Bull Environ Contam Toxicol (2011) 86:384–388
DOI 10.1007/s00128-011-0231-1

Effect of Pesticides on Plant Growth Promoting Traits of Greengram-Symbiont, *Bradyrhizobium* sp. strain MRM6

Munees Ahemad · Mohammad Saghir Khan

Received: 11 December 2010 / Accepted: 17 February 2011 / Published online: 26 February 2011

Abstract The aim of this study was to investigate the toxicity of herbicides (metribuzin and glyphosate), insecticides (imidacloprid and thiamethoxam) and fungicides (hexaconazole, metalaxyl and kitazin) at the recommended and the higher dose rates on plant growth promoting activities of *Bradyrhizobium* sp. under in vitro conditions. The *Bradyrhizobium* sp. strain MRM6 was isolated from nodules of greengram plants. Pesticide-concentration dependent progressive-decline was observed in plant growth promoting traits of the strain MRM6 apart from exo-polysaccharides which increased consistently on increasing pesticide concentrations. Generally, the highest toxicity to plant growth promoting characteristics of the *Bradyrhizobium* sp. strain MRM6 was observed when the strain MRM6 was grown with three times the recommended field rates of glyphosate, imidacloprid and hexaconazole.

Keywords *Bradyrhizobium* · Pesticide · Plant growth promoting rhizobacteria · Toxicity · Tolerance · Greengram

The industrial progress in recent times has led to the production of diverse agrichemicals such as fertilizers, pesticides, soil conditioners and phytohormones. These chemicals are applied extensively and indiscreetly to optimize the food production. A major fraction of these plant protection agents especially the pesticides is accumulated in soils due to the repeated use and consequently, adversely affects the metabolic activities of soil microflora leading to the losses in soil fertility (Ahemad et al. 2009). For example, the changes in aerobic bacteria, autotrophic nitrifiers, respiration and nitrification in soils treated with herbicide, cinosulfuron at 42 (field rate) and 4,200 μg/kg occurred one and 4 weeks after incubation under laboratory conditions. Of the measured parameters, only nitrification was slightly inhibited by cinosulfuron application at the field rate. Cinosulfuron at 100 mg/L negatively affected the growth of aerobic bacteria, fungi and *Azotobacter* strains under conditions similar to those of soil environment (Allievi and Gigliotti 2001). Therefore, understanding the toxic effects of pesticides on rhizobia and symbiotic N_2-fixation is important in order to evade the possible damage caused by the pesticide application. In other study, Wani et al. (2005) reported that insecticide phorate (applied at 100 and 500 μg/mL) substantially reduced the indole acetic acid production by phosphate solubilizing bacteria belonging to genera *Serratia*, *Pseudomonas* and *Bacillus,* isolated from various rhizospheric soils. In contrast, phosphate solubilizing activity of these bacteria was marginally affected in the presence of phorate. Similarly, Dunfield et al. (2000) assessed the effects of captan and thiram (fungicides) at rates of 0.25–2 g a.i./kg seed on the survival and phenotypic characteristics of the *Rhizobium leguminosarum* bv. *viceae* strain C1. Captan and thiram significantly reduced the numbers of rhizobia recovered from seeds and altered the FAME and Biolog profiles of recovered rhizobia. However, only the highest concentrations of captan affected nodulation and the legume growth. Contact with some seed-applied fungicides can also significantly change the phenotypic characteristics of rhizobia. Therefore, from a range of toxic pesticides, pesticides with fewer or without toxicity should be selected for further use.

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

Studies on the effect of various pesticides have largely been focused on changes in diversity of soil microorganisms while the information concerning the impact of agrochemicals on plant growth promoting activities of bradyrhizobia is poorly investigated.

The present study was therefore designed to evaluate the effects of herbicides (metribuzin and glyphosate), insecticides (imidacloprid and thiamethoxam) and fungicides (hexaconazole, metalaxyl and kitazin) at the recommended, double and three times the recommended field rates on the survival and in vitro plant growth promoting activities of *Bradyrhizobium* sp.

