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Nodulation and Yield Response of Chickpea (*Cicer arietinum* L) to Pre and Post Emergence Herbicides

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A B S T R A C T

To assess the integrated effect of herbicide mixtures on nodulation, yield attributes and yields of chickpea and to determine the economic feasibility of different weed management practices in chickpea, a field experiment was conducted at ARS, Kalaburagi during *rabi* season. Nine treatments were applied and arranged in a randomized block design with three replications. The results revealed that higher weed dry biomass and lower plant height were obtained in weedy check plots. The plants weed free check recorded higher nodules than herbicide treated. Higher number of pod plant^{-1}, number of seeds pod^{-1} and yield^{-1} ha were observed in weed free check (Hand weeding). The weed management practices revealed that hand weeding (Weed free check) gave the highest nodule dry weight (43.73mg plant^{-1}) followed by pendimethalin + inter cultivation (35 mg plant^{-1}) at 60 DAS. The maximum grain yield (997 kg ha-1) was recorded in weed free check followed by pendimetheline + inter cultivation (974 kg ha^{-1}). Therefore, managing the weeds with the application of herbicides recorded lower nodulation, plant parameters (*viz.*, plant height, dry weight, and pods per plant) and yield. However, in a situation of unavailability of herbicide on time, hand weeding and inter cultivation seemed to be an alternate weed management practice.

Keywords

Chickpea, Grain yield, Herbicide, Nodulation, Weed.

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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 (Ali and Kumar, 2006).

Grown principally for its protein rich edible seeds, this crop can be used for both seeds and forage production.

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*.

Chickpea is the world's second-largest cultivated food legume. The 95 per cent legume production and consumption occurs in developing countries. Most chickpeas are grown in South Asia, which accounts for more than 75 per cent of the world chickpea area. India is by far the largest chickpea producing country. Over the period of 1978-2010, the area under chickpea in India increased marginally from 7.6 million hectares to 7.9 million hectares, but production increased by 65 per cent of world's annual production (from 4.8 to 6.8 million metric tons). Other important chickpea producing countries are Pakistan, Turkey, Mexico, Canada and Australia (Anon., 2012).

Weeds compete with the crop plants for nutrients, moisture, and light and thus, reduce the yields considerably. Among pesticides, herbicides are the plant protection agents which are used in high input agricultural practices to kill the unwanted weeds, thus to prevent yield losses due to these noxious plants (Cork and Krueger, 1992). To get higher yield it is essential to control weeds at appropriate time with suitable methods. Due to easiness and labour scarcity to control weeds particularly at the critical period, use of herbicides has become very common.

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 agriculture, weeds often pose a major threat to crop yield and therefore, herbicides are widely used. Regular application leads to their accumulation in the soil and changes its microfloral composition. Herbicides when added to bacterial cultural in excess of recommended rates, generally reduced the growth of *Rhizobium* species and a differential tolerance between fast and slow growing *Rhizobium* species has been reported for some but not all herbicides (Tomar *et al.*, 2003).

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.

Nonetheless, Chickpea is believed to be more sensitive to herbicides than other leguminuos species. Therefore, herbicide combination is applied to broaden the spectrum of weeds controlled and sometimes combinations can give spectacularly good control at doses considerably below those normally applied in single application. The use of herbicide combinations is not new, but it has not received the attention and input that is necessary to fully understand and implement the practice. The present study therefore is aimed at to assess the herbicides impact on nodulation, yield attributes and yields of chickpea.

Materials and Methods

Description of the study area

The experiment was conducted at ARS, Kalaburagi during the 2012-13 at *Rabi* season. The total rainfall received during the crop season was 720 mm with mean maximum and minimum temperatures of 31.86-27.59 °C and 25.89 and 19.64 °C, respectively

Treatments and experimental design

Nine treatments, planting patterns of chickpea is 30x10cm. Selected four herbicides two pre emergence herbicides (pendimethalin @ 3.3 ml/l and chlormuron ethyl2.3 ml/l) two post emergence herbicides (quizalofop ethyl@ 1.5 ml/1 and phenaxoprop ethyl @ 0.1 ml/l) two treatments receiving combination of pre and post emergence herbicides (pendimethalin + quizalofop ethyland pendimethalin + phenaxoprop ethyl) one treatment is made to receive pendimethelin and inter cultivation, weed free (Hand weeding) and weedy check were maintained

The treatments were laid out in Randomized Complete Block Design with three replications. The plan of layout and treatment details (herbicide application) is shown in table 1. Pre-emergence herbicides were sprayed on the day of sowing of chickpea whereas, post emergence herbicides were applied/ sprayed 20 days after sowing.

Enumeration of beneficial microflora *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 28º C. Colonies that appear on the YEMA medium were enumerated and expressed in terms of CFU per gram of soil on dry weight basis

Plant growth parameters

The observations on nodulation, plant growth parameters, N and P content in plant, shoot, root dry matter content were recorded at 30, 60 and 90 DAS. Observations pertaining to yield and yield parameters were recorded at harvest.

