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# Impact of herbicide application on Beneficial soil microbial community, Nodulation and Yield parameters of chickpea (*Cicer arietinum* L.)

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**ABSTRACT**

*The effect of pre and post emergent herbicides [Pendimethalin (PRE), Chlormuron Ethyl (PRE), Propaquizafop Ethyl (POE), Oxyfluorfen (PRE), Imazethapyr (POE)]on beneficial soil microflora [Azotobacter, Rhizobium and PSB (phosphorous solublising)], nodulation and yield parameters was assessed over a different growth stages of chickpea crop plant [30 Days after sowing (DAS), 60 DAS and at Harvest (90 DAS)]. Soil samples from ARS, Kalaburagi,were treated with herbicides at recommended rates.. Azotobacter, Rhizobium and PSB populations decreased upon treatment with herbicides when compared to the weed free (Hand weeding) and Weedy check. Obtained results indicated that soils treated with weed free (Hand weeding) hadthe highest microfloral population followed by weedy check. Similarly, noticed highest nodulation (41 nodules/ plant @ 60 DAS), growth and yield parameters (837.67 grams / plot) of chickpea.*

***KEY WORDS:*** *Pre emergent, Post emergent, Herbicide, Nodulation and Chickpea yield*

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## INTRODUCTION

Chickpea (*Cicer arietinum* L.) occupies an important position due to its nutritive values (17-23% protein) in large vegetarian population of the country In addition to proteins, it is a good source of carbohydrates, minerals and trace elements. Apart from human and animal nutrition, chickpea plays an important role in sustaining soil fertility with its unique ability to fix atmospheric nitrogen through symbiosis with *Rhizobium*. There are more than 75 weed species that infest chickpea fields. These species are mostly dicotyledonous and belong to 26 different families (El-Brahli, 1988). In closer-row sown crops, such as legumes, use of herbicides for controlling weeds is considered to be the most effective method. Depending upon the properties of herbicides they can be used as pre-sowing, pre-emergence or post-emergence herbicides (Anderson, 1996). 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.

## MATERIAL AND METHODS

The field experiment was conducted to study the effect of Pre and Post emergence herbicides on soil microflora, nodulation, growth and yield of chickpea under rainfed condition during the rabi season of 2012-13 at Agricultural Research Station, Kalaburagi. The treatments were laid out in Randomized Complete Block Design with three replications. Pre-emergent herbicides were sprayed on the day of sowing of chickpea whereas, post emergent herbicides were applied/ sprayed 20 days after sowing.

The soil samples were collected from the field (30 days after sowing (DAS), 60 DAS and at harvest (90 DAS) by the standard method described by Jackson (1967). Soil samples collected from different treatment plots were used forenumeration of beneficial soil microorganisms (*Rhizobium*, *Azotobacter*, Phosphorus Solubilizing Microorganism (PSM)) at different stages of crop growth by using suitable laboratory media (Table.2).
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.

Table 1. Treatment details

| Treatment | Herbicides | Mode/ method of application | Dosage |
|---|---|---|---|
| 1 | $T_1$: Pendimethalin | Pre emergence | 3.3 ml/l |
| 2 | $T_2$: Chlormuron Ethyl | Pre emergence | 2.3 ml/l |
| 3 | $T_3$: Oxyfluorfen | Pre emergence | 2.0 ml/l |
| 4 | $T_4$: Propaquizafop Ethyl | Post emergent | 1 ml/l |
| 5 | $T_5$:Oxyfluorfen | Post emergent | 1 ml/l |
| 6 | $T_6$: Imazethapyr | Post emergent | 1ml/l |
| 7 | $T_7$: Weedy check | Control | Control |
| 8 | $T_8$:Weed free | Herbicides were not imposed | Hand weeding |

**Table 2. Laboratory media use to enumerate the following microbial population.**

| Sl. No. | Enumeration of microorganism | Medium used for enumeration |
|---|---|---|
| 1 | Azotobacter | Waksman No. 77. |
| 2 | Rhizobium | Yeast extract mannitol agar (YEMA). |
| 3 | PSB (Phosphorous solublizing microorganism) | Pikovskaya's media. |

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 $70^{o}$ C to constant weight and the average weight was expressed as milligrams (mg) per plant and chickpea seeds were separated from pods and expressed in grams.

