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ORIGINAL ARTICLE

Effects of pesticides on plant growth promoting traits of *Mesorhizobium* strain MRC4

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Tolerance;
Toxicity

Abstract The objective of this study was to assess the effect of selected pesticides [herbicides (metribuzin and glyphosate), insecticides (imidacloprid and thiamethoxam) and fungicides (hexaconazole, metalaxyl and kitazin)] at recommended and higher dose rates on plant growth promoting activities of the *Mesorhizobium* sp. isolated from chickpea-nodules. A total of 50 rhizobial strains recovered from the nodules of chickpea root systems were identified following morphological, biochemical and host-specificity tests and tested for pesticide-tolerance. Among these strains, the *Mesorhizobium* sp. strain MRC4 was specifically selected due to the highest tolerance levels for all selected pesticides and the maximum production of plant growth promoting substances. Strain MRC4 produced indole acetic acid (44 μg ml^{-1}), siderophores [salicylic acid (35 μg ml^{-1}) and 2,3-dihydroxy benzoic acid (19 μg ml^{-1})], exo-polysaccharides (21 μg ml^{-1}), HCN and ammonia. Under pesticide-stress, pesticide-concentration dependent progressive-decline in all plant growth promoting traits of the *Mesorhizobium* sp. strain MRC4 exposed was observed except for exo-polysaccharides which consistently increased with exceeding the concentration of each pesticide from recommended dose. For instance, hexaconazole at three times the recommended dose elicited the maximum stress on siderophore-biosynthesis by the *Mesorhizobium* sp. strain MRC4 and decreased

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salicylic acid and DHBA by 40% and 47%, respectively and the greatest stimulatory effect on exopolysaccharides secretion was shown by imidacloprid which stimulated the *Mesorhizobium* sp. strain MRC4 to secrete EPS by 38%, compared to control. Generally, the maximum toxicity to plant growth promoting traits of *Mesorhizobium* was shown by glyphosate, thiamethoxam and hexaconazole, at three times the recommended rate among herbicides, insecticides and fungicides, respectively. This study revealed an additional aspect of the toxicological mechanisms of the pesticides through which they suppress the plant growth.

1. Introduction

Pulses are important source of dietary proteins, and have unique property of maintaining and restoring soil fertility through biological N_2 fixation (BNF) as well as conserving and improving physical properties of soil by virtue of their deep root system and leaf fall. Pulse crops add a reasonable quantity of nitrogen (upto 30 Kg N/ha) to soils. Of the different legumes grown around the world, chickpea [*Cicer arietinum* (L.)] is one of the most widely grown legumes. In India, chickpea occupies 7.1 million ha with a production of 5.75 million tones, accounting for 31% and 31% of total pulse area and production, respectively (ICAR, 2006). Chickpea replenish nitrogen in soils by forming specific symbiosis with its cognate N_2 fixing bacterium, *Mesorhizobium* that convert atmospheric N_2 to ammonia and other compounds and transport it to the growing plants (Wani et al., 2008). The efficiency of this approach, however, depends on principally maximizing symbiotic N_2 fixation (SNF) and plant yield to resupply organic and inorganic nitrogen and other nutrients to soils. Rhizobial inoculants as bio-fertilizers are therefore, applied to soils/seeds of legumes to ensure effective nodulation and subsequent N_2 fixation and consecutively, to increase the nitrogen pool of soils (Dudeja and Singh, 2008).

The inoculants are often used together with agrochemicals, which besides containing essential nutrients also contain contaminants and toxic elements. The exposure of these chemicals to field-grown plants could be either intentional (e.g. by spraying the legumes with pesticides) or through residues remaining from previous applications (Khan et al., 2004). Of these chemicals, pesticides and their microbially degraded products interact with soils and rhizosphere microorganisms including rhizobia and cause DNA, protein, oxidative or membrane damage (Pham et al., 2004). In addition, the common use of pesticides in agricultural practices has been shown to affect N_2 fixation adversely, either directly by affecting the rhizobia (Mallik and Tesfai, 1985; Anderson et al., 2004) or disrupting the signaling between legume-derived phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors (Fox et al., 2007) or indirectly by reducing photosynthate allocation to the nodules for N_2 fixation (Sprout et al., 1992; Koopman et al., 1995; Datta et al., 2009) or by restricting root growth and hence reduce the number of sites available for infection (Eberbach and Douglas, 1991). Additionally, pesticides that persist in soils may have a long-lasting impact on rhizobial survival and function (Eberbach and Douglas, 1989; Mårtensson and Nilsson, 1989; Eliason et al., 2004).

