EFFECT OF PRE-EMERGENCE APPLICATION OF SOME HERBICIDES ON NODULATION, NITROGEN FIXATION AND GROWTH OF SOYBEAN[1]

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ABSTRACT - Aretit at the recommended rate (XI) and treflan, especially at high concentration (x 5 folds), stimulated nodulation of soybean 'Clark', while planavin, cobex or linuron, especially at high concentration inhibited nodule formation and even, in some instances, prevented plant growth. In all herbicidal treatments, the dry matter content of whole plant on the second harvest (92 days or pod filling) was lower, even those which showed stimulation at the first harvest (49 days or full bloom) than the control; the yields of pods obtained on maturity were also lower. In general, total-N contents of shoots, roots and pods as well as yield of total-N seemed to be affected by the application of herbicides. The effect differed according to herbicides concentration and growth stages of the plant. Nitrogenase activity as a measure of biological fixation of atmospheric nitrogen using the acetylene-reduction technique was also carried out for root nodules to investigate effect of aretit or treflan on the symbiotic N-fixation processes. The dehydrogenase (DHA) activity in nodule homogenate was also determined in presence or absence of succinate, citrate or ethanol to investigate the effect of herbicidal treatments on respiratory chain of bacteroids. This was claimed to thraw light on effect of herbicidal treatments on capacity of the legume plant (soybean 'Clark') to supply the symbiont (*R. japonicum*) in the root nodules with some products (hydrogen doners) of the Kreb's cycle and consequently thus affects nitrogen fixation efficiency of root nodules.

EFEITO DA APLICAÇÃO DE ALGUNS HERBICIDAS À PRÉ-EMERGÊNCIA SOBRE A NODULAÇÃO, A FIXAÇÃO DE NITROGÊNIO E O CRESCIMENTO DA SOJA

RESUMO - O aretit na dose recomendada (x1), e o treflan, mormente em alta concentração (5 dobras), estimularam a nodulação da soja 'Clark'. O planavin ou o linuron, mormente em alta concentração, inibiram a formação de nódulos e, em certos casos, até anteciparam o crescimento da planta. Em todos os tratamentos com herbicidas, a quantidade de matéria seca na segunda colheita (92º dia, período de enchimento da vagem) foi menor do que na testemunha, mesmo em se tratando de plantas que mostraram estimulação na primeira colheita (49º dia, plena floração). Também as produções de vagens foram menores. Em geral, parece que os conteúdos de N-total dos brotos, raízes e vagens e a própria produção de N-total são favorecidos com o uso de herbicidas. O efeito diferiu de acordo com o grau de concentração dos herbicidas e de acordo também com o estádio de desenvolvimento da planta. Para investigar o efeito do aretit ou do treflan sobre os processos simbióticos de fixação de nitrogênio, foi efetuada, para os nódulos radiculares, no 49º e 92º dias, a atividade de nitrogenase, como medida de fixação biológica de nitrogênio atmosférico. Também foi efetuada a atividade de deidrogenase (DHA) em nódulo homogeneizado, em presença ou ausência de succinato, citrato, ou etanol, para investigação do efeito dos tratamentos com herbicidas na corrente respiratória dos bacteróides. Ela foi necessária para se esclarecer o efeito dos tratamentos com herbicidas sobre a capacidade da soja 'Clark' de fornecer o simbionte (*R. japonicum*) nos nódulos radiculares com alguns produtos (doadores de hidrogênio) do ciclo Kreb e, conseqüentemente, de tornar mais eficiente a fixação de nitrogênio por parte dos nódulos radiculares.

INTRODUCTION

The sensitivity of soybean plants to mechanical control of weeds and the rapid increase in the labour loans has obviously brought the application of herbicides to forefront. On the other hand, herbicides may affect the beneficial activities of soil micro-organisms, e.g. the symbiotic nitrogen fixation (Dunigan et al. 1970 and 1972; Gafar 1976 and Herzallah 1980).

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The aim of the present study, however, is to gain some informations about effect of pre-emergence application of some herbicides (at different rates) on nodulation, nitrogen fixation and growth of soybean.

MATERIALS AND METHODS

A pot experiment was carried out (in triplicates) using 12 kg portions of Nile silt soil amended with 5 g calcium superphosphate/pot. Six seeds of soybean (*Glycine max*) variety 'Clark' obtained from the Department of Legume Crops Res., Ministry of Agric., Giza, Egypt., were planted to each pot. Inoculation with *Rhizobium japonicum* strain 3407 "CB 1802" (kindly supplied by Dr. P.S. Nutman, Dept. Soil Microbiol., Rothamsted Expt. St., U.K.) and grown in medium "79" broth (Allen 1959) for seven days was carried out directly after planting using 20 ml/pot.