## RESULTS

The Microfloral populations of soil samples varied at different stages of crop growth. In general, beneficial microbial population (*Azotobacter,Rhizobium* and Phosphorous solublizing bacteria (PSB)) of soil samples varied at different stages of chickpea crop growth. In general, *Azotobacter, Rhizobium* and PSB population of rhizosphere soil samples of different treatments was maximum at 60 DAS compared to other stages of plant growth as shown in table.
The observations recorded on the *Azotobacter, Rhizobium* and PSB population of soil samples collected before implementation of the treatments indicated that, there was no significant variation in the *Azotobacter,Rhizobium* and PSB population.
In the present investigation, 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. The population of beneficial microflora at different growth stages was determined after the implementation of the herbicide treatments.

### Impact of herbicide application on *Azotobacter* population.

The observations recorded on the *Azotobacter* population of soil samples collected before implementation of the treatments indicated that, there was no significant variation in the *Azotobacter* population. *Azotobacter* population ranged from 2.10 x $10^4$ to 2.33 x $10^4$ cfu per gram of soil.
At 30 DAS, the highest *Azotobacter* population of 5.02 x $10^4$ cfu per gram of soil was noticed in the plots without the application of herbicides. Whereas, among pre emergence herbicides treatments, the highest population of *Azotobacter* were observed in the pendimethalin formulation treated plots (3.42 x $10^4$ cfu per gram of soil) and lowest population of *Azotobacter* was noticed in the oxyfluorfen treated plots (3.20 x $10^4$ cfu per gram of soil).
In 60 DAS,the soil samples of different treatments, more number of *Azotobacter* was noticed in herbicide free plots (5.86 x $10^4$ cfu per gram of soil).

In general, among the pre and post emergence herbicides, the post emergence herbicides reduced the population of *Azotobacter*. Among the pre emergence herbicides, significantly higher population of *Azotobacter* was observed in pendimethalin applied treatment (4.93 x $10^4$ cfu per gram of soil) and significantly lowest population of *Azotobacter* was found in oxyfluorfen (PRE) applied treatment (4.33 x $10^4$ cfu per gram of soil). Whereas, among post emergence herbicides, more number of *Azotobacter* population were observed in Propaquizafop Ethyl (POE) treated plot (4.60 x $10^4$ cfu per gram of soil) and lowest population were noticed in Oxyfluorfen (PRE) treatment (4.33 x $10^4$ cfu per gram of soil).

At 90 DAS, population of *Azotobacter* was more in herbicide free plots compared to herbicide sprayed plots. Among the pre emergence herbicides, significantly higher population of *Azotobacter* was observed in pendimethalin applied treatment (3.44 x $10^4$ cfu per gram of soil) and significantly lowest population of *Azotobacter* was found in oxyfluorfen applied treatment (3.21 x $10^4$ cfu per gram of soil). Whereas, among post emergence herbicides, more number of *Azotobacter* population were observed in Propaquizafop Ethyl (POE) treated plot (3.41 x $10^4$ cfu per gram of soil) and lowest (3.26 x $10^4$ cfu per gram of soil) was recorded in oxyfluorfen treated plot.

**Impact of herbicide application on *Rhizobium* populations.**

*Rhizobium* populations 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.

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 $10^4$cfu per gram of soil.

At 30 DAS,The 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 $10^4$cfu per gram of soil).

In 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 Imazethapyr (POE)treated plot (6.33 x $10^4$ cfu per gram of soil) and lowest population of *Rhizobium* (3.16 x $10^4$ cfu per gram of soil) were noticed in oxyfluorfen treatment.

In 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 followed by inter cultivation treatment (4.20 x $10^4$ cfu per gram of soil) 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) and lowest in oxyfluorfen treated plot with a population of 2.15 x $10^4$ cfu per gram of soil.

**Impact of herbicide application on Phosphorus solubilising microorganism (PSB) population**

The PSB populations of soil samples varied at different stages of crop growth. In general PSB population of soil samples of different treatments was maximum at 60 DAS compared to other stages of plant growth as shown in table.

The observations recorded before sowing,on the PSB population of soil samples collected before implementation of the treatments indicated that, there was no significant variation in the PSB population and the population ranged from 1.52 x $10^3$ to 2.17 x $10^3$ cfu per gram of soil before sowing of chickpea.