An alternative to overcome the deleterious effects of pesticides on plants could be the treatment of seeds with rhizobia as a bio-inoculant which displays a wide range of tolerance to pesticides and exhibit PGP activities including their inherent N_2-fixing attribute under pesticide-stress (Wani et al., 2005; Ahemad and Khan, 2010). Therefore, identifying rhizobia possessing multiple plant growth promoting activities and exhibiting pesticide tolerance will be useful in optimizing the yields of legumes in stressed production systems (Ahemad and Khan, 2011a).

Studies on the effect of various pesticides have largely, been focused on changes in populations of soil microorganisms and the effect of these agrochemicals on plant growth promoting (PGP) activities of rhizobia remains uninvestigated. 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 recommended, double and three times the recommended field rates on the survival and *in vitro* PGP activities of *Mesorhizobium* sp.

2. Materials and methods

2.1. Rhizobial strains and pesticide-tolerance

A total of 50 rhizobial strains were recovered from the root nodules of chickpea (*C. arietinum*) plants grown in pesticide-contaminated agricultural fields of the Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh (27°29′ latitude and 72°29′ longitude), India using yeast extract mannitol (YEM) medium (g l^{-1}: mannitol 10; K_2HPO_4 0.5; $MgSO_4{\cdot}7H_2O$ 0.2; NaCl 0.1; yeast extract 1; $CaCO_3$ 1 and pH 7) (Vincent, 1970). The strains were maintained on the same medium until use. The rhizobial strains were referred to as rhizobia following *Bergey's Manual of Determinative Bacteriology* (Holt et al., 1994) and identified as mesorhizobia through host specificity test (plant infection test) following Somasegaran and Hoben (1994). The strains were tested for their sensitivity/tolerance to chemically and functionally diverse pesticides (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; $MgSO_4{\cdot}7H_2O$ 0.2; $CaCl_2{\cdot}2H_2O$ 0.02; $FeSO_4{\cdot}7H_2O$ 0.01 and pH 6.5). The freshly prepared agar plates were amended separately with increasing concentrations of pesticides (0 to 3200 μg ml^{-1}; at a two-fold dilution interval). Later, plates were spot inoculated with 10 μl of 10^8 cells ml^{-1} mesorhizobial strains. Plates were incubated at 28 ± 2 °C for 7 days and the highest concentration of each pesticide supporting mesorhizobial growth was defined as the maximum tolerance level (MTL).

2.2. Growth patterns of mesorhizobia

For the determination of growth kinetics, 0.1 ml of the culture (10^8 cells ml^{-1}) of freshly grown mesorhizobia were inoculated

Table 1 Pesticides used in the present study.

Category	Common name	Grade (purity)	Chemical name	Chemical family	Recommended dose	Source
Herbicides	Metribuzin	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}	Singhal Pesticides, Mumbai, India
	Glyphosate	Commercial (71% w/w)	*N*-(Phosphonomethyl)glycine	Organophosphate	1444 μg kg^{-1}	Excel Crop Core LTD., Mumbai, India
Insecticides	Imidacloprid	Technical (100% EC)	(*E*)-1-(6-Chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine	Pyridylmethylamine	100 μg l^{-1}	Parijat Agrochemicals, New Delhi, India
	Thiamethoxam	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}	Parijat Agrochemicals, New Delhi, India
Fungicides	Hexaconazole	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}	Parijat Agrochemicals, New Delhi, India
	Metalaxyl	Commercial (35% w/w)	Methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate	Anilide	1500 μg kg^{-1}	Tropical Agrosystem Ltd., Chennai, India
	Kitazin	Commercial (48% EC)	*O*,*O*-Bis(1-methylethyl) *S*-phenylmethyl phosphorothioate	Organophosphate	96 μg kg^{-1}	P.I. Industries Ltd., Rajasthan, India

into 10 ml mineral salt medium containing 0 (control), the recommended dose (1×), two times the recommended dose (2×) and three times the recommended dose (3×) of each pesticide (Table 1) as the sole source of carbon and nitrogen. The cultures were incubated at 28 ± 2 °C on rotary shaker. At regular time intervals, the optical density at 540 nm was measured using a spectrophotometer (Spectronic 20, USA). The growth curves were obtained by plotting the optical density as a function of time.

