Effect of Pendimethalin and Imazethapyr on Nodulation, Nitrogen Fixation and Nitrogenase Activity and Yield in Groundnut (*Arachis Hypogaea* L.)

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Article History

Manuscript No. c429a
Received in 27th November, 2012
Received in revised form 25th May, 2013
Accepted in final form 5th June, 2013

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Keywords

Groundnut, nitrogen fixation, nitrogenase activity, imazethapyr

Abstract

A field experiment was conducted at College of Agriculture, Rajendranagar, Hyderabad in *kharif* 2011 to study effect of Pendimethalin and Imazethapyr on nodulation, nitrogen fixation and nitrogenase activity in groundnut. Results indicated that Seed treatment with *Rhizobium* in groundnut significantly influence the total nodule number up to 45 DAS and active nodules throughout the crop growth period. Application of Pendimethalin doses did not adversely affect the number of total nodules or active nodule count. But, application of Imazethapyr at double dosage resulted in significantly lower total nodule and active nodule up to 45 DAS. Nitrogenase activity in groundnut increased up to 60 DAS and thereafter decreased gradually. Imazethapyr double dose adversely affected the nitrogenase activity up to 45 DAS which later on recovered from 60 DAS. Despite better weed control achieved in Imazethapyr double dose treatment, highest pod yield was recorded in Imazethapyr at recommended dose where seed inoculation was done compared to Imazethapyr double dose.

1. Introduction

Groundnut (*Arachis hypogaea* L.) is one of the most important oilseed crops in India. Groundnut contributes nearly 65% to the vegetable oil produced in India and holds the key to the fluctuating fortunes of vegetable oil industry. The most commonly used herbicides for weed management in groundnut crop cultivated in Andhra Pradesh are Pendimethalin as pre-emergence and Imazethapyr as early post-emergence spray. Application of Pendimethalin as pre-emergence herbicide or Imazethapyr as early post-emergence herbicide (15-20 DAS) is recommended for effective weed management in groundnut (DWSR, 2009). Use of herbicides for weed control in legumes and especially in groundnut has certainly contributed to the increased yield and improved quality. However, detrimental effects caused by these herbicides on soil microorganism's growth and metabolism have also been reported in several studies.

2. Materials and Methods

This experiment was conducted during *kharif*, 2011 on sandy clay loam soil at College of Agriculture, Rajendranagar, Hyderabad which is in Southern Telangana agro-climatic zone of Andhra Pradesh, India. The experiment was laid out in split plot design with 2 main treatments as *Rhizobium* un-inoculated and inoculated at recommended dose (1 kg ha^{-1}). Sub-treatments as herbicide dosages viz., weedy check, Pendimethalin at two doses 750 g a.i. ha^{-1} and 1500 g a.i. ha^{-1}, Imazethapyr at two doses 75 g a.i. ha^{-1} and 150 g a.i. ha^{-1}. Five plants that were collected were used to count number of nodules and the number of active nodules (nodules having pink colour pigmentation) at an interval of 15, 30, 45, 60 and 90 DAS. The nitrogenase activity was obtained by measuring the ethylene by oxidizing it to formaldehyde and determining the formaldehyde colorimetrically. Oxidant solution which was prepared by mixing 80 ml of 0.05 m $NaIO_4$ and 10 ml of 0.005 M $KMnO_4$ pH adjusted to 7.5 using KOH and diluted to 100 ml about 1.5 ml of oxidant solution kept in a conical flask sealed with rubber serum cap. The Chamber from which the nodules were incubated taken and about 5 ml of gas containing 1µ mole of ethylene was transferred to the conical flask where oxidant solution was kept and agitated using shaker at 300 rpm for about 90 minutes. Then about 0.25 ml of 4 M $NaAsO_2$ and 0.25 ml of 4 N sulphuric acid were added for destroying the excess oxidant. One ml of Nash reagent was added and absorbance was measured at 412 nm after 60 minutes. The absorbance was proportional to ethylene from 0.1 µ mole to 1 µ mole $sample^{-1}$.

