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Growth suppression of legumes in pyriproxyfen stressed soils: A comparative study

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Abstract

Insecticides are commonly used to combat economically important insect pests in agriculture. These plant-protecting agents severely decline crop productivity by affecting different plant growth parameters. The assessment of phytotoxicity of insecticides are generally, confined to one plant species and broad studies concomitantly evaluating the insecticide effect on more than one crop specifically, the legume are scarce. Hence, this study was designed to assess the effect of technical grade insecticide, pyriproxyfen simultaneously on legumes like chickpea, pea, lentil and greengram. Pyriproxyfen showed the highest toxicity to root and shoot dry biomass, leghaemoglobin, chlorophyll content and seed protein in chickpea, nodule numbers in pea, shoot nitrogen and root phosphorus in greengram, and nodule biomass, root nitrogen, root phosphorus, shoot phosphorus and seed yield in lentil. For instance, pyriproxyfen decreased the number of nodules (percent decline over controls) in each legume in an order: pea (44) > greengram (14) > chickpea (5) = lentil (5). Similarly, pyriproxyfen mediated percent decline in leghaemoglobin occurred in the order like: chickpea (69) > lentil (25) > pea (18) > greengram (12). Generally, pyriproxyfen affected most adversely the growth of both chickpea and lentil. It is concluded that the extent of phyto-toxicity of insecticide and the type of plant organs affected might differ among plant species.

Key words: Insecticide, Pyriproxyfen, Toxicity, Legume, Soil

Introduction

In agricultural fields and farms, considerable amount of pesticides are being used to increase the agricultural production, by controlling insect pests, diseases and weeds as these chemicals act on pests that are detrimental to agricultural output (Ahemad and Khan, 2011a). Pesticides including insecticides accumulated in soils disturb the natural ecological balance by producing toxic effects in recipient environments (Skevas et al., 2012; Pal et al., 2006). The behavior of pesticides in the environment depends on its stability, physico-chemical properties, the nature of the medium into which it is applied, the organisms present, and the prevailing climatic conditions (Ahemad et al., 2009; Ismail et al., 2009; Abou Ayana et al., 2011). Some of the negative effects of pesticides include low crop yield (Fox et al., 2007; Ahemad and Khan, 2012a), destruction of soil micro-fauna and flora (Chowdhury et al., 2008) and their beneficial physiological activities (Madhaiyan et al., 2006; Ahemad and Khan, 2010), undesirable residue accumulation in food crops (Mattina et al., 2000) and decreased soil fertility (Abdalla et al., 2009). Further, they affect the soil microbial communities by adversely decreasing protein synthesis and inhibiting various metabolic enzymes (Boldt and Jacobsen, 1998; Ahemad and Khan, 2012b; Ahemad and Khan, 2012c). Moreover, they also damage the structural proteins by geno-toxicity and by altering the membrane composition (Pham et al., 2004; Kumar et al., 2010).

Among the pesticides used in modern agronomical practices, pyriproxyfen [4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether] (CAS No. 95737-68-1), a broad spectrum insecticide belonging to juvenile hormone mimics is being used in crop production to control many insect pests (e.g., locusts, ticks, whiteflies, houseflies and mosquitoes) both at larval and adult

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stages (Ahemad and Khan, 2011b). Previous studies of assessment of phyto-toxicity of insecticides are generally, restricted to any single crop and comprehensive data evaluating the impact of any specific insecticide on more than one legume in parallel is rare. Experimentation with large number of legume crops with respect to specific insecticide would remove the ambiguity in the reported results consequently would be more informative. Hence, this study aims to evaluate the effect of pyriproxyfen on four commonly grown legumes like, chickpea (*Cicer arietinum* L.), pea (*Pisum sativum*), lentil (*Lens esculentus*) and greengram (*Vigna radiata* L. Wiclzek) simultaneously, to characterize the effects of pyriproxyfen on legumes.