2.3. Quantitative assay of indole acetic acid

Indole-3-acetic acid (IAA) synthesized by mesorhizobial strains was quantitatively evaluated by the method of Gordon and Weber (1951), later modified by Brick et al. (1991). For this activity, the mesorhizobial strains were grown in Luria Bertani broth (g l^{-1}: tryptone 10; yeast extract 5; NaCl 10 and pH 7.5). Luria Bertani (LB) broth (100 ml) having a fixed concentration of tryptophan (100 μg ml^{-1}) and supplemented with 0, 1×, 2× and 3× of each pesticide was inoculated with 0.1 ml culture (10^8 cells ml^{-1}) of mesorhizobial strains and incubated for 7 days at 28 ± 2 °C with shaking at 125 rpm. After seven days, a 5 ml culture from each treatment was centrifuged (9000*g*) for 15 min and an aliquot of 2 ml supernatant was mixed with 100 μl of orthophosphoric acid and 4 ml of Salkowsky reagent (2% 0.5 M $FeCl_3$ in 35% per-chloric acid) and incubated at 28 ± 2 °C in darkness for 1 h. The absorbance of developed pink color was read at 530 nm. The IAA concentration in the supernatant was determined using a calibration curve of pure IAA as a standard.

2.4. Qualitative and quantitative estimation of siderophores

The mesorhizobial strains were further tested for siderophore production using Chrome Azurol S (CAS) agar medium following the method of Alexander and Zuberer (1991). Chrome Azurol S agar plates supplemented with 0, 1×, 2× and 3× of each pesticide were prepared separately and divided into equal sectors and spot inoculated with 10 μl of 10^8 cells ml^{-1} and incubated at 28 ± 2 °C for 5 days. Development of yellow orange halo around the bacterial growth was considered as positive test for siderophores-biosynthesis. The production of siderophores by the test strains was further detected quantitatively using Modi medium (K_2HPO_4 0.05%; $MgSO_4$ 0.04%; NaCl 0.01%; mannitol 1%; glutamine 0.1% and NH_4NO_3 0.1%) (Reeves et al., 1983). Modi medium amended with 0, ×, 2× and 3× of each pesticide, was inoculated with 10^8 cells ml^{-1} of bacterial cultures and incubated at 28 ± 2 °C for 5 days. Catechol type phenolates were measured on ethyl acetate extracts of the culture supernatant using a modification of the ferric chloride-ferricyanide reagent of Hathway (Reeves et al., 1983). Ethyl acetate extracts were prepared by extracting 20 ml of supernatant twice with an equal volume of the solvent at pH 2. Hathway's reagent was prepared by adding one milliliter of 0.1 M ferric chloride in 0.1 N HCl to 100 ml of distilled water, and to this, was added 1 ml of 0.1 M potassium ferricyanide (Reeves et al., 1983). For the assay, one volume of the reagent was added to one volume of the sample and the absorbance was determined at 560 nm for salicylic acid (SA) with sodium salicylate as a standard and at 700 nm for dihydroxy phenols with 2,3-dihydroxy benzoic acid (DHBA) as a standard.

2.5. Assay of hydrogen cyanide (HCN), ammonia and exopolysaccharides

Hydrogen cyanide production by mesorhizobial strains was detected by the method of Bakker and Schipper (1987). For HCN production, all mesorhizobial strains were grown on an HCN induction medium (g l^{-1}: tryptic soy broth 30; glycine 4.4 and agar 15) supplemented with 0, 1×, 2× and 3× of each pesticide at 28 ± 2 °C for 4 days. Further, 100 μl of 10^8 cells ml^{-1} of each mesorhizobial strain was placed in the center of the petri plates. A disk of Whatman filter paper No. 1 dipped in 0.5% picric acid and 2% Na_2CO_3 was placed at the lid of the petri plates. Plates were sealed with parafilm.

After 4 days incubation at 28 ± 2 °C, an orange brown color of the paper indicating HCN production was observed. For ammonia assessment, the mesorhizobial strains were grown in peptone water with 0, 1×, 2× and 3× of each pesticide and incubated at 28 ± 2 °C for 4 days. One milliliter of Nessler reagent [potassium iodide 50 g; distilled water (ammonia free) 35 ml; add saturated aqueous solution of mercuric chloride until a slight precipitate persists; potassium hydroxide 400 ml; dilute the solution to 1000 ml with ammonia free distilled water; allow it to stand for 1 week, decant supernatant liquid and store in a tightly capped amber bottle] was added to each tube and the development of yellow color indicating ammonia production was recorded following the method of Dye (1962). The exo-polysaccharide (EPS) produced by the mesorhizobial strains was determined as suggested by Mody et al. (1989). For this, the bacterial strains were grown in 100 ml capacity flasks containing basal medium [Luria Bertani (LB) broth ($g\,l^{-1}$: tryptone 10; yeast extract 5; NaCl 10 and pH 7.5)] supplemented with 5% sucrose and treated with 0, 1×, 2× and 3× of each pesticide. Inoculated flasks were incubated for 5 days at 28 ± 2 °C on rotary shaker (100 rpm). Culture broth was spun (5433 g) for 30 min and EPS was extracted by adding three volumes of chilled acetone (CH_3COCH_3) to one volume of supernatant. The precipitated EPS was repeatedly washed three times alternately with distilled water and acetone, transferred to a filter paper and weighed after overnight drying at room temperature.

