DISCUSSION

Soil microbial communities play pivotal roles in various biogeochemical cycles and influence the fertility of soils. In addition, the soil microflora influence above-ground ecosystems by providing nutrients to plants; improve soil structures and consequently, affect soil health (Ahemad et al., 2009). Rhizosphere microflora is also involved in many other soil processes e.g. decomposition of organic matter, nutrient mobilization, mineralization, mineral phosphate solubilization, denitrification, bioremediation of pollutants and suppression of soil borne phytopathogens (Rameshkumar and Nair, 2009; Khan et al., 2010; Ahemad and Khan, 2011b). Thus the plant rhizosphere is a versatile and dynamic ecological environment of intense microbes–plant interactions for harnessing essential micro- and macro-nutrients from a limited nutrient pool (Jeffries et al., 2003).

The widespread application of chemical fertilizers, herbicides, and pesticides in modern agriculture is severely modifying and polluting the natural environment (Punja, 1997). The potential negative effects of chemical fertilizers on the global environment together with its increased cost have promoted the research and application of microbial inoculants which offer an environment-friendly means to increasing crop productivity and soil health in an integrated plant nutrient management.

In the present investigation, seven PGPR isolates were evaluated for their plant growth promoting activities. Firstly, we compared the microbial population in rhizospheric vs non-rhizospheric soil. Serial dilution of the rhizospheric soil from oil seeds mustard, sesame and flax plants revealed. The results, (i.e. R/S ratio=10-20) clearly indicates that rhizospheric region has much higher number of bacteria than that of non-rhizospheric region of soil. This difference in population could be because of the exudates secretion by plant roots along the rhizospheric region. Similar findings were reported by Hiltner, (1904) which suggested that that the rhizosphere is much richer in bacteria, against bulk soil. This rhizosphere effect is thought to be caused by root exudate-dependent growth of rhizosphere microorganisms. The plant’s root exudates (carbon compounds) release from plants into the rhizosphere increases microbial biomass and activity (Bashan and de-Bashan, 2005).

In soil, despite of large reservoir of P, the amount of available forms to plants is generally low. This low availability of phosphorous to plants is because the majority of soil P is found in insoluble forms, while the plants absorb it only in two soluble
forms, the monobasic ($H_2PO_4^{-1}$) and the diabasic ($HPO_4^{2-}$) ions (Bhattacharyyya and Jha, 2012). To overcome the P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. Plants absorb fewer amounts of applied phosphatic fertilizers and the rest is rapidly converted into insoluble complexes in the soil (Mckenzie and Roberts, 1990). Besides, regular application of phosphate fertilizers is not only costly but is also environmentally undesirable. Only 0.1% of the total P is available to plant because of poor solubility and its fixation in soil (Illmer et al., 1995), which could be improved by the application of isolated PSB strains DMR 1c, TSR 7c, TSR 10a, TSR 23a, DVR 20f, PCR 25d and LRS 6a strains. Our results show that strains confer p-sol activity for both organic and inorganic phosphate. The bacterial biochemical ability to produce and release organic acids determines the p-sol activity (Kpomblekou et al., 1994). Goldstein (1987) proposed direct glucose oxidation to GA as a major mechanism for mineral phosphate solubilization. This could be a possible reason for the P-sol activity by isolated rhizobacterial strains.

Organic P can constitute between 30 and 50% of the total P of the soil, a high proportion of it corresponding to phytate (Borie et al., 1989; Turner et al., 2002). Previously, there are only a few bacteria capable of producing phytase enzymes for the mineralization of phytates (Lim et al., 2007; Jorquera et al., 2008b). Our results in suggested that the isolated bacterial strains were capable of solubilizing organic P.

To exert beneficial effects in the rhizospheric environment, bacteria have to be rhizosphere competent, i.e., able to compete well with other rhizosphere microbes for nutrients secreted by the root and for sites that can be occupied on the root. Even, the motility was reported as essential factor for chemotaxis toward root exudates (Lugtenberg and Kamilova, 2009). In the present study we observed that the rhizospheric isolates were motile.

We also found that the isolated strains produce IAA. The rhizobacteria synthesize IAA from tryptophan by different pathways, although these bacteria can also synthesize IAA via tryptophan-independent pathways, though in lower quantities (Spaepen et al., 2007). Similar, results were observed with the PGPR strains isolated from the oil seed and medicinal plants. These strains were able to synthesize IAA with and without the addition of tryptophan that indicated the synthesis of IAA both by tryptophan independent and tryptophan dependent pathways. The occurrence of similar pathways in *Azospirillum brasilense* was suggested by Prinsen et al. (1993).
Plant growth-promoting rhizobacteria that fix N\textsubscript{2} in non-leguminous plants are also called as diazotrophs capable of forming a non-obligate interaction with the host plants (Glick et al., 1999). The fixation of atmospheric N\textsubscript{2} and ammonia production was observed in all the isolated strains. These findings are quite similar with N\textsubscript{2} fixing bacteria such as \textit{Rhizobium} and \textit{Bradyrhizobium} which were reported to form nodules on roots of leguminous plants such as soybean, pea, peanut, and alfalfa, in which they convert N\textsubscript{2} into ammonia, which in contrast to N\textsubscript{2} can be used by the plant as a nitrogen source (Van and Vanderleyden, 1995). Ammonification, an important step in the transformation of organic nitrogen to ammoniacal form, would enhance soil nitrogen content by the ammonifying character of the PGPR isolates (Dey et al., 2004). Hence, involvement of ammonification trait of isolated strains might be significant. In the present study we have observed that all the seven bacterial strains were positive for ammonia production activity.

