A Comparative Analysis of the Growth Parameters of Different Legume Crops Grown Inquizalafop-p-ethyl Applied Soils

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Abstract: Background: In current agronomic practices, herbicides are repeatedly used to control weeds and consequently to improve the plant productivity. However, the intensive and injudicious application of herbicides leads to their accumulation in soils and deteriorate the soil fertility. Previous studies concerning the effect of herbicides are commonly, confined to any particular legume and hence, reports about the concurrent impact of any specific herbicide on more than one legume are rare. **Objective:** The present study was therefore, navigated to assess the effect of herbicide quizalafop-p-ethyl simultaneously on four commonly grown food legumes (chickpea, pea, lentil and greengram). **Results:** In this study, the growth of the tested legumes grown in soils amended with the recommended field rate of quizalafop-p-ethyl decreased significantly. The root and shoot biomass, the symbiotic attributes (numbers, dry weight and leghaemoglobin content in nodules), nutrient-uptake (nitrogen and phosphorus), seed protein and yields of the tested legumes varied significantly in the presence of quizalafop-p-ethyl. The maximum toxicity of quizalafop-p-ethyl was observed on the root P content and the root growth in greengram, shoot growth, shoot N and seed protein in chickpea and nodulation, root N, shoot P and seed yield in lentil. **Conclusion:** The degree of phytotoxicity of any herbicide to plants and the type of plant organs affected in response to herbicide exposure may differ from one plant genotype to another.

Key words: Herbicide, quizalafop-p-ethyl, toxicity, legume, soil, phosphorus, nitrogen

INTRODUCTION

Unparalleled and incessant augmentation in crop yield would be a constructive stride to nurture the rapidly increasing human population, whose overall food consumption is predicted to be doubled in subsequent fifty years (Fox *et al.*, 2007). Currently, the extensive application of agrichemicals specifically pesticides of various chemical families to control the diverse phytopathogens detrimental for crop health has resulted into increased crop yield (Ahemad and Khan, 2011). However, this approach to ameliorate plant production at an unprecedented rate is unsustainable since the most important long term effect of the agricultural intensification and the continuous cropping with indiscriminate application of pesticides is that this results into decreased plant beneficial rhizobacterial communities (Srinivas *et al.*, 2008) and the soil fertility (Ahemad *et al.*, 2009) which then drives increased usage of pesticides.

Among pesticides, herbicides are the plant protection agents which are used in high input agricultural practices to kill the unwanted weeds thus to prevent yield losses due to these noxious and plants (Cork and Krueger, 1992). However, once accumulated into soils, the herbicides may

leach down polluting groundwater or if remained immobile, they would persist on the upper surface of agricultural soils (Ayansina and Oso, 2006) and affect negatively the crop growth (Ahemad *et al.*, 2009). Thus, there is an increasing need among users of pesticides, consumers and policy makers to obtain substantial scientific data concerning the risk of pesticides to the environment, specifically herbicides to non-target plants. Attempts are under process at both the international and national levels to address safety to non-target plants and herbicide-toxicity assessment for the protection of non-target plants is now required for the registration of pesticides (Follak and Hurle, 2003).

Quizalafop-p-ethyl [ethyl (RS)-2-(4-6-chloroquinoxolin-2-yloxy) phenoxy] propionate] (CAS No. 76578-14-8) belonging to chemical family aryloxyphenoxy, is a selective grass killer for control of many annual and perennial grass weeds, pampas grass, couch, wild oats, volunteer cereals, Indian doab, onion twitch, ryegrasses etc. in both legume and non-legume crop production. Its mode of action on the target plants is through the inhibition of fatty acid synthesis. Most of the earlier studies of phyto-toxicity of herbicides are generally, restricted to any single crop and comprehensive data assessing the impact of any specific herbicide on more

than one legume in parallel is rare. Hence, the present study was designed to evaluate the effect of quizalafop-p-ethyl on four commonly grown legumes like, chickpea (*Cicer arietinum* L.), pea (*Pisum sativum*), lentil (*Lens esculentus*) and greengram (*Vigna radiata* L. Wiczek) together so that a firm conclusion can be drawn about the phyto-toxicology of this herbicide.

MATERIALS AND METHODS

Herbicide treatment: The technical grade (a.i., 98%) quizalafop-p-ethyl was obtained from Parijat Agrochemicals (New Delhi, India). To prevent the degradation, the stock solution was prepared just prior to each experiment by dissolving herbicide in solvent (acetone). The recommended field dose ($40 \mu\text{g kg}^{-1}$ soil) of quizalafop-p-ethyl was used for the experiments.