Each individual experiment was repeated three times.

2.6. Statistical analysis

The experiments were conducted in three replicates using the same treatments. The difference among treatment means was compared by high range statistical domain (HSD) using Tukey test ($p \leq 0.05$).

3. Results

3.1. Characterization, identification and pesticide-tolerance

In the present study, a total of 50 rhizobial strains recovered from the nodules of chickpea root systems were identified on the basis of morphological, biochemical and host-specificity tests for nodulation in sterile soils and monitored further for pesticide-tolerance. Among these strains, the *Mesorhizobium* sp. strain MRC4 was specifically selected due to the highest MTL for all selected herbicides, insecticides and fungicides (Fig. 1, Table 2) and maximum production of PGP substances (siderophores, IAA, EPS, HCN and ammonia) (Table 3).

3.2. Siderophore production under pesticide-stress

Production of siderophores by the pesticide-tolerant the *Mesorhizobium* sp. strain MRC4 was determined on CAS agar plates supplemented with varying concentrations of the pesticides (Table 3). The *Mesorhizobium* sp. strain MRC4 displayed siderophores-producing potential by forming an orange zone of 12 mm size on pesticide free CAS agar medium. In general, addition of pesticides to the medium significantly ($p \leq 0.05$) reduced the siderophore-zone formed by pure culture of the *Mesorhizobium* sp. strain MRC4. At recommended rate, the effect of all the pesticides was marginally inhibitory to siderophore-zone except fungicide, hexaconazole which reduced significantly ($p \leq 0.05$) the siderophore-zone to the highest degree by 25% over control. In addition, the degree of zone-inhibition was not co-related with the concentration of pesticides. However, maximum decline was observed at the highest tested dose of each pesticide (Table 3).

Furthermore, the ethyl acetate extraction from culture supernatant of the *Mesorhizobium* sp. strain MRC4 grown in the Modi medium devoid of pesticides yielded SA and DHBA type siderophores significantly ($p \leq 0.05$) (Table 3). Pesticide-concentration dependent progressive decline for both iron-binding molecules was observed. Nevertheless, degree of pesticide-mediated decrease for SA and DHBA differed from the type and functional group of each pesticide. Within herbicide group, glyphosate showed the highest toxicity to the synthesis of SA and DHBA. For instance, glyphosate at 3× decreased significantly ($p \leq 0.05$) SA and DHBA secretion by 17% and 32%, respectively, compared to the control. Among insecticides, thiamethoxam at 3× showed the most deleterious effect on SA and DHBA synthesis which significantly ($p \leq 0.05$) decreased by 34% and 58%, respectively, over control in the presence of the same concentration of thiamethoxam. Among fungicides, hexaconazole at three times the recommended dose elicited maximum stress on siderophore-biosynthesis by the *Mesorhizobium* sp. strain MRC4 and decreased significantly ($p \leq 0.05$) SA and DHBA by 40% and 47%, respectively, above control. Among all pesticides, hexaconazole and thiamethoxam at three times of the recommended dose in general, displayed the most toxic effect on SA and DHBA synthesis, respectively (Table 3).

3.3. Indole acetic acid production under pesticide-stress

The effect of three concentrations of each pesticide on IAA synthesized by the *Mesorhizobium* sp. strain MRC4 varied considerably (Table 3). In the medium unsupplied with pesticides, the *Mesorhizobium* sp. strain MRC4 produced significant amount ($p \leq 0.05$) of IAA (44 $\mu g\ ml^{-1}$). In contrast, the quantity of IAA released by the *Mesorhizobium* sp. strain MRC4, however, decreased progressively with graded-increment of each pesticide in LB broth. Of herbicides, insecticides and fungicides, most severe effect on IAA synthesis was evident in the presence of glyphosate, imidacloprid and hexaconazole, respectively. For example, glyphosate decreased IAA significantly ($p \leq 0.05$) by 14%, 18% and 25%, imidacloprid by 9%, 16% and 20% and hexaconazole by 36%, 43% and 52% at 1×, 2× and 3×, respectively. While comparing the concentrations and types of pesticides, hexaconazole in general had the most toxic effect on IAA biosynthesis by the *Mesorhizobium* sp. strain MRC4 (Table 3).

