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Effect of Herbicides on Weeds, Nodulation and Growth of *Rhizobium* in Summer Blackgram (*Vigna mungo*)

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Being a short duration and initially slow growing, blackgram suffers heavily due to infestation of weeds. Depending upon intensity and nature of weed flora, an average yield reduction of 30-50% has been reported due to weed competition (Mishra, 1997). Use of herbicides is advantageous and economical. Herbicides could affect root formation (Garcia and Jordan, 1969) and also change the effectiveness of the rhizobia, especially after long-term exposure to residues of persistent herbicides. A number of herbicides have been evaluated for their efficacy against weeds in blackgram, however, the information on the effect of these herbicides on nodulation and soil microflora is lacking. Therefore, the present investigation was undertaken.

Field and laboratory experiments were conducted at the National Research Centre for Weed Science, Jabalpur (Madhya Pradesh) during summer season of 2005. The soil was clay loam in texture, low in organic carbon (0.65%) and available nitrogen (230 kg ha^{-1}), medium in available phosphorus (18 kg P ha^{-1}) and high in available potassium (280 kg K ha^{-1}) with neutral pH (7.1) and EC (0.35 dSm^{-1}). Fluchloralin, imazethapyr, metolachlor, pretilachlor, pendimethalin, quizalofop and fenoxaprop applied at different doses and stages of application alongwith weedy and weed-free were evaluated in randomized block design with three replications (Tables 1 and 2). Blackgram variety (TU 98-14) was sown in rows 25 cm apart on February 19, 2005. All the herbicides were applied with knapsack sprayer fitted with flat fan nozzle using spray volume of 500 l ha^{-1}. Population and dry matter of weeds were taken at 60 days after sowing (DAS). The data on number and dry weight of weeds were subjected to square-root transformation using $\sqrt{(X+0.5)}$. Number of nodules and their dry weight were recorded at 20, 40 and 60 DAS.

To see the effect of herbicides on growth of rhizobia, nodules were collected at 40 DAS from weed-free plots where no herbicide was used. *Rhizobium* from root nodules was isolated in the laboratory as per the methods suggested by Mostara *et al.* (2000). Desired quantities of herbicides (Table 2) were added in the conical flasks containing 100 ml of freshly prepared and sterilized yeast extract mannitol broth. Thereafter, 1.0 ml of freshly prepared bacterial culture was added in each flask and inoculated flasks were incubated at 28±1 °C for 24 h. Experiment was replicated thrice in a completely randomized design. After 24 h of incubation, bacterial enumeration was done by spectrophotometer method (optical density) and viable counting and growth of bacteria were seen in both liquid and solid media. Number of colonies in each plate was counted by colony counter and colony forming units (cfu) were calculated using following formula :

$$\text{No. of cells/ml} = \frac{\text{No. of colonies x Dilution factor}}{\text{Size of sample (ml)}}$$

The experimental field was infested with ground cherry (*Physalis minima* L.) (77.4%) and spurge (*Euphorbia geniculata* Orteg.) (22.6%). Application of herbicides significantly reduced the population of *P. minima* as compared to weedy check (Table 1). Among different herbicides imazethapyr at 0.10 kg ha^{-1} applied 20 DAS and pretilachlor, pendimethalin and metolachlor applied as pre-emergence controlled *P. minima* more effectively than other herbicides. Imazethapyr at 0.10 kg ha^{-1} (as PPI or Post-em.) was most effective against *E. geniculata*. All the herbicidal treatments, except quizalofop, significantly reduced the total weed dry weight. However, metolachlor being at par with pretilachlor was most effective in reducing the total weed dry weight.

Table 1. Effect of herbicides on weeds and blackgram

Treatment	Dose ($g\ ha^{-1}$)	Application stage	Weed population (No. m^{-2})			Total weed dry weight ($g\ m^{-2}$)	Seed weight ($g\ plant^{-1}$)	1000-seed weight (g)	Seed yield ($kg\ ha^{-1}$)
			P. minima	*E. geniculata*	Total				
Fluchloralin	1000	PPI	6.36 (40.0)	4.81 (22.6)	7.93 (62.4)	7.60 (57.3)	0.91	36.32	289
Pendimethalin	1000	Pre-em.	2.80 (7.3)	7.13 (50.3)	7.61 (57.4)	7.68 (58.5)	0.94	35.39	973
Imazethapyr	100	PPI	6.14 (37.2)	3.06 (8.9)	6.79 (41.1)	6.64 (43.6)	1.48	34.84	1190
Imazethapyr	100	Post-em.	2.11 (4.0)	3.62 (12.6)	4.14 (16.6)	6.72 (44.7)	0.93	37.10	770
Quizalofop	50	Post-em.	4.44 (19.2)	6.25 (38.6)	7.68 (58.5)	8.65 (74.3)	0.73	32.37	370
Metolachlor	1000	Pre-em.	2.91 (8.0)	6.54 (42.3)	7.19 (51.2)	5.46 (29.3)	0.73	35.33	617
Pretilachlor	750	Pre-em.	2.54 (6.0)	6.14 (37.2)	6.62 (43.3)	5.68 (31.8)	1.15	31.78	483
Fenoxaprop	90	Pre-em.	5.69 (31.9)	4.36 (18.5)	7.11 (50.1)	7.36 (53.7)	0.70	32.37	484
Weedy			8.06 (64.5)	5.00 (24.5)	9.30 (86.0)	9.46 (89.0)	0.40	34.14	457
Weed-free			0.71 (0)	0.71 (0)	0.71 (0)	0.71 (0)	1.70	35.65	1263
LSD (P=0.05)			0.53	1.18	1.04	1.24	0.27	4.14	173

