Ecological Performance of Multifunctional Pesticide Tolerant Strains of Mesorhizobium Sp. In Chickpea With Recommended Pendimethalin, Ready-mix of Pendimethalin and Imazethpyr, Carbendazim and Chlorpyrifos Application

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Abstract

The present study was designed to screen the *Mesorhizobium* strains (50) for tolerance in four recommended pesticides for chickpea. It was followed by in-vitro development of robust pesticide tolerant strains by growth in pesticides amended media over several generations. Further, verification of the multifunctional traits of pesticide tolerant mesorhizobia under pesticide stress was conducted *in-vitro*. Among different pesticides, significantly high tolerance in *Mesorhizobium* strains was observed with recommended doses of pendimethalin (37%) and ready-mix (36%) followed by chlorpyrifos (31%) and carbendazim (30%), on an overall basis. Based on multifunctional traits, *Mesorhizobium* strains viz. MR2, MR17 and recommended MR33 were the most promising. Ecological performance of the potential *Mesorhizobium* strains alone and in dual-inoculation with recommended PGP rhizobacterium strain RB-1 (*Pseudomonas argentitensis* JX239745.1) was further analyzed in field following standard pesticide application in PBG-7 and GPF-2 chickpea varieties for two consecutive *rabi* seasons (2015 and 2016). Dual-inoculant treatments; recommended RB-1+MR33 (4.1%) and RB-1+MR2 (3.8%) significantly increased the grain yield over *Mesorhizobium* alone treatments viz MR33 and MR2, respectively. Grain yield in PBG7 variety was significantly affected (7.3%) by the microbial inoculant treatments over GPF2 variety. Therefore, the potential pesticide tolerant strains MR2 and MR33 can be further explored as compatible dual-inoculants with recommended RB-1 for chickpea under environmentally stressed conditions (pesticide application) at multiple locations. Our approach using robust multifunctional pesticide tolerant *Mesorhizobium* for bio-augmentation of chickpea with might be helpful in the formulation of effective bio-inoculants consortia in establishing successful chickpea–*Mesorhizobium* symbiosis.

1. Introduction

Chickpea is the most important *rabi* pulse crop of India with an increase in production from 6.5 million tonnes to 10.3 million tonnes and grain yield from 630 to 935 Kg ha⁻¹ during the past 35 years (Singh et al. 2018). Inoculation of chickpea seeds with a selected strain of *Mesorhizobium* sp. is under wide practice to improve the yield by enhancing root nodulation and N uptake of the plant. This helps in reducing the dependency on chemical fertilizers (Brigido et al. 2017). Co-inoculation of plant growth-promoting (PGP) bacteria with *Rhizobium* synergistically improves the legume growth, symbiotic nitrogen fixation (SNF), yield, and overall plant's performance by absorption of nitrogen, phosphorus and other macro-and micronutrients (Gopalakrishnan et al. 2015). Therefore, identification and manipulation of their relationships with PGP bacteria might fulfill modern demands of agricultural, economic, social, and environmental sustainability (Fatnassi et al. 2015). Chickpea seeds are susceptible to varied fungal pathogens (Ascochyta blight and Botrytis grey mold), insect, termites and weeds. Under a recommended strategy of maximizing productivity, farmers often treat the seeds with fungicides (carbendazim) followed by insecticides (chlorpyrifos) to protect them from seed-borne pathogens and then inoculate them with a nitrogen (N₂) fixing strain to use the natural resources. Also, there are two recommended herbicides viz. pre-emergence (pendimethalin) and pre-plant (ready-mix which is a combination of pendimethalin + imazethapyr) herbicides for controlling broad-leaved weeds in chickpea (Anonymous 2013). However, the problems arise when we use chemical pesticide treatments in conjunction with *Mesorhizobium*/ *Mesorhizobium* + rhizobacterium as bio-fertilizer. Adverse effects of pesticides on legume-rhizobia symbiosis include inhibition of cell division and nodule formation, limiting carbohydrate supply to the nodules, blocking the initial attachment of complementary rhizobia to lectins by protecting recognition sites present on root hairs and inhibition of symbiotic signaling between host plant and rhizobia (Meena et al. 2020). One of the major constraints faced in *Rhizobium* inoculant development technology is the selection of isolates that are symbiotically efficient and adapted to the local environmental conditions loaded with various types of stress (Porter et al. 2019). Previous studies have largely focussed on the effects of few pesticides in a limited number of mesorhizobia on SNF in chickpea. However, there is scarce information on pesticide tolerant mesorhizobia in chickpea. The present investigation was designed to screen indigenous *Mesorhizobium* strains for intrinsic tolerance to recommended pesticides followed by verification of their *in-vitro* multifunctional traits under pesticide stress. Further, the robust strains of *Mesorhizobium* with multifunctional traits were explored for ecological performance on plant growth, SNF and yield in chickpea crop raised with the recommended application of pesticides under field conditions.

