Growth Behavior, Nodulation and Rhizobium Population, as Affected by Combined Application of Herbicide and Insecticide In Soybean (*Glycine max* L.)

Dujeshwer Kurrey[1], Rajendra Lakpale[1] and Rahul Singh Rajput[2]

[1]Department of Agronomy, Indira Gandhi Krishi Vishwavidyalaya., Raipur - 492 012, India.
[2]Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya., Raipur - 492 012, India.

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A field experiment was conducted at Research Farm of IGKV, Raipur during *Kharif* 2013 to evaluate the combined effect of herbicide and insecticide on growth, nodulation and Rhizobium population in soybean. All the herbicidal treatments recorded significantly at par to improving growth characters viz. LAI, CGR and RGR. Highest Nodule number (74.2 plant^{-1}) and nodule dry weight (74.2 mg plant^{-1}) was resulted by Quizalophop ethyl 5 EC @ 1.0 l ha^{-1} as sole or combination with insecticides. Whereas all the pesticidal treatments found negative impact on microbial count which was recorded superior (62.1 x10^{6} g^{-1} soil) under Untreated Check. The highest seed yield (2323 kg ha^{-1}), stover yield (2943 kg ha^{-1}), net income (63655 ^{1} /ha) and B:C ratio (3.09) was recorded under Imazathapyr 10 SL@1.0 l ha^{-1}.

Keywords: Herbicide, Insecticide, Soybean, nodulation and Rhizobium.

Soybean is one of the most important leguminous oil seed crops of great economic value, occupying an important position in the world trade as it is important in the soil by fixing atmospheric nitrogen through *Rhizobium* bacteria that lives in their root nodules (Stewart 2009). In Chhattisgarh, soybean occupies 0.147 million ha with production of 0.134 million tone and average productivity of 915 kg ha^{-1} (www.sopa.org/REK2014.pdf, 2014). It grows well during the kharif or monsoon, season (July-October) in the dry-land areas of peninsular India. In kharif season due to continuous rains there will be high weed infestation and high weed competition is one of the most of important causes of yield loss in soybean and is estimated to be 22-77 % [Kuruchania *et al.*, 2001]. The costly and unavailability of labours coupled with unfavourable weather conditions offer an opportunity for the chemical weed control. (Amaregonda *et al.*, 2013). Soybean is very much susceptible to insect attack from seedling to mature stage. All parts of the plant including plant leaves, stems and pods are subjected to attack by different species of insect in India. Both the constraints drastically reduces the growth of the soybean results in lower crop yield. The *Rhizobium* inoculants are commonly applied to seeds of legume crops to ensure effective nitrogen fixation by *Rhizobium*, thereby making the one essential nutrients available to the crop. The use of pesticides has become an integral and economically essential part of agriculture. There are reports which suggest that herbicides when applied indiscriminately have variable effects on legume Rhizobium symbiosis (Khan *et al.*, 2004). Herbicides may have negative effects on growth of rhizobia. Considering the above facts we tried

* To whom all correspondence should be addressed.
E-mail: dkurrey73@gmail.com

to control the weed and pests in a single spray and to evaluate the effect on growth behavior, nodulation and Rhizobium count in soybean.

MATERIALS AND METHODS

A field experiment was conducted to evaluate the effect of herbicide and insecticide combination on growth, nodulation and Rhizobium population in soybean at Instructional cum Research Farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during *kharif* 2013. The experiment was laid out in Randomized Block Design (RBD) with four replication and twelve treatments which included rynaxypyre 20 EC @100 ml ha^{-1}, indoxacarb 14.5 EC @ 300 ml ha^{-1}, quinolphos 25 EC @1.5 l ha^{-1}, imazathapyr 10 SL @1.0 l ha^{-1}, quizalophop ethyl 5 EC @ 1.0 l ha^{-1} as alone and with combination of herbicide and insecticide and Untreated Check. All the treatments were applied at 20 DAS (Day after sowing) as a tank mix at time of spraying. Soybean variety JS-335 was sown with spacing of 30 X 7 cm and seed rate of 65 kg ha^{-1} was used. Seed was treated by Rhizobium culture @10 g kg^{-1} seed at the time of sowing. The study on Leaf area index observed at 30, 60 and 90 DAS. The leaf area existing on unit area was proposed by Watson (1952) as an appropriate measure of crop growth. This measures is known as leaf area index. It is dimensionless ratio and calculated by following formula-

$$\text{Leaf area index (LAI)} = \frac{\text{Total leaf area of plant}^{-1}\ (\text{cm}^2)}{\text{Total ground area of plant}^{-1}\ (\text{cm}^2)}$$