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 70^{o} C 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).

Grain yield and yield parameters

Number of pods, dry weight and grain yield 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, Dry weight and grain yield 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.

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

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 and Discussion

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 10^4 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 10^4cfu per gram of soil).

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 pendimethalinxtra formulation applied treatment (5.20 x 10^4cfu per gram of soil). Whereas, among post emergence herbicides, highest *Rhizobium* population were observed in phenaxoprop ethyl treated plot (x 10^4cfuper gram of soil). In case of pre followed by post emergence herbicides sprayed treatments, the highest *Rhizobium* population was noticed in pendimethalin + phenaxoprop ethyl applied treatment (3.16 x 10^4 cfu per gram of soil) and lowest *Rhizobium* population was found in pendimethalin (PRE) + oxyfluorfen (POE) treated plot (3.00x 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 followed by inter cultivation treatment (4.20 x 10^4cfu per gram of soil) and significantly lowest population of *Rhizobium*, was noticed in oxyfluorfen applied treatment (1.50 x 10^4cfu per gram of soil). Among the post emergence herbicides, more number of *Rhizobium* population were observed in phenaxoprop ethyl treated plot (3.30 x 10^4cfu per gram of soil) and lowest in oxyfluorfen treated plot with a population of 2.15 x 10^4cfu per gram of soil. In dual application of pre followed by post emergence herbicide, the highest *Rhizobium* population was noticed in pendimethalin (PRE) + phenaxoprop ethyl (POE) applied treatment (3.14 x 10^4 cfu per gram of soil) and lowest count of *Rhizobium* was found in pendimethalin + oxyfluorfen treated plot (2.98 x 10^4 cfu per gram of soil). 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.

Effect of pre and post emergence 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 3.

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 Chlormuron Ethyl applied treatment (21 per plant). Whereas, among post emergence herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (24 per plant). In combined application of herbicides treatments (pre followed by post), the highest nodules (25 per plant) was noticed in pendimethalin + phenaxoprop ethyl.

Observations of nodule number at 60 days after sowing ranged from 21 to 41 nodules per plant and the highest was noticed inweed free check (41 per plant). Whereas, weedy check recorded lowest count of nodules per plant. Among the pre emergence herbicides, significantly higher nodules were 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). Among the combined application of herbicides (pre and post) treatments, the highest nodules (29 per plant) was noticed in pendimethalin + phenaxoprop ethyl.

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 inweed free (40 per plant) and lowest nodule was recorded in weedy check (15 per plant). Among the pre emergence herbicides, significantly higher nodules were observed in pendimethalin applied treatment (22 per plant).

Table.1 Treatment details imposed on chickpea using different herbicides

Treatment	Herbicides	Mode/ method of application	Dosage
1	Pendimethalin	Pre emergence	2.3 ml/l
2	Chlormuron Ethyl	Pre emergence	7.5 g/l
3	Quizalofop Ethyl	Post emergent	1.5 ml/1
4	Phenaxoprop Ethyl	Post emergent	0.1 ml/l
5	Pendimethalin + Quizalofop Ethyl	$T_1 + T_3$	2.3 ml/l+ 1.5 ml/1
6	Pendimethalin + Phenaxoprop Ethyl	$T_1 + T_4$	2.3 ml/l+ 0.1 ml/l
7	Pendimethalin + Intercultivation	Pre emergence + Intercultivation	2.3 ml/l + intra cultivation
8	Weedy check	Control	Control
9	Weed free	Herbicides were not imposed	Hand weeding

Table.2 Effect of pre and post emergence herbicides on *Rhizobium* population at different growth stages of chickpea

Treatments	**x 10^4Cfu /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: Quizalofop Ethyl (POE)	2.10	4.10	4.06	3.10
T_4: Phenaxoprop Ethyl (POE)	2.33	4.12	4.08	3.30
T_5:Pendimethalin + Quizalofop Ethyl	2.23	3.15	3.12	3.10
T_6:Pendimethalin + Phenaxoprop Ethyl	2.35	3.17	3.16	3.14
T_7: Pendimethalin + intercultivation (IC)	2.15	3.21	6.21	4.20
T_8: Weedy check (WC)	2.12	4.02	6.33	4.31
T_9: 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.3 Effect of pre and post emergence herbicides on nodule number and nodule dry weight at different growth stages of chickpea