2. Materials And Methods

2.1 Procurement and purity of reference cultures

Seventy-five strains of *Mesorhizobium* sp. including recommended cultures of MR33 (*Mesorhizobium cicer* sp. LGR33) and PGP rhizobacterium strain RB-1 (*Pseudomonas argentitensis* JX239745.1) were procured from the culture collection of Pulses Research Laboratory, Department of Plant Breeding and Genetics, PAU, Ludhiana. The purity of cultures was tested using the Gram staining technique (Ouma et al. 2016). Further, for differentiation of *Mesorhizobium* from *Agrobacterium* and other contaminants, different

confirmatory tests were performed on the Hoffer's alkaline (Ansari et al., 2014), the ketolactose, (Finer et al. 2016), the congo red yeast extract mannitol agar (CRYEMA) and the glucose peptone agar media (Gaur and Sen 1981). The cultures of mesorhizobia and rhizobacterium were maintained on the Yeast Extract Mannitol Agar (YEMA) and the Nutrient Agar (NA) media slants, respectively and stored at 4°C for further use during the study period.

2.2 *In-vitro* screening of strains of *Mesorhizobium* sp. for pesticide tolerance

In this experiment, four recommended pesticides *viz.* pre-emergence (pendimethalin) and pre-plant (ready-mix which is a combination of pendimethalin + imazethapyr), insecticide (chlorpyrifos) and fungicide (carbendazim) were tested with selected mesorhizobia strains for tolerance to single (1×) and double (2×) the recommended doses of pesticides (Table 1) using the disc diffusion method. Sterile filter paper discs were soaked in pesticide solutions in methanol (based on their optimal solubility) at concentrations equivalent to single and double the recommended doses when applied under field conditions (Table 1). Bacterial cultures were grown in YEM broth until they reached the mid-exponential growth phase (OD 600 = 0.8) before inoculation. Pesticide discs were placed on the minimal salt agar medium plates (Ahemad and Khan 2009) and were uniformly spread with the bacterial cultures. The plates were kept overnight at 4°C to allow diffusion of the pesticide, followed by incubation at 28 °C for 3–5 days. Growth of individual *Mesorhizobium* sp. with different pesticides were compared with control discs dipped in sterile water without pesticides.

Table 1
Concentrations of recommended pesticides at different doses

Pesticides	Mode of pesticide application	Pesticide Dose: Recommended single doses ((1×) and double (2×) the recommended doses	Concentration of recommended pesticides under field conditions	Equivalent concentration of pesticides in methanol	Equivalent concentration of pesticides in Parts/million
				(µL ml ⁻¹) *(µg ml ⁻¹)	(ppm)
Pendimethalin 30 EC	pre-emergence	1×	0.75 Kg a.i. ha ⁻¹	6	9× 10 ⁴
	herbicide	2×	1.5 Kg a.i. ha ⁻¹	12	18× 10 ⁴
Ready mix combination of imazethapyr + pendimethalin 48 EC	pre-plant herbicide	1×	1.2 Kg Kg a.i. ha ⁻¹	6	2.4 × 10 ⁴
		2×	2.4 Kg a.i. ha ⁻¹	12	4.8× 10 ⁴
Chlorpyrifos 20 EC	insecticide for seed treatment	1×	10 ml a.i. Kg ⁻¹ seed	1.8	7.4 × 10 ⁴
		2×	20 ml a.i. Kg ⁻¹ seed	3.6	14.8×10 ⁴
Carbendazim 50 % WP	fungicide for seed treatment	1×	3 g a.i. Kg ⁻¹ seed	*3	3 × 10 ³
		2×	6 g a.i. Kg ⁻¹ seed	*6	9 × 10 ³

Further, the selected bacterial cultures were grown in different pesticides supplemented minimal salt broth media for upto 6 months using sub-culturing technique in 10 mL tubes at 28 ± 2°C for 48 h and 130 rpm in a rotatory shaker. Mixed culture of each bacterial strain from four different pesticide amended media were taken in LB medium broth incubated for 48 h at 28 ± 2°C. This technique was conducted to induce possible mutations in bacterial cultures for intrinsic tolerance to pesticides while maintaining stable multifunctional traits. Further experiments were conducted to assess the consistent performance of robust pesticide tolerant strains thus developed *in-vitro*, for the multifunctional traits.

2.3 Effect of different pesticides on multifunctional traits in strains of *Mesorhizobium* sp. (*in-vitro*)

Indole acetic acid (IAA) production of robust pesticide tolerant strains of *Mesorhizobium* sp. developed *in-vitro* was determined by the method as demonstrated by Gordon and Weber (1951) later modified by Brick et al. (1991). For this, Luria Bertani (LB) medium broth cultures of the selected pesticide tolerant strains of *Mesorhizobium* sp. in (10ml) tubes, each having a fixed concentration of tryptophan (100 µgml⁻¹) were supplemented with recommended doses of different pesticides (Table 1). It was followed by standard estimation of IAA using orthophosphoric acid and 4ml of Salkowsky's reagent (2% 0.5M FeCl₃ in 35% perchloric acid). Selected pesticide tolerant

strains of *Mesorhizobium* sp. were further tested for siderophore production using the Chrome Azurol S (CAS) agar medium supplemented with recommended doses of different pesticides following the method of Schwyn and Neilands (1987). For hydrogen cyanide (HCN) production, all the pesticide tolerant strains of *Mesorhizobium* sp. were grown on the HCN induction medium (Bakker and Schipper 1987) amended with the recommended dose of individual pesticides. Cell wall degrading enzyme *viz.* cellulase and protease production by pesticide tolerant strains of *Mesorhizobium* sp. was analyzed by the formation of clear zones on the carboxymethylcellulose (CMC) agar and skimmed milk (SM) agar medium, respectively (Vijayabharathi et al. 2018) supplemented with recommended doses of pesticides.