Materials and Methods

A total of 50 rhizobial strains were isolated from root nodules of greengram [*Vigna radiata* (L.) Wilczek] plants grown in experimental fields of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh (27° 29′ latitude and 72° 29′ longitude), India using yeast extract mannitol (YEM) medium (gL^{-1}: mannitol 10; K_2HPO_4 0.5; MgSO4·7H2O 0.2; NaCl 0.1; yeast extract 1; $CaCO_3$ 1; pH 7) (Vincent 1970). The experimental soil was an alluvial sandy clay loam (sand 667 g kg^{-1}, silt 190 g kg^{-1}, clay143 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 cmol kg^{-1} and 5.1 cmol kg^{-1} anion exchange capacity). The rhizobial strains were characterized and identified as *Bradyrhizobium* sp. following Bergey's Manual of Determinative Bacteriology (Holt et al. 1994) and nodulation test using greengram as the host plant in sterile soils (Somasegaran and Hoben 1994). The bradyrhizobial strains were tested for sensitivity/tolerance to metribuzin, glyphosate, imidacloprid, thiamethoxam, hexaconazole, metalaxyl and kitazin, by agar plate dilution method using minimal salt agar medium (g l^{-1}: KH_2PO_4 1, K_2HPO_4 1, NH_4NO_3 1, $MgSO_4·7H_2O$ 0.2, $CaCl_2·2H_2O$ 0.02, $FeSO_4$·7H2O 0.01, pH 6.5). The freshly prepared agar plates were amended separately with increasing concentrations (0–3200 μg mL^{-1}; at a two-fold dilution interval) of pesticides. Plates were spot inoculated with 10 μl of 10^8 cells mL^{-1} bradyrhizobial strains and incubated at 28 ± 2°C for 72 h. The highest concentration of each pesticide supporting bradyrhizobial growth was defined as the maximum tolerance level (MTL).

For quantitative assay of indole acetic acid, the bradyrhizobial strains were grown in Luria–Bertani (LB) broth. Luria–Bertani broth (100 mL) containing fixed concentration of tryptophan (100 μg/mL) supplemented with 1×, 2× and 3× of the recommended rate of each pesticide and without pesticide (control) was inoculated with one ml culture (10^8 cells/mL) of bacterial isolates and was incubated for 7 days at 28 ± 2°C with shaking at 125 rpm. Indole acetic acid concentration in the supernatant was determined by the method of Gordon and Weber (1951), later modified by Brick et al. (1991). The bradyrhizobial strains were also tested for siderophore [salicylic acid and 2,3-dihydroxybenzoic acid] production using chrome azurol S agar medium and Modi medium supplemented with 0, 1×, 2× and 3× of pesticides following the method of Alexander and Zuberer (1991) and Reeves et al. (1983), respectively. Hydrogen cyanide (Bakker and Schipper 1987), ammonia (Dye 1962) and exo-polysaccharides (Mody et al. 1989) production by bradyrhizobial strains was also determined.

Each experiment was replicated three times using the same treatments. The difference among treatment means was compared by honestly significant difference (HSD) using Tukey test at 5% probability level.

Results and Discussion

A total of 50 rhizobial isolates recovered from the nodules of greengram were tested to assess their tolerance to herbicides, glyphosate and metribuzin; insecticides, imidacloprid and thiamethoxam, and fungicides, hexaconazole, metalaxyl and kitazin (Table 1) on minimal salt agar medium. Of these, strain MRM6 was Gram negative rods and showed a variable biochemical reaction (Table 2). The rhizobial strain MRM6 was identified as *Bradyrhizobium* sp. on the basis of nodulation test. Strain MRM6 had the highest MTL to all herbicides, insecticides and fungicides and produced the plant growth promoting substances like, siderophores, indole acetic acid, exo-polysaccharides, hydrogen cyanide and ammonia, in substantial amounts (Table 3). In addition, the tolerance level of the strain MRM6 against the regularly increasing concentration of each pesticide in minimal salt agar medium was considerably higher (Table 3).

