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EFFECTS OF HERBICIDES ON BIOLOGICAL NITROGEN FIXATION IN GRAIN AND FORAGE LEGUMES - A REVIEW

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ABSTRACT

Legumes are an integral part of the farming systems. They are grown sole as well as inter/ mixed crops. They not only provide food for human beings and fodder for animals but also fix atmospheric nitrogen in the soil. Nitrogen so fixed by various legumes in association with different species of rhizobia results in increased soil fertility. Everyone is interested in higher amounts of nitrogen fixation by legumes but some factors limit their efficiency. One of these factors could be the herbicides. Though herbicides are applied for controlling weeds yet indirectly some of them may influence their (legumes') nitrogen-fixing activity. Herbicidal effect on nitrogen fixation may be either beneficial or deleterious. In this paper an attempt has been made to review the literature on the effects of herbicides on biological nitrogen fixation in some of the commonly grown legumes, either for food or forage.

MAJOR LEGUMES

The family Fabaceae (Leguminosae) is estimated to contain 16000-19000 species in about 750 different genera (Allen and Allen, 1981). Some plants are of more importance than the others. There are hundreds of legumes of varied uses in the world (Duke, 1981). Grain legumes provide protein-rich food for the human beings and forage legumes are a good source of fodder for the animals. They have the capacity to fix atmospheric nitrogen in their root nodules. Thus, they enrich the soil with nitrogen - the most limiting nutrient element for plant growth. The inclusion of various legumes in various cropping systems is essential for sustainable agriculture.

There are many grain legumes grown in various parts of the world. Chickpea, pigeonpea, fieldpea, fababean, soybean, lentil, common bean, limabean, cowpea, mungbean, urdbean, winged bean, lupin and groundnut are the important grain legumes. However, there are some other grain legumes which have their importance locally. Adzuki bean, tepary bean, moth bean, jack bean, sword bean, bambara groundnut, horse gram, hyacinth bean, Indian pea and rice bean are some of the less utilized grain legumes. Lucerne, clovers and lupin are the important forage legumes.

HERBICIDES COMMONLY USED IN LEGUMES FOR CONTROLLING WEEDS

Any plant growing where it is not wanted is a weed (Anderson, 1996). Weeds compete with the crop plants for nutrients, moisture, and light and thus, reduce the yields considerably. 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. In closer-row sown crops, such as legumes, use of herbicides for controlling weeds is considered to be the most effective method. Depending upon the properties of herbicides they can be used either pre-sowing, pre-emergence or post-emergence.

Pendimethalin has been found to control weeds effectively and increase yield of peas (Tripathi *et al.*, 1993), urdbean (Mishra and Singh, 1993) and chickpea (Balyan *et al.*, 1991). Oxadiazon at 0.5-1.0 kg and pendimethalin at 1.5 kg/ha resulted in the greatest weed control efficiency as well as grain yields of fieldpea (Gogoi *et al.*, 1991). In mungbean, urdbean and fieldpea pre-sowing spray of fluchloralin at 0.67 kg and the pre-emergence application of pendimethalin at 0.75 kg/ha was quite effective for controlling weeds (Sekhon *et al.*, 1993). Pre-plant incorporated fluchloralin and pre-emergence application of pendimethalin and oxadiazon reduced weed population density and weed dry weight and increased seed yield of chickpea (Singh and Bajpai, 1992). Pre-emergence pronamide (propyzamide), post-emergence sethoxydim and fluazifop-butyl, all applied at 0.5 kg a.i./ha, controlled grassy weeds and improved yields in chickpea and lentil (Yasin *et al.*, 1995). In urdbean (De and Modak, 1993) and pigeonpea (Patel *et al.*, 1993) fluchloralin, alachlor and pendimethalin reduced the weed biomass considerably and increased yields. Pre-emergence application of alachlor proved to be superior over hand weeding in controlling weed dry weight and improving yield of pigeonpea (Nagaraju *et al.*, 1995). Alachlor (Ramamoorthy *et al.*, 1995) and fluchloralin (Tuteja *et al.*, 1995; Satao and Chandurkar 1994) gave the highest seed yield of soybean. Sethoxydim showed promising results in controlling weeds and increasing yield of soybean (Jha *et al.*, 1993). Trifluralin as pre-sowing and simazine, trietazine+simazine, linuron, prometryne and terbuthylazine + terbutryne as pre-emergence are recommended in various legumes (O' Keeffe *et al.*, 1978).

