms are sensitive to particular herbicide, its application will interfere with vital metabolic activities of microbes (Oliveira and Pampulha, 2006) [19], thus affect the availability of nutrients in the soil (Nautiyal, 2006) [18]. Numerous studies have shown the effect of herbicides on soil micro-organism populations that ultimately affect the drastic reduction in nodule number and their fresh as well as dry weight has been reported with the use of simazine. Leghaemoglobin did not develop at all in the nodules of simazine treated plants. However, prometryne at the same rates was found to be less damaging to nodulation and it improved nitrogen fixation (Kumar *et al.,* 1981) [16]. Thus there is a need to test different 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 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 flora, 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.

Materials and Methods

The field experiment was conducted to study the effect of Pre and Post emergence herbicides on soil microflora, nodulation and yield of chickpea under rainfed condition during the Rabi season of 2012-13 at Agricultural Research Station, Kalaburgi. The materials used and the methods followed are presented as below in Table 1.

Table 1: Treatment details imposed on chickpea using different herbicides Treatment

Sl. No.	Herbicides	Mode/ method of application	Dosage
1	Pendimethalin	Pre emergence	2.3 ml/l
2	Chlormuron Ethyl	Pre emergence	7.5 g/l
3	Oxyfluorfen	Pre emergence	2.0 ml/l
4	Quizalofop Ethyl	Post emergent	1.5 ml/1
5	Phenaxoprop Ethyl	Post emergent	0.1 ml/l
6	Weedy check	Control	Control
7	Weed free	Herbicides were not imposed	Hand weeding

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 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 per gram of soil on dry weight basis (Bunt and Rovira, 1955) [3].

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 28 ºC. 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.

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

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.

N content

Nitrogen content was estimated by modified Micro kjeldahl method (Jackson, 1967) [14] at 30, 60 DAS and at harvest. Powdered plant sample of 0.5 g was digested with 5 ml of concentrated H_2SO_4 and 200 mg digestion calalyst (K_2SO_4:$CuSO_4$:Selenium in 100:10:1 ratio) until the contents become clear. After cooling, the volume was made upto 25 ml with distilled water. Then 5 ml of aliquot was transferred to micro kjeldhal distillation unit. An aliquot of 10 ml of 40 per cent sodium hydroxide was added and steam distilled. Ammonia evolved was collected over 2 per cent boric acid (20 ml) containing 2 drops of double indicator (83.3 mg bromocresol green and 16.6 mg methyl red indicator dissolved in 10 ml of 95% ethanol) and back titrated against 0.05 N H_2SO_4. Nitrogen uptake was expressed as percentage at different growth stages. The total N uptake was calculated for each treatment separately using the following formula.

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

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.

$$\text{Grain yield (kg ha}^{-1}) = \frac{\text{Grain yield (kg) per plot}}{\text{Area harvested (m}^2)} \text{ x } 1000$$

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.

Results

The present study emphasized evaluation of different pre and post emergence herbicides on the population of total

microorganisms and beneficial microflora at different growth stages of chickpea, apart from determining nodulation, growth and yield parameters under field condition at ARS Kalaburagi, University of Agricultural Sciences, Raichur, Karnataka during the year 2012-2013. The results of the field investigation conducted are furnished in the Tables.

The Microbial populations of soil samples varied at different stages of crop growth. In general, bacterial, fungal, actinomycetes and *Rhizobium* population of soil samples of different treatments, were maximum at 60 DAS compared to other stages of plant growth, is presented in Table. Treatments receiving pre emergence herbicide inhibited their population at 30 DAS and treatment receiving post emergence herbicide inhibited their population at 60 DAS whereas, in weed check and weed free finds more microbial population when compared with all the treatments (PRE and POE herbicides), at different growth stages (30. 60 and 90 DAS).