2.4 Compatibility of pesticide tolerant strains of *Mesorhizobium* sp. and *Rhizobacterium* RB-1 for functional traits with recommended pesticides

2.4.1 Seed germination and vigor index (*in-vitro*)

Before the field experiment, compatibility of pesticide tolerant strains of *Mesorhizobium* sp. with rhizobacterium strain RB-1 was tested for growth index using disc plate technique. Lawn of the strain RB-1 was prepared by spreading culture on the NA medium. The sterile discs were dipped in different mesorhizobial cultures and then placed on a lawn of the strain RB-1. The zone of inhibition around the individual disc showed the incompatibility of two cultures. For seed germination experiment, seven treatments were maintained *viz.* recommended *Mesorhizobium cicer* strain MR33, *Mesorhizobium* sp. strain MR2, *Mesorhizobium* sp. strain MR17, recommended RB-1 + MR33, RB-1 + MR2, RB-1 + MR17 and un-inoculated control (with pesticides application as per recommendation, alone) in Factorial Randomized Block Design (FRBD) with three replications. In co-inoculation treatments, different *Mesorhizobium* sp. strains along with rhizobacterium strain RB-1 were applied to chickpea seeds in equal ratios (1:1). For seed treatment, healthy seeds of two chickpea varieties *viz.* PBG7 and GPF2 were surface-sterilized according to Vincet (1970). Surface sterilized seeds were initially treated with fungicide carbendazim (50% WP @ 3 g Kg⁻¹ seed) and insecticide chlorpyrifos (20EC @10 ml Kg⁻¹ seed) followed by microbial inoculants as per recommended practice for seed treatment in chickpea. Pendimethalin (9× 10⁴ ppm) and ready-mix (2.4× 10⁴ ppm) were incorporated in water agar medium simulating their field application @ 0.75 Kg and 1.2 Kg ha⁻¹, respectively. Treated seeds were allowed to germinate in Petri dish containing 0.7% water agar with incubation at 28°C. After 15 days, percent seed germination, fresh weight of seedling, root and shoot length were recorded.

Seedling vigor indices were determined by the following formula:

Seed vigor index I (SVI)=(mean of the root length + shoot length)×% germination

Seed vigor index II (SVII)=(mean of the fresh weight of root + shoot)×% germination

2.4.2 Treatment structure for field experiment

The field investigation was conducted for two consecutive winter seasons (2014-15 and 2015-16) in Factorial Randomized Block Design (FRBD) with three replications. The location of the experiment was at Pulse Research Farm and Microbiology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. In total, 42 plots were designed with 4 rows each, having row to row distance of 30 cm, plant to plant distance of 10 cm and total plot size of 4×2.4 = 9.6 sq.m. Chickpea seeds of *desi* PBG1 and GPF2 varieties were procured from the Pulses Section, Department of Plant Breeding and Genetics, PAU, Ludhiana. The seeds were inoculated with potential pesticide tolerant *Mesorhizobium* sp. strains (MR2 and MR17) and recommended *Mesorhizobium cicer* strain MR33 individually and in combination with the recommended culture of PGP rhizobacterium (RB-1) as per treatment. Monoculture treatment comprised of 20g charcoal inoculant per kg of chickpea seeds. For co-inoculation treatments, a cocktail of *Mesorhizobium* sp. strains and rhizobacterium (RB-1) was made in the ratio of 1:1 for application to chickpea seeds. Before sowing, inoculated seeds were air-dried at room temperature under the shade and sown within two hours. Total 7 microbial inoculant treatments were maintained *viz.* recommended *Mesorhizobium cicer* sp. strain MR33, *Mesorhizobium* sp. strain MR2, *Mesorhizobium* sp. strain MR17, recommended RB-1 + MR33, RB-1 + MR2, RB-1 + MR17 and un-inoculated control (with recommended pesticides application alone). All the standard agronomic practices were followed for raising the chickpea crop. Chickpea seeds were initially treated with the carbendazim and chlorpyrifos followed by microbial inoculants (Table 1). Readymix and pendimethalin were applied as pre-plant and pre-emergence herbicides (Table 1), respectively in the plots to control the broad-leaved weeds according to recommended management practices.

2.4.4 Plant growth parameters

Emergence count was recorded at days after sowing (10 DAS) from central rows of each plot after leaving two border rows on each side and calculated from number of emerged seedlings per meter row length. Observations for plant height, dry weight of roots and shoots, chlorophyll and carotenoid contents were taken after 60 and 90 DAS. For measuring plant height, three plants were randomly selected from each plot and uprooted. Roots were removed from shoots and the height of shoots was measured from the base in cm. Similarly for dry weight of shoots, three randomly selected plants were uprooted from each plot. Roots were detached from shoots, sun dried and then oven dried at 60°C for 2 days. Dry weight of shoot and root was recorded in g. Chlorophyll and carotenoid contents were determined as per the methods of Witham et al. (1971) and Dere et al. (1998), respectively.