This study demonstrated that the *Bradyrhizobium* sp. strain MRM6 had a wide-range of tolerance (MTL: 1,800–3,200 μg mL^{-1}) towards the selected pesticides. The tolerance or resistance among microorganisms against pesticides is a complex process, regulated at physiological and genetic level. And hence, the microorganisms that develop resistance to a pesticide are capable of frequently degrading it (Kumar et al. 1996; Ortiz-Hernández and Sánchez-Salinas 2010). The temporary resistance (tolerance) against pesticides in general, is however, attributed to physiological changes that induce the microbial metabolism leading to the formation of a new metabolic pathway to bypass a biochemical reaction that could otherwise be inhibited by a specific pesticide (Bellinaso et al. 2003).

Table 1 Pesticides used in the present study

Common name	Grade (purity)	Chemical name	Chemical family	Recommended dose
Metribuzin (H)	Commercial (70%w/w)	4-amino-6-*tert*-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one	Triazinone	850 μg kg^{-1}
Glyphosate (H)	Commercial (71% w/w)	*N*-(phosphonomethyl)glycine	Organophosphate	1444 μg kg^{-1}
Imidacloprid (I)	Technical (100% EC)	(*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine	Pyridylmethylamine	100 μg L^{-1}
Thiamethoxam (I)	Technical (100%w/w)	(*EZ*)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine	Thiazole	25 μg L^{-1}
Hexaconazole (F)	Technical (100%w/w)	(*RS*)-2-(2,4-dichlorophenyl)-1-(1*H*-1,2,4-triazol-1-yl)hexan-2-ol	Conazole	40 μg kg^{-1}
Metalaxyl (F)	Commercial (35%w/w)	Methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate	Anilide	1500 μg kg^{-1}
Kitazin (F)	Commercial (48% EC)	O,O-Bis(1-methylethyl) S-phenylmethyl phosphorothioate	Organophosphate	96 μg kg^{-1}

H herbicides, *I* insecticides, *F* fungicides

Table 2 Morphological and biochemical characteristics of the *Bradyrhizobium* sp. strain MRM6

Characteristics	Strain MRM6
Morphology	
Gram reaction	−
Shape	Rods
Biochemical reactions	
Citrate utilization	−
Indole	+
Methyl red	+
Nitrate reduction	+
Oxidase	−
Voges proskauer	+
Carbohydrate utilization	
Dextrose	−
Lactose	−
Mannitol	+
Sucrose	−
Hydrolysis	
Starch	+
Gelatin	−

+ indicates positive and − indicates negative reactions

Permanent resistance, on the other hand, depends on genetic modifications, inherited by the subsequent generation of microbes (Herman et al. 2005; Johnsen et al. 2001).

The siderophores-production by the pesticide-tolerant strain MRM6 was determined on the chrome azurol S agar plates supplemented with varying concentration of each pesticide. The *Bradyrhizobium* sp. strain MRM6 revealed the siderophores-producing ability by forming an orange colored zone of 13 mm on pesticide-free chrome azurol S agar medium. Addition of pesticides to the medium substantially reduced the size of siderophore-zone. At the recommended rate, there was no inhibitory effect of any pesticide except hexaconazole and metalaxyl, which reduced the siderophore-zone by 8%, compared to control. Maximum decline in the zone diameter was observed at the 3× of each pesticide (Table 3).

The ethyl acetate extraction of the culture supernatant of the strain MRM6 grown in the Modi medium in the absence of pesticide produced 32 and 18 μg/mL salicylic acid and 2,3-dihydroxybenzoic acid. Pesticide-concentration dependent progressive decline for both types of the siderophores was observed. Decrease in salicylic acid and 2,3-dihydroxybenzoic acid released by the *Bradyrhizobium* sp. strain MRM6 however, varied with the types of pesticides. Among herbicides, glyphosate had a maximum inhibitory effect on salicylic acid and 2,3-dihydroxybenzoic acid. For instance, 3× of glyphosate decreased salicylic acid and 2,3-dihydroxybenzoic acid by 28% over control. Imidacloprid at 3× decreased salicylic acid and 2,3-dihydroxybenzoic acid by 72 and 33%, respectively, while 3× of hexaconazole reduced salicylic acid and 2,3-dihydroxybenzoic acid maximally by 37 and 39%, respectively compared to the control. Among the selected pesticides, hexaconazole at 3× generally, exhibited the highest toxicity to salicylic acid and 2,3-dihydroxybenzoic acid synthesis (Table 3). In the aerobic environment, iron occurs principally as Fe^{3+} and is likely to form insoluble hydroxides and oxyhydroxides, thus making it generally inaccessible to microorganisms. To acquire sufficient iron, the most common strategy adopted by bacteria is the secretion of siderophores, low-molecular mass iron chelators with high association constants for complexing iron. Thus, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation (Miethke and Marahiel 2007).