Crop growth rate was calculated from the dry weight taken at different time intervals. It denotes overall growth rate of the crop plant and it is measured after fix period of the time, irrespective of the previous growth rate. The value was calculated by using the following formula suggested by Leopold and Kridermann (1975)-

$$\text{Crop growth rate (CGR)}\ (\text{g plant}^{-1}\ \text{day}^{-1}) = \frac{W_2 - W_1\ (\text{Difference in oven dry biomass at the time interval})}{t_2 - t_1\ (\text{Time interval in days})}$$

The relative growth rate indicates the increase in dry weight per unit of original dry weight over any specific time interval. The values were computed by using the following formula suggested by Leopold and Kridemann (1975)-

$$\text{Relative growth rate (RGR)}\ (\text{g g plant}^{-1}\ \text{day}^{-1}) = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Where, ln = Logarithm at base (natural log)

The number of nodules were recorded from three randomly selected plants in each plot. The uprooting of sample were preformed with the help of core cutting equipment along with the soil upto effective root zone. The roots of the plant were washed in sieve with running water and effective root nodules were separated and counted. The counted nodules were dried at 60°C for 48 hours in hot air oven thereafter dry weight of nodules was recorded by an electronic digital balance and average dry weight of nodule $plant^{-1}$ was worked out. Analysis of rhizobium population was done by serial dilution plating method (Subba Rao 1988). The sampling of soil was done from 5-10 cm depth at, 50 DAS. Yield and yield attributes were recorded at harvest. The economics of soybean crop production pertaining to each of the treatment has been worked out in terms of cost of cultivation. Gross return (Rs. ha^{-1}) was obtained by converting the harvest into monetary terms at the prevailing market rate during the course of studies for every treatment. Net return (Rs. ha^{-1}) was obtained by deducting cost of cultivation from gross return.

RESULTS AND DISCUSSION

Effect on growth behavior

Leaf area index

Leaf area index (LAI) is the important physiological parameter for growth and yield. LAI of soybean showed increasing trend upto 60 DAS at higher pace and there after increased at slower pace (Fig. 1). Maximum LAI was recorded under the treatment Rynaxypyre 20 EC @100 ml ha^{-1} + Quizalophop ethyl 5 EC @ 1.0 l ha^{-1}, followed by Imazathapyr 10 SL @1.0 l ha^{-1} and Rynaxypyre 20 EC @100 ml ha^{-1} + Imazathapyr 10 SL @1.0 l ha^{-1}. Minimum LAI was recorded under the treatment of Indoxacarb 14.5 EC @ 300 ml ha^{-1}. Increased leaf area might have enhanced the photosynthesis due to which plant dry matter accumulation was higher under these treatments. There was lower weed competition in terms of dry matter of weeds, higher number of branches, leaves and suitable environment might led to higher value of leaf area, which allowed soybean to absorb required amount of nutrient, water and sunlight for expanding the leaf to the plant potential. Similar trends were also

Table 1. Effect of herbicide and insecticide on nodulation and Rhizobium population in soybean

Treatments	Number of nodule plant^{-1}		Dry weight of nodule population (mg plant^{-1})		Rhizobium (x 10^6 g^{-1} soil)
	40 DAS	60 DAS	40 DAS	60 DAS	50 DAS
T$_1$-Rynaxypyre 20 EC @ 100 ml/ha	30.3	55.4	150	520	43.9
T$_2$- Indoxacarb 14.5 EC @ 300 ml/ha	29.4	54.3	190	510	45.4
T$_3$- Quinolphos 25 EC @ 1.5 l/ha	27.9	61.3	180	510	51.1
T$_4$- Imazathapyr 10 SL @ 1.0 l/ha	35.4	69.3	160	640	52.8
T$_5$- Quizalophop ethyl 5 EC @1.5 l/ha	29.0	74.2	160	560	33.5
T$_6$- Rynaxypyre 20 EC @ 100 ml/ha + Imazathapyr 10 SL @ 1.0 l/ha	28.9	56.1	150	480	39.3
T$_7$- Rynaxypyre 20 EC @ 100 ml/l + Quizalophop ethyl 5 EC @ 1.0 l/ha	40.0	71.2	200	560	45.8
T$_8$- Indoxacarb 14.5 EC @ 300 ml/ha + Imazathapyr 10 SL @ 1.0 l/ha	28.1	70.1	140	580	50.9
T$_9$- Indoxacarb 14.5 EC @ 300 ml/ha+ Quizalophop ethyl 5 EC @ 1.0 l/ha	25.4	72.4	160	560	58.2
T$_{10}$- Quinolphos 25 EC @ 1.5 l/ha + Imazathapyr 10 SL 1.0 l/ha	32.4	66.0	160	570	50.8
T$_{11}$- Quinolphos 25 EC @ 1.5 l/ha + Quizalophop ethyl 5 EC @ 1.0 l/ha	30.1	65.7	170	760	48.1
T$_{12}$-Untreated check	29.0	62.1	170	580	62.1
SEm (±)	1.4	3.5	10.0	50.0	2.1
CD (P=0.05)	4.1	10.0	NS	140.0	6.1