2.4.5 Antioxidant enzymes activities in root exudates

For collection of root exudates, frozen root and shoot tissues of each treatment were homogenized at 4°C in an ice-chilled mortar in QB buffer per gram of tissue (Kumar et al. 2009). Crude homogenates were centrifuged at 15000 *g* for 15 min at 4 °C, and the supernatant fractions were frozen at -20 °C. Superoxide dismutase (SOD) activity and catalase (CAT) activity were recorded at 120 DAS by the methods of Aebi (1983) and Marklund and Marklund (1974), respectively.

2.4.6 Symbiotic nitrogen fixation

Observations of nodule number, dry weight of nodules and leghaemoglobin content were recorded at 60 and 90 DAS. For evaluation of symbiotic parameters, three randomly selected plants were uprooted from each plot with their root system being intact. The roots were washed in running tap water and nodules carefully detached with forceps. The number of nodules per plant was recorded by taking average. The detached nodules were oven dried at 60°C for 2 days and the dry weight of nodules per plant was recorded in mg. Leghaemoglobin content was estimated according to Wilson and Reisenauer (1963) by reading absorbance of clear nodular tissue extract using Drabkin's solution at 540 nm.

2.4.7 Soil health

Soil enzyme activities were analyzed at 90 DAS. Estimation of Dehydrogenase activity of soil was based on production of Triphenyl formazan (TPF) from Triphenyl Tetrazolium Chloride (Klosse and Tabatabai 2000). Soil Urease activity was activity was calculated as μg of ammonium N released g^{-1} of soil 2hr^{-1} (Tabatabai 1982).

2.4.8 Yield attributing traits, seed protein content and grain yield

Yield attributing traits, Seed protein content and Grain yield were determined at harvesting stage. The number of pods per plant was recorded by taking average of three plants were randomly selected at harvesting stage. The number of seeds per pod was recorded by taking average of seeds from three randomly selected plants at harvesting stage. Crop was sickle harvested and grains were thrashed. Hundred-seed weight was measured on a per-plot basis by taking a sample of the harvested seed in each plot. Total protein content of seeds was determined by Kjeldahl's technique with slight modification (McKenzie and Wallace 1954).

2.5 Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) based on the pooled mean of two years with SAS Statistical Package Version 9.3. Further, mean separation of treatment effects was accomplished using the Tukey test. The least significant difference (LSD) was calculated at the 5% probability level.

3. Results

3.1 Tolerance of strains of *Mesorhizobium* sp. with recommended pesticides (*in-vitro*)

Confirmatory tests based on Hoffer's alkaline medium and ketolactose tests showed white translucent colonies in 50 strains of *Rhizobium* where as red colonies indicated *Agrobacterium*. as contaminant. All the rhizobia showed growth at 28°C on YEMA medium after 48–72 hrs and considered as *Mesorhizobium*. All the strains of *Mesorhizobium* sp. (50) were further subjected to screening for pesticide tolerance by the disc diffusion method. The growth inhibition zones around pesticide discs allowed us to divide the bacterial strains under study into three categories *viz.* luxuriant, moderate and poor tolerant. The highest growth index was mainly expressed in terms of percentage (%) of luxuriantly tolerant strains, whereas the lowest growth index was expressed in terms of poorly tolerant strains. Among different pesticide treatments at recommended doses, higher growth index was observed with pendimethalin (37%) as compared to ready-mix (36%), chlorpyrifos (31%) and carbendazim (30%) (Plate 1 and Fig. 1). An overall decline in growth index was

noticed with 2×recommended doses of all the pesticides. viz. overall 8% decline in luxuriantly tolerant strains with 2×pendimethalin and 2×ready-mix each, followed by 5% with 2×chlorpyrifos and 10% with 2×carbendazim. However, the maximum decline in growth index was witnessed for chlorpyrifos. Based on this experiment, 20 strains of *Mesorhizobium sp.* were found to have the potential to be selected as pesticide tolerant strains. This test showed that among different pesticides, the highest inhibitory effect on the growth of *Mesorhizobium* strains was exhibited by insecticide (chlorpyrifos) followed by fungicide (carbendazim) while both the herbicides (pendimethalin and ready-mix of pendimethalin + imazthpyr) were comparatively less toxic to the growth of selected *Mesorhizobium* strains.