(Larue and Kurz, 1972). The nitrogenase activity was measured at intervals of 30, 60 DAS and at harvest in Pendimethalin plots and at 0, 15, 30, 60 DAS and at harvest in Imazethapyr plots. At maturity, grain yields and haulm yields from the net plot in different treatments were recorded after thorough sun drying and the yields were expressed in kg ha^{-1}.

3. Results and Discussion

The total number of nodules $plant^{-1}$ increased up to 60 DAS with a rapid increase between 30 and 60 DAS. After 60 DAS the increase in nodule number was low up to 90 DAS (Table 1 and Figure 1-4). Application of Pendimethalin doses did not adversely affect the total nodule count or active nodule count and application Imazethapyr at double dosage resulted in significantly lower total nodule and active nodule up to 45 DAS. Thereafter, the total number of nodules increased in Imazethapyr treatments. Even though the total or active nodules were significantly higher up to 45 DAS in Pendimethalin treatment in the later stages of the crop growth increase in nodule number was lesser compared to Imazethapyr treatments which could be due to increased weed competition after 45 DAS in these treatments. Un-weeded control treatment recorded lowest number of total nodule and active nodules throughout the crop growth period. *Rhizobium* seed inoculation had positive impact on total number of root nodules up to 45 DAS and positive effect could be witnessed in case of active nodules through crop growth period. Fox et al. 2004 reported that some pesticide can mimic naturally occurring biochemical's and thereby interfere with natural biochemical signalling process between Rhizobia and host plants as a consequence early nodulation events were disrupted. Pahwa and Prakash (1992) also reported similar variations in nodule number and fresh weight of nodule in Pendimethalin treatments compared to control plots. Chaudary and Abbasi (2000) reported inferior nodule number in groundnut with the increase in dosage of Pendimethalin from 0.75 kg ha^{-1} to 1.5 kg ha^{-1}. In the present study also it was observed that Pendimethalin application at both doses did not have any adverse influence on nodule initiation.

However, the weed control efficiency of the Pendimethalin reduced with increase in the age of the crop resulting in higher weed competition, lower nutrient supply to the symbiotic *Rhizobium* which might have consequently resulted in lower nodulation at 60 and 90 DAS. In the no herbicide treatment plant intensive competition between weeds and crop plants might have resulted in low nodulation. Gonzalez et al. (1996) reported that, the action of herbicides on Rhizobia could involve inhibition of initial steps of symbiosis process and

Table 1: Number of active nodules and total nodules at different intervals as influenced by inoculation and herbicide treatments

Treatment	30 DAS		45 DAS		60 DAS		90 DAS	
	Active Nodule	Total Nodule	Active Nodule	Total Nodule	Active Nodule	Total Nodule	Active Nodule	Total Nodule
M_1S_1	4.2	6.2	9.8	16.9	20.9	21.4	22.1	26.7
M_1S_2	7.3	10.6	17.4	29.3	28.3	36.9	30.9	41.5
M_1S_3	6.7	9.8	17	28.6	30.2	38.2	29.3	40.8
M_1S_4	5.1	7.4	14	24.2	32.4	40.7	31.6	43.1
M_1S_5	4.0	5.8	11.2	20.6	34.6	42.1	30.8	42.3
Mean	5.4	7.9	13.8	23.9	29.2	35.8	28.9	38.8
M_2S_1	6.7	9.8	13.5	22.8	22.4	26.8	22.8	27.5
M_2S_2	8.3	12.8	23.9	36.3	32.8	40.8	31.4	43.2
M_2S_3	8.7	12.7	23	35.7	33.6	41	30.2	42.6
M_2S_4	6.0	8.9	22.6	34.3	37.4	40.8	31.4	43.8
M_2S_5	4.9	7.2	15.4	28.8	35.4	41.5	30.2	43.2
Mean	6.9	10.2	19.7	31.6	31.3	38.1	29.2	40.6
SEm±	0.05	0.64	0.89	0.10	0.75	0.85	0.17	0.45
SEm±	0.41	0.97	0.71	1.14	1.52	0.67	0.84	1.17
SEm±	0.12	1.44	1.98	0.22	1.67	1.92	0.38	1.02
SEm±	0.51	1.39	1.27	1.44	2.06	1.20	1.08	1.56
CD (p=0.05) Main factor	0.32	1.21	5.48	0.62	N.S	N.S	N.S	N.S
CD (p=0.05) Sub-factor	1.21	2.92	2.14	3.42	4.55	2.01	2.53	3.51
CD (p=0.05) S×M	NS	NS	NS	NS	NS	NS	NS	NS
CD (p=0.05) M×S	NS	NS	NS	NS	NS	NS	NS	NS