The isolated bacterial strains were positive for siderophore production. The siderophores were reported to play a key role in the control of phyto-pathogenic fungus. Ajilogba and Babalola 2013, reported that the PGPR make use of mechanisms such as siderophore production, systemic resistance induction and antifungal volatile production to control or inhibit the growth of plant pathogens e.g. inhibition of \textit{Fusarium} sp. Further, siderophore release by PGPR could be involved in plant growth promotion, since siderophore release increase Fe availability (Van Loon et al., 1999, 2007). Plants assimilate iron from bacterial siderophores by means of different mechanisms, for instance, chelate and release of iron, the direct uptake of siderophore-Fe complexes, or by a ligand exchange reaction (Schmidt, 1999; Crowley and Kraemer, 2007). Similarly, the Fe-pyoverdine complex synthesized by \textit{P. fluorescens} C7 was taken up by \textit{Arabidopsis thaliana} plant, leading to an increase of iron inside plant tissues and to improved plant growth (Vansuyt et al., 2007).

All the seven isolated PGPR strains were checked to exhibit broad spectrum biocontrol against the \textit{Fusarium} sp., \textit{Rhizoctonia} sp., \textit{Alternaria} sp. \textit{Rosellinia} sp. and \textit{Pythium} sp by dual culture method. To check the broad spectrum application of PSB as biocontrol agents, isolated bacteria were checked against the five plant pathogenic fungi. The major pattern and extent of inhibition against \textit{Fusarium} sp., \textit{Rhizoctonia} sp., \textit{Alternaria} sp., \textit{Rosellinia} sp. and \textit{Pythium} sp. by all of the PSB strains was different. Maximum percentage of inhibition (71.42\%) against \textit{Fusarium} sp. and \textit{Alternaria} sp. was shown by LRS 6a strain. Maximum percentage of inhibition (82.22\%) against
*Rhizoctonia* sp. was shown by *Burkholderia* sp. strain DVR 20f. Maximum percentage of inhibition (about 83%) against *Rosellinia* sp. was shown by *Burkholderia* spp. strains DVR 20f and PCR 25d. Maximum percentage of inhibition (74.44%) against *Pythium* sp. was shown by *Burkholderia* sp. strain PCR 25d.

The antifungal activity of the test isolates might be due to the production of siderophore and HCN or synergistic interaction of these two or with other metabolites. However, role of HCN was not expected as these isolates were negative for HCN production. Several studies have demonstrated that production of siderophores, other secondary metabolites and lytic enzymes by *Pseudomonas* strains was most effective in controlling the plant root pathogens including *F. oxysporum* and *R. solani* (Ahmad *et al.*, 2008). *B. cereus* strain UW85, *P. fluorescens* strains CHA0 and Pf5 produce numerous antibiotics with different degrees of action against specific pathogenic fungi (Raaijmakers *et al.*, 2002).

We further went on to test the application of isolated strains in our seedling model. Seed bacterization with three isolates (DMR 1c, TSR 7c, and TSR 23a) increased the root length, shoot length, number of lateral roots, fresh weight and dry weight of seedlings significantly over control consistently under invitro conditions. Moreover, the application of these strains to the maize seeds confirmed the ability of strains to enhance plant growth alone and in the presence of pathogenic fungus. The positive effect of inoculation with *Burkholderia* spp. DMR 1c, TSR 7c and TSR 23a strains on maize seedlings was found to inhibit *Fusarium* sp. Previously, bacterization of maize seeds with *B. cepacia* MCI 7 resulted in a significant (P~0.05) increase of maize plant growth in both uninfested soil and soil infested with *F. moniliforme* ITEM-504, as compared to untreated plants (Bevivino *et al.*, 2000).

Seed bacterization with TSR 23a of black gram, gram also showed antagonism of fungus but the overall response towards shoot growth and side roots formation was found to be different in all cases. These observations suggest that it could be due to the differential degree of tolerance of the crops towards *Fusarium* sp. Our results are also in agreement with previous studies which suggest that biocontrol efficiency of Bcc strains varied among cultivars mainly due to the differences in their susceptibility to the fungal pathogens (Hebbar *et al.*, 1998; Bevivino *et al.*, 2000).