Legume-growth measurement: Seeds of the commonly grown legumes like, chickpea var. C235, pea var. arakle, lentil var. K75 and greengram var. K851 were obtained from Indian Agricultural Research Institute (IARI), Pusa, New Delhi, India. Seeds of the legumes were surface sterilized with 70% ethanol, 3 min; 3% sodium hypochlorite, 3 min; rinsed six times with sterile water and dried. A total of 10 seeds of each legume were sown in clay pots (25 cm high, 22 cm internal diameter) using 3 kg unsterilized soils [sandy clay loam, organic carbon (C) 0.4%, Kjeldahl nitrogen (N) 0.75 g kg^{-1} , Olsen phosphorus (P) 16 mg kg^{-1} , pH 7.2 and water holding capacity 0.44 mL g^{-1} , cation exchange capacity $11.7 \text{ cmol kg}^{-1}$ and 5.1 cmol kg^{-1} anion exchange capacity) with a control (without quizalafop-p-ethyl) and a treatment with the recommended field rate of quizalafop-p-ethyl (in three replicates for each legume). Seeds of chickpea, lentil, greengram and pea were sown in October, November, March and November, respectively. Plants in each pot were thinned to three plants 10, 10, 7 and 7 Days after Sowing (DAS) of chickpea, lentil, greengram and pea, respectively. The pots were watered with tap water when required and were maintained in an open field.

Biomass production and symbiotic attributes: All plants for each treatment were removed at 135 DAS (at harvest stage) of chickpea, 120 DAS (at harvest stage) for both pea and lentil and 80 DAS (at harvest stage) for greengram. The root and shoot of each legume were carefully washed and oven dried at 80°C and weighed. The nodulation in chickpea, pea and lentil was recorded at 90 DAS (pod fill stage) and that of greengram at 50 DAS (pod fill stage). Nodules from the root systems of each legume were separated, counted, oven dried at 80°C

and weighed. The Leghaemoglobin (Lb) content in fresh nodules recovered from the root system of each legume crop was quantified at 90 DAS each for chickpea and pea and lentil and 50 DAS for greengram, respectively, by the method of Sadasivam and Manikam (1992). Briefly, fresh nodules were crushed with the help of mortar and pestle in 5 mL sodium phosphate buffer (pH 7.4) and filtered through two layers of cheese cloth. The nodule debris was discarded. The turbid reddish brown filtrate was clarified by centrifugation at 10000 g for 30 min. The supernatant was diluted to 10 mL with sodium phosphate buffer (pH 7.4). The extract was divided equally into two glass tubes (5 mL/tube) and equal amount of alkaline pyridine reagent was added to each tube. The haemochrome formed was read at 556 and 539 nm after adding a few crystals of potassium hexacyanoferrate and sodium dithionite, respectively. The leghaemoglobin content was calculated using the formula:

$$\text{Lb content (mM)} = \frac{[A_{556} - A_{539}] \times 2D}{23.4}$$

where, D is initial dilution.

Total chlorophyll, nitrogen and phosphorus contents:

The total chlorophyll content in fresh foliage of each experimental legume crop was quantified at 90 DAS each for chickpea, pea and lentil and 50 DAS for greengram by the method of Amon (1949). Briefly, one gram of fresh leaves of each legume was grinded in 40 mL of 80% acetone with the help of mortar and pestle. The suspension was decanted in Buchner funnel having Whatman filter paper No. 1. The residue was grounded three times with acetone and the resulting suspension was filtered again. Contents in mortar-pestle was washed with 80% acetone and filtered. The filtrate was transferred to 100 mL volumetric flask and volume was made upto 100 mL. The absorbance was read at 645 and 663 nm using double beam UV-Visual spectrophotometer (Electronics Corporation of India Limited, India). The total chlorophyll content was calculated as:

$$\text{Total chlorophyll} = \frac{[20.2(\text{OD}_{645}) + 8.02(\text{OD}_{663})] \times V}{1000 \times W}$$

Where:

OD_{645} = Optical density at 645 nm

OD_{663} = Optical density at 663 nm

V = Final volume of chlorophyll extract in 80% acetone and

W = Fresh weight of tissue extracted

The total N and P content in roots and shoots of chickpea (135 DAS), lentil (120 DAS), pea (120 DAS) and

greengram (80 DAS) were measured by the micro-Kjeldahl method of Iswaran and Marwah (1980) and the method of Jackson (1967), respectively.