The post-emergence application of bentazone, MCPB salt, dinoseb-amine and dinoseb-acetate can be made in various leguminous crops (O' Keeffe *et al.*, 1978). The proper time of application of herbicides is very important. Too early application of a herbicide can damage the crop and on the other hand too delayed application will not be effective in controlling weeds as they may become tolerant at advanced age. Imazethapyr resulted in effective weed control and high yields in urdbean (Chin and Pandey, 1991) and soybean (Angiras and Rana, 1995).

In pigeonpea-sorghum intercropping system pre-emergence oxadiazon at 0.75 kg/ha gave more effective control of weeds and was less phytotoxic than pendimethalin at 0.75-1.00 kg (Shelke and Bhosle, 1989). However, in the same intercropping system, Ali (1991) found pre-emergence application of 1.5 kg/ha pendimethalin as the most effective followed by alachlor at 2 kg/ha.

EFFECT OF HERBICIDES ON BIOLOGICAL NITROGEN FIXATION

Herbicides are used for controlling weeds in the crops. However, some herbicides may adversely affect biological nitrogen fixation in legumes. On the other hand some herbicides have been reported to favourably influence nitrogen fixation in some legumes. Adverse or beneficial effect of herbicides can be either on nodulation or nitrogen-fixing efficiency of the plants. Effect of herbicides on nitrogen fixation varies with the crop variety, soil type, environmental conditions, *Rhizobium* strain, and the herbicide, its dose and application time.

Grain Legumes

In chickpea, Kumar *et al.* (1981) reported drastically reduced nodule number and their fresh as well as dry weight with 1.6 and 3.2 kg/ha simazine. Leghaemoglobin did not develop at all in the nodules of simazine treated plants. However, prometryne at the same rates was found to be less damaging to nodulation and it improved nitrogen fixation. In comparison to weed free or hand weeding twice chlorbromuron, methabenzthiazuron and terbutryne at 1.5-3.5, 2.5-3.5 and 2-4 kg/ha, respectively did not significantly affect number of nodules and their fresh as well as dry weight per plant in chickpea (Khokhar and Malik, 1988). By carefully looking their data it was noted that though the treatments were statistically at par, however, as compared to some of the herbicidal treatments values of all these parameters were more than double in case of hand weeding done twice. In a pot trial comprising pre-sowing incorporated 0.5-1.5 kg fluchloralin, pre-emergence application of 0.25-0.75 kg metribuzin, and 0.75-1.25 kg/ha pendimethalin, nodulation in chickpea was not adversely affected by lower doses and there was significant increase in leghaemoglobin content of nodules (Pahwa and Prakash, 1992). Nitrogen fixation activity in herbicidal treated plants was higher as compared to the control plants.

Sandhu *et al.* (1991) reported that the number of nodules and their dry weight per plant and nitrogenase activity/g nodule dry weight in lentil was highest in plots hand-weeded twice whereas pre-emergence application of oxyfluorfen, linuron, metribuzin and oxadiazon reduced all these parameters. In comparison to weedy check Yadav *et al.* (1990) got higher number of nodules/plant, nodule dry weight and nitrogenase activity in lentil with pre-sowing incorporation of 1.5 kg fluchloralin or pre-emergence application of 1.0 kg pendimethalin or hand weeding. However, as compared to herbicidal treatments hand weeding gave the highest nodule dry weight and nitrogenase activity. Metribuzin, when sprayed 8 days after planting lentil, had significant negative effect on the number of nodules and acetylene reduction activity (Sprout *et al.*, 1992).

Alachlor, diclofop-methyl and tri-allate at 1, 2 or 4 times the commercial rates had no significant effect on nodulation and nitrogen fixation in peas (Pomela and Callihan, 1986). Metribuzin at 0.5-1.0 lb and trifluralin at 1.0-2.0 lb/acre decreased nodulation and nitrogen fixation but MCPA at 1.0 lb/acre increased nitrogen fixation. Prometryne at 6 mg/kg of soil reduced green plant weight, nitrogenase activity, nodule size and leghaemoglobin contents (Paromenskaya and Lebskii,1985). Prakash and Pahwa (1984) studied the effect of 1-2 kg methabenzthiazuron, 0.6-1.2 kg diclofop-methyl, 0.5-1 kg fluchloralin, 0.1-0.2 kg oxyflourfen and 1-2 kg pendimethalin in pea and reported that herbicides, in general, decreased root length but not shoot length. They also found increased nodulation and number of leaves/plant with herbicides compared with the untreated control.

