Bull Environ Contam Toxicol (2009) 82:761–766
DOI 10.1007/s00128-009-9692-x

Toxicity Assessment of Herbicides Quizalafop-*p*-Ethyl and Clodinafop Towards *Rhizobium* Pea Symbiosis

Munees Ahemad · Mohammad Saghir Khan

Received: 22 August 2008 / Accepted: 26 February 2009 / Published online: 17 March 2009
© Springer Science+Business Media, LLC 2009

Abstract In modern conventional agriculture, herbicides are frequently used to prevent yield losses due to weeds. Herbicides also affect negatively the productivity of legumes. With these considerations, we evaluated the effects of soil applications of different concentrations of quizalafop-*p*-ethyl and clodinafop on the performance of *Rhizobium* inoculated pea, grown in clay pots. In this study, the concentration of herbicides higher than the recommended rates of quizalafop-*p*-ethyl and clodinafop adversely affected the dry matter accumulation, symbiotic properties, grain yield and nutrient status of pea plants. Toxicity of quizalafop-*p*-ethyl and clodinafop to pea plants increased progressively with increase in rates of herbicides. Of the two herbicides, quizalafop-*p*-ethyl was more toxic than clodinafop. In contrast, when herbicide tolerant *Rhizobium* strain MRP1 was also used with herbicide, it increased the measured parameters at all concentrations. A maximum increase of 11%, 17%, 46%, 33%, 21% and 7% in the root N, shoot N, root P, shoot P, seed yield and seed protein, respectively, was observed when MRP1 was used with 120 μg quizalafop-*p*-ethyl kg^{-1} soil while with 1,200 μg clodinafop kg^{-1} soil it increased the root N, shoot N, root P, shoot P, seed yield and seed protein by 20%, 9%, 56%, 56%, 29% and 7%, respectively, compared with the un-inoculated but herbicide treated control. This study suggested that the toxic effects of herbicides on pea plants could be attenuated by applying growth promoting herbicide tolerant strain of *Rhizobium* under herbicide stressed soil environment.

M. Ahemad · M. S. Khan (✉)
Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, UP, India
e-mail: khanms17@rediffmail.com

Keywords Herbicide · Pea · *Rhizobium* · Symbiosis · Nutrient status

In modern agronomic practices, herbicides are often used to control weeds and consequently to improve plant productivity. However, the intensive and injudicious application of herbicides lead to their accumulation in soils and may deteriorate the quality of soil and water and also affect the microbial population of soils (Aamil et al. 2004; Javier Benitez et al. 2006; Nomal 2006). The rhizospheric microorganisms form an important component of soil and play a pivotal role in augmenting the plant growth and protecting the crop plants from stress factors and deleterious plant pathogens. These organisms are also metabolically inactivated by the excessive application of herbicides to soils (Singh and Wright 2002a). The phytotoxic effects of various herbicides on legumes including pea (*Pisum sativum*), chickpea (*Cicer arietinum*), lentil (*Lens esculentus*) and fababean (*Vicia faba*) have been reported (Avola et al. 2004; Abbate et al. 2001; Wall 1996). Severity of these effects depends upon the type and concentration of herbicides, the *Rhizobium* species, and plant genotypes (Sawicka and Selwet 1998; Khan et al. 2004). However, reports of herbicidal effects on legumes and its symbionts (rhizobia) are contradictory. For instance, a significant reduction in the rhizobial growth has been observed for some herbicides (Santos et al. 2005) while two times of the field dose rates of herbicides did not influence rhizobial growth but reduced the subsequent ability of rhizobia to form nodules. However, the growth of rhizobia is only one aspect of symbiosis, which also depends on nodule formation, nodule growth and nodule function; all these processes can be affected by the herbicide applications. In other study, herbicides have shown a considerable reduction in the symbiotic efficiency and nitrogenase