Seed yield and grain protein: Chickpea, pea, lentil and greengram were finally harvested at 135, 120, 120 and 80 DAS, respectively and seed yield was measured. The protein content in grains of each legume was estimated by the method of Lowery *et al.* (1951).

Statistical analysis: The experiments were conducted for two consecutive years with the identical environmental conditions and with the same herbicide treatment to ensure the reproducibility of the results. Since the data of the measured parameters obtained were homogenous, they were pooled together and subjected to analysis of variance. The difference among treatment means was compared by Tukey test at 5% probability level.

RESULTS

In this study, the growth of four legume crops chickpea, pea, greengram and lentil exposed to herbicide quizalafop-p-ethyl at the recommended field rate was evaluated. The root and shoot biomass and the symbiotic attributes (numbers, dry weight and leghaemoglobin content in nodules) of the tested legumes varied significantly in the presence of quizalafop-p-ethyl.

Generally, quizalafop-p-ethyl adversely affected the biomass accumulation in both root and shoot of all legumes. Among the tested legumes, the greengram plants suffered the maximum decline in the root biomass under the herbicide-stress while the highest toxicity of the same agrichemical on the shoot biomass accumulation was observed for the chickpea plants over the untreated control. Moreover, the toxic effect of quizalafop-p-ethyl on root biomass accumulation in pea and chickpea plants was nearly similar. On the other hand, both pea and lentil

plants exposed to the herbicide-stress displayed an approximately similar level of decrease in shoot biomass accumulation. The order of the degree of toxicity (percent decline over the controls) of quizalafop-p-ethyl to the root biomass accumulation was observed as: greengram (62)>lentil (53)>pea (33)>chickpea (30). In contrast, the herbicide-mediated percent reduction in the shoot dry matter accretion in legumes over their controls manifested in the following array: chickpea (64)>pea (50)>lentil (49)>greengram (43) (Table 1).

Nodulation in legumes is one of the most important growth parameters. Hence symbiotic attributes of the tested legumes were determined under herbicide-stress. The recommended dose of quizalafop-p-ethyl had a detrimental effect on the nodule development for each legume. The effect of quizalafop-p-ethyl on nodulation of lentil plants was so noxious that not a single nodule was recovered from their roots as quizalafop-p-ethyl completely ceased the nodule formation in lentil. Quizalafop-p-ethyl decreased the nodules (percent decline over controls) in each legume in an order: lentil (100)>pea (74)>greengram (29)>chickpea (14). While comparing two or more legume species, evaluation of nodule numbers does not provide an accurate inference because size of nodules varies from one legume species to another. Both nodule dry biomass and the most importantly their Lb content are the precise parameters to assess the actual impact of any stress factor on nitrogen fixation. Therefore, these two symbiotic characteristics for each legume were also determined. The toxic effect of quizalafop-p-ethyl on dry biomass (percent decline over controls) was observed as: Lentil (100)>chickpea (54)>pea (48) = greengram (48). Moreover, quizalafop-p-ethyl affected Lb content in nodules of the tested legumes in the following sequence (percent decline over controls): Lentil (100)>chickpea (77)>pea (41)>greengram (25) (Table 1).

Furthermore, the impact of quizalafop-p-ethyl on total chlorophyll in fresh leaves of each legume was also

Table 1: Effect of quizalafop-p-ethyl on dry biomass and symbiotic properties of legume crops

Legumes	Dose rate ($\mu\text{g kg}^{-1}$ soil)	Dry biomass (g plant^{-1})		Nodulation		
		Root	Shoot	No./plant	Nodule biomass (mg plant^{-1})	Leghaemoglobin content (m Mg^{-1} FM)
Chickpea	0 (control)	0.91 ^a	3.80 ^a	21 ^b	180 ^b	0.13 ^b
	40	0.64 ^b	1.36 ^d	18 ^c	83 ^d	0.03 ^e
Pea	0 (control)	0.92 ^a	2.07 ^b	27 ^a	283 ^a	0.17 ^a
	40	0.62 ^c	1.04 ^e	7 ^e	176 ^e	0.10 ^d
Greengram	0 (control)	0.47 ^a	2.08 ^b	21 ^b	66 ^e	0.08 ^e
	40	0.18 ^e	1.19 ^e	15 ^d	41 ^f	0.06 ^f
Lentil	0 (control)	0.55 ^d	1.97 ^c	19 ^c	30 ^e	0.12 ^c
	40	0.26 ^f	1.17 ^f	-	-	-
LSD ($p \leq 0.05$)	0.05	0.03	2.3	3.2	0.004	
F-value	74	21.6	38.9	43.5	51.4	