3.4. Production of exo-polysaccharides, HCN and ammonia under pesticide stress

Unlike other PGP substances produced by the *Mesorhizobium* sp. strain MRC4 exposed to pesticidal stress, the amount of EPS synthesized increased significantly ($p \leq 0.05$) with gradual addition of each pesticide in culture medium. Among all tested pesticides, the greatest stimulatory effect on EPS secretion was shown by imidacloprid which stimulated significantly ($p \leq 0.05$) the *Mesorhizobium* sp. strain MRC4 to secrete EPS by 38% compared to control (Table 3). Interestingly,

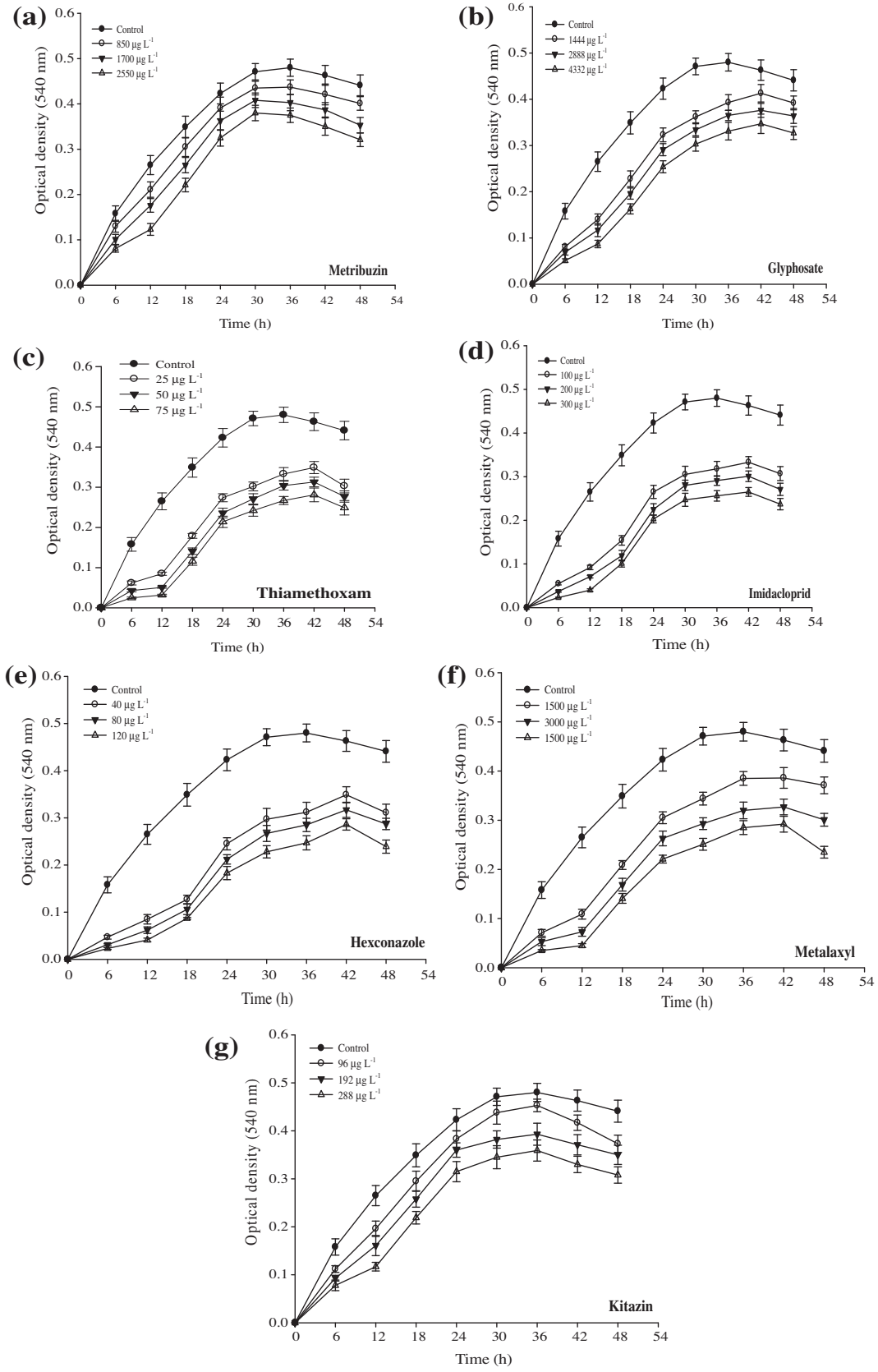

Figure 1 Impact of the recommended (open circle), double (inverted triangle) and three times (open upright triangle) the recommended rates of metribuzin (a), glyphosate (b), imidacloprid (c), thiamethoxam (d), hexaconazole (e), metalaxyl (f) and kitazin (g) on the *Mesorhizobium* strain MRC4 grown in minimal salt agar medium.

Table 2 Morphological and biochemical characteristics of the *Mesorhizobium* sp. strain MRC4.

Characteristics	Strain MRC4
Morphology	
Gram reaction	–
Shape	rods
Biochemical reactions	
Citrate utilization	–
Indole	+
Methyl red	+
Nitrate reduction	+
Oxidase	–
Voges Proskaur	+
Carbohydrate utilization	
Dextrose	–
Lactose	–
Mannitol	+
Sucrose	–
Hydrolysis	
Starch	+
Gelatin	–
Maximum tolerance level (MTL) to	
Metribuzin	3200 μg ml^{-1}
Glyphosate	3000 μg ml^{-1}
Imidacloprid	2400 μg ml^{-1}
Thiamethoxam	2800 μg ml^{-1}
Hexaconazole	2200 μg ml^{-1}
Metalaxyl	2800 μg ml^{-1}
Kitazin	3200 μg ml^{-1}

'+' Indicates positive and '–' indicates negative reactions.

the three concentrations of each herbicide, insecticide and fungicide did not affect negatively HCN and ammonia synthesis by the *Mesorhizobium* sp. strain MRC4 (Table 3).

4. Discussion

In our study, the *Mesorhizobium* sp. strain MRC4 portrayed abnormally higher tolerance to selected pesticides of various chemical groups. The MTL values of pesticides ranged from 2200 to 3200 μg ml^{-1}. Tolerance or resistance in microorganisms against pesticides is a complex process which is regulated both at physiological/genetic level of microorganism. And hence, the microorganisms that developed resistance to pesticides are frequently capable of biodegrading them (Kumar et al., 1996; Ortiz-Hernández and Sánchez-Salinas, 2010). The temporary