After their application effect of certain herbicides changes with time. Venkateswarlu *et al.* (1988) reported that in urdbean at 20 days after sowing (DAS) pre-sowing incorporated fluchloralin at 1.5 kg/ha and pre-emergence application of pendimethalin at 1.0 kg/ha depressed nodulation and nodule dry weight but at 40 DAS and at harvest significantly more nodules were found on herbicide treated plots as compared with hand weeded and unweeded plots. Gupta and Surat (1987) are of the opinion that in mungbean nitrofen at 1 ppm is the optimum dose for maximum number and biomass of nodules and nitrogen fixation and higher than 1.5 ppm decrease all the symbiotic parameters.

In soybean, pendimethalin and trifluralin incorporated into a silt loam at 1.1, 1.7 and 2.2 kg/ha decreased nodule number, dry weight and nitrogen fixation at all herbicide rates (Bollich *et al.*, 1988). They found reduced nodulation and lower levels of nitrogen fixation in one year as compared to the other, possibly as a result of drought conditions. In Thailand, application of alachlor, metolachlor, fluazifop-butyl and sethoxydim did not reduce the level of nitrogen fixation but paraquat dichloride significantly reduced nitrogen fixation (Kucey *et al.*, 1988).

Soil type influence herbicidal effect on nitrogen fixation. In soybean in a sandy loam soil 0.3 kg metribuzin, 2.2 kg alachlor and 0.6 kg/ha prometryne reduced ethylene production/plant/h but in a Mhoon silt loam 0.6 kg/ha metribuzin increased ethylene production, and no herbicide affected ethylene production, nodule number and nodule dry weight in a Commerce silt loam (Bollich *et al.*, 1985).

In greenhouse experiments trifluralin and PCNB applied as pre-emergence at 2.8 l/ha and 15.5 kg/ha or post-emergence application of bentazone and sethoxydim at 2.8 and 29.4 l/ha did not adversely affect acetylene rate in soybean (Yueh and Hensley, 1993). It is important to mention here that the above rates were 3-fold the manufacturers rates. They also found that trifluralin, not only at 3-fold recommended concentration but even at the recommended rate depressed nodulation. However, in another study on soybean, soil incorporated trifluralin at 0.8-2.2 kg/ha did not affect nodule number, nodule weight and nitrogen fixation capacity significantly (Ozair and Moshier, 1988a). In another study, Ozair and Moshier (1988b) found reduced number of nodules 2 weeks after acifluorfen application but none of the herbicides (acifluorfen, bentazone, chloramben and 2,4-DB) reduced acetylene reduction activity after 4 weeks of application.

Nodulation extent depends upon the dose of herbicides. A slight stimulatory effect of fluchloralin on nodulation in groundnut was noted at 6 ppm but at 60-960 ppm there was a reduction in number and weight of nodules (Durgesha and Lakshminarasimhan, 1989). In groundnut, fluchloralin increases nodulation and nitrogenase activity whereas benefin (benfluralin), dinitramine and nitralin adversely affect these parameters (Durgesha, 1994). Terbutryne, dinitramine and alachlor at the recommended rates have no adverse effect on nodulation and nitrogenase activity in groundnut (Kishinevsky *et al.*, 1988). They further reported that up to 94 g/g the herbicides had no inhibitory effect on growth of *rhizobium*. Sidhu *et al.* (1985) did not find any significant effect of 0.36 or 0.72 kg fluchloralin, 0.25 or 0.50 kg oxadiazon and 0.375 or 0.75 kg/ha pendimethalin on nodule numbers and their weight per plant in case of groundnut.

In fababean at double the recommended dose nodulation was suppressed by 38% by linuron and 20% by dinoseb-acetate and both herbicides reduced nitrogen fixation by 40% (Haider *et al.*, 1991). Interestingly, sub-lethal doses of methabenzthiazuron (175 and 220 g/ha) increased nodulation and nitrogenase activity in fababean (Vidal *et al.*, 1992).

Bentazone applied at 12.5-62.5 ml/litre to 4-week old plants did not influence leghaemoglobin concentration and nitrogenase activity in nodules of kidney bean (*rajmash*) (Schnelle and Hensley, 1989). It is important to observe the effect of herbicides on nitrogen fixation at different intervals after applying herbicides. These researchers in their another experiment (Schnelle and Hensley, 1990) found that bentazone at 6.7 kg/ha consistently depressed nitrogen fixation rates within 48 hours of application but after six days nitrogen fixation rates were similar to the control plants. Nodulation was affected neither at the label nor 3-times the label rates. However, nodule growth was inhibited by alachlor, trifluralin and combinations of alachlor+trifluralin and EPTC+trifluralin (Nkwen-Tamo *et al.*, 1989).