At 30 DAS,the highest PSB population of 2.45 x $10^3$ cfu per gram of soil was observed in the plots without application of herbicides. Among the pre emergence herbicides, highest population of PSB was found in the pendimethalin treated plots (2.25 x $10^3$ cfu per gram of soil) and the lowest population of PSB was noticed in the oxyfluorfen treated plots (1.86 x $10^3$ cfu per gram of soil).

At 60 DAS, PSB count was more in the herbicides free plots (2.84 x $10^3$ cfu per gram of soil). Among the pre and post emergence herbicides, the post emergence herbicides reduced the population of PSB. Among the pre emergence herbicides, significantly higher population of PSB was observed in pendimethalin applied treatment (2.43 x $10^3$ cfu per gram of soil) and significantly lowest population of PSB was found in oxyfluorfen applied treatment (2.23 x $10^3$ cfu per gram of soil). Among post emergence herbicides, more number of PSB population were observed in phenaxoprop ethyl treated plot (2.37 x $10^3$ cfu per gram of soil) and less number of population of PSB (2.26 x $10^3$ per gram of soil) were noticed in oxyfluorfen sprayed plot.

At harvest (90 DAS), highest PSB population was noticed in herbicide free plots. Among the pre emergence herbicides, significantly higher population of PSB was observed in pendimethalin followed by inter cultivation (2.41 x $10^4$ cfu per gram of soil), followed by pendimethalin sprayed treatment (2.37 x $10^3$ cfu per gram of soil) and significantly lowest population of PSB was found in oxyfluorfen sprayed treatment (2.26 x $10^3$ cfu per gram of soil). Among post emergence herbicides sprayed samples more number of PSB population were observed in Imazethapyr (POE)applied plot (2.25 x $10^3$ cfu per gram of soil) while, lesser number of PSB population was observed in oxyfluorfen (2.12 x $10^3$ cfu per gram of soil) applied plot.

**Table 3. Effect of pre and post emergence herbicides on *Azotobacter* population at different growth stages of chickpea**

| Treatments | x $10^3$Cfu /g of soil | | | |
|---|---|---|---|---|
| | Before sowing | 30 DAS | 60 DAS | At harvest |
| $T_1$: Pendimethalin (Xtra formulation) (PRE) | 2.25 | 3.42 | 4.93 | 3.44 |
| $T_2$: Chlormuron Ethyl (PRE) | 2.23 | 3.38 | 4.38 | 3.40 |
| $T_3$: Oxyfluorfen (PRE) | 2.13 | 3.20 | 4.33 | 3.21 |
| $T_4$: Propaquizafop Ethyl (POE) | 2.10 | 4.81 | 4.60 | 3.41 |
| $T_5$:Oxyfluorfen (POE) | 2.10 | 4.87 | 4.34 | 3.26 |
| $T_6$: Imazethapyr (POE) | 2.35 | 4.96 | 4.43 | 3.34 |
| $T_7$: Weedy check (WC) | 2.20 | 4.81 | 5.81 | 4.00 |
| $T_8$: Weed free check (WF) | 2.33 | 5.02 | 5.86 | 4.04 |
| **S.Em±** | 0.15 | 0.59 | 0.40 | 0.41 |
| **C.D at 0.05%** | NS | 1.69 | 1.19 | 1.20 |

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

**Table 4. Effect of pre and post emergence herbicides on *Rhizobium* population at different growth stages of chickpea**

| Treatments | x $10^4$Cfu /g of soil | | | |
|---|---|---|---|---|
| | Before sowing | 30 DAS | 60 DAS | At harvest |
| $T_1$: Pendimethalin (Xtra formulation) (PRE) | 2.37 | 3.33 | 5.20 | 3.39 |
| $T_2$: Chlormuron Ethyl (PRE) | 2.30 | 2.65 | 3.63 | 2.43 |
| $T_3$: Oxyfluorfen (PRE) | 2.08 | 4.09 | 4.05 | 3.06 |
| $T_4$: Propaquizafop Ethyl (POE) | 2.15 | 4.13 | 3.16 | 2.15 |
| $T_5$:Oxyfluorfen (POE) | 2.25 | 4.10 | 3.18 | 2.67 |
| $T_6$: Imazethapyr (POE) | 2.12 | 4.02 | 6.33 | 4.31 |
| $T_7$: Weedy check (WC) | 2.35 | 4.13 | 6.54 | 4.53 |
| $T_8$: Weed free check (WF) | 2.35 | 4.13 | 6.54 | 4.53 |
| **S.Em±** | 0.31 | 0.33 | 0.43 | 0.47 |
| **C.D at 0.05%** | NS | 0.96 | 1.26 | 1.37 |