In the absence of pesticide, the *Bradyrhizobium* sp. strain MRM6 produced a maximum (38 μg/mL) amount of indole acetic acid, which however, decreased progressively with increasing concentration of each pesticide. The toxic effect of pesticides on indole acetic acid was in the order: hexaconazole > glyphosate > imidacloprid. Hexaconazole, glyphosate and imidacloprid at 3×, decreased the indole acetic acid by 42, 39 and 29%, respectively over untreated control (Table 3). The indole acetic acid so produced by rhizobacteria is known to promote root growth by directly stimulating plant cell elongation or cell division (Khan et al. 2010).

In contrast to other plant growth regulating substances, exo-polysaccharides produced by the strain MRM6 increased gradually with consistent increase in pesticide dose. The greatest stimulatory effect on exo-polysaccharides production was recorded for glyphosate, which increased it by 10, 24 and 38% at 1×, 2× and 3×, respectively compared to the control (Table 3). Why exo-polysaccharides increased on increasing pesticide-stress? The reason is unknown.

Table 3 Plant growth promoting activities of the *Bradyrhizobium* strain MRM6 in the presence of varying concentrations of pesticides

Pesticides	Dose (μg/L)	Plant growth promoting activities						
		Siderophores				EPS[f] (μg/mL)	Ammonia	HCN[g]
		Zone on CAS[a] agar (mm)	SA[b] (μg/mL)	DHBA[c] (μg/mL)	IAA[d] (μg/mL) 100T[e]			
Metribuzin	850	13 ± 1.5^{a}	28 ± 1.2^{c}	16 ± 0.9^{c}	30 ± 2.6^{gh}	21 ± 1.1^{h}	+	+
	1,700	13 ± 1.0^{a}	27 ± 2.1^{d}	15 ± 1.3^{d}	27 ± 1.5^{j}	22 ± 2.3^{gh}	+	+
	2,550	13 ± 1.2^{a}	24 ± 1.5^{gh}	12 ± 1.2 g	26 ± 2.0^{jk}	25 ± 1.4^{d}	+	+
Glyphosate	1,444	13 ± 1.6^{a}	29 ± 2.2^{bc}	16 ± 1.4^{c}	28 ± 2.5^{i}	23 ± 0.9^{f}	+	+
	2,888	13 ± 1.4^{a}	26 ± 1.7^{ef}	14 ± 1.1e	25 ± 1.4^{k}	26 ± 1.5^{cd}	+	+
	4,332	13 ± 0.8^{a}	23 ± 2.4^{h}	13 ± 1.1^{fg}	23 ± 1.5^{l}	29 ± 1.6^{a}	+	+
Imidacloprid	100	13 ± 1.4^{a}	28 ± 1.5^{c}	16 ± 0.8^{c}	32 ± 2.2^{ef}	21 ± 2.3^{h}	+	+
	200	13 ± 1.3^{a}	25 ± 2.2^{f}	14 ± 2.0^{e}	30 ± 1.4^{gh}	23 ± 2.4^{f}	+	+
	300	12 ± 0.6^{ab}	23 ± 1.5^{h}	12 ± 1.3^{g}	27 ± 1.5^{j}	25 ± 1.5^{d}	+	+
Thiamethoxam	25	13 ± 1.3^{a}	30 ± 1.3^{ab}	17 ± 2.1^{bc}	35 ± 1.2^{bc}	22 ± 2.1^{gh}	+	+
	50	12 ± 1.2^{ab}	29 ± 1.2^{bc}	16 ± 1.8^{c}	33 ± 2.2^{d}	23 ± 1.5^{f}	+	+
	75	11 ± 1.0^{b}	27 ± 1.5^{d}	15 ± 1.3^{d}	31 ± 1.7^{fg}	26 ± 2.3^{cd}	+	+
Hexaconazole	40	12 ± 1.0^{ab}	26 ± 2.0^{ef}	16 ± 2.4^{c}	28 ± 2.1^{i}	22 ± 2.1^{gh}	+	+
	80	11 ± 1.0^{b}	23 ± 2.3^{h}	14 ± 1.7^{e}	24 ± 1.6^{k}	25 ± 1.6^{d}	+	+
	120	11 ± 0.8^{b}	20 ± 1.4^{i}	11 ± 0.9^{h}	22 ± 1.4^{l}	27 ± 2.3^{b}	+	+
Metalaxyl	1,500	12 ± 1.4^{ab}	28 ± 1.3^{c}	17 ± 1.3^{bc}	35 ± 2.3^{bc}	22 ± 0.9^{gh}	+	+
	3,000	12 ± 1.2^{ab}	27 ± 2.2^{d}	15 ± 2.1^{d}	32 ± 1.4^{ef}	24 ± 1.4^{ef}	+	+
	4,500	11 ± 0.9^{b}	25 ± 1.4^{f}	13 ± 1.7^{fg}	29 ± 1.6^{hi}	26 ± 1.5^{cd}	+	+
Kitazin	96	13 ± 1.0^{a}	30 ± 2.5^{ab}	17 ± 2.2^{bc}	36 ± 2.4^{b}	21 ± 1.6^{h}	+	+
	192	13 ± 1.2^{a}	28 ± 2.1^{c}	16 ± 2.6^{c}	34 ± 2.1^{cd}	22 ± 1.3^{gh}	+	+
	288	13 ± 1.0^{a}	26 ± 1.5^{ef}	14 ± 1.8^{e}	31 ± 1.7^{fg}	24 ± 2.4^{ef}	+	+
Control (without pesticide)		13 ± 0.8^{a}	32 ± 1.8^{a}	18 ± 1.3^{a}	38 ± 1.8^{a}	21 ± 1.6^{h}	+	+
LSD ($p \leq 0.05$)		0.57	1.15	1.09	1.19	1.10	–	–
F value (treatment)		58.3	167.5	241.4	351.5	279.3	–	–