Forage Legumes

Effect of herbicides on lucerne and nodule-forming bacteria symbiosis varies according to the chemical and the activity of the bacterial strain. When inoculated lucerne plants were grown in aseptic cultures in the presence of 0, 5.5 and 55 μM of chlorsulfuron, only 55 μM of the herbicide inhibited nodulation (Martensson and Nilsson, 1989). In lupin, cyanazine, simazine and trifluralin cause a significant increase in acetylene reduction activity (Pozuelo *et al.*, 1989). However, Felipe *et al.* (1987) reported that simazine altered nodule cells by causing vesicle formation, degeneration of bacteria, and by decreasing number of nitrogen-fixing bacteroids and found reduced acetylene reduction assay values.

In white clover, paraquat and MCPB lowered the number of nodules, and bentazone showed the least toxicity to nodulation and nitrogen fixation under bacteriologically-controlled conditions and higher toxicity on plants grown in soil (Clark and Mahanty, 1991). Nodule development was inhibited at increased levels of bentazone, chlorsulfuron and glyphosate (Martensson, 1992a). Dinoseb reduced the levels of nitrogenase activity of red clover plants up to 18 days after treatment (Lindstrom *et al.*, 1985). Martensson and Nilsson (1989) reported that chlorsulfuron at 55 μM and not at 5.5 μM inhibited nodulation in red clover. As the concentration of amitrole, diclofop-methyl and glyphosate in the rooting environment increased from 0 to 20 mg a.i./ha nodulation in subterranean clover decreased linearly and 2, 4-D, atrazine, chlorsulfuron, diquat, paraquat and trifluralin at the same concentration were even more damaging to nodulation (Eberbach and Douglas, 1989).

HOW HERBICIDES AFFECT BIOLOGICAL NITROGEN FIXATION

Herbicides may affect biological nitrogen fixation either by affecting plant growth or by directly affecting nitrogen-fixing rhizobia. There are a complex of processes which are affected by herbicides. The more important could be photosynthesis, respiration and protein synthesis. The overall effect of herbicides is reflected in dry matter production. Either above-ground plant growth or root growth or both can be affected by the herbicides.

Effect of herbicides on plant growth

On the basis of their results Khokhar and Malik (1988) pointed out that in chickpea decrease in specific nitrogenase activity by terbutryne at 4.0 kg/ha as compared to 3.0 kg/ha might be due to partial loss of effectivity by *Rhizobium* and phytotoxic effect on photosynthesis which may limit food supply to *Rhizobium*. Except fluchloralin; benefin (benfluralin), dinitramine and nitralin decreased nodulation as well as nitrogenase activity in all groundnut cultivars except Kadiri 3. However, CO_2 exchange rate was not affected by herbicides in any cultivar (Durgesha, 1994). In a pot experiment dinoseb inhibited nitrogenase activity of red clover when sprayed on the leaves, but not when added to the growth medium and thus, dinoseb did not act directly on nodules but affected nitrogen fixation by damaging the photosynthetic parts of the plant (Lindstrom *et. al.*, 1985).

Mafluidide at 0.28 and 0.42 kg/ha reduced shoot and nodule weight of soybean (Ozair and Moshier, 1988), indicating a relationship between photosynthesis and nodule size. Similarly Matensson (1992b) reported that at higher levels bentazone and chlorsulfuron inhibit nodule development and adversely affect dry matter production of *Trifolium* plants, indicating disturbance in nodule function by herbicides by affecting photosynthesis.

As less than 0.2% of foliarly applied metribuzin is translocated to the root so the detrimental effects of metribuzin on plants are mainly due to direct effects on the plant, which result in indirect effects on nodulation and nitrogen fixation (Sprout *et al.*, 1992). They also reported significant negative effect of metribuzin on plant weight and tap root growth which might had adversely affected number of nodules and acetylene reduction activity.

Prakash and Pahwa (1984) tested pendimethalin, methabenzthiazuron, fluchloralin, oxyflourfen and diclofop-methyl and observed that, in general, herbicides decreased root length but diclofop-methyl at 0.6 and 0.9 kg/ha increased the total root growth and dry matter accumulation.

Pahwa and Prakash (1992) reported reduced root and shoot dry weight recorded at 30-75 DAS with higher rates of fluchloralin, metribuzin and pendimethalin. At 75 DAS they also found decreased number of nodules and their dry weight with all herbicides except fluchloralin at lower rate. Similarly in chickpea Kumar *et al.* (1981) applied simazine and prometryne at 1.6 and 3.2 kg/ha and reported reduced nodulation especially with simazine and reduced root dry weight and they were of the view that reduction in nodulation with both the herbicides appeared to be primarily a case of general root growth reduction.