resistance (tolerance) against pesticides in general, is attributed to physiological changes that induce the microbial metabolism for the formation of a new metabolic pathway to bypass a biochemical reaction inhibited by a specific pesticide (Bellinaso et al., 2003). Permanent resistance, on the other hand, depends upon genetic modifications, inherited by the subsequent generation of microbes (Johnsen et al., 2001; Herman et al., 2005).

In similar studies, Gram negative bacteria have also shown tolerance to other pesticides. For instance, the maximum tolerant concentrations of different organophosphorus pesticides for both *Pseudomonas* and *Flavobacterium* species isolated from polluted sites were 250, 4000 and 8000 μg ml^{-1} of guthion, methyl parathion and dimethoate, respectively (Nazarian and Mousawi, 2005). Likewise, both *Rhizobium* sp. specific to chickpea and *Rhizobium* sp. specific to greengram tolerated aldrin upto 2000 μg ml^{-1} (Juneja and Dogra, 1978). Moreover, Boldt and Jacobsen (1998) also reported a variation in the MTL of *Pseudomonas* strains to sulfonylurea herbicides (e.g. metsulfuron methyl, chlorsulfuron and thifensulfuron methyl). Among the herbicides, metsulfuron methyl was more toxic compared to other herbicides and order of toxicity was: metsulfuron methyl > chlorsulfuron > thifensulfuron methyl. The variation in tolerance to pesticide by rhizobacteria could probably be due to the fact that rhizobacteria adopt different strategies to overcome the toxic effects of pesticides and such mechanisms included biodegradation (Yang and Lee, 2008) and enzymatic hydrolysis (Dumas et al., 1989; Herman et al., 2005) of pesticide. For instance, organophosphorus hydrolase (OPH), an enzyme isolated from *Pseudomonas diminuta* MG and *Flavobacterium* sp. strain ATCC 27551 possesses the ability to hydrolyze different organophosphorus insecticides (Dumas et al., 1989). Hydrolysis of organophosphorus compounds by OPH dramatically reduced their toxicity (DiSoudi et al., 1999). Similarly, dicamba monooxygenase (DMO), an enzyme extracted from *Pseudomonas maltophilia* strain DI-6, completely inactivated the herbicidal activity of dicamba (Herman et al., 2005). Our study however, showed that the tolerance levels of the mesorhizobial strain against the selected pesticides was considerably high.