activity of *Rhizobium leguminosarumin* bv. trifolii, *Sinorhizobiuni melilotii* and *Badyrhizobium* sp. during symbiosis with their host plants (Niewiadomska and Klama 2005). However, the adverse effects of herbicides on nodulation and nitrogen fixation of legume plants observed at concentrations not normally expected to occur under field conditions could, probably be due to their direct toxic effect on plant growth without adversely affecting the symbiotic partner (Singh and Wright 2002b). Pea is a leading legume of the world and is cultivated over an area of 5.9 m ha with a production of about 11.7 m t. In India, pea is grown over an area of 0.7 m ha accounting for about 0.6 m t and contributes 8% to total pulse area and about 5% in total pulses production (Ahlawat 2000). Though, a large number of reports on the effects of herbicides on legume-*Rhizobium* symbiosis are available, yet there is discrepancy in the reported results. And, hence, a firm conclusion on the phytotoxic effects of herbicides on legumes-*Rhizobium* symbiosis can not be drawn. Moreover, the reports on toxicity of quizalafop-*p*-ethyl and clodinafop on legumes are scarce. Due to lack of adequate data and conflicting reports on the effects of herbicides on legumes, the present study was conducted to assess the impact of technical grade herbicides, quizalafop-*p*-ethyl [ethyl (*RS*)-2-(4-6-chloroquinoxolin-2-yloxy) phenoxy] propionate] and clodinafop [(*R*)-2-[4-(5-chloro-3-fluoro-2-pyridyloxy) phenoxy] propionic acid], on biological and chemical properties of *Rhizobium* inoculated pea plants, grown in sandy clay loam soils, treated with or without herbicides.

Materials and Methods

Strains of *Rhizobium* were isolated from nodules borne on the root system of pea plants grown in experimental fields of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India, using yeast extract mannitol (YEM) medium. The rhizobial strains were tested for their sensitivity/resistance to technical grade quizalafop-*p*-ethyl and clodinafop (a.i. 98% for each herbicide; Parijat Agrochemicals, New Delhi, India) by agar plate dilution method using minimal salt agar medium. Indole-3-acetic acid (IAA) produced by rhizobial strain was quantitatively assayed by the method of Gordon and Weber (1951) later modified by Brick et al. (1991). The rhizobial strains were further screened for production of siderophores on the Chrome azurol S (CAS) agar medium by the method of Alexander and Zuberer (1991). The siderophore produced by the test strain was also quantitatively assayed for salicylic acid (SA) and 2,3 dihydroxybenzoic acid (DHBA) in the supernatant by the method of Reeves et al. (1983). Moreover, the exo-polysaccharide (EPS) production by the rhizobial strains was evaluated under in vitro conditions as described by Mody et al. (1989).

The experimental soil was an alluvial sandy clay loam (sand 667 g kg^{-1}, silt 190 g kg^{-1}, clay 143 g kg^{-1}, organic matter 6.2 g kg^{-1}, Kjeldahl N 0.75 g kg^{-1}, Olsen P 16 mg kg^{-1}, pH 7.2 and water holding capacity 0.44 mL g^{-1}, cation exchange capacity 11.7 and 5.1 cmol kg^{-1} anion exchange capacity). Seeds of pea var. C 235 were surface sterilized with 70% ethanol, 3 min followed by 3% sodium hypochlorite, 3 min, washed six times with sterile water and dried. The sterilized seeds were inoculated with *Rhizobium* strain MRP1, grown in YEM broth, by dipping the seeds in liquid culture medium for 2 h using 10% gum arabic as adhesive to deliver $\sim 10^8$ cells per seed. The non-coated sterilized seeds were soaked in sterile water only and served as control. The non-inoculated and inoculated seeds (10 seeds per pot) were sown in clay pots (25 cm high, 22 cm internal diameter) using 3 kg unsterilized soils with control (without quizalafop-*p*-ethyl and clodinafop) and three treatments with 40 (recommended), 80 and 120 μg kg^{-1} soil of quizalafop-*p*-ethyl and 400 (recommended), 800 and 1,200 μg kg^{-1} soil of clodinafop. For each treatment, six pots were used and arranged in a complete randomized design. Three plants were maintained in each pot 1 week after emergence. The pots were watered with tap water when required and were maintained in an open field conditions. The experiments were repeated for two successive years to ensure the reproducibility of the results. All plants in three pots for each treatment were removed 90 days after seeding (DAS) and were observed for the extent of nodulation. The roots were carefully washed and nodules were detached, counted, oven dried (at 80°C) and weighed. Plants uprooted at 90 DAS were oven-dried at (80°C) and the dry matter was measured. The leghaemoglobin (Lb) content in fresh nodules removed from the root system of plants was quantified at 90 DAS (Sadasivam and Manikam 1992). Total N and P content in roots and shoots was measured at 120 DAS by micro-Kjeldahl (Iswaran and Marwah 1980) and Jackson (1967) method, respectively. The remaining pots (three pots) for each treatment having three plants per pot were maintained until harvest (120 DAS) and seed yield and grain protein (Sadasivam and Manikam 1992) was determined. Since the experiment was conducted for 2 consecutive years under the identical environmental conditions using the same treatments and the data obtained were homogenous, the data of the measured parameters were pooled together and subjected to analysis of variance. The difference among treatment means was compared by high range statistical domain (HSD) using Tukey test at 5% level of probability.