Values are mean of 3 replicates where each replicate constituted 3 plants/pot. Mean values followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test; FM: Fresh biomass

Table 2: Effect of quizalafop-p-ethyl on biological and chemical properties of legume crops

Legumes	Dose rate ($\mu\text{g kg}^{-1}$ soil)	Chlorophyll content (mg g^{-1})	N content (mg g^{-1})		P content (mg g^{-1})		Seed protein (mg g^{-1})	Seed yield (g plant^{-1})
			Root	Shoot	Root	Shoot		
Chickpea	0 (control)	1.96 ^a	18 ^e	27 ^e	0.17 ^d	0.21 ^d	241 ^b	2.7 ^a
	40	1.45 ^b	14 ^g	20 ^f	0.13 ^f	0.17 ^e	185 ^g	1.2 ^g
Pea	0 (control)	0.75 ^d	34 ^b	45 ^b	0.21 ^b	0.28 ^b	224 ^a	7.4 ^a
	40	0.68 ^f	29 ^e	37 ^d	0.17 ^d	0.23 ^c	220 ^f	5.3 ^b
Greengram	0 (control)	0.82 ^c	36 ^a	50 ^a	0.27 ^a	0.36 ^a	261 ^a	7.4 ^a
	40	0.74 ^e	27 ^d	38 ^e	0.18 ^c	0.28 ^b	235 ^c	3.6 ^e
Lentil	0 (control)	0.32 ^g	17 ^f	45 ^b	0.21 ^b	0.28 ^b	232 ^d	3.0 ^d
	40	0.25 ^h	12 ^h	38 ^e	0.16 ^e	0.21 ^d	221 ^f	1.3 ^f
LSD ($p \leq 0.05$)		0.04	1.4	1.8	0.004	0.007	3.6	0.06
F-value		65	128.0	285.2	112.5	74.9	349.6	186.4

Values are mean of 3 replicates where each replicate constituted 3 plants/pot. Mean values followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test

assessed. Generally, the chlorophyll content in leaves of each legume significantly decreased when grown in quizalafop-p-ethyl amended soils. The most toxic effect of the herbicide was observed on the total chlorophyll of chickpea plants wherein the chlorophyll content declined 26% compared to control. Moreover, quizalafop-p-ethyl mediated decline in the photosynthetic molecules of lentil was found to be 22% above control. In addition, the percent reduction in the total chlorophyll of pea and greengram was approximately similar (9 and 10%, respectively) (Table 2).

Estimation of N content in roots and shoots of each tested legume revealed that quizalafop-p-ethyl decreased the root N maximum (29%) in lentil followed by greengram (25%), chickpea (22%) and pea (15%) compared to their respective controls. Conversely, the decline in the shoot N of each legume exposed to the herbicide-stress was found to follow the trend as: chickpea (26%) > greengram (24%) > pea (18%) > lentil (16%) (Table 2). Besides, the root P of the tested legumes in response to quizalafop-p-ethyl exposure decreased in the order: greengram (33%) > chickpea (24%) = lentil (24%) > pea (19%) while the quizalafop-p-ethyl mediated shoot P reduction declined over controls as: lentil (25%) > greengram (22%) > chickpea (19%) > pea (18%) (Table 2).

Further, quizalafop-p-ethyl also significantly decreased the seed protein of chickpea and greengram by 23 and 10%, respectively, compared to control with marginal inhibitory effect on pea and lentil. Moreover, seed yield of each legume was also adversely affected in the presence of quizalafop-p-ethyl and the following decreasing trend (percent decline over controls) was documented: lentil (57%) > greengram (51%) > chickpea (29%) > pea (28%).