In the present study, the *Mesorhizobium* sp. strain MRC4 exhibited plant growth promoting traits like production of siderophores, phytohormone and exo-polysaccharides in substantial amount in both the absence and presence of pesticide-stress. 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 commonly found strategy in 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).

The phytohormone, IAA synthesized from transamination and decarboxylation of tryptophan, primarily in young leaves and seeds, controls cell division, root initiation, phototropism, geotropism and apical dominance in plants (Khan et al., 2010). In general, the IAA produced by rhizobacteria promotes root growth by directly stimulating plant cell elongation or cell division. A low level of IAA produced by rhizosphere bacteria promotes primary root elongation whereas a high level of IAA stimulates lateral and adventitious root formation but inhibit primary root growth (Ma et al., 2009).

The EPS helps to protect bacteria against desiccation, phagocytosis and phage attack besides supporting N_2 fixation by preventing high oxygen tension (Tank and Saraf, 2003). Owing to abundance, highly charged nature, and extracellular location, EPS also plays an important role in the attachment of bacterial cells to varied surfaces; osmoregulation and ion transport (Spaink, 2000). In addition, the role of EPS in legume–*Rhizobium* interaction (symbiosis) is well reported. For example, *Rhizobium leguminosarum* has been shown to produce large amounts of acidic EPS which is essentially required for nodule invasion and, therefore, for successful nitrogen-fixing symbioses with many legumes like, *Medicago, Pisum, Trifolium* and *Vicia* spp. (Becker and Pühler, 1998; Per-

Table 3 Plant growth promoting activities of the *Mesorhizobium* strain MRC4 in the presence of varying concentrations of pesticides.

Pesticides	Dose rate (μg l^{-1})	Plant growth promoting activities						
		Siderophores			IAA[D](μg ml^{-1})	EPS[F](μg ml^{-1})	Ammonia	HCN[G]
		Zone on CAS[A] agar (mm)	SA[B](μg ml^{-1})	DHBA[C](μg ml^{-1})	100T[E]			
Metribuzin	850	11 ± 1b	33 ± 1.4b	18 ± 1.5ab	41 ± 2.1ab	22 ± 2.5e	+	+
	1700	10 ± 2bc	32 ± 1.3bc	17 ± 1.1b	39 ± 1.9b	24 ± 2.2d	+	+
	2550	10 ± 2bc	30 ± 1.1d	15 ± 1.4d	37 ± 1.7c	27 ± 1.8b	+	+
Glyphosate	1444	12 ± 1a	32 ± 1.6bc	16 ± 1.2cd	38 ± 2.3bc	23 ± 1.6e	+	+
	2888	11 ± 1b	31 ± 1.2c	15 ± 1.5d	36 ± 2.3d	25 ± 2.3c	+	+
	4332	11 ± 1b	29 ± 1.3ef	13 ± 1.3f	33 ± 1.5f	26 ± 2.6b	+	+
Imidacloprid	100	12 ± 2a	29 ± 1.5ef	18 ± 1.2ab	40 ± 1.2b	26 ± 1.7b	+	+
	200	11 ± 1b	27 ± 1.5gh	15 ± 1.3d	37 ± 1.5c	28 ± 2.5ab	+	+
	300	11 ± 1b	23 ± 1.0kl	15 ± 1.5d	35 ± 1.5d	29 ± 2,3a	+	+
Thiamethoxam	25	12 ± 1a	33 ± 1.4b	13 ± 1.2f	42 ± 2.0ab	24 ± 2.1d	+	+
	50	12 ± 1a	26 ± 1.3h	10 ± 1.1hi	39 ± 1.6b	25 ± 1.9c	+	+
	75	12 ± 1a	23 ± 1.4kl	8 ± 1.3i	36 ± 1.2d	27 ± 2.4b	+	+
Hexaconazole	40	9 ± 1cd	26 ± 1.1h	14 ± 1.4ef	28 ± 2.8h	23 ± 1.5e	+	+
	80	8 ± 1d	24 ± 1.3jk	12 ± 1.5g	25 ± 1.9i	26 ± 2.3b	+	+
	120	8 ± 2d	21 ± 1.2l	10 ± 1.2hi	21 ± 1.5j	28 ± 1.3ab	+	+
Metalaxyl	1500	12 ± 1a	28 ± 1.2f	16 ± 1.4cd	34 ± 2.0ef	23 ± 1.5e	+	+
	3000	10 ± 1bc	25 ± 1.2i	15 ± 1.2d	31 ± 2.3g	25 ± 1.6c	+	+
	4500	9 ± 2cd	22 ± 1.1l	14 ± 1.4ef	29 ± 2.2h	26 ± 1.4b	+	+
Kitazin	96	12 ± 1a	31 ± 1.2c	17 ± 1.3b	37 ± 2.5c	22 ± 1.6e	+	+
	192	10 ± 2bc	28 ± 1.3f	15 ± 1.4d	35 ± 2.1d	24 ± 2.4d	+	+
	288	9 ± 2cd	26 ± 1.1h	14 ± 1.3ef	32 ± 1.8g	27 ± 2.2b	+	+
Control (without pesticide)		12 ± 1a	35 ± 1.5a	19 ± 1.7a	44 ± 2.4a	21 ± 2.3f	+	+
F value (treatment)		57.9	345.6	285.5	459.2	619.4	–	–