3.2 Effect of recommended pesticides on multifunctional traits in strains *Mesorhizobium sp.*

The selected robust strains of *Mesorhizobium sp.* with intrinsic tolerance to different pesticides developed over several generations *in-vitro* were further analyzed for multifunctional traits viz. IAA, siderophore and HCN production with recommended pesticide treatments. For IAA production, the selected strains of *Mesorhizobium sp.*, differed significantly with all the pesticides in presence (Trp+) and absence (Trp-) of tryptophan (Figs. 2a-d). Additionally, IAA production significantly increased with Trp + over Trp- in different treatments. Among different pesticide treatments, significantly high IAA production was observed for *Mesorhizobium sp.* strain MR2 (48.5 µgml⁻¹) with pendimethalin (Plate 2) followed by ready-mix, carbendazim and chlorpyrifos (39.8, 25.2 and 23.2 µg ml⁻¹, respectively). However, an overall decline in IAA production by 1.2 fold was recorded with ready-mix followed by carbendazim (1.6 fold) and chlorpyrifos (2.3 fold) over the control. Further, siderophore production by the *Mesorhizobium* strains with recommended pesticides was shown by the appearance of clear zones around the bacterial colonies due to the chelation of iron bound to CAS dye (Plate 3a-c). Among 20 pesticide tolerant *Mesorhizobium sp.*, only the strains MR2, MR17 and recommended MR33 were tested positive for siderophore production on CAS medium amended with recommended doses of pendimethalin and ready-mix treatments, whereas, with chlorpyrifos only strain MR2 produced siderophores. However, carbendazim amended CAS agar medium was inhibitory for siderophore production by *Mesorhizobium sp.* strains MR2, MR17 and recommended strain MR33. The remaining strains of *Mesorhizobium sp.* along with *Mesorhizobium sp.* strains MR2, MR17 and recommended MR33 appeared negative for siderophore production with the pesticides (pendimethalin, ready-mix and chlorpyrifos). Further for cell wall degrading enzyme production, among all the pesticide tolerant strains, only *Mesorhizobium sp.* strain MR17 was positive for protease production in presence of pendimethalin and ready-mix while it presented cellulase production with only pendimethalin amended SM agar medium (Plates 4a-c). However, there was an absence of any cell wall degrading enzyme production in the remaining *Mesorhizobium sp.* strains. Based on the *in-vitro* evaluation of multifunctional traits, it was observed that insecticide (chlorpyrifos) exhibited a highest inhibitory effect on metabolism of *Mesorhizobium sp.* strains followed by fungicide (carbendazim) while both the herbicides (pendimethalin and ready-mix of pendimethalin + imazthpyr) were less toxic towards selected *Mesorhizobium* strains, accounting for a comparatively better exhibition of multifunctional traits.

3.3 Compatibility of pesticide tolerant strains of *Mesorhizobium sp.* and *Rhizobacterium RB-1* for functional traits with recommended pesticides

3.3.1 Seed germination and vigor index (*in-vitro*)

Based on multifunctional traits, two strains of *Mesorhizobium sp.* viz. MR2, MR17 along with recommended strain MR33 were selected as potential pesticide tolerant strains. *In-vitro* compatibility test of individual potential pesticide tolerant strains of *Mesorhizobium sp.* with the recommended culture of RB-1 revealed that all the dual-inoculants were compatible (Plates 5a-g). The effects of dual-inoculant treatments on seed germination in chickpea were significantly different. The highest percentage seed germination (92%), radical length (4.3cm), plumule length (9.8cm), fresh weight of seedling (0.49g), vigor index I (1367.1) and vigor index II (44.9) were found with *Mesorhizobium sp.* strain MR2 as compared to un-inoculated control treatment (89%, 4.3cm, 7.0 cm, 0.39g, 1012.0, 34.8, respectively). Dual inoculation of MR2 with RB1 further increased the seed germination (1.1 fold), radical length (1.9 fold), plumule length (1.6 fold), fresh weight of seedling (1.4 fold), vigor index I (1.8 fold) and vigor index II (1.5 fold) over un-inoculated control treatment (Tables 2 and 3). However, PBG7 and GPF2 chickpea varieties were at par for seedling germination. All the potential *Mesorhizobium* strains under study significantly improved the vigor index (1.2–1.3 fold) and germination (2.2–3.4%) which further increased by 1.7–1.8 fold and by 3.4–6.7%, respectively, with individual dual-inoculant treatments over un-inoculated control. However, RB-1 + MR2 performed at par with recommended RB-1 + MR33 for enhancement of seed germination and fresh weight of seedling and vigor index II, whereas, for an increase in plumule length RB-1 + MR17 was at par with recommended RB-1 + MR33. This experiment further confirmed the potential of selected pesticide tolerant strain of *Mesorhizobium sp.* viz. MR2 and MR17 along with recommended strain MR33 for enhancement of growth and yield in chickpea. However, the ecological performance of all the selected *Mesorhizobium* strains alone and individual dual

inoculation treatments with RB-1 was assessed under field conditions for a better understanding of chickpea-*Mesorhizobium* symbiosis under pesticide stressed field conditions.