Treatments	30 DAS		60 DAS		At harvest	
	Nodule no./plant	Nodule dry weight/plant	Nodule no./plant	Nodule dry weight/plant	Nodule no./plant	Nodule dry weight/plant
T_1:Pendimethalin (Xtra formulation) (PRE)	24	27.83	28	30.90	22	21.88
T_2:Chlormuron Ethyl (PRE)	21	23.56	25	26.63	19	18.58
$T_{3:}$ Quizalofop Ethyl (POE)	23	25.85	27	28.92	20	20.76
$T_{4:}$ Phenaxoprop Ethyl (POE)	24	26.67	28	29.74	21	20.79
T_5:Pendimethalin + Quizalofop Ethyl	23	25.70	27	28.77	23	25.37
T_6:Pendimethalin + Phenaxoprop Ethyl	25	29.97	29	33.04	26	31.77
T_7: Pendimethalin + intercultivation (IC)	26	32.11	30	35.18	31	34.00
T_8: Weedy check (WC)	17	20.36	21	23.43	15	14.18
T_9: Weed free check (WF)	37	40.66	41	43.73	40	42.23
S.Em±	0.68	**0.39**	0.71	**0.55**	0.45	**0.42**
C.D at 0.05%	2.04	**1.17**	2.10	**1.60**	1.30	**1.22**

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

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

Treatments	N content in Plant (%)		
	30 DAS	60 DAS	At harvest
T_1:Pendimethalin (Xtra formulation) (PRE)	2.44	2.60	2.72
T_2:Chlormuron Ethyl (PRE)	2.24	2.40	2.53
$T_{3:}$ Quizalofop Ethyl (POE)	2.39	2.55	2.67
$T_{4:}$ Phenaxoprop Ethyl (POE)	2.40	2.55	2.68
T_5:Pendimethalin + Quizalofop Ethyl	2.45	2.62	2.74
T_6:Pendimethalin + Phenaxoprop Ethyl	2.46	2.63	2.76
T_7: Pendimethalin + intercultivation (IC)	2.55	2.72	2.84
T_8: Weedy check (WC)	2.04	2.22	2.32
T_9: Weed free check (WF)	2.60	3.10	3.29
S.Em±	0.05	0.16	0.21
C.D at 0.05%	0.15	0.48	0.68

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

Table.5 Effect of pre and post emergence herbicides on grain yield of chickpea

Treatments	Grain yield (kg/ha)
T_1:Pendimethalin (Xtra formulation) (PRE)	881
T_2:Chlormuron Ethyl (PRE)	651
$T_{3:}$ Quizalofop Ethyl (POE)	800
$T_{4:}$ Phenaxoprop Ethyl (POE)	848
T_5:Pendimethalin + Quizalofop Ethyl	895
T_6:Pendimethalin + Phenaxoprop Ethyl	942
T_7: Pendimethalin + intercultivation (IC)	974
T_8: Weedy check (WC)	564
T_9: Weed free check (WF)	997
S.Em±	61
C.D at 0.05%	172

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

Whereas, among post emergence herbicides, more number of nodules were observed in phenaxoprop ethyl treated plot (21 per plant) and among the combined application of herbicides (pre and post) treatments, the highest nodules was noticed in pendimethalin + penaxoprop ethyl applied treatment (26 per plant).

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 pre, post and combination of pre followed by post emergence herbicides treated plots. Same trend followed as observed during nodule count recorded observations.

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 4. 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%) and among post emergence herbicides, highest N content (2.40%) was recorded in phenaxoprop ethyl. Whereas, in both pre followed by post emergence herbicides, the highest N content (2.46%) was noticed in pendimethalin (PRE) + phenaxprop ethyl (POE).

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. Whereas, in both pre followed by post emergence herbicides, the highest N content (2.63%) was noticed in pendimethalin + phenaxprop 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. Whereas, in combined application of pre followed by post emergence herbicides, highest was recorded (2.76%) in pendimethalin + phenaxprop ethyl (POE). Similarly Khan *et al.*, (2006) 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.

Chickpea yield parameters: Grain yield (kg/ha)

Grain yield differed significantly due to different weed control treatments. Grain yield per plant ranged from 564 to 997 kg/ha.

Significantly highest grain yield (997 kg/ha) was recorded in weed free followed by Pendimethalin (PRE) + intercultivation (974 kg/ha) and Pendimethalin (PRE) + Phenaxoprop Ethyl (POE) (942 kg/ha) and lowest grain yield (564 kg/ha) was recorded in the weedy check as presented in the table 5. Among pre emergence herbicide, highest grain yield per plot (881 kg/ha) was noticed in pendimethalin and among post emergence herbicides, highest grain yield per plant (848 kg/ha) was noticed in phenaxoprop ethyl and lowest (628 kg/ha) in oxyfluorfen. Whereas, in both pre and post emergence herbicides, the lowest (695 kg/ha) grain yield per plot was recorded in pendimethalin + oxyfluorfen and highest (942 kg/ha) was recorded in pendimethalin + phenaxoprop ethyl.

The various yield components were significantly influenced by different weed control treatments (Table 5). 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) and Vyas *et al.,* (2003). 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.

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