Five herbicides namely aretit (dinoseb, acetate), planavin (nitralen), cobex, linuron (afalon, larox) or treflan (triffuralin) were chosen to represent the most recommended for pre-emergence application to soybean in Egypt (Herzallah 1980). Each herbicide was applied one day after sowing either at the field rate of application, twice or five-folds. The pots were kept in a wire proof greenhouse and watered when necessary. After three weeks, the plants were thinned to three seedlings/pot. After 49 or 92 days of sowing (full bloom stage or pod filling) the plants were gently uprooted and the following determinations were carried out:

1. Numbers of nodules, dry weight of shoots, roots and pods;
2. Total-N contents of shoots, roots or pods using the micro-Kjeldahl method (Jackson 1958);
3. Nitrogenase activity of root nodules using the acetylene-reduction technique (Dart 1972); a PYE UNICAM gas liquid chromatograph model 104 was used;
4. Dehydrogenase activity of root nodule homogenate in presence or absence of succinate, citrate or ethanol (Alaa-Eldin et al. 1978).

The mature dry pods were collected weighed and analyzed for total-N content.

RESULTS AND DISCUSSION

Data on effect of pre-emergence herbicides (applied either at field-rate x 1 or x 5 folds) namely aretit, planavin, cobex, linuron or treflan on numbers of nodules, growth (dry matter) and yield of pods of soybean 'Clark' are presented in Table 1. The results generally indicated the presence of high numbers of nodules on roots of soybean (49 or 92 days) grown in pots treated with either aretit at field application (x 1) or treflan, especially the high concentration (x 5 folds), as compared with control. Stimulative effect of the two herbicides on plant growth, as indicated by the detection of higher dry matter content of whole plants on the 49th day after soybean sowing (but not on 92nd day) was also observed. On the other hand, all other herbicides, especially at high concentrations including aretit (x 5 folds) seemed to be inhibitory to nodule formation and even in some instances, i.e. cobex or linuron prevented plant growth. These results, however, may agree with those of Lucietta (1969), Tewfik et al. (1975) and Gafar (1976) who observed that treflan at concentrations up to (6L/Feddan = 4.200 m^2) did not toxicate the nodule bacteria and its efficiency to fix nitrogen, while linuron even at lower rates (0.375 kg/Feddan) was of harmful effect. In all pre-emergence herbicides treatments, the dry matter content of soybean plant on the second harvest (92 days) were lower, even those which showed stimulation on the first harvest (49 days), than the control. Furthermore, the yield of soybean pods (numbers and dry weight) obtained on maturity (Table 1) were lower as compared with control. This may be due to the inhibitory effect of the secondary by-products of degraded herbicides which some times are more toxic to plants and micro-organisms than the original herbicide (Rahal 1979).

Total-N contents in shoots, roots (and pods) as well as yield of total-N in whole plants (mg/pot) were determined after 49 or 92 days after sowing (Table 2) and in the final yield of dry pods (Table 3). The results generally show that on the second harvest (92nd day), the plant exhibited lower content of nitrogen in shoots and roots as compared with first harvest (49th day). This may be attributed to translocation of nitrogen from shoots and roots into the pods as indicated by the presence of higher yield of total-N (mg/pot) in the whole plant on the 92nd day than on the 49th day. In this respect, all herbicidal treatments showed lower yield of total-N as compared with control, with the exception of aretit at the recommended rate (on 49th day) and treflan at the high concentration (on 92nd day). On maturity, the yield of

TABLE 1. Effect of pre-emergence herbicides on numbers of nodules, growth (dry weight) and yield of pods of soybean 'Clark'.