Bacterial population

The bacterial populations of soil samples varied at different stages of crop growth. In general, bacterial population of soil samples of different treatments, were maximum at 60 DAS compared to other stages of plant growth and is presented in Table 2. Before sowing the observations recorded on the bacterial population of soil samples collected before implementation of the treatments indicated that there was no significant variation in the bacterial population.

At 30 DAS, highest bacterial population of 8.37 x 10^6 cfu per gram of soil was noticed in the plots without application of herbicides. Whereas, among pre emergence herbicides, the highest population of bacteria was observed in the pendimethalin treated plot (7.43x 10^6 cfu per gram of soil). At 60 DAS, among the soil samples of different treatments, more number of bacteria was noticed in plots where herbicides were not imposed (8.77 x 10^6 cfu per gram of soil). At harvest (90 DAS), among the different treatments, more number of bacteria was noticed in herbicides free plots, when compared to herbicides treated plots. Bacterial population ranged from 4.30 x 10^6 to 6.16 x 10^6 cfu per gram of soil.

In general, among the herbicides tested, pre emergence herbicides recorded highest bacterial population compared to post emergence herbicides. Among the pre emergence herbicides, significantly higher population of bacteria was observed in pendimethalin. Whereas, among post emergence herbicides, highest bacterial population were observed in phenaxoprop ethyl treated plot (5.27 x 10^6 cfu per gram of soil).

Fungal population

In general, fungal population in soil samples varied at different stages of crop growth as observed with bacterial population. The fungal population of soil samples of different treatments was maximum at 60 DAS compared to other stages of plant growth as shown in Table 2.

The observations recorded on the fungal population of soil samples collected before implementation of the treatments indicated that, there was no significant variation in the fungal population and the population ranged from 1.93 x 10^3 to 2.30 x 10^3 cfu per gram of soil.

At 30 DAS, the highest fungal population of 3.93 x 10^3 cfu per gram of soil was noticed in the plots, where herbicides were not treated. Whereas, among pre emergence herbicides, highest fungal population was observed in the pendimethalin treated plots (3.62 x 10^3 cfu per gram of soil) and lowest fungal population was noticed in the oxyfluorfen treated plots (2.86 x 10^3 cfu per gram of soil). The fungal population ranged from 2.80 x 10^3 to 3.93 x 10^3 per gram of soil. At 60 DAS, among the soil samples of different treatments, more number of fungal populations was noticed in herbicides free plots (4.27 x 10^3 cfu per gram of soil). In general, among the pre and post emergence herbicides, the post emergence herbicides reduced the fungal population. Among the pre emergence herbicides, significantly higher population of fungi was observed in pendimethalin applied treatment (3.93 x 10^3 cfu per gram of soil) and significantly lowest population of fungi was found in Quizalofop Ethyl (POE) applied treatment (3.31 x 10^3 cfu per gram of soil). Whereas, among post emergence herbicides, more number of fungal populations was observed in phenaxoprop ethyl treated plot (3.32 x 10^3 cfu per gram of soil).

At harvest (90 DAS), more number of fungal populations was noticed in herbicides free plots, when compared to herbicide sprayed plots. Among the pre emergence herbicides, significantly higher population of fungi was observed in pendimethalin applied treatment (3.05 x 10^3 cfu per gram of soil) and significantly lowest population of fungi was found in oxyfluorfen applied treatment (2.65 x 10^3 cfu per gram of soil). Whereas, among post emergence herbicides, more number of fungal population was observed in phenaxoprop ethyl treated plot (2.97 x 10^3 cfu per gram of soil).

Actinomycetes population

The actinomycetes populations in soil samples vary at different stages of crop growth. In general actinomycetes population was maximum at 60 DAS compared to other stages of plant growth as presented in Table 2.

Before sowing, observations recorded on the actinomycetes population of soil samples collected before implementation of the treatments indicated there was no significant variation in the actinomycetes population. The population of actinomycetes ranged from 1.87 x 10^3 to 2.17 x 10^3 cfu per gram of soil.