Maximum tolerance levels (MTL) against metribuzin, glyphosate, imidacloprid, thiamethoxam, hexaconazole, metalaxyl and kitazin were 3,200, 3,000, 2,200, 2,400, 1,800, 2,800 and 3,200 μg mL^{-1}. Values indicate mean of three replicates. Mean values (±S.D.) followed by different letters are significantly different within a row or column at $p \leq 0.05$ according to Tukey test

[a] Chrome azurol S agar

[b] Salicylic acid

[c] 2,3 Dihydroxy benzoic acid

[d] Indole acetic acid

[e] Tryptophan concentration (μg ml^{-1})

[f] Exopolysaccharides

[g] Hydrogen cyanide; + indicates positive reaction

It may however probably be due to the fact that pesticides might have induced the exo-polysaccharides secretion by the *Bradyrhizobium* sp. strain MRM6 which led strain MRM6 to produce excessive amounts of exo-polysaccharides in order to protect itself from pesticide-stress. Moreover, exo-polysaccharides protect bacteria from desiccation, phagocytosis and phage attack and also promote N_2 fixation process (Tank and Saraf 2003). No pesticide in this study, however, suppressed the hydrogen cyanide and ammonia synthesis by strain MRM6 (Table 3). Rhizobacteria protect the growing plants from pathogen attack by directly killing parasites by producing hydrogen cyanide (Kang et al. 2010). The ammonia released by the rhizobacterial strain plays a signaling role in the interaction between rhizobacteria and plants and also increase the glutamine synthetase activity (Chitra et al. 2002). The reduction in plant growth promoting activities of strain MRM6 when grown in the presence of different pesticides thus could probably be due to the impairment of various metabolic activities, as reported for other bacteria (Boldt and Jacobsen 1998; Kumar et al. 2010).

In conclusion, the selected herbicides, insecticides and fungicides when used independently showed a pronounced toxicity at the highest tested rates to plant growth promoting traits except exo-polysaccharides of the *Bradyrhizobium* sp. strain MRM6. The study revealed that a careful screening of pesticides should be carried out in laboratory before their field application.