In kidney bean photosynthesis and RNA synthesis are inhibited about 75% at 1 μM bentazone after 30 min treatment and at 10 μM bentazone, protein and lipid synthesis are also inhibited (Al-Mendoufi and Ashton, 1984). Earlier Bethlenfalvay *et al.* (1979) had also reported that in kidney bean inhibition of nitrogen fixing capacity was not caused by bentazone directly but indirectly through limiting the availability of photosynthate to support root nodule activity.

Simazine affected the photosynthetic apparatus not only by considerably reducing the size of the chloroplasts but also by affecting the grana structure (Felipe *et al.*, 1987) and this could be the reason for reduced nitrogen fixation in *Lupinus albus*. In mungbean dichlorprop-methyl at 0.6-1.5 kg, pendimethalin at 1-2 kg and oxyflourfen at 0.1-0.2 kg/ha as pre-emergence were tested and were found to reduce shoot length initially (Prakash and Pahwa, 1982). However, at later stages only pendimethalin at 2 kg/ha reduced growth. In studies with isolated kidney bean leaf cells, photosynthesis, respiration and synthesis of lipids and particularly RNA and protein were inhibited by glophosate at 5 mM/ dm^3 (Villiers and Koch, 1982).

Effect of herbicides on nitrogen fixing rhizobia

Madhavi *et al.* (1994) studied biotoxic effects of pesticides (herbicides, insecticides and fungicides) on symbiotic properties of three rhizobial species (*Rhizobium sp.* IC 3342, nodulating pigeonpea; *Rhizobium leguminosarum* 2001, nodulating lentil, and *Rhizobium meliloti* 4013 nodulating alfalfa). Out of these three cultures symbiotic properties of *Rhizobium* sp IC 3342 were susceptible to all pesticidal treatments. Among pesticides herbicides (butachlor, simazine and oxyfluorfen) showed more adverse effect than insecticides and fungicides. They also found (Madhavi *et al.*, 1993) that plants infested with herbicide treated *Rhizobium* had less dry weight and total nitrogen content due to reduced growth and nitrogen fixing capacity.

Yueh and Hensley (1993) reported that though nitrogen fixation of soybean and limabean was not significantly influenced by trifluralin yet nodulation was adversely affected. Based on a disc inhibition study trifluralin was found to be non-toxic to *Bradyrhizobium* and *Rhizobium* spp. In *in vitro* studies the order of toxicity on various *Rhizobium* strains infesting groundnut in decreasing order was dinitramine, benefin, nitralin and fluchloralin (Durgesha, 1994). Different strains of *Rhizobium leguminosarum, R. meliloti* and *R. loti* were able to multiply when they were exposed to the equivalent to 1-3 kg/ha bentazone and glyphosate and 0.5-3.5 kg MCPA and bacterial exposure to herbicides did not influence the nodulating ability of the bacteria except for glyphosate (Martensson, 1992b). Bacterial growth in pure culture was unaffected by addition of 0.55 and 5.5 μM chlorsulfuron (Martensson and Nilsson, 1989) and they concluded that the inhibition of nodulation and nitrogenase activity of nodules of alfalfa and red clover is probably due to adverse effects of the herbicide on the plant growth and development rather than on the rhizobia.

In nutrient broth concentration of 2 mg a.i./litre of paraquat and chlorsulfuron and 10 mg a.i./litre of glyphosate significantly reduced the growth of *Rhizobium trifolii* TA 1 (Eberbach and Douglas, 1989). At a concentration of 6 mg prometryne/kg of soil, Paromenskaya and Lebskii (1985), reported destruction of the cytoplasm and nucleous of plant cells and lysis of the bacteroids, which might be responsible for reduced nodule size, nodule leghaemoglobin and nitrogenase activity in pea.

Kumar *et al.* (1981) reported that the growth of the rhizobia (*Rhizobium leguminosarum*) in culture was reduced by 2-9% with simazine and by 4-11% with prometryne treatments of 1-20 mg/l. However, Felipe *et al.* (1987) did not find any adverse effect of cyanazine and simazine on the growth of *Bradyrhizobium*, used for lupin. So the response of different *Rhizobium* strains to different chemicals can vary. While working with different strains of rhizobia Martensson (1992b) found that rhizobial strains differed in their tolerance to the growth in the presence of various agrochemicals including chlorsulfuron and MCPA.

CONCLUSIONS

The effect of herbicides on biological nitrogen fixation in different legumes varies with the herbicides, their doses and application time. Different crops, cultivars and *Rhizobium* strains show differential response to herbicides. Soil conditions and environmental factors may modify the effect of herbicides on nitrogen fixation. Herbicidal effect on nitrogen fixation can be either through their effect on above-ground plant parts or on under-ground plant parts or directly on nitrogen-fixing rhizobia.

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