Herbicides	Rate of applic. Fed.*	Number of nodules/pot				Dry matter of whole plant (shoots + roots)				Yield of pods/pot			
		49		92 days		49		92 days		Numbers		Dry weight	
		Numb.	% ±	Numb.	% ±	g/pot	% ±	g/pot	% ±	Numb.	% ±	Numb.	% ±
Control	0.0	188.3	± 0.0	259.3	± 0.0	22.1	± 0.0	70.0	0.0	78.3	± 0.0	17.5	± 0.0
Aretit	2 kg	216.0	+ 14.7	276.0	+ 6.4	26.2	+ 18.7	47.2	- 23.6	24.0	- 69.3	3.3	- 69.7
	10 kg	119.0	- 36.8	139.7	- 46.1	16.2	- 26.5	38.1	- 45.6	41.0	- 47.0	8.4	- 52.0
Planavin	1.5 kg	5.7	- 97.0	40.0	- 84.6	31.1	+ 40.6	14.2	- 79.7	60.0	- 23.4	16.5	- 5.7
	7.5 kg	1.3	- 99.3	0.0	- 100.0	1.1	- 95.1	-	-	No	Growth		
Cobex	1.5 L	58.3	- 69.0	81.0	- 68.8	13.3	- 39.9	32.6	- 53.4	15.0	- 80.8	3.6	- 79.4
	3 L	0.0	- 100.0	-	-	3.2	- 85.5	-	-	-	-	-	-
	7.5 L	0.0	- 100.0	-	-	-	-	-	-	-	-	-	-
Linuron	0.75 kg	51.0	- 72.9	174.0	- 32.9	14.2	- 35.8	29.0	- 58.5	46.7	- 40.4	10.5	- 40.0
	1.5 kg	26.0	- 86.2	-	-	7.2	- 67.4	-	-	51.0	- 34.9	14.0	- 20.0
	3.75 kg	0.0	- 100.0	-	-	-	-	-	-	-	-	-	-
Treflan	1 L	189.0	+ 0.4	201.3	- 22.4	20.3	- 7.9	44.8	- 35.9	40.7	- 48.0	10.7	- 38.9
	5 L	328.3	+ 74.3	324.0	+ 25.2	26.5	+ 20.2	55.2	- 21.2	60.3	- 23.0	14.7	- 16.0

* Feddan = 4.200 m^2 = Ca.one million kg to 6 inches depth.

TABLE 2. Total-N contents and yield of nitrogen of soybean 'Clark' as affected by pre-emergence herbicides.

Herbicides	Rate of applic. Fed.	Total-N contents % 49 shoots	49 roots	96 shoots	96 roots	pods	Yield of total-N of whole plant 49 mg/pot	49 % ±	92 mg/pot	92 % ±
Control	0.0	4.1	2.6	1.8	1.0	3.6	868.6	± 0.0	1392.7	± 0.0
Aretit	2 kg	3.5	2.2	2.8	1.4	3.2	871.3	+ 0.31	1259.2	- 9.6
	10 kg	3.4	1.8	2.9	1.5	3.5	494.2	- 43.1	1000.7	- 28.1
Planavin	1.5 kg	2.9	2.2	2.6	1.6	0.0	429.4	- 50.6	333.2	- 76.1
	7.5 kg	3.3	1.8	No	Growth		39.1	- 95.5	0.0	- 100.0
Cobex	1.5 L	3.3	1.7	3.2	1.0	3.5	413.5	- 52.4	899.1	- 35.4
	3.0 L	2.0	1.3	No	Growth		58.0	- 93.3		
Linuron	7.5 L	No	Growth							
	0.75 K	3.4	1.8	2.7	1.2	3.3	453.7	- 47.8	755.7	- 24.2
	1.5 K	2.8	1.3				168.1	- 80.6		
	3.75 K	No	Growth							
Treflan	1 K	3.0	1.8	2.7	1.2	3.3	566.6	- 34.8	1159.6	- 16.7
	5 L	2.5	1.4	2.9	1.2	3.4	586.6	- 32.5	1416.9	+ 1.7

TABLE 3. Total-N contents and yield of nitrogen in soybean pods as affected by pre-emergence herbicides.

Herbicides	Rate	% N	Yield of N mg/pot	Yield of N % ±
Control	0.0 kg	4.78	779.2	± 0.0
Aretit	2 kg	3.63	197.3	- 74.7
	10 kg	4.05	339.6	- 56.4
Planavin	1.5 kg	4.17	684.0	- 12.2
Cobex	1.5 L	3.90	140.4	- 82.0
Linuron	0.75 kg	4.20	447.5	- 42.6
	1.5 kg	4.61	645.5	- 17.2
Treflan	1 L	5.00	544.2	- 30.2
	5 L	4.13	602.4	- 22.7

total-N (mg/pot) as well as the percentage of total-N content in soybean pods were lower in all herbicidal treatments than in control. This is not in harmony with Dunigan et al. (1970 and 1972) and Gafar (1976), who reported that treflan at the recommended rate was not determined for N-accumulation. The authors, however, found that linuron, even at lower rates (0.375 kg/Fed.), inhibited growth and nitrogen fixation.