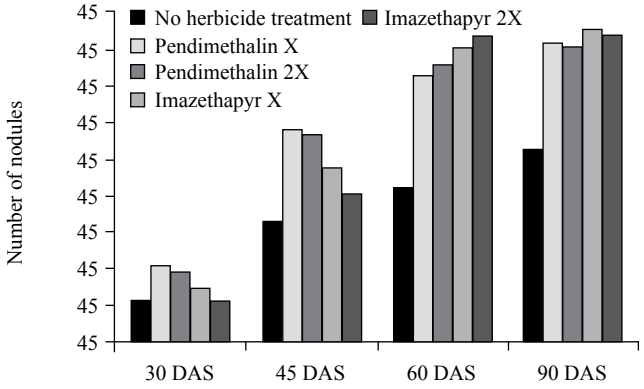

Figure 1: Influence of herbicide on total nodules of main treatment M_1 at different intervals

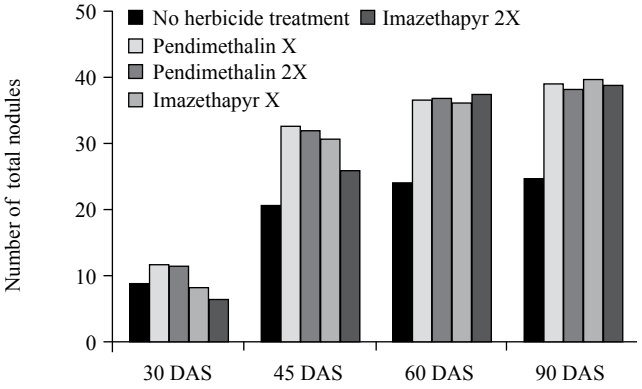

Figure 2: Influence of herbicide on total nodules of main treatment M_2at different intervals

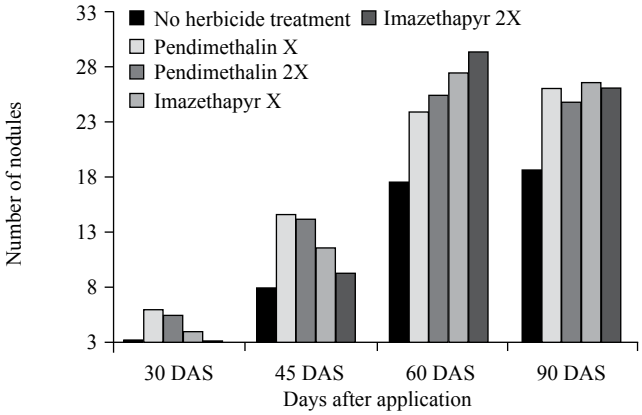

Figure 3: Influence of herbicide on active nodules of main treatment M_1 at different intervals

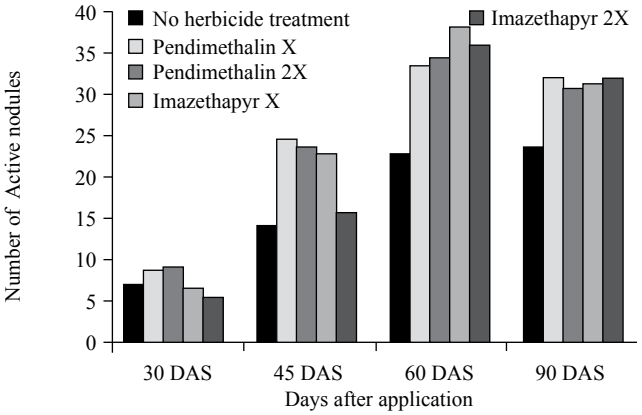

Figure 4: Influence of herbicide on active nodules of main treatment M_2 at different intervals