Table 2. Effect of herbicide and insecticide on yield and economics in soybean

Treatment	Seed yield (kg/ha)	Stover yield (kg ha^{-1})	Harvest index (%)	Net income (¹ /ha)	B:C ratio
T$_1$- Rynaxypyre 20 EC @ 100 ml/ha	1550	2171	41.67	36828	1.88
T$_2$- Indoxacarb 14.5 EC @ 300 ml/ha	1513	2031	42.70	35785	1.86
T$_3$- Quinolphos 25 EC @ 1.5 l/ha	1548	2213	41.20	37540	1.99
T$_4$- Imazathapyr 10 SL @ 1.0 l/ha	2323	2943	43.84	63655	3.09
T$_5$- Quizalophop ethyl 5 EC @1.5 l/ha	2201	2684	45.09	60026	3.05
T$_6$- Rynaxypyre 20 EC @ 100 ml/ha + Imazathapyr 10 SL @ 1.0 l/ha	2205	2756	44.28	57938	2.63
T$_7$- Rynaxypyre 20 EC @ 100 ml/l + Quizalophop ethyl 5 EC @ 1.0 l/ha	2247	2835	44.24	60387	2.86
T$_8$- Indoxacarb 14.5 EC @ 300 ml/ha + Imazathapyr 10 SL @ 1.0 l/ha	2049	2612	43.77	52726	2.44
T$_9$- Indoxacarb 14.5 EC @ 300 ml/ha+ Quizalophop ethyl 5 EC @ 1.0 l/ha	2030	2484	47.11	52833	2.55
T$_{10}$- Quinolphos 25 EC @ 1.5 l/ha + Imazathapyr 10 SL 1.0 l/ha	2254	2887	43.94	60524	2.85
T$_{11}$- Quinolphos 25 EC @ 1.5 l/ha + Quizalophop ethyl 5 EC @ 1.0 l/ha	2255	2845	44.29	61417	3.02
T$_{12}$- Untreated check	1521	1978	43.50	37270	2.08
SEm (±)	138	145	1.39	-	-
CD (P=0.05)	381	416	NS	-	-

recorded by (Amaregonda *et al.*, 2013).

Crop growth rate

Crop growth rate showed increasing trend upto 60 DAS and declined thereafter till harvest depicted in Fig. 2. During 0-30 DAS, comparatively similar crop growth rate value were recorded under all the treatments. After 30 DAS, maximum crop growth rate was recorded under Imazathapyr 10 SL @1.0 l ha^{-1} followed by Quizalophop ethyl 5 EC @ 1.0 l ha^{-1}. Minimum crop growth rate was observed under non herbicidal treatments during entire growth period in soybean. Declined crop growth rate was caused by senescence of leaves probably owing to competition from weeds for solar radiation and also due to density of weeds higher in these periods. (Jakhar and Sharma, 2015) also reported that weed free plots recorded higher value of CGR.

Relative growth rate

Relative growth rate (RGR) increased at higher pace from sowing to 90 DAS thereafter increased at slower pace (Fig. 3). The rate of RGR was recorded differently under different period of time. During 0-30 DAS, numerically maximum RGR was observed under treatment Indoxacarb 14.5 EC @ 300 ml ha^{-1} + Imazathapyr 10 SL @1.0 l ha^{-1} but During 30-60 DAS, maximum RGR was observed under treatment Quizalophop ethyl 5 EC @ 1.0 l ha^{-1}. Rynaxypyre 20 EC @100 ml ha^{-1} recorded highest RGR during 60-90 DAS and Untreated Check recorded highest from 90 DAS to at harvest. Relative growth rate of soybean in above treatments was higher because of comparatively less crop-weed and pest competition. The increased sink size, stored the photosynthates very effectively and ultimately transformed in the shape of more dry matter accumulation which resulted in higher relative growth rate.