Results and Discussion

The *Rhizobium* inoculated and non-inoculated pea plants grown in soil treated with quizalafop-*p*-ethyl and

clodinafop demonstrated a variable plant growth. In the absence of bioinoculant, 40, 80 and 120 μg kg^{-1} quizalafop-*p*-ethyl significantly ($p \leq .05$) decreased the dry matter by 51%, 66% and 74% at 90 DAS while 44%, 57% and 65% at 120 DAS, respectively (Table 1). Similarly, the sole application of clodinafop substantially declined the dry mass by 8%, 33% and 39% (at 90 DAS) and by 14%, 32% and 45% (at 120 DAS) at 400, 800 and 1,200 μg kg^{-1} soil, respectively, relative to the control. When *Rhizobium* strain MRP1 was also used with quizalafop-*p*-ethyl at 40, 80 and 120 μg kg^{-1} soil and clodinafop at 400, 800 and 1,200 μg kg^{-1} soil, the dry matter decreased by 32%, 41% and 48% (for quizalafop-*p*-ethyl) and 9%, 21% and 41% (for clodinafop), respectively, at 90 DAS and 23%, 49% and 59% (for quizalafop-*p*-ethyl) and 12%, 19% and 38% (for clodinafop), respectively, at 120 DAS. In general, with increase in concentration of each herbicide, a progressive decrease in the measured parameters was observed. Moreover, the bioinoculant when used with higher concentration of quizalafop-*p*-ethyl (120 μg kg^{-1} soil) significantly ($p \leq .05$) subsided the toxic effects on pea plants and increased the dry matter accumulation by 140% while 800 μg kg^{-1} clodinafop showed a maximum increase of 57% in dry matter production at 90 DAS compared to non-inoculated but treated with the same dose of clodinafop. Similarly, when used with inoculant, quizalafop-*p*-ethyl increased plant dry matter maximally by 58% at 40 μg kg^{-1} of soil and clodinafop increased maximum dry matter accumulation at a concentration of 800 μg kg^{-1} soil by 34% at 120 DAS (Table 1). Quizalafop-*p*-ethyl at 40 μg kg^{-1} soil decreased the nodule numbers by 74% while at 80 and 120 μg kg^{-1} soil, it completely inhibited the nodule formation. In comparision, clodinafop at 400, 800 and 1,200 μg kg^{-1} soil decreased the nodule numbers by 30%, 39% and 59%, respectively, relative to control at 90 DAS. A trend similar to 90 DAS was observed for 120 DAS with all concentrations of the two herbicides except 40 μg kg^{-1} of quizalafop-*p*-ethyl that completely diminished nodule formation. Interestingly, the two herbicides though reduced the symbiosis even in the presence of bioinoculant but the effect was lesser compared to only herbicide treated plants. For instance, in the presence of inoculant MRP1, quizalafop-*p*-ethyl (80 μg kg^{-1} soil) and clodinafop (1,200 μg kg^{-1} soil) decreased nodule numbers maximally by 84% and 65%, respectively at 90 DAS. At 120 DAS, all concentrations of quizalafop-*p*-ethyl inhibited nodulation whereas 800 μg kg^{-1} soil of clodinafop decreased nodule number by 53%, compared to control. While comparing the effects of each concentration of the two herbicides used either alone or in the presence of MRP1, a maximum increase of 85% and 26% in nodule numbers was observed when strain MRP1 was used with 40 μg kg^{-1} quizalafop-*p*-ethyl and 400 μg kg^{-1} clodinafop, respectively, at 90 DAS compared to the sole application of herbicides (Table 1). The trend similar to nodule numbers was observed for nodule mass. For example, quizalafop-*p*-ethyl when used alone, decreased nodule dry mass by 38% at 40 μg kg^{-1} soil while clodinafop decreased the nodule mass by 11% at 400 μg kg^{-1} soil, compared to control at 90 DAS. In