interfere with chemo-tactical mobility between legume root and bacteria which could disrupt sequential exchange of signals both parameters. In the study also the toxic effects of Imazethapyr when applied at double the recommended dose were evident from the phytotoxic observations. This phyto-toxic effect in combination with disrupted signalling process between plant and *Rhizobium* might have resulted in impaired nodulation in earlier stages after Imazethapyr application (30-45 DAS). As the herbicide induced toxicity reduced with the increasing age of the crop the crop recovered and the nodule number recorded in the later stages of the crop growth have also improved (60 and 90 DAS). Increase in the nodulation at 60 and 90 DAS in the Imazethapyr higher dose treatments can be attributed to the efficient weed control achieved in these plots and the reducing herbicide induced toxicity with the increasing age of the crop.

At 30 DAS, lowest nitrogenase activity was observed in Imazethapyr double dose treatment which was significantly lower compared to all other herbicide treatments but at par with no-herbicide treatment, which indicated the negative impact of Imazethapyr on nitrogenase activity. However, at 45 DAS the nitrogenase activity in Imazethapyr double dose treatment was statistically on par with the recommended dose (S_4). But, the nodule nitrogenase activity observed in Pendimethalin treatments (S_2 and S_3) was significantly superior over Imazethapyr double dose (S_5), at 45 DAS. At 60 DAS, significantly increased nitrogenase activity in Imazethapyr treatments (S_4 and S_5) compared to Pendimethalin treatments (S_2 and S_3) and no herbicide treatment (S_1) was noticed. Influence of *Rhizobium* seed inoculation was non-significant on root nodule nitrogenase activity. Significant reduction in nodule nitrogenase activity was observed in all the treatments at harvest stage of the crop. Sawicka and Selvet (1998) concluded that, Imazethapyr application as post emergence herbicide lowered the nitrogenase activity and in some cases the reduction was about 90% of the un-sprayed plots. The phyto-toxicity symptoms exhibited by the plants also indicate herbicide induced stress on the plant might have hampered the symbiotic relationship between plant and *Rhizobium*. In the advanced stages of the crop growth (60 DAS onwards) the increased weed competition in Pendimethalin sprayed plots might have resulted in stress on nutrient supply to the symbiont consequently resulting in reduced nitrogenase activity.

Highest pod yield was recorded with Imazethapyr at

recommended dosage of inoculated main treatment. *Rhizobium* seed inoculation did not influence the pod yield. In spite of better weed control in Imazethapyr double dose treatment the phyto-toxicity observed during 1-2 weeks after spray might have resulted in lower nodulation, nitrogen fixation and consequently lower yields (Table 3). Lowest mean pod yield was recorded in no herbicide sprayed plots (730 kg ha^{-1}). Even though the crop establishment was in relatively weed free situation in Pendimethalin treatments compared to Imazethapyr treatments, reduced weed control efficiency during later stages of crop growth and consequent higher weed competition might have adversely affected the supply of nutrients to sink. Interaction was found to be non significant. Similar trend was noticed with haulm yield. *Rhizobium* seed inoculation did not significantly influence the haulm yield. Highest haulm yield was noticed in Imazethapyr double the recommended dose (2610 kg ha^{-1}). Least haulm yield was found in no herbicide treatment (575 kg ha^{-1}).

Table 2: Effect of inoculation and herbicide treatments on Nitrogenase activity (µ mole plant^{-1} hr^{-1}) at 30, 45, 60 DAS and harvest.

Treatments	Days after application (DAS)			Harvest
	30	45	60	
Inoculation treatments				
Un-inoculated	3.52	7.1	10.92	1.23
Inoculated with *Rhizobium*	3.7	7.62	11.68	1.25
SEm±	0.07	0.11	0.270	0.01
CD (*p*=0.05)	NS	NS	NS	NS
Herbicide treatments				
S_1	3.05	4.750	7.500	1.223
S_2	4.40	8.600	11.200	1.245
S_3	4.00	8.200	10.900	1.235
S_4	3.80	7.750	13.000	1.260
S_5	2.80	7.500	13.900	1.252
SEm±	0.11	0.17	0.19	0.02
CD (*p*=0.05)	0.33	0.51	0.58	NS
Inoculation at same level of herbicide				
SEm±	0.15	0.25	0.36	0.03
CD (*p*=0.05)	NS	NS	1.73	NS
Herbicides at same level of inoculation				
SEm±	0.15	0.25	0.60	0.03
CD (*p*=0.05)	NS	NS	1.31	NS