Acknowledgments The authors thank to Dr. Nakhat Ara Naqvi, Parijat Agrochemicals, New Delhi, India, for providing technical grade pesticides and University Grants Commission (UGC), New Delhi for financial support.

References

Ahemad M, Khan MS, Zaidi A, Wani PA (2009) Remediation of herbicides contaminated soil using microbes. In: Khan MS, Zaidi A, Musarrat J (eds) Microbes in sustainable agriculture. Nova Science Publishers Inc., New York, pp 261–284

Alexander DB, Zuberer DA (1991) Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biol Fertil Soils 12:39–45

Allievi L, Gigliotti C (2001) Response of the bacteria and fungi of two soils to the sulfonylurea herbicide cinosulfuron. J Environ Sci Health Part B 36:161–175

Bakker AW, Schipper B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biol Biochem 19:451–457

Bellinaso ML, Greer CW, Peralba MC, Henriques JA, Gaylarde CC (2003) Biodegradation of the herbicide trifluralin by bacteria isolated from soil. FEMS Microb Ecol 43:191–194

Boldt TS, Jacobsen CS (1998) Different toxic effects of the sulphonylurea herbicides metsulfuron methyl, chlorsulfuron and thifensulfuron methyl on fluorescent pseudomonads isolated from an agricultural soil. FEMS Microbiol Lett 161:29–35

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

Chitra RS, Sumitra VC, Yash DS (2002) Effect of different nitrogen sources and plant growth regulators on glutamine synthetase and glutamate synthase activities of radish cotyledons. Bulg J Plant Physiol 28:46–56

Dunfield KE, Siciliano SD, Germida JJ (2000) The fungicides thiram and captan affect the phenotypic characteristics of *Rhizobium leguminosarum* strain C1 as determined by FAME and Biolog analyses. Biol Fertil Soils 31:303–309

Dye DW (1962) The inadequacy of the usual determinative tests for the identification of *xanthomonas* spp. Nat Sci 5:393–416

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

Herman PL, Behrens M, Chakraborty S, Crastil BM, Barycki J, Weeks DP (2005) A three component dicamba O-demethylase from *Pseudomonas maltiphilia* strain DI-6: gene isolation, characterization and heterologous expression. J Biol Chem 280:24759–24767

Holt JG, Krieg NR, Sneath PHA, Staley JT, Willams ST (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams and Wilkins, USA

Johnsen K, Jacobsen CS, Torsvik V, Sorensen J (2001) Pesticide effects on bacterial diversity in agricultural soils-a review. Biol Fertil Soils 33:443–453

Kang BG, Kim WT, Yun HS, Chang SC (2010) Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnol Rep. doi:10.1007/s11816-010-0136-1

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi-current perspective. Arch Agron Soil Sci 56:73–98

Kumar S, Mukerji KG, Lal R (1996) Molecular aspects of pesticide degradation by microorganisms. Critic Rev Microbiol 22:1–26

Kumar N, Anubhuti Bora JI, Amb MK (2010) Chronic toxicity of the triazole fungicide tebuconazole on a heterocystous, nitrogen-fixing rice paddy field cyanobacterium, *Westiellopsis prolifica* Janet. J Microbiol Biotechnol 20:1134–1139

Miethke M, Marahiel MA (2007) Siderophore-based iron acquisition and pathogen control. Microbiol Mol Biol Rev 71:413–451

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

Ortiz-Hernández ML, Sánchez-Salinas E (2010) Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. Rev Int Contam Ambient 26:27–38

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

Somasegaran P, Hoben HJ (1994) Handbook for rhizobia: methods in legume *Rhizobium* technology. Springer, New York

Tank N, Saraf M (2003) Phosphate solubilization, exopolysaccharide production and indole acetic acid secretion by rhizobacteria isolated from *Trigonella foenum-graecum*. Ind J Microbiol 43:37–40

Vincent JM (1970) A manual for the practical study of root nodule bacteria, IBP handbook no. 15. Blackwell Scientific Publications, Oxford, UK

Wani PA, Zaidi A, Khan AA, Khan MS (2005) Effect of phorate on phosphate solubilization and indole acetic acid releasing potentials of rhizospheric microorganisms. Ann Pl Protec Sci 13:139–144