Materials and Methods

Insecticide

The technical grade (a.i. 98%) pyriproxyfen was obtained from Parijat Agrochemicals (New Delhi, India). To prevent the degradation, the stock solution was prepared just prior to each experiment by dissolving insecticide in solvent [dimethyl sulfoxide (DMSO)]. The recommended field dose (1300 µg/ kg soil) of pyriproxyfen was used for the experiments.

Plant growth evaluation

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 and surface sterilized with 70% ethanol, 3 min.; 3% sodium hypochlorite, 3 min.; rinsed six times with sterile water and dried. The soil used in pot experiments had the following properties: sandy clay loam, 0.4% organic carbon (C), 0.75 g kg^{-1} Kjeldahl nitrogen (N), 16 mg kg^{-1} Olsen phosphorus (P), 0.44 ml g^{-1} water holding capacity (WHC), 11.7 cmol kg^{-1} cation exchange capacity, 5.1 cmol kg^{-1} anion exchange capacity and pH 7.2. A total of ten seeds of each legume were sown in clay pots (25 cm high, 22 cm internal diameter) using three kg unsterilized soils with a control (without pyriproxyfen) and a treatment with the recommended field rate of pyriproxyfen (in three replicates for each legume). Seeds of chickpea, lentil, greengram and pea were sown in October (2007), November (2007), March (2008) and November (2007), 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 and were maintained in an open field.

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 Manickam (1992).

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 Arnon, (1949). 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. 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 Lowrey (1951).

Statistical analysis

The experiments were repeated the next year (2008-2009) with the similar environmental conditions and with the same insecticide treatments 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 (ANOVA). The difference among treatment means was compared by Tukey test (one-way ANOVA) at 5% probability level by statistical software, SPSS 10.

Results and Discussion

The growth parameters of chickpea, pea, greengram and lentil assessed concurrently in the presence of the recommended field rate of pyriproxyfen showed that the root and shoot biomass and the symbiotic attributes (numbers, dry weight and leghaemoglobin content in nodules) varied significantly in pyriproxyfen-amended soils.

In general, pyriproxyfen had a detrimental effect on root and shoot growth. Pyriproxyfen decreased the root biomass of chickpea by highest degree over control. In contrast, the pea plants suffered the least reduction in root biomass

compared to control. Pyriproxyfen declined (percent decline above control) the root biomass of the legumes in the following order: chickpea (46) > greengram (45) > lentil (27) > pea (13). Similarly, the percent reduction in shoot biomass in the presence of pyriproxyfen was observed as: chickpea (46) > greengram (45) > lentil (27) > pea (13).

Nodulation in legumes is an important growth parameter. Therefore, the symbiotic attributes of the tested legumes were also assessed under insecticide-stress. The recommended dose of pyriproxyfen negatively affected on the nodule development for each legume. Pyriproxyfen decreased the number of nodules (percent decline over controls) in each legume in an order: pea (44) > greengram (14) > chickpea (5) = lentil (5). Comparative evaluation of nodule numbers of legume species does not provide an accurate assessment 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 nodulation. Therefore, these two symbiotic characteristics for each legume were also determined. The toxic effect of pyriproxyfen on dry biomass (percent decline over controls) was observed in an array as: lentil (33) > chickpea (24) > greengram (19) > pea (17). On the contrary, pyriproxyfen decreased Lb content in nodules of the tested legumes in the following order (percent decline over controls): chickpea (69) > lentil (25) > pea (18) > greengram (12) (Table 1).

The decline in growth of legumes following pyriproxyfen application in our study could be due to the toxic effects of this insecticide 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 insecticide application may possibly be due to (i) the inhibition of enzymes involved in growth metabolisms (Zablotowicz and Reddy, 2004; Ahmad et al., 2003) (ii) disruption of signaling between (legume derived) phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors (Fox et al., 2007). Moreover, plants produced phytoestrogens discourage herbivores and attract insects and also act as recruitment signals for rhizobia. Pesticides have been reported to obstruct the phytoestrogen signaling system that regulates symbiosis between legume plants and rhizobia (Fox et al., 2004). Additionally, pesticides not only inhibit the biochemical signaling between the hosts and the cognate rhizobia but also block the initial attachment of complementary rhizobia to lectins present on root hairs as the recognition sites by protecting them (Musarrat and Haseeb, 2000). As a result, pesticides also adversely affect the legume-*Rhizobium* symbiosis due to competition of pesticides for the rhizobial-binding sites (lectins) on the surface of the legume root hairs.