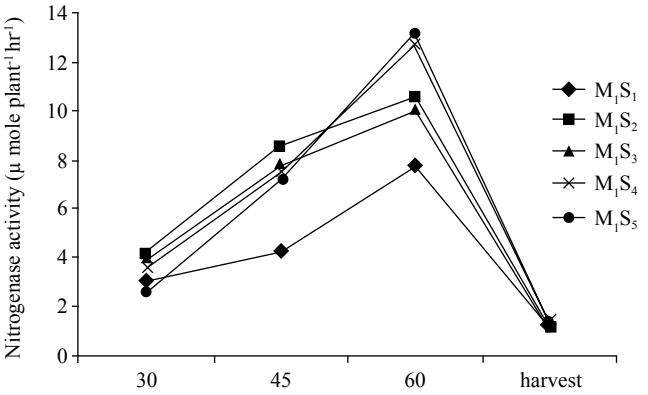

Figure 5: Influence of herbicide treatment on Nitrogenase activity (µ mole C_2H_4 plant^{-1} hr^{-1}) of main treatment M_1 at different intervals

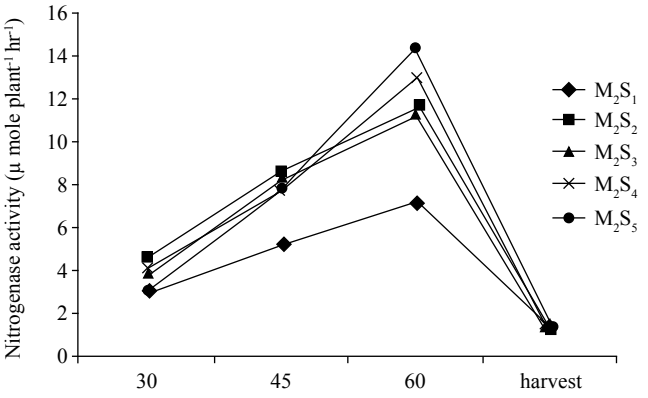

Figure 6: Influence of herbicide treatment on nitrogenase activity (µ mole C_2H_4 plant^{-1} hr^{-1}) of main treatment M_2 at different intervals

Table 3: Pod yield and haulm yield of groundnut as influenced by inoculation and herbicide treatments

Inoculation treatments (M)	Pod yield (in kg ha^{-1})						Haulm yield (in kg ha^{-1})					
	Herbicide treatments (S)					Mean	Herbicide treatments (S)					Mean
	S_1	S_2	S_3	S_4	S_5		S_1	S_2	S_3	S_4	S_5	
Un-inoculated (M_1)	640	1490	1540	2120	2080	1574	560	2025	2160	2440	2590	1955
Inoculated (M_2)	820	1610	1640	2285	2160	1703	590	2080	2265	2460	2630	2005
Mean	730	1550	1590	2202	2120		575.0	2052.5	2212.5	2450	2610	
	SEm±			CD (*p*=0.05)			SEm±			CD (*p*=0.05)		
Main (M)	21.80			N.S			38.3			N.S		
Sub (S)	37.63			112.8			27.7			83.3		
S at same level of M	48.74			N.S			85.8			N.S		
M at same level of S	52.35			N.S			52.0			N.S		

4. Conclusion

Application of Pendimethalin doses did not adversely affect either total or active nodule count. But, application of Imazethapyr at double dosage resulted in significantly lower total nodule and active nodule count up to 45 DAS, also adversely affected the nitrogenase activity up to 45 DAS which later on recovered from 60 DAS. Despite better weed control achieved in Imazethapyr double dose treatment, highest pod yield was recorded in Imazethapyr at recommended dose where seed inoculation was done compared to Imazethapyr double dose.

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