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

**Table 5. Effect of pre and post emergence herbicides on Phosphorus solubilising microorganism population at different growth stages of chickpea**

| Treatments | x $10^3$Cfu /g of soil | | | |
|---|---|---|---|---|
| | Before sowing | 30 DAS | 60 DAS | At harvest |
| $T_1$: Pendimethalin (Xtra formulation) (PRE) | 1.82 | 2.25 | 2.43 | 2.37 |
| $T_2$: Chlormuron Ethyl (PRE) | 1.73 | 1.93 | 2.25 | 2.13 |
| $T_3$: Oxyfluorfen (PRE) | 2.17 | 2.39 | 2.29 | 2.27 |
| $T_4$: Propaquizafop Ethyl (POE) | 2.13 | 2.36 | 2.26 | 2.12 |
| $T_5$:Oxyfluorfen (POE) | 1.79 | 2.38 | 2.28 | 2.15 |
| $T_6$: Imazethapyr (POE) | 1.74 | 2.40 | 2.72 | 2.56 |
| $T_7$: Weedy check (WC) | 1.52 | 2.45 | 2.84 | 2.62 |
| $T_8$: Weed free check (WF) | 1.52 | 2.45 | 2.84 | 2.62 |
| **S.Em±** | 0.41 | 0.08 | 0.07 | 0.07 |
| **C.D at 0.05%** | NS | 0.24 | 0.21 | 0.22 |

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

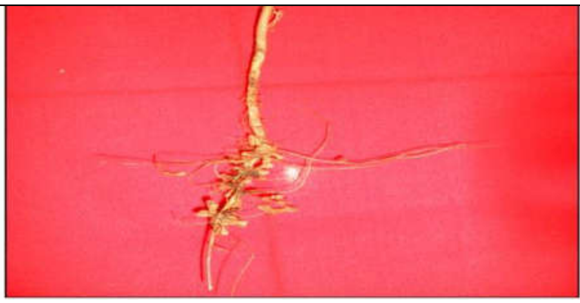
**Table 6. Effect of pre and post emergence herbicides on grain yield of chickpea**

| Treatments | Grain yield (g /plot) |
|---|---|
| $T_1$: Pendimethalin (PRE) | 739.67 |
| $T_2$: Chlormuron Ethyl (PRE) | 547.00 |
| $T_3$: Oxyfluorfen (PRE) | 507.67 |
| $T_4$: Propaquizafop Ethyl (POE) | 660.33 |
| $T_5$: Oxyfluorfen (POE) | 527.67 |
| $T_6$: Imazethapyr (POE) | 609.97 |
| $T_7$: Weedy check (WC) | 474.67 |
| $T_8$: Weed free check (WF) | 837.67 |
| **S.Em±** | 89.98 |
| **C.D at 0.05%** | 258.60 |

PRE = Pre-emergence herbicide POE = post-emergence herbicide

**DISCUSSION**

The changes in microbial activity were greater in the herbicide treated treatments than herbicide free control (Simonida and Mirjana., 2006). 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) 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) 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) suggested that, the direct effects of herbicides on the plant, decreased the number of nitrogen-fixing bacteroids.



$T_1$: Pendimethalin (PRE)

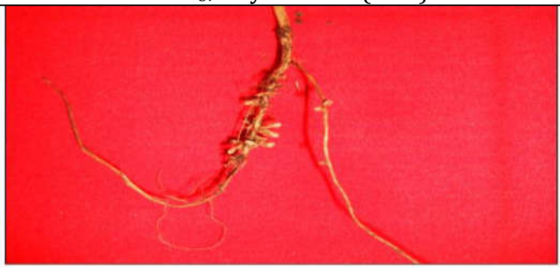
$T_2$: Chlormuron Ethyl (PRE)

$T_3$: Oxyfluorfen (PRE)

$T_4$: Propaquizafop Ethyl (POE)

$T_5$: Oxyfluorfen (POE)

$T_6$: Imazethapyr (POE)



$T_7$: Weedy check (WC)

$T_8$: Weed free check (WF)

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