Data subjected to square root transformation. Values in parentheses are original.

Table 2. Effect of herbicides on nodules in blackgram and growth of *Rhizobium* in liquid and solid media

Treatment	Dose ($g\ ha^{-1}$)	Application stage	No. of nodules $plant^{-1}$ DAS			Dry weight of nodules ($mg\ plant^{-1}$) DAS			No. of colonies Cfu ($X\ 10^7$)		Optical density
			20	40	60	20	40	60	Solid media	Liquid media	
Fluchloralin	1000	PPI	10.3	15.7	16.7	4.17	6.10	7.40	145	122	0.45
Pendimethalin	1000	Pre-em.	13.3	11.5	08.7	6.23	5.06	4.17	198	181	0.66
Imazethapyr	100	PPI	11.6	08.4	07.6	4.53	4.03	3.53	024	015	0.64
Imazethapyr	100	Post-em.	14.5	10.7	10.6	6.03	4.96	4.90	-	-	-
Quizalofop	50	Post-em.	14.5	11.1	08.7	6.93	5.06	4.36	056	049	0.53
Metolachlor	1000	Pre-em.	13.0	10.3	08.3	5.33	4.86	4.06	031	024	0.47
Pretilachlor	750	Pre-em.	12.5	09.7	06.3	6.43	4.06	3.20	056	038	0.63
Fenoxaprop	90	Post-em.	15.1	09.5	05.4	7.53	4.00	2.80	168	154	0.54
Weedy			14.9	10.0	07.7	6.76	4.13	3.80	-	-	-
Weed-free			11.5	11.0	10.5	4.53	5.00	4.93	149	129	0.63
LSD (P=0.05)			00.6	00.5	00.6	0.40	0.21	0.38	009	016	0.04

DAS–Days after sowing.

Infestation of weeds throughout the season caused 63.8% reduction in seed yield over weed-free check (Table 1). The highest seed yield was recorded with weed-free check (1263 kg ha^{-1}) closely followed by pre-plant incorporation of imazethapyr (1190 kg ha^{-1}) and pre-emergence application of pendimethalin (973 kg ha^{-1}). Pre-plant incorporation of imazethapyr was superior to its post-emergence application in increasing seed yield.

Irrespective of the treatments, the nodule number and their dry weight (Table 2) declined with advancement in plant age except in fluchloralin where both number and dry weight of nodules increased upto 60 DAS. Among pre-emergence herbicides, pendimethalin recorded the maximum number of nodules and nodule dry weight at 20 DAS followed by metolachlor and pretilachlor. At 40 DAS when the nodulating bacterial activity is expected to be maximum, fluchloralin treated plants showed maximum number and dry weight of nodules. Murti *et al.* (2004) also reported that root nodules and dry weight of nodules/plant in chickpea were enhanced significantly by fluchloralin application. Pendimethalin, imazethapyr and quizalofop did not affect the nodule number and dry weight. However, metolachlor, pretilachlor, imazethapyr, fenoxaprop resulted in reduced number as well as dry weight of nodules. This might be due to inhibitory effect of these herbicides on *Rhizobium*. Royuela *et al.* (2000) also reported that imazethapyr significantly reduced plant growth and nodule initiation in pea.

Pendimethalin and fenoxaprop significantly increased the *Rhizobium* count in both solid and liquid media (Table 2). Imazethapyr, metolachlor, pretilachlor and quizalofop significantly decreased the bacterial count compared to control. Fluchloralin did not affect the *Rhizobiurn* count. Gupta and Pandey (1992) also observed the least toxic effect of fluchloralin on *Rhizobium* in blackgram. Pendimethalin which gave maximum bacterial count in both the media did not affect the optical density significantly as compared to control. The effect of imazethapyr and pretilachlor in optical density was at par with pendimethalin. The other herbicides significantly reduced the optical density.

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