At 30 DAS, highest actinomycetes population of 4.02 x 10^4 cfu per gram of soil was noticed in the plots without the application of herbicides. Among the pre emergence herbicides, significantly higher population of actinomycetes was observed in the pendimethalin treated plots (2.90 x 10^4 cfu per gram of soil). Lowest population of actinomycetes was noticed in the oxyfluorfen treated plots (2.37 x 10^4 cfu per gram of soil). At 60 DAS, the Actinomycetes count was more (4.14 x 10^4 cfu per gram of soil) in the plots, where herbicides were not imposed. Among the pre and post emergence herbicides, the post emergence herbicides reduced the population of actinomycetes. Among the pre emergence herbicides, significantly higher population of actinomycetes was observed in pendimethalin applied treatment (3.95 x 10^4 cfu per gram of soil) and significantly lowest population of actinomycetes was found in oxyfluorfen
applied treatment (3.54 x 10^4 cfu per gram of soil). Whereas, among post emergence herbicides, more number of actinomycetes populations were observed in phenaxoprop ethyl treated plot (3.88 x 10^4 cfu per gram of soil). At harvest, highest actinomycetes population of 3.42 x 10^3 cfu per gram of soil was noticed in the herbicides free plots. Among the pre emergence herbicides, significantly higher population of actinomycetes was observed in pendimethalin and significantly lowest population of actinomycetes was found in oxyfluorfen applied treatment (2.70 x 10^4 cfu per gram of soil). Whereas, among post emergence herbicides, more number of actinomycetes population were observed in

phenaxoprop ethyl treated plot (3.20 x 10^4 cfu per gram of soil) and among the different herbicides treatment, the count of actinomycetes was more in weed free (4.04 x 10^4 cfu per gram of soil) and weedy check (3.84 x 10^4 cfu per gram of soil) plots, when compared to herbicides treated plots.

***Rhizobium* population**

The *Rhizobium* populations of soil samples varied at different stages of chickpea crop growth. In general, *Rhizobium* population of rhizosphere soil samples of different treatments was maximum at 60 DAS compared to other stages of plant growth as shown in Table 2.

Before sowing, the observations recorded on the *Rhizobium* population of soil samples collected before implementation of the treatments indicated that, there was no significant variation in the *Rhizobium* population and population ranged from 2.08 x 10^4 to 2.37 x 10^4cfu per gram of soil.

At 30 DAS, highest *Rhizobium* population of 4.13 x 10^4 cfu per gram of soil was noticed in the plots without application of herbicides. Whereas, among pre emergence herbicides, highest population of *Rhizobium* was observed in the pendimethalin treated plots (3.33 x 10^4 cfu per gram of soil). The lowest population of *Rhizobium* was noticed in the oxyfluorfen treated plots (2.05 x 10^4cfu per gram of soil).

At 60 DAS, rhizosphere soil samples of different treatments at 60 DAS, more number of *Rhizobium* was noticed in herbicides free plots (6.54 x 10^4 cfu per gram of soil). Among the pre emergence herbicides, significantly higher population of *Rhizobium* was observed in pendimethalin applied treatment (5.20 x 10^4 cfu per gram of soil) and significantly lowest population of *Rhizobium* was found in oxyfluorfen applied treatment (3.14 x 10^4 cfu per gram of soil). Whereas, among post emergence herbicides, highest *Rhizobium* population were observed in phenaxoprop ethyl treated plot (4.08 x 10^4 cfu per gram of soil).

At harvest (90 DAS), among the different herbicide treatments, the *Rhizobium* population was more in herbicide free plots compared to herbicides treated plots. Among the pre emergence herbicides, significantly highest *Rhizobium* population was observed in pendimethalin and significantly lowest population of *Rhizobium*, was noticed in oxyfluorfen applied treatment (1.50 x 10^4 cfu per gram of soil). Among the post emergence herbicides, more number of *Rhizobium* population were observed in phenaxoprop ethyl treated plot (3.30 x 10^4 cfu per gram of soil).