Further, PGP traits of PGPR strains were cheked in the presence of chlorpyrifos. Chlorpyrifos, which is used throughout the world to control a variety of chewing and sucking insect pests and mites on a range of economically important crops, including
citrus fruit, bananas, vegetables, potatoes, coffee, cocoa, tea, cotton, wheat and rice etc. (Thengodkar and Sivakami., 2010). It was observed that the isolated strains DMR 1c, TSR 7c, TSR 23a and DVR 20f strains degrade 94%, 88%, 95% and 87% of chlorpyrifos (50mg/L of concentration) respectively, after 72 h. Similarly, Zhang et al. (2014) reported the degradation of triazine herbicide metribuzin upto 73.5% in 120 h by Bacillus sp. with an initial concentration of 20 mg/L. To date, several chlorpyrifos-degrading bacterial strains including Enterobacter strain B-14 (Singh et al., 2004), Stenotrophomonas sp. strain YC-1 (Yang et al., 2006), Sphingomonas sp. strain Dsp-2 (Li et al., 2007), Paracoccus sp. strain TRP (Xu et al., 2008), Bacillus pumilus strain C2A1 (Anwar et al., 2009), and Bacillus laterosporus strain DSP (Zhang et al., 2012) have been isolated from diverse sources; however, only Paracoccus sp. strain TRP and Bacillus pumilus strain C2A1 were able to degrade both chlorpyrifos and TCP. One recently isolated cyanobacterium, Synechocystis sp. strain PUPCCC 64, was also capable of degrading chlorpyrifos (Singh et al., 2011).

The tolerance or resistance of PGP activity of PGPR against pesticides is a complex process regulated at both physiological and genetic level (Herman et al., 2005). This study however, documented abnormally high tolerance levels (up to maximum 4 g/L) of four isolated Burkholderia spp. strains against the selected pesticides amended in minimum salt medium lacking any other carbon source.

The reports on in vitro PGP activities of phosphate (P) solubilizing Burkholderia spp. in the presence of pesticides are rare. Considering these scientific gaps, the present study was, therefore, carried out to evaluate the effects of insecticides (chlorpyrifos, profenofos, cypermethrin and imidacloprid) and fungicides (carbendazim, myclobutanil, hexaconazole and mancozeb) on PGP activities of rhizobacterial Burkholderia spp. In the present study, Burkholderia spp. strains were checked for their potential to degrade chlorpyrifos.

In general, phosphate solubilizing property is due to a drop in pH, which has been associated with the secretion of low molecular weight organic acids such as gluconic, 2-ketogluconic, oxalic, citric, acetic, malic, and succinic. (Zaidi et al., 2009). In our study, the Burkholderia spp. strains (DMR 1c, TSR 7c, TSR 23a, DVR 20f) solubilized the inorganic phosphate considerably even in the presence of 100 mg/l doses of pesticides. It was observed that phosphate solubilization zone on Pikovskaya’s medium by the Burkholderia sp. strain TSR 7c remained almost unaffected by pesticides.
In our study, substantial IAA (7-14 µg/ml) was produced by the *Burkholderia* spp. strains (DMR 1c, TSR 7c, TSR 23a, and DVR 20f) even when exposed to 100mg/l dose of each pesticide. Furthermore, nitrogen fixation and ammonia production by the *Burkholderia* spp. strains (DMR 1c, TSR 7c, TSR 23a, and DVR 20f) remained unaffected whether pesticides were added into media or not.

Siderophores, low-molecular mass iron chelators with high association constants for complexing iron, act as solubilizing agents for minerals or organic compounds under iron limitation conditions (Indiragandhi *et al*., 2008). The siderophores-production by the pesticide-tolerant strains (DMR 1c, TSR 7c, TSR 23a, and DVR 20f) were determined on the chrome azurol S agar plates supplemented with varying concentration of each pesticide. At the concentration 1mg/L rate, there was no inhibitory effect of any pesticide, as compared to control. Maximum decline in the zone diameter was observed at the concentration of 100mg/l of each pesticide.

It is important to mention that there indeed is a reduction in some plant growth promoting activities of test strains, when grown in the presence of different pesticides. This could probably be due to the impairment of various metabolic activities, as reported for other bacteria (Boldt and Jacobsen 1998; Kumar *et al.* 2010). At the same time none of the activity is reduced by 40% even in the presence of 100mg/l of pesticide.

Interestingly, in plantae experiments in the presence of pesticide showed that the measured parameters (seed germination, shoot length, root length, number of lateral roots, fresh weight and dry weight of seedlings) increased following inoculants (*Burkholderia* spp. strains DMR 1c, TSR 7c, TSR 23a) application to chlorpyrifos treated maize seedlings. This increment in growth parameters may be attributed either to the detoxification of fungicides (Yang and Lee, 2008) by the *Burkholderia* spp. strains (Zaidi *et al*., 2009). The pesticide-degrading potential of the strains DMR 1c, TSR 7c, TSR 23a and DVR 20f were also supported by the growth of these strains on minimal media having chlorpyrifos as only C source.

This study inferred that the DMR 1c, TSR 7c, TSR 23a and DVR 20f strains with multiple PGP traits can be used as bio-inoculant to increase the productivity of crops in pesticide contaminated soils. In addition to this, our results also suggested the necessity to use pesticide-tolerant PGPR strains as bio-inoculants, in order to ensure that, in the presence of different pesticides, the PGP activities can still be beneficial to the plants.