Table 1 Effect of three concentrations of quizalafop-*p*-ethyl and clodinafop on biological properties of pea plants grown in soil inoculated with and without *Rhizobium* sp. strain MRP1

Herbicides	Dose rate (μg kg^{-1} soil)	Dry weight (g $plant^{-1}$)				Nodulation			
						No. $plant^{-1}$			
		90 DAS		120 DAS		90 DAS		120 DAS	
		UI	I	UI	I	UI	I	UI	I
Control		1.71a(0.05)	2.07a(0.07)	2.99a(0.12)	3.39a(0.14)	27a(2.0)	36a(3.0)	15a(1.2)	19a(1.3)
Quizalafop-*p*-ethyl	40	0.84c(0.04)	1.40d(0.04)	1.66d(0.08)	2.62b(0.10)	7d(0.5)	13c(1.0)	–	–
	80	0.58d(0.05)	1.22e(0.05)	1.28e(0.09)	1.74c(0.07)	–	6d(0.5)	–	–
	120	0.45d(0.03)	1.08f(0.03)	1.04f(0.06)	1.38d(0.04)	–	–	–	–
Clodinafop	400	1.58a(0.06)	1.89b(0.06)	2.58b(0.07)	3.00a(0.07)	19b(1.0)	24b(1.4)	9a(0.5)	18a(1.0)
	800	1.14b(0.04)	1.63c(0.05)	2.05c(0.05)	2.75c(0.06)	16b(1.2)	20b(1.6)	–	9b(0.7)
	1,200	1.04b(0.05)	1.23e(0.04)	1.63d(0.04)	2.11d(0.07)	11c(0.7)	13c(1.1)	–	–
F value		231.8	248.8	1,104.2	1,088.9	42.9	96.4	3.5	117.3

In this and succeeding tables, values are mean of three replicates. Mean values followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test. Values in parenthesis indicate standard deviation. UI = un-inoculated; I = inoculated. Strain MRP1 tolerated quizalafop-*p*-ethyl and clodinafop 1,600 and 2,400 μg L^{-1}, respectively, when grown in minimal salt agar medium. Strain MRP1 produced 32, 28, 25 and 21 μg L^{-1} of salicylic acid (SA); 22, 20, 18 and 15 μg L^{-1} of 2,3 dihydroxy benzoic acid (DHBA) and 32, 30, 28 and 25 μg mL^{-1} IAA at 0, 400, 800 and 1,200 μg L^{-1} clodinafop and 32, 22, 19 and 14 μg L^{-1} of SA; 22, 15, 14 and 10 μg L^{-1} of DHBA and 32, 23, 21 and 18 μg L^{-1} of IAA at 0, 40, 80 and 120 μg L^{-1} quizalafop-*p*-ethyl, respectively

contrast, quizalafop-*p*-ethyl without inoculant completely diminished nodule formation and hence, no nodule dry mass was recovered while clodinafop decreased nodule dry mass by 25% at 400 μg kg^{-1} soil compared to control and at subsequent concentrations of clodinafop no dry mass was obtained due to failure of nodule formation at 120 DAS. When used with MRP1, quizalafop-*p*-ethyl and clodinafop at double the recommended rate, decreased nodule dry mass by 67% and 28%, respectively, at 90 DAS while MRP1 when used with the normal rates of quizalafop-*p*-ethyl and clodinafop decreased nodule dry mass by 42% and 54%, respectively, compared to control at 120 DAS. A strong correlation was found between nodule numbers and nodule mass when pea was grown in the absence ($r = 0.91$) and the presence ($r = 0.94$) of bioinoculant at 90 DAS (Table 2).