Table 2: Effect of herbicides on microcosm (Bacteria, Fungi, Actinomyceteses and *Rhizobium*) at different growth stages of chickpea.

Treatments	Before sowing	30 DAS	60 DAS	At harvest	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: Pendimethalin (PRE)	3.10	7.43	8.21	5.90	2.20	3.62	3.93	3.05	1.87	2.90	3.95	3.30	2.37	3.33	5.20	3.39
T_2: Chlormuron Ethyl (PRE)	3.30	6.37	7.27	4.50	2.20	2.83	3.73	2.85	1.90	2.40	3.58	2.78	2.30	2.65	3.63	2.43
T_3: Oxyfluorfen (PRE)	3.40	6.27	7.23	4.30	2.30	2.86	3.61	2.65	2.00	2.37	3.54	2.70	2.25	2.05	3.14	1.50
T_4: 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	2.10	4.10	4.06	3.10
T_5: 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	2.33	4.12	4.08	3.30
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	2.12	4.02	6.33	4.31
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	2.35	4.13	6.54	4.53
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	0.31	0.33	0.43	0.47
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	NS	0.96	1.26	1.37

DAS = Days after sowing PRE = Pre-emergence herbicide, POE = post-emergence herbicide

Effect of herbicides on nodule number and nitrogen percent

Observations recorded on the nodule number and nitrogen percent of chickpea at different growth stages (30, 60 DAS and at harvest) are presented in Table 3 respectively.

Nodule number

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.

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 the pre emergence herbicides, significantly higher nodules was observed in pendimethalin applied treatment (24 per plant) and significantly the lowest nodules was found in oxyfluorfen applied treatment (18 per plant). Whereas, among post emergence herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (24 per plant).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. Among the pre emergence herbicides, significantly higher nodules was observed in pendimethalin applied treatment (28 per plant). Whereas, among post emergence herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (28 per plant).

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). Among the pre emergence herbicides, significantly higher nodules was observed in pendimethalin applied treatment (22 per plant) and significantly lowest nodules was found in oxyfluorfen applied treatment (18 per plant). Whereas, among post

emergence herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (21 per plant).

Nitrogen content

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 3. 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 pre emergence herbicide, pendimethalin observed highest N content (2.44%) Among post emergence herbicides, highest N content (2.40%) was recorded in phenaxoprop ethyl. 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 pre emergence herbicide, pendimethalin observed the highest N content (2.60%) and lowest N content (2.25%) was recorded in weedy check. Among post emergence herbicides, highest N (2.55%) content was recorded in phenaxoprop ethyl. 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 pre emergence herbicide, pendimethalin observed highest N content (2.72%) and lowest N content (2.38%) was recorded with weedy check. Among post emergence herbicides, highest N (2.68%) content was recorded in phenaxoprop ethyl.

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 3. Among pre emergence herbicide, highest grain yield per plot (881 kg/ha) was noticed in pendimethalin and lowest (604 kg/ha) in oxyfluorfen. Among post emergence herbicides, highest grain yield per plant (848 kg/ha) was noticed in phenaxoprop ethyl.

Table 3: Effect of herbicides on Nodulation, N content and Yield of chickpea

Treatments	No. of Nodules / Plant			N content in Plant (%)			Yield
	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest	kg/ha
T_1: Pendimethalin (PRE)	24	28	22	2.44	2.60	2.72	881
T_2: Chlormuron Ethyl (PRE)	21	25	19	2.24	2.40	2.53	651
T_3: Oxyfluorfen (PRE)	18	22	18	2.11	2.25	2.38	604
T_4: Quizalofop Ethyl (POE)	23	27	20	2.39	2.55	2.67	800
T_5: Phenaxoprop Ethyl (POE)	24	28	21	2.40	2.55	2.68	848
T_6: Weedy check (WC)	17	21	15	2.04	2.22	2.32	565
T_7: Weed free check (WF)	37	41	40	2.60	3.10	3.29	997
S.Em±	0.68	0.71	0.45	0.05	0.16	0.21	61
C.D at 0.05%	2.04	2.10	1.30	0.15	0.48	0.68	172