Table 2
Effect of potential pesticide tolerant strains of *Mesorhizobium* sp. and rhizobacterium RB-1 on seedling germination

Treatments	Seed germination (%)			Plumule length (cm)			Radical length (cm)			Fresh weight of seedling (g)		
	PBG7	GPF2	Mean	PBG7	GPF2	Mean	PBG7	GPF2	Mean	PBG7	GPF2	Mean
Control	90 ± 1.76	88 ± 2.08	89 ± 1.28a	7.3 ± 0.27	6.8 ± 0.26	7.0 ± 0.20a	4.5 ± 0.26	4.2 ± 0.23	4.3 ± 0.17a	0.41 ± 0.02	0.37 ± 0.03	0.39 ± 0.02a
MR33	92 ± 1.20	91 ± 1.73	91 ± 0.95f	9.3 ± 0.29	8.7 ± 0.29	9.0 ± 0.23f	5.2 ± 0.27	4.4 ± 0.23	4.8 ± 0.24f	0.46 ± 0.03	0.43 ± 0.03	0.44 ± 0.02abc
MR2	93 ± 1.76	92 ± 0.88	92 ± 0.91e	9.6 ± 0.35	9.9 ± 0.32	9.8 ± 0.22e	5.2 ± 0.23	4.9 ± 0.29	5.1 ± 0.18e	0.51 ± 0.03	0.46 ± 0.03	0.49 ± 0.02abc
MR17	91 ± 1.53	91 ± 1.67	91 ± 1.01fg	9.2 ± 0.35	8.4 ± 0.32	8.8 ± 0.29g	5.1 ± 0.26	4.7 ± 0.37	4.9 ± 0.22fg	0.47 ± 0.03	0.42 ± 0.03	0.44 ± 0.02abc
RB-1 + MR33 (Recommended)	96 ± 0.88	95 ± 0.67	95 ± 0.54b	11.1 ± 0.32	10.6 ± 0.35	10.9 ± 0.24c	7.9 ± 0.29	7.4 ± 0.19	7.7 ± 0.19d	0.55 ± 0.02	0.54 ± 0.03	0.55 ± 0.02bc
RB-1 + MR2	95 ± 1.45	94 ± 0.58	94 ± 0.71bc	11.5 ± 0.44	10.8 ± 0.35	11.2 ± 0.29b	8.4 ± 0.26	8.0 ± 0.17	8.2 ± 0.17b	0.58 ± 0.03	0.54 ± 0.03	0.56 ± 0.02b
RB-1 + MR17	93 ± 1.45	92 ± 1.15	92 ± 0.84d	11.1 ± 0.44	10.4 ± 0.39	10.8 ± 0.28cd	8.1 ± 0.21	7.9 ± 0.17	8.0 ± 0.17c	0.53 ± 0.04	0.47 ± 0.04	0.50 ± 0.03abc
Mean	93 ± 1.43	92 ± 1.25		9.9 ± 0.3	9.4 ± 0.3		6.3 ± 0.26	5.9 ± 0.26		0.50 ± 0.03	0.47 ± 0.04	
Variety (V)	0.60			0.74			0.82			0.73		
Treatment (T)	1.1			0.14			0.15			0.14		
V×T	NS			0.19			0.22			NS		
Values presented are means ± SE: (*)=significant difference between mean values of two varieties, (NS) = non-significant, (SE) = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P=≤0.05 according to Tukey's least significance difference (LSD)												

Table 3
Effect of potential pesticide tolerant strains of *Mesorhizobium* sp. and rhizobacterium RB-1 on vigour index

Treatments	Vigor Index I			Vigor Index II		
	PBG7	GPF2	Mean	PBG7	GPF2	Mean
CONTROL	1053.9 ± 68.06	970.0 ± 66.65	1012.0 ± 46.55a	36.8 ± 2.61	32.7 ± 3.63	34.8 ± 2.21a
MR33	1330.5 ± 68.01	1193.9 ± 69.98	1262.2 ± 53.26f	42.2 ± 3.00	38.9 ± 3.73	40.6 ± 2.26f
MR2	1376.5 ± 79.38	1357.7 ± 68.55	1367.1 ± 47.09e	47.4 ± 3.38	42.5 ± 3.31	44.9 ± 2.37e
MR17	1303.0 ± 76.12	1189.4 ± 79.46	1246.2 ± 55.38f	42.6 ± 3.58	38.2 ± 3.62	40.4 ± 2.48fg
RB-1 + LGR33 (Recommended)	1821.9 ± 74.40	1710.9 ± 60.56	1766.4 ± 49.56d	53.0 ± 2.75	51.5 ± 3.58	52.2 ± 2.05bc
RB-1 + MR2	1885.9 ± 94.97	1774.1 ± 62.45	1830.0 ± 56.59b	55.3 ± 3.30	50.5 ± 3.40	52.9 ± 2.38b
RB-1 + MR17	1781.1 ± 87.09	1684.9 ± 72.62	1733 ± 55.8g	48.9 ± 4.51	43.6 ± 4.32	46.3 ± 3.03d
Mean	1508 ± 78.3*	1412 ± 68.6		46.6 ± 3.30*	42.6 ± 3.66	
Variety (V)	13.9			0.68		
Treatment (T)	26.1			1.3		
V×T	36.8			NS		
Values presented are means ± SE: (*)=significant difference between mean values of two varieties, (NS) = non-significant, (SE) = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P≤0.05 according to Tukey's least significance difference (LSD)						

3.3.2 Plant growth promotion

Seedling's emergence count has been expressed as percentage (%) germination of seeds. This revealed a significant variation among all the microbial inoculant treatments in respect of the un-inoculated control. With the mono-inoculant treatments, seed germination significantly enhanced, ranged from 5.6–8.7% over the un-inoculated control. The highest germination was recorded with recommended *Mesorhizobium cicer* strain MR33 (92%) followed by *Mesorhizobium* sp. strain MR2 (91%) and *Mesorhizobium* sp. strain MR17 (89%) as compared to the un-inoculated control (84%). The germination significantly enhanced with dual-inoculant treatment of recommended RB-1 + MR33 (4.3%) followed by RB-1 + MR2 (3.3%) over MR33 and MR2 alone treatments, respectively. However, recommended RB-1 + MR33 (96%) performed at par with RB-1 + MR2 (94%), whereas RB-1 + MR17 resulted in the minimum germination (92%). The pooled mean of plant height revealed that the PBG7 variety (2.2%) was significantly better over the GPF2 chickpea variety. Significant interaction was not observed between the varieties and treatments on emergence count.