DISCUSSION

In this study, quizalafop-p-ethyl adversely affected the growth of all tested legumes and significantly

decreased their root and shoot growth and symbiotic attributes. The decline in growth of legumes following quizalafop-p-ethyl application in this study could be due to the toxic effects of this herbicide on plant organs, especially the function of nodules which consequently disrupts the legume-*Rhizobium* symbiosis and hence, the N_2 fixation and in turn the overall plant growth (Evans *et al.*, 1991). In addition, the inhibitory effect of the herbicide application may possibly be due to the inhibition of enzymes involved in growth and metabolisms (Zablotowicz and Reddy, 2004) or due to disruption of signaling between (legume derived) phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors that is necessary for initiation of nodulation and N_2 fixation (Fox *et al.*, 2007). Reports on the effect of herbicides on symbiotic attributes of legumes are, however, contradictory. For example, sethoxydim, alachlor, fluazifop butyl and metolachlor at recommended rates did not result in detrimental effects on seed yields or symbiosis in soybean while paraquat significantly reduced the amount of N_2 fixed as measured by ^{15}N dilution methods (Kucey *et al.*, 1989). Similarly, the adverse effects of terbutryn/terbuthylazine and bentazone on the performance of pea (Singh and Wright, 2002) and the phytotoxic effects of chlorimuron-ethyl on *Bradyrhizobium japonicum* inoculated soybean (Zawoznik and Tomaro, 2005) are reported.

However, the degree of toxicity of quizalafop-p-ethyl to the parameters to each legume varied considerably in this study. For instance, the selected herbicide completely suppressed the nodule formation in lentil plants while nodulation in greengram plants affected the least in the presence of quizalafop-p-ethyl. The variable response of the tested legumes to quizalafop-p-ethyl is due to the fact that extent of toxicity of any specific herbicide to the plants depends upon the both genetics and physiology of plants which varies from one plant species to another (Ahmad *et al.*, 2009). In yet other report Anderson *et al.* (2004) claimed that herbicides may negatively affect the nodulation in legumes by:

- Directly affecting root and shoot biomass of the host plant thereby limiting the number of available sites for the cognate rhizobia to attach to or by decreasing the carbohydrate supply to existing nodules
- Directly affecting rhizobial survival or growth that leads to a decreased potential for rhizobial infection on root hairs
- Inhibiting or inactivating the biochemical signaling that plants require to initiate nodule development- this inhibition could affect either rhizobia or plants
- Inhibiting nodule development by reducing the capacity for cell division

Moreover, quizalafop-p-ethyl, in this study, also significantly decreased chlorophyll content measured at pod fill stage of chickpea (90 DAS), pea (90 DAS), greengram (50 DAS) and lentil (90 DAS) plants. As reported by Boldt and Jacobsen (1998) that the pesticides adversely affect the metabolic enzymes, therefore, it seems probable that pesticides employed in this study might have inversely inhibited the functioning of the enzymes of Photosynthetic Carbon Reduction (PCR) cycle, such as Rubisco, 3-PGA kinase, NADP, NAD-Glyceraldehyde-3-P-dehydrogenase and aldolase. Nitrogen and P content of the legume plants is one of the most important aspects of legume growth. The nitrogen content in roots and shoots determined at different stages of chickpea, lentil, pea and greengram differed among treatments. The decrease in N contents of legumes might have been due to the reduction in legume-*Rhizobium* symbiosis, as indicated by a decline in the nodulation in this study. In agreement to this finding, Fox *et al.* (2007) concluded that agrichemicals including herbicides induce a symbiotic phenotype that inhibits or delays recruitment of rhizobia to host plant roots, fewer root nodules produced, lowers the rate of nitrogenase activity which in turn reduces N content and decrease the overall plant yields at harvest. A similar reduction in N uptake in chickpea plants following herbicides application has been reported (Khan *et al.*, 2006). However, the reduction in P content and seed attributes following herbicide application could probably, be due to inhibition of the enzymes and functional proteins of metabolic pathways involved in protein synthesis and P-uptake (Boldt and Jacobsen, 1998). In addition, it is reported that application of increased concentration of herbicides affect the photosynthetic pigments and photo-system II electron transport and therefore, the process of photosynthesis which in turn inversely affect the vegetative growth and seed yield of crops (Eilers *et al.*, 1992; Grossman, 2000; Khan *et al.*, 2006).

CONCLUSION

In this study, quizalafop-p-ethyl showed a varying toxicity to the tested legumes. The highest toxicity of quizalafop-p-ethyl was observed on the root P content and the root growth in greengram, shoot growth, shoot N and seed protein in chickpea and nodulation, root N, shoot P and seed yield in lentil. In general, the most adverse impact of quizalafop-p-ethyl was manifested on the growth parameters of lentil plants. These findings evidently revealed that an outcome of the phytotoxic effects of a specific herbicide on a specific plant genotype can not be generalized for all plant species. The degree of toxicity of any herbicide and the type of plant organs affected may differ from one plant species to another.

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