Table 1. Effect of pyriproxyfen on dry biomass and symbiotic properties of legume crops.

Legumes	Dose rate (µg/ kg soil)	Dry biomass (g/ plant)		Nodulation		
		Root	Shoot	No./ plant	Nodule biomass (mg/ plant)	Leghaemoglobin content [mM (g.f.m.)$^{-1}$]
Chickpea	0 (control)	0.91b	3.80a	21b	180c	0.13c
	1300	0.49e	1.93c	20c	136d	0.04h
Pea	0 (control)	0.92a	2.07b	27a	283a	0.17a
	1300	0.80c	1.84d	15f	236b	0.14b
Greengram	0 (control)	0.47f	2.08b	21b	66e	0.08f
	1300	0.26h	1.33f	18e	54f	0.07g
Lentil	0 (control)	0.55d	1.97c	19d	30g	0.12d
	1300	0.40g	1.66e	18e	20h	0.09e
LSD ($p \leq 0.05$)		0.008	0.05	0.67	5.31	0.003
F value		12.6	106	263.3	489.5	27.9

Values are mean of three replicates where each replicate constituted three 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; (g.f.m.)$^{-1}$ = (gram fresh biomass)$^{-1}$

Reports on the effect of insecticides on symbiotic attributes of legumes are, however, contradictory. For instance, Fox et al. (2007) reported a considerable decrease in nodulation, total plant biomass and nitrogenase activity of alfalfa (*Medicago sativa* L.), when grown in soil treated separately with methyl parathion, DDT, bisphenol A and pentachlorophenol. In contrast, monocrotophos, quinolphos and cypermethrin at lower concentration were stimulatory to ammonification process in agricultural soils while toxic at higher concentration (Rangaswamy and Venkateswarlu, 1993). A comparable observation on the effect of insecticides on legumes has been reported. For example, effects of afugan, brominal, gramoxone, selecron and sumi oil on growth and nodulation of soybean (*Glycine max*) were determined by Abd-Alla et al. (2000). Growth of cowpea (*Vigna sinensis* L.), common bean (*Phaseolus vulgaris* L.) and lupin (*Lupinus albus* L.) was inhibited by afugan, brominal, gramoxone and selecron application. However, the effect of insecticides varied with the pesticide applied and plant species. In addition, Aggarwal et al. (1986) evaluated the effect of carbamate on nodulation in *Pisum sativum* and *Vigna sinensis*. They reported that the low concentrations of the insecticides had little effect on nodulation whereas higher concentrations adversely affected it. In a similar study, Alonge (2000) evaluated the phytotoxicity of imazaquin on the growth of soybean plants and found that imazaquin reduced chlorophyll content in leaves, root nodules, shoot growth, whole plant dry weight and grain yield.

In our study, the degree of toxicity of pyriproxyfen to the parameters to each legume however, varied considerably. The variable response of the tested legumes to pyriproxyfen is because the extent of toxicity of any specific insecticide to the plants depends upon the both genetics and physiology of plants that varies from one plant species to another (Ahemad and Khan, 2011c). According to Anderson et al. (2004), insecticides negatively affect the nodulation in legumes by limiting the number of available sites on host plants for the cognate rhizobia by decreasing the carbohydrate supply to existing nodules. Thus, insecticides decrease the rhizobial survival and growth, inactivate the biochemical signaling required to initiate nodule development in plants and inhibit the nodule development by reducing cell division.