In this experiment, the nodules on the root system of pea plants raised in soil treated with quizalafop-*p*-ethyl and clodinafop had considerably a lower concentration of Lb. In contrast, bio-inoculant increased the Lb content under the influence of quizalafop-*p*-ethyl and clodinafop. Lb content of nodules in the absence of bioinoculant progressively decreased as the doses of both quizalafop-*p*-ethyl and clodinafop was increased and a maximum decline was recorded at 120 μg kg^{-1} soil for quizalafop-*p*-ethyl and at 1,200 μg kg^{-1} soil for clodinafop in comparison to un-inoculated control. In contrast, in presence of bioinoculant, the Lb content decreased maximally by 43% at 80 μg kg^{-1} soil of quizalafop-*p*-ethyl and by 9% at 800 μg kg^{-1} soil of clodinafop relative to control. The bioinoculant increased the Lb content substantially when compared to the treatments having only herbicides. For instance, bioinoculant increased the Lb content maximally by 130% and 50% in the presence of 80 and 800 μg kg^{-1} soil of quizalafop-*p*-ethyl and clodinafop, respectively. The correlation between nodule mass and Lb content was more pronounced in the absence of inoculant ($r = 0.96$) compared to inoculanted treatments ($r = 0.79$). The total chlorophyll contents in fresh foliage of pea plants were decreased consistently as the rates of both herbicides was increased. Generally, quizalafop-*p*-ethyl and clodinafop declined the chlorophyll content marginally in this study, both in the presence or absence of bioinoculant. A maximum increase of 6% and 7% in the chlorophyll content was observed when strain MRP1 was also used with three times the recommended rates of quizalafop-*p*-ethyl and clodinafop compared to the uninoculated but treated with the same rates of two herbicides (Table 2).

The reduction in growth of pea plants following herbicide application observed in this study could be due to the adverse effects of quizalafop-*p*-ethyl and clodinafop on plant organs, especially the function of nodules which consequently diminishes the N_2 fixation. Such inhibitory effect following herbicide applications may possibly be due to the inhibition of enzymes involved in growth and metabolisms or due to disruption of signaling between legume (host) plant-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 effective symbiosis of rhizobia with the legume host plants are, however, contradictory. For example, sethoxydim, alachlor, fluazifop butyl and metolachlor at recommended rates did not result in detrimental effects on seed yields or N_2 fixation in soybean while paraquat significantly reduced the amount of N_2 fixed as measured by ^{15}N dilution methods (Kucey et al. 1988). Similarly, the adverse effects of terbutryn/terbuthylazine and bentazone on the performance of pea (Singh and Wright 2002b) and the phytotoxic effects of chlorimuron-ethyl on *Bradyrhizobium japonicum*

Table 2 Effect of three concentrations of quizalafop-*p*-ethyl and clodinafop on biological and chemical properties of pea plants grown in soil inoculated with and without *Rhizobium* sp. strain MRP1

Herbicides	Dose rate (μg kg^{-1} soil)	Nodulation				Lb content (mM)		Total chlorophyll (mg g^{-1})	
		Dry mass (mg $plant^{-1}$)							
		90 DAS		120 DAS		UI	I	UI	I
		UI	I	UI	I				
Control		283a(14)	373a(20)	65a(7.2)	124a(9.5)	0.17a(0.02)	0.23a(0.03)	224a(3.6)	240a(7.5)
Quizalafop-*p*-ethyl	40	176d(11)	190e(13)	–	–	0.10 cd(0.01)	0.16b(0.02)	220a(3.0)	232ab(4.5)
	80	–	123f(15)	–	–	–	0.13 cd(0.01)	218a(3.5)	230ab(3.8)
	120	–	–	–	–	–	–	216a(2.0)	228b(2.5)
Clodinafop	400	253b(19)	290b(27)	49b(5.5)	57b(4.7)	0.16ab(0.03)	0.22a(0.01)	224a(3.5)	239a(5.8)
	800	210c(15)	270c(16)	–	50c(3.0)	0.14ab(0.02)	0.21ab(0.01)	223a(2.5)	238ab(6.3)
	1,200	170d(14)	236d(12)	–	–	0.13ab(0.01)	0.19ab(0.02)	221a(3.0)	237ab(6.0)
F value		2,615.6	1,197	575.6	1,575	11.3	22.5	1.2	4.6