The nitrogenase activity as a measure of biological fixation of atmospheric nitrogen using the acetylene reduction technique was carried out for root nodules of soybean 'Clark' on 49th and 92nd day to investigate the effect of aretit or treflan (each applied either at field rate x 1 or x 5 folds) on the symbiotic-N fixation processes. The specific nitrogenase activity (μ mole C_2H_4/E fresh nodules/h) and the calculated nitrogen fixation rate (μgm N_2 fixed/pot/h) as well as percentages of losses or gains to the control untreated are presented in Table 4. On 49th day, aretit at the recommended field rate (x 1) stimulated N_2-ase activity of root nodules by + 7.3% over the control while the high dose (x 5) treatment showed increased values reached to + 43.8% over the control, although this high dose (x 5) of aretit decreased numbers of nodules "-36.8% lower than control" (Table 1). The rate of nitrogen fixation was generally lower in aretit (x 1 and x 5 folds) treatments (-35.5% or -17.8% respectively) than control. This may indicate that aretit negatively

TABLE 4. Nitrogenase activity (C_2H_4 - reduction) and dehydrogenase activity (μ mol H_2/g fresh nodules/h, difference to endogenous dehydrogenase) of nodule homogenate of soybean as affected by different pre-emergence herbicides (after 49 or 92 days after planting).

Herbicide	Rate of application	Nitrogenase				Endogenous absolute value	Dehydrogenase (Δ to endogenous)					
		μ mole C_2H_4/g fresh nodules/h		μg N_2 fixed per pot/h			Succinate		Citrate		Ethamol	
		Absolute	% ±	Absolute	% ±		Δ	%	Δ	%	Δ	%
						49 Days after planting						
Control	0.0	52.1	± 0.0	418.7	± 0.0	59.0	+10.3	+17.5	+ 5.8	+ 9.8	+13.2	+22.4
Aretit	2 kg	55.9	+ 7.3	269.9	- 35.5	28.9	+13.6	+47.1	- 0.6	- 2.1	+ 3.2	+11.1
	10 kg	74.9	+ 43.8	344.2	- 17.8	-	-	-	-	-	-	-
Treflan	1 L	65.0	+ 24.8	299.8	- 28.4	13.1	+ 8.0	+16.1	+ 6.0	+45.8	+ 5.7	+43.5
	5 L	135.4	+159.9	1132.0	+170.4	43.6	+ 7.9	+18.1	+ 1.4	+ 3.2	+ 0.0	+ 0.0
						92 Days after planting						
Control	0.0	42.4	± 0.0	1249.0	± 0.0	35.4	- 8.8	- 24.9	- 11.3	- 31.9	+13.0	+36.7
Aretit	2 kg	29.4	- 30.6	862	- 31.0	27.1	- 1.6	- 5.9	+11.8	+43.5	+ 2.7	+10.0
	10 kg	38.2	- 9.9	818	- 34.5	32.6	- 9.4	- 28.8	+ 6.3	+19.3	+ 6.6	+20.2
Treflan	1 L	14.3	- 66.6	589	- 52.8	21.0	- 3.5	- 16.7	+ 7.0	+33.3	+ 4.5	+12.4
	5 L	32.3	- 23.9	2421	+ 93.8	21.7	- 5.5	- 25.3	+11.3	+52.1	- 4.9	- 22.6

affects nodulation and formation of nodular tissue but stimulates nitrogenase activity of formed nodules proportionally to concentration applied (Herzallah 1980). The determined nitrogenase activities of root nodules (on 49th day) were generally higher in treflan (x 1 or x 5 folds) treatments (+ 24.8% or + 159.9% respectively) over than control. However, treflan especially at high dose was found to stimulate nodulation (Table 1).

On the second harvest (92nd day), the determined N_2-ase activities in all herbicidal treatments were lower as compared with those on the first harvest (49 days). This was expected since the maximal N_2-ase activity is always recorded during the early stages of flowering (Herzallah 1980). Soybean variety 'Clark' used in this investigation starts flowering one month after planting. The second harvest occurred during the pod filling stage when less nitrogen is fixed. However, the results indicated that N_2-ase values determined on the second harvest (Table 4) were lower in all herbicidal treatments although higher numbers of nodules were found in aretit treatment applied at the recommended rate as well as in treflane-treatment applied in high concentration (x 5 folds).