For enhancement of plant height at 60 DAS, all the mono-inoculant treatments varied significantly by 10.2–19.1% over the un-inoculated control (Table 4). The effect of dual-inoculant treatments on plant height further increased by 25.0–33.9% over the control. In similarity with emergence count, dual-inoculant treatments of pesticide tolerant *Mesorhizobium* sp. strains with rhizobacterium RB-1 significantly improved the plant height over *Mesorhizobium* alone treatments. The highest improvement was obtained with recommended RB-1 + MR33 (13.6%) over MR33 alone followed by RB-1 + MR2 (7.8%) over MR2 alone treatment. Further, PBG7 variety performed significantly better by 10.1% over GPF2 chickpea variety for enhancement of the plant height. A similar trend was followed at 90 DAS, where there was significant variation in plant height from 8.9–17.1% with the mono-inoculant treatments over un-inoculated control. All the dual-inoculant treatments, further increased the plant height ranged from 16.5–22.7% over un-inoculated control, however, RB-1 + MR2 was at par with RB-1 + MR17. Recommended RB-1 + MR33 presented the most abundant increase in plant height (11.0%) over MR33 alone treatment. Moreover, plant height significantly increased with PBG7 variety over GPF2 variety by 10.5%. The interaction between variety and treatment was not significant at 60 and 90 DAS, respectively.

Table 4
Effect of potential pesticide tolerant strains of *Mesorhizobium* sp. and rhizobacterium RB-1 on plant growth

Treatments	Plant Height (cm plant ⁻¹)			Dry weight of shoot (g plant ⁻¹)			Dry weight of root (g plant ⁻¹)		
	60 DAS (90 DAS)			60 DAS (90 DAS)			60 DAS (90 DAS)		
	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean
Control	33.4 ± 1.81 (48.3 ± 0.78)	31.4 ± 1.27 (45.2 ± 1.42)	32.4 ± 1.09ac (46.7 ± 0.90 a)	2.23 ± 0.18 (3.57 ± 0.41)	2.04 ± 0.17 (2.84 ± 0.24)	2.14 ± 0.12a (3.20 ± 0.25a)	0.94 ± 0.09 (1.96 ± 0.17)	0.83 ± 0.06 (1.71 ± 0.12)	0.89 ± 0.04a (1.84 ± 0.11a)
MR33	41.4 ± 1.29 (55.1 ± 1.40)	35.0 ± 2.10 (48.1 ± 0.99)	38.2 ± 1.54e (51.6 ± 1.34f)	2.70 ± 0.16 (4.21 ± 0.34)	2.22 ± 0.19 (3.25 ± 0.19)	2.46 ± 0.14e (3.73 ± 0.24e)	1.07 ± 0.10 (2.10 ± 0.15)	0.92 ± 0.06 (1.87 ± 0.16)	0.99 ± 0.05a (1.98 ± 0.11e)
MR2	40.8 ± 1.23 (57.8 ± 1.45)	36.4 ± 1.58 (51.6 ± 1.36)	38.6 ± 1.16ef (54.7 ± 1.34cde)	2.75 ± 0.16 (4.28 ± 0.35)	2.13 ± 0.23 (3.30 ± 0.19)	2.44 ± 0.16ef (3.79 ± 0.24ef)	1.03 ± 0.09 (2.10 ± 0.16)	0.87 ± 0.07 (1.90 ± 0.16)	0.95 ± 0.05a (2.00 ± 0.11ef)
MR17	37.9 ± 2.20 (55.6 ± 1.33)	33.5 ± 1.56 (46.3 ± 2.03)	35.7 ± 1.46g (50.9 ± 1.81g)	2.34 ± 0.16 (3.75 ± 0.40)	1.98 ± 0.18 (2.93 ± 0.26)	2.16 ± 0.13a (3.34 ± 0.26a)	0.95 ± 0.09 (2.01 ± 0.15)	0.84 ± 0.06 (1.77 ± 0.14)	0.90 ± 0.04a (1.89 ± 0.11a)
RB-1 + MR33 (Recommended)	45.3 ± 2.09 (58.3 ± 0.72)	41.4 ± 1.48 (56.4 ± 2.42)	43.4 ± 1.30b (57.3 ± 1.24b)	3.16 ± 0.18 (4.49 ± 0.41)	2.52 ± 0.21 (3.94 ± 0.33)	2.84 ± 0.16b (4.21 ± 0.26bc)	1.28 ± 0.13 (2.47 ± 0.09)	1.08 ± 0.11 (2.07 ± 0.12)	1.18 ± 0.07a (2.27 ± 0.09bc)
RB-1 + MR2	43.2 ± 1.60 (57.1 ± 0.75)	40.0 ± 1.21 (52.0 ± 2.05)	41.6 ± 1.09c (54.5 ± 1.35c)	3.16 ± 0.21 (4.56 ± 0.41)	2.59 ± 0.22 (4.02 ± 0.32)	2.88 ± 0.17bc (4.29 ± 0.26b)	1.23 ± 0.14 (2.46 ± 0.11)	1.02 ± 0.10 (2.13 ± 0.13)	1.13 ± 0.07a (2.29 ± 0.09b)
RB-1 + MR17	42.4 ± 2.02 (56.5 ± 1.61)	38.6 ± 1.43 (52.4 ± 1.77)	40.5 ± 1.38cd (54.4 ± 1.30cd)	3.06 ± 0.21 (4.40 ± 0.38)	2.31 ± 0.19 (3.90 ± 0.31)	2.69 ± 0.18bcd (4.15 ± 0.25bcd)	1.14 ± 0.12 (2.35 ± 0.13)	0.95 ± 0.10 (1.96 ± 0.10)	1.05 ± 0.06a (2.16 ± 0.10bcd)
Mean	40.7 ± 1.40* (55.6 ± 1.31)*	36.6 ± 1.34 (50.3 ± 1.47)		2.77 ± 0.14 (4.18 ± 0.14)	2.26 ± 0.088 (3.45 ± 0.19)		1.09 ± 0.05 (2.21 ± 0.08)	0.93 ± 0.04 (1.91 ± 0.06)	