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. '+' Indicates positive reaction.

[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.

ret et al., 2000). However, EPS-deficient mutants of rhizobia, *R. leguminosarum* for example, fail to nodulate their host plants (Spaink, 2000) or induce formation of ineffective nodules (Rolfe et al., 1996; van Workum et al., 1997). Rhizobacteria protects the growing plants from pathogen attack by directly killing parasites by producing HCN (Kang et al., 2010). In agreement to our report, Devi et al. (2007) also reported the excretion of HCN by the rhizobacterial strains into the rhizosphere. 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).

Each PGP trait of bacteria is the result of sequential metabolic reactions mediated by various specific functional proteins (enzymes) along the defined metabolic pathway. The metabolic pathways for any specific PGP trait may be more than one depending upon the type of the PGP substances and bacterial genera/species. Pesticides adversely affect protein synthesis and the metabolic enzymes (Kapoor and Arora, 1996; Boldt and Jacobsen, 1998). Therefore, it seems probable that pesticides employed in this study might have inhibited the functioning of the enzymes participating in different metabolic pathways of PGP traits (SA, DHBA and IAA) in the *Mesorhizobium* sp. strain MRC4. Therefore, SA, DHBA and IAA production declined under pesticide-stress. Since In contrast to other PGP traits in this study, the amount of EPS secreted by the *Mesorhizobium* sp. strain MRC4 increased progressively with gradual increment in pesticide-concentrations. The reason for this abnormal trend is unknown. Nevertheless, the increase in EPS following increased concentration of each pesticide suggested that the pesticides might have acted as inducers of EPS biosynthesis. EPS provides protection to soil bacteria against environmental stresses (Tank and Saraf, 2003); hence it is possible that rhizobia secreted more EPS under pesticide-stress to shield themselves against these chemicals in a proportion to the pesticide-concentrations. Further, HCN and ammonia act as signaling molecules. Moreover, pesticides employed in this study might have induced mesorhizobial metabolism of HCN and ammonia production to form new metabolic pathways to bypass biochemical pathways inhibited by pesticides (Bellinaso et al., 2003). Therefore, HCN and ammonia prduction by *Mesorhizobium* sp. strain MRC4 remained unaffected under pesticide-stress.

Pesticides vary in their toxicology to organisms owing to their functional groups and a great degree of variability occurs even among pesticides of similar functional groups (Ahemad and Khan, 2011b). Most of the pesticides in our study have different functions groups. In our study, degree of inhibition of phyto-beneficial traits of *Mesorhizobium* sp. strain MRC4 under pesticide stress hence differs from one pesticide to another. Additionally, pesticides not only damage structural proteins essential for the growth of the organism but also responsible for geno-toxicity (Pham et al., 2004) and eventually leads to the decreased functioning and survival of organisms exposed to high concentration of pesticides (Kumar et al., 2010).

5. Conclusion

This study has shown that the pesticides not only affect the growth of rhizobia but also have an adverse impact on their PGP activities. These findings evidently revealed an additional aspect of the toxicological mechanisms of the pesticides through which they decline the plant growth. The study showed that a careful screening of pesticides should be carried out in laboratory before their field application. Further research on pesticide-rhizobacteria interaction at molecular level is needed to identify which enzymes or genes are affected in rhizobacteria under pesticide-stress.

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