Generally, the chlorophyll content in leaves of each legume significantly decreased when grown in pyriproxyfen-amended soils. The most toxic effect of the insecticide was observed on the total chlorophyll of chickpea plants wherein the chlorophyll content decreased by 14% above control. Moreover, pyriproxyfen mediated reduction in the chlorophyll content of lentil was found to be 9% compared to control. Furthermore, marginal decline in the total chlorophyll of pea and greengram was observed (4 and 7% respectively, compared to control) (Table 2).

Table 2. Effect of pyriproxyfen on biological and chemical properties of legume crops.

Legumes	Dose rate (µg/ kg soil)	Chlorophyll content (mg/g)	N content (mg/g)		P content (mg/g)		Seed protein (mg/g)	Seed yield (g/plant)
			Root	Shoot	Root	Shoot		
Chickpea	0 (control)	1.96a	18e	27e	0.17d	0.21f	241c	2.7d
	1300	1.68b	15g	24f	0.17d	0.20g	228e	1.8e
Pea	0 (control)	0.75d	34b	45b	0.21b	0.28c	224f	7.4a
	1300	0.72e	29d	42cd	0.19c	0.25d	223f	7.2a
Greengram	0 (control)	0.82c	36a	50a	0.27a	0.36a	261a	7.4a
	1300	0.76d	31c	43c	0.22b	0.31b	251b	5.5b
Lentil	0 (control)	0.32f	17f	45b	0.21b	0.28c	232d	3.0c
	1300	0.29g	14h	41d	0.17d	0.22e	225f	1. 8e
LSD ($p \leq 0.05$)		0.018	0.72	1.53	0.01	0.005	2.7	0.35
F value		115.4	244	361.8	71.3	117.8	178.5	102.3

Values are mean of three replicates where each replicate constituted three 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.

Pyriproxyfen decreased the root N by 17, 15, 14 and 18% in chickpea, pea, greengram and lentil respectively, compared to respective controls. Conversely, the decline in the shoot N of each legume exposed to the insecticide-stress was found to follow the trend as: greengram (14%) > chickpea (11%) > lentil (9%) > pea (7%) (Table 2). In addition, the root P of the tested legumes in response to pyriproxyfen exposure decreased in the following order: greengram (19%) = lentil (19%) > pea (19%) > chickpea (0%) while pyriproxyfen declined shoot P over controls in an array: lentil (21%) > greengram (14%) > pea (11%) > chickpea (5%) (Table 2).

Moreover, pyriproxyfen also significantly decreased the seed protein of chickpea and greengram by 5% and 4%, respectively, compared to control while seed protein in pea and lentil plants was marginally decreased (1% and 3%, respectively over controls). Generally, seed yield of each legume was also adversely affected under pyriproxyfen-stress. The lentil suffered the maximum reduction in seed yield while the least decline in seed yield was observed for pea in the presence of pyriproxyfen. The following decreasing trend (percent decline over controls) was observed: lentil (40) > chickpea (33) > greengram (26) > pea (3).

As reported by Boldt and Jacobsen (1998) that the pesticides adversely affect the metabolic enzymes, therefore, it seems probable that insecticide employed in this study might has 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 insecticides 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. However, the reduction in P content and seed attributes following insecticide 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; Nare et al., 2010; Ahemad and Khan, 2011a).

Conclusions

Pyriproxyfen showed varying degree of toxicity to the selected legumes. The highest toxicity of pyriproxyfen was observed on root dry biomass, shoot dry biomass, leghaemoglobin chlorophyll content and seed protein in chickpea; nodule numbers in pea; shoot N and root P in greengram; and nodule biomass, root N, root P, shoot P and seed yield in lentil. In general, the most adverse impact of pyriproxyfen was observed on the growth parameters of chickpea and lentil. These findings demonstrate that an outcome of the effects of a specific insecticide on a specific crop plant species cannot be generalized. The degree of toxicity of any insecticide and the type of plant organs affected may differ from one plant species to another.

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