The calculated rates of nitrogen fixation (μ gm N_2/pot/h) in root nodules of soybean (49 or 92 days) in all herbicidal treatments, with the exception of treflan (x 5 folds), were lower than the control. Treflan-treatment (x 5 folds), on the other hand, showed stimulative effects as indicated by + 170.4% or + 93.8% (on 49 or 92 days respectively) over the control. However, the calculated rates of nitrogen fixation may be in accordance with the nitrogen yield of the whole plants (Table 2) estimated on 92nd day when treflan treatment (x 5 folds) showed increased values.

The inhibition or stimulation of N_2-ase activity found in the pre-emergence herbicidal treatments may be attributed to inhibition or stimulation of either the formation of N_2-ase enzyme system or/and formation of leghaemoglobin in the nodules formed (Gafar 1976; Johnson & Hume 1973). Application of herbicides may affect the supplementation of root nodules with photosynthesis products essential for symbiotic nitrogen fixation, thus may be a reason for the different nitrogenase activities recorded (Herzallah 1980). However, little and conflicting informations on such hypothesis were reported (Gafar 1976 and Johnson & Hume 1973), and therefore, further research is needed to elucide mechanism of affecting nitrogenase activity in root nodules.

The dehydrogenase activity (DHA) in nodule homogenate was also determined in presence or absence of succinate, citrate or ethanol to investigate effect of herbicidal-treatment on the respiratory chain of the bacteroids. This is claimed to throw some light on nitrogen fixation efficiency of root nodules (Alaa-Eldin et al. 1978). The use of succinate, citrate or ethanol substrates is believed to give idea about effect of herbicidal-treatment on capacity of a legume plant to supply the symbiont (Rhizobia) in the root nodules with some products of the Kreb's cycle (Herzallah 1980). In Table 4, the recorded DHA values of succinate, citrate or ethanol were referred to those of endogenous DHA (μ mole H_2/g fresh nodules/h) which were generally lower in herbicidal treatments either on the 49th or the 92nd day after soybean sowing (Herzallah 1980).

In aretit treatment, the stimulation recorded on 49th day, especially in presence of succinate, may indicate the shortage of supplementation with succinate of the soybean plant to the symbiont (bacteroids). This may be attributed to the inhibition of the capacity of soybean plant to supply the nodules with succinate so that succinate added (from outside) increased dehydrogenase activity. In case of treflan treatment, especially at field rate application (x 1), citrate or ethanol also exhibited similar trend of stimulation. This may be in accordance with the results of N_2-ase (Table 4) activity where the recommended rate of treflan showed lower N_2-ase activity than the higher concentration (x 5).

On 92nd day, application of succinate showed no positive effect on DHA as compared to the endogenous one but citrate increased the DHA than the endogenous in all herbicidal treatments where control treatment exhibited lower citrate DHA (- 31.9%) than the endogenous one. This changing effect of the herbicidal treatments by affecting the capability of soybean plant to supply the bacteroids in the root nodules with certain substrate (succinate) on 49th day, then another

substrate (citrate) on 92nd day may be attributed to biodegredation products of herbicides or to mutation of bacteroids in root nodules. Some authors, however, found that application of certain pesticides, even those which were tolerated by rhizobia, resulted in increased mutagenic activity of rhizobia with or without affecting their efficiency (Gillberg 1971 and Nameo & Dube 1973). In general, however, little but conflicting informations concerning the effect of herbicides on DHA of root nodules (Dorosinsky et al. 1966 and 1971; Kretovich et al. 1969 and Dunigan et al. 1970) or on the complex relation between succinate, citrate, ethanol and endogenous DHA of root nodules on one hand and nitrogen fixation efficiency on the other hand were recently published (Alaa-Eldin et al. 1976 and 1978; Hamdi et al. 1978 and Tewfik et al. 1978).

The debatable relation between numbers of nodules, dry weight of shoots, roots and pods, total-N content of plant, dehydrogenase activity of nodule homogenate in presence or absence of different hydrogen doners as well as nitrogenase activity of root nodules of soybean 'Clark' and nitrogen fixation efficiency of root nodules as affected by different pre-emergence herbicides could not be confirmed in the present study. Together with further investigation (Herzallah 1980) concerning, in addition, constitutive and nitrate inducible nitrate reductase activity in top leaves and in root nodule homogenate, dehydrogenase activity as well as potential nitrogenase activity of soil planted to soybean 'Clark' treated with several herbicides, may give limited idea on such complexed relations but more studies are required.

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