inoculated soybean (Zawoznik and Tomaro 2005) is reported. Plant growth promoting rhizobacteria including symbiotic N_2 fixers can affect plant development either indirectly by circumventing the toxic effects of pesticides (Yang and Lee 2008) or directly by synthesizing the plant growth regulating substances (Wani et al. 2008). Inoculation of quizalafop-*p*-ethyl and clodinafop tolerant and phytohormone producing *Rhizobium* strain MRP1 in this study stimulated the growth of pea when strain MRP1 was applied as seed inoculant in herbicide treated soil. Generally, the measured parameters were increased when inoculant strain was used with quizalafop-*p*-ethyl and clodinafop compared to plants grown in soils treated solely with quizalafop-*p*-ethyl and clodinafop. The present investigation suggested that the quizalafop-*p*-ethyl and clodinafop tolerance of the strain MRP1 might have provided protection to pea plants against the inhibitory effects of quizalafop-*p*-ethyl and clodinafop possibly due to entrapment of herbicides within the exo-polysachharides, released by the inoculant strain. In addition, the synthesis of siderophore and IAA by the strain MRP1 might also have enhanced root growth and uptake of soil minerals by the host plant. Moreover, the bioinoculant significantly increased the nodulation compared to un-inoculated control consolidating the fact that the strain MRP1 might have reduced the toxicity of quizalafop-*p*-ethyl and clodinafop in sandy loam soil, as was evident through the growth of this strain on minimal media using quizalafop-*p*-ethyl and clodinafop as C source.

Nitrogen (N) and P content, seed yield (SY) and grain protein (GP) decreased progressively with increase in the rates of quizalafop-*p*-ethyl and clodinafop (Table 3) both in the presence and absence of MRP1 strain. A maximum reduction in the measured parameters was observed at the highest rate of each herbicide. For example, at 120 µg quizalafop-*p*-ethyl kg^{-1} soil, the percent decrease was 24, 36, 39, 36, 50 and 4 for root N, shoot N, root P, shoot P, SY and GP, respectively, compared to the control. Similarly, for 1,200 µg clodinafop kg^{-1} soil, the percent decrease was 15, 29, 24, 18, and 14 for root N, shoot N, root P, shoot P, and SY, respectively, compared to the control. Similar trend was also observed when inoculant strain was used along with herbicides. However, the inoculant strain significantly ($p < 0.05$) increased the shoot and root N and P, SY and GP at all concentration of herbicides compared to the sole application of herbicides. For example, the inoculant strain when used with herbicide, increased the root N, shoot N, root P, shoot P, SY and GP by 11%, 17%, 46%, 33%, 21% and 7%, respectively, at 120 µg quizalafop-*p*-ethyl kg^{-1} soil and 20%, 9%, 56%, 56%, 29% and 7%, respectively, at 1,200 µg clodinafop kg^{-1} soil compared with only herbicide treated soils (Table 3). A comparable observation on the effect of herbicides on legumes and

Table 3 Effect of three concentrations of quizalafop-*p*-ethyl and clodinafop on nitrogen and phosphorus uptake, seed yield and seed protein in pea plants grown in soil inoculated with and without *Rhizobium* sp. strain MRP1