DAS = Days after sowing PRE = Pre-emergence herbicide POE = post-emergence herbicide

Discussion

The use of pesticides is an integral and essential part of agricultural production. Soil represents a major environmental compartment on which most of applied pesticides are finally deposited, and it is estimated that three million reported case of pesticide associated acute poisoning occurs annually. Most synthetic pesticides are accumulating in the soil and ground water, where they threaten the health of entire ecosystem. Heavy use of pesticide in agriculture is associated with significantly undesirable side effects (Adil *et al*., 2012) [2] on beneficial microorganisms *viz*., *Azotobacter*, *Rhizobium* and Phosphorus solublising microorganism etc.

Herbicides may affect soil microflora, beneficial microflora and biological nitrogen fixation either by affecting plant growth or by directly affecting nitrogen-fixing *Rhizobia*. There are complex processes which are affected by herbicides. The overall effect of herbicides is reflected in dry matter production. Either above-ground plant growth or root growth or both can be affected by the herbicides.

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

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 micro flora (Bacteria, fungi and actinomycetes). 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. At 30 DAS samples when the pre emergence herbicides were imposed and the soil sample was analyzed for general microbial population, the results found that the general microbial population was lesser than untreated samples while, herbicide free check recorded highest population. 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 and whereas, in combined application of pre followed by post the population was lowest when compared to individual application of pre or post emergence herbicide. Overall

herbicides applied treatments recorded lesser population. 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) [22] determined the effects of herbicides might affect the microbial population and microbial community structure in agricultural soils (Changpeng *et al*., 2010) [5]. 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 2 µg/g metolachlor. Alachlor caused a reduction in bacterial counts at 7 and 14 days after treatment with 2 µg/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.

The population of beneficial microflora at different growth stages was determined after the implementation of the herbicide treatments, among the treatments, combined application of pre followed by post emergence herbicides recorded lowest microbial population when compared to individual application of herbicides. The changes in microbial activity were greater in the herbicide treated treatments than herbicide free control (Simonida and Mirjana., 2006) [23]. In the present investigation, the population of beneficial mcroflora (*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) = 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) [25] 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 bacteroids.

Observations recorded on the nodule number and nodule dry weight of chickpea at different growth stages (30, 60 DAS and at harvest) is presented in Table 9. 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. Among herbicide applied treatments, treatment receiving combination of pre followed by post (pendimethalin followed by oxyflurofen) recorded lowest number of (23 per plant) nodule per plant and (27.70 mg per plant) nodule dry weight at 60 DAS. Gupta *et al.*, (2002) [12] 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) [15] 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 in pendimethalin with Imazethapyr applied treatments.

The various yield components were significantly influenced by different weed control treatments (Table 18). 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) [4] and Vyas *et al.* (2003) [28]. 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.

Among the treatments, treatment receiving pre emergence herbicide oxyflurofen, recorded lowest yield when compared to herbicide free treatments. Thus herbicide affected the nitrogen-fixing bacteroids, plant growth, nutrient uptake and also delayed flowering and maturity. Similar observations were observed by Taran *et al.* (2013) [26] 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.

Application of (pre followed by post) pendimethalin with oxyflurofen recorded lowest yield, this is because of both pre and post emergence herbicides are treated at different growth stages. Hence, the lower population of *Rhizobia* was recorded. 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 pendimethalin + inter cultivation 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. From the above experiment the following conclusions can be drawn, that the population of the general microflora, beneficial microflora (*Rhizobium*) 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. Among the different treatments, weed free observed highest general and beneficial microflora population and plant growth and yield parameters of chickpea followed by pre emergence Pendimethalin. Among the treatments, significantly higher weed control was noticed in weed free followed by pre emergence Pendimethalin.

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