EFFECT ON NODULATION

The applied herbicides did not show adverse effects on the number and dry weight of root nodules reported earlier by (Jha *et al*, 2014) and depicted in (Table 1). At 40 DAS, significantly maximum number of root nodules and dry weight of nodule was observed under treatment of Rynaxypyre 20 EC @100 ml ha^{-1} + Quizalophop ethyl 5 EC @ 1.0 l ha^{-1}, however at 60 DAS, Quizalophop ethyl 5 EC @ 1.0 l ha^{-1} recorded highest root nodule which was at par with all herbicidal treatments but Quinolphos 25 EC @1.5 l ha^{-1} + Quizalophop ethyl 5 EC @ 1.0 l ha^{-1} recorded highest dry weight of nodule. Increased number of root nodules and dry weight may be due to the favorable microclimate after suppression of weeds near the root zone and greater infection of *Rhizobium* in the growing roots of soybean crop. Higher nodulation fixed the atmospheric nitrogen which ultimately supported in higher crop growth of soybean. (Jha *et al*, 2014) and (Kandaki *et al*, 2015) reported that weed free treatments enhances nodule number and nodule dry weight.

Effect on Rhizobium population (x 10^6 g^{-1} soil)

It is reported that application of pesticides both in crop and soil is known to affect microbial activity. (Shankar *et al*. 2012). Significantly

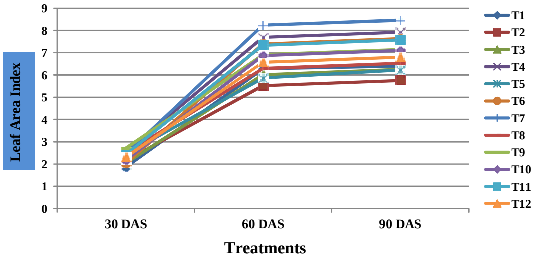

Fig. 1. Leaf area index as affected by combined use of herbicide and insecticide

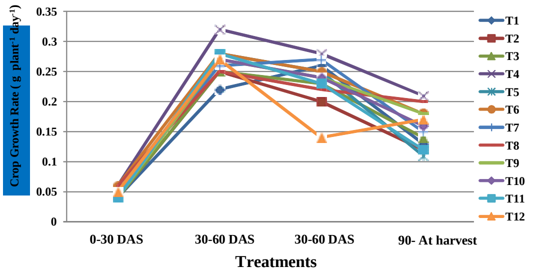

Fig. 2. Crop growth rate (g plant $^{-1}$ day^{-1}) of soybean as affected by combined use of herbicide and insecticide

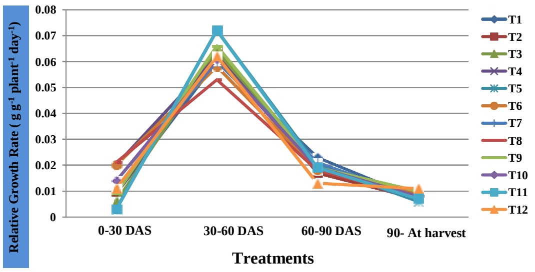

Fig. 3. Relative growth rate (g g^{-1} plant $^{-1}$ day^{-1}) of soybean as affected combined use of herbicide and insecticide

maximum *rhizobial* population was observed under treatment Untreated Check (Table 1), which was at par with treatment Indoxacarb 14.5 EC @ 300 ml ha^{-1} + Quizalophop ethyl 5 EC @ 1.0 l ha^{-1}. The highest *Rhizobial* population observed under Untreated Check might be due to the provision of food in the form of organic matter by crop as well as weeds and by secretion of organic acids, beneficial for *rhizobium* bacteria. Similar findings were also reported by Gupta *et al.* (2013).

Effects on yield and economics

All herbicidal treatment significantly increased the yield and yield component like seed yield, straw yield, harvest index, net income and B:C ratio in soybean (Table 2). The significantly highest seed yield, straw yield, net income and B:C ratio was recorded under Imazathapyr 10 SL @1.0 l ha^{-1} which was comparable with all herbicidal treatments. However Indoxacarb 14.5 EC @ 300 ml ha^{-1} + Quizalophop ethyl 5 EC recorded highest harvest index. The higher seed yield under this treatment were might be due to better efficacy of herbicide at initial stage of crop growth providing weed free environment to the crop. Similar results was also reported by Venkatesha *et al*. (2008), Goud *et al*. (2013) and Sangeetha *et al*. (2013).

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