Herbicides	Dose rate (µg kg^{-1} soil)	N uptake (mg g^{-1})				P uptake (mg g^{-1})				Seed yield (g $plant^{-1}$)		Seed protein (mg g^{-1})	
		Shoot		Root		Shoot		Root					
		UI	I	UI	I	UI	I	UI	I	UI	I	UI	I
Control		45a(2.5)	52a(2.2)	34a(3.2)	40a(2.0)	0.28a(0.03)	0.36a(0.04)	0.21a(0.02)	0.30a(0.04)	7.4a(0.3)	9.6a(0.6)	224a(4.0)	240a(5.0)
Quizalafop-*p*-ethyl	40	37bc(2.0)	43bc(2.0)	29ab(2.4)	35ab(4.0)	0.23a(0.02)	0.29ab(0.03)	0.17a(0.02)	0.24ab(0.03)	5.3b(0.3)	6.5b(0.4)	220a(3.5)	232ab(7.0)
	80	35bc(2.5)	39bc(1.5)	27c(2.5)	33ab(3.0)	0.21a(0.03)	0.27ab(0.02)	0.15a(0.01)	0.22ab(0.03)	4.8b(0.4)	5.7bc(0.2)	218a(4.0)	230ab(4.4)
	120	29c(3.1)	34c(2.5)	26c(2.0)	29c(3.0)	0.18a(0.02)	0.24b(0.02)	0.13a(0.02)	0.19b(0.01)	3.7c(0.3)	4.5c(0.2)	216a(3.7)	228b(5.5)
Clodinafop	400	42ab(2.2)	49a(3.1)	34a(1.8)	39a(2.5)	0.28a(0.03)	0.33a(0.03)	0.19a(0.02)	0.29a(0.03)	7.1a(0.5)	9.3a(0.3)	224a(2.2)	239ab(6.4)
	800	39bc(2.4)	44ab(2.1)	32ab(2.0)	37ab(2.0)	0.26a(0.03)	0.31ab(0.02)	0.18a(0.01)	0.27ab(0.01)	6.9a(0.4)	8.8a(0.2)	223a(2.5)	238ab(7.5)
	1,200	32bc(2.7)	35bc(1.8)	29ab(1.5)	35ab(2.3)	0.23a(0.02	0.28ab(0.02)	0.16a(0.02)	0.25ab(0.04)	6.4a(0.2)	8.3a(0.1)	221a(2.0)	237ab(5.7)
F value		6.7	12.7	4.6	3.5	2.7	2.0	1.9	3.8	36.7	38.1	1.1	4.1

Strain MRP1 produced 20, 21, 23 and 26 µg mL^{-1} EPS at 0, 400, 800 and 1,200 µg L^{-1} clodinafop and 20, 20, 21 and 23 µg L^{-1} of EPS at 0, 40, 80 and 120 µg L^{-1} quizalafop-*p*-ethyl, respectively

other crops has been reported (Khan et al. 2006a, b). The reduction in the bio-chemical properties of inoculated or uninoculated pea could possibly be due to the inactivation of nitrogenase (Zablotowicz and Reddy 2004).

In this study, we demonstrated the deleterious effects of quizalafop-*p*-ethyl and clodinafop on the performance of pea plants, grown in herbicide treated alluvial soils. The inoculation of *Rhizobium* sp. strain MRP1 used as seed inoculant, however, not only protected the pea plants from the toxicity of these herbicides but also increased the growth, symbiotic properties, nutrient status and quantity and quality of pea seeds. The increased growth of inoculated pea plants even in the presence of herbicides as observed in this study might have possibly been due to the synthesis and release of plant growth promoting substances, and siderophores by strain MRP1 and reduced availability of herbicides to plants due to EPS secreted by rhizobial strain in the soil environment in addition to its natural ability of fixing N. The rhizobial strain endowed with such multiple properties of growth promotion could be exploited as bioinoculant for the better performance of pea even under herbicide stressed soil environment.

Acknowledgments We are grateful to Dr. Naqvi, Parijat Agrochemicals, New Delhi, India, for providing technical grade herbicides.

References

Aamil M, Zaidi A, Khan MS (2004) Effects of herbicide dose rate on nodule bacteria and growth, nodulation and yield of chickpea (*Cicer arietinum* L.). Ann Pl Protec Sci 12:186–191

Abbate V, Avola G, Tuttobene R, Barbera A (2001) Valutazione di variata di fava, cece, pisello proteico, lenticchia e cicerchia, Inf. Agrar 39:67–75

Ahlawat IPS (2000) Pea. In: Rathore PS (ed) Technique and management of filed crop production. Agrobios Publications, New Delhi

Alexander DB, Zuberer DA (1991) Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol Fert Soils 12:39-45 doi: 10.1007/BF00369386

Avola G, Gresta F, Patane C, Abbate V (2004) Chemical weeds control and sowing depth in grain legumes. In Proceedings of the VIII International Congress of European Society of Agronomy, Copenhagen, Denmark, 571–572

Brick JM, Bostock RM, Silversone SE (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl Environ Microbiol 57:535–538

Fox JE, Gulledge J, Engelhaupt E, Burow ME, McLachlan JA (2007) Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants. PNAS 104:10282–10287. doi:10.1073/pnas.0611710104

Gordon S, Weber RP (1951) The colorimetric estimation of IAA. Plant Physiol 26:192–195

Iswaran V, Marwah TS (1980) A modified rapid Kjeldahl method for determination of total nitrogen in agricultural and biological materials. Geobios 7:281–282

Jackson ML (1967) Soil chemical analysis. Prentice-Hall of India, New Delhi, pp 134–144

Javier Benitez F, Real FJ, Acero JL, Garcia C (2006) Photochemical oxidation processes for the elimination of phenyl-urea herbicides in waters. J Hazard Mater B 138:278–287. doi:10.1016/j.jhazmat.2006.05.077

Khan MS, Aamil M, Zaidi A (2004) Influence of herbicides on Chickpea-*Mesorhizobium* symbiosis. Agronomie 24:123–127. doi:10.1051/agro:2004009

Khan MS, Chaudhry P, Wani PA, Zaidi A (2006a) Biotoxic effects of the herbicides on growth, seed yield, and grain protein of greengram. J Appl Sci Environ Mgt 10:141–146

Khan MS, Zaidi A, Rizvi PQ (2006b) Biotoxic effects of herbicides on growth, nodulation, nitrogenase activity, and seed production in chickpeas. Commu Soil Sci Pl Anal 37:1783–1793. doi:10.1080/00103620600710645

Kucey RMN, Chaiwanakupt P, Arayangkool T, Snitwongse P, Siripaibool C, Wadisirisuk P, Boonkerd N (1988) Effect of herbicides and water application schedule. Plant Soil 108:87–92. doi:10.1007/BF02370103

Mody BR, Bindra MO, Modi VV (1989) Extracellular polysaccharides of cowpea rhizobia: compositional and functional studies. Arch Microbiol 1:2–5

Niewiadomska A, Klama J (2005) Pesticide side effect on the symbiotic efficiency and nitrogenase activity of Rhizobiaceae bacteria family. Pol J Microbiol 54:43–48

Nomal SS (2006) Allelopathy in ecological sustainable agriculture. In: Reigosa MJ, Pedrol N, Gonzalez L (eds) Allelopathy: a phsiological processes with ecological implications. Springer, Dordrecht, pp 537–564

Reeves MW, Pine L, Neilands JB, Balows A (1983) Absence of siderophore activity in *Legionella species* grown in iron-deficient media. J Bacteriol 154:324–329

Sadasivam S, Manikam A (1992) Biochemical methods for agricultural sciences. Wiley Eastern Limited, New Delhi

Santos JB, Ferreira EA, Kasuya MCM, Silva AA, Procopio SO (2005) Tolerance of *Bradyrhizobium* strains to glyphosate formulations. Crop Prot 24:543–547. doi:10.1016/j.cropro.2004.10.007

Sawicka A, Selwet M (1998) Effect of active ingredients on *Rhizobium* and *Bradyrhizobium* Legume dinitrogen fixation. Pol J Environ Studies 7:317–320

Singh G, Wright D (2002a) In vitro studies on the effects of herbicides on the growth of rhizobia. Lett Appl Microbiol 35:12–16. doi:10.1046/j.1472-765X.2002.01117.x

Singh G, Wright D (2002b) Effects of herbicides on nodulation and growth of two varieties of peas (*Pisum sativum*). Acta Agronom Hung 50:337–348. doi:10.1556/AAgr.50.2002.3.11

Wall DA (1996) Lentil (*Lens culineris*) and faba bean (*Vicia faba*) tolerance to post emergence application of imazethapyr. Can J Plant Sci 76:525–529

Wani PA, Khan MS, Zaidi A (2008) Effect of metal-tolerant plant growth-promoting *Rhizobium* on the performance of pea grown in metal-amended soil. Arch Environ Contam Toxicol. doi 10.1007/s00244-007-9097-y

Yang C, Lee C (2008) Enrichment, isolation, and characterization of 4-chlorophenol-degrading bacterium *Rhizobium* sp. 4-CP-20. Biodegradation 19:329–336

Zablotowicz RM, Reddy KN (2004) Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate-resistant transgenic soybean: a minireview. J Environ Qual 33:825–831

Zawoznik MS, Tomaro ML (2005) Effect of chlorimuron-ethyl on *Bradyrhizobium japonicum* and its symbiosis with soybean. Pest Manage Sci 61:1003–1008. doi:10.1002/ps.1077