Values presented are means ± SE: (*)=significant difference between mean values of two varieties, (NS) = non-significant, (SE) = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P≤0.05 according to Tukey's least significance difference (LSD)

Treatments	Plant Height (cm plant ⁻¹)			Dry weight of shoot (g plant ⁻¹)			Dry weight of root (g plant ⁻¹)		
	60 DAS (90 DAS)			60 DAS (90 DAS)			60 DAS (90 DAS)		
	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean
Variety (V)	0.8 (1.4)			0.93 (0.16)			0.38 (0.71)		
Treatment (T)	1.4 (2.6)			0.17 (0.30)			0.72 (0.13)		
V×T	NS (NS)			NS (NS)			NS (NS)		
Values presented are means ± SE: (*)=significant difference between mean values of two varieties, (NS) = non-significant, (SE) = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P≤0.05 according to Tukey's least significance difference (LSD)									

The dry weight of shoot 60 DAS, revealed significant variation among all the mono-inoculant treatments over un-inoculated control apart from the strain MR17 (Table 4), however, recommended MR33 (14.9%) was at par with MR2 (14.0%). Similarly, dual-inoculant treatment of RB-1 + MR2 performed at par with recommended RB-1 + MR33 for enhancement of dry weight of shoot. However, the highest increase was significantly obtained with RB-1 + MR2 (18.0%) over MR2 alone followed by recommended RB-1 + MR33 (15.4%) over MR33 alone treatment. The effect of treatments on varieties was not significant, while both the chickpea varieties were at par for enhancement of dry weight of shoot. Similar trend was observed at 90 DAS, where the highest dry weight of shoot was significantly recorded with strain MR2 (18.4%) followed by recommended strain MR33 (16.5%) over un-inoculated control. Dual-inoculant treatment of RB-1 + MR2 and recommended RB-1 + MR33 led to significantly enhanced dry weight of shoot by 34.1% and 31.6%, respectively over un-inoculated control. Further, RB-1 + MR2 (13.2%) and recommended RB-1 + MR33 (12.9%) significantly increased the dry weight of shoot over MR2 and MR33 alone treatments, respectively. Both chickpea varieties viz PBG7 and GPF2 were at par for the dry weight of shoot at 90 DAS.

In terms of the dry weight of root at 60 DAS, significant differences were not observed among all the treatments. However, at 90 DAS, all the mono-inoculant treatments resulted in significantly improved root dry weight over un-inoculated control apart from *Mesorhizobium* sp. strain MR17 (Table 4). The increase was witnessed with strain MR2 (8.7%) followed by recommended MR33 (7.6%) over un-inoculated control. All the dual-inoculant treatments significantly increased the dry weight of root ranged from 17.4%-24.5% over un-inoculated control. However, recommended RB-1 + MR33 (14.6%) produced the highest root dry weight over MR33 alone followed by RB-1 + MR2 (14.5%) over MR2 alone and RB-1 + MR17 (14.3%) over MR17 alone.

For improvement of the chlorophyll content, all the treatments were at par with each other at 60 DAS (Fig. 3a). Further at 90 DAS, among all the treatments, significant enhancement in chlorophyll content was seen with RB-1 + MR2 (26.6%) and recommended RB-1 + MR33 (23.8%) over un-inoculated control. However, RB-1 + MR2 and recommended RB-1 + MR33 were at par with each other and also with MR2 and MR33 alone treatments, respectively. The pooled mean analysis also revealed a significant interaction between the varieties and the treatments. However, chickpea variety PBG7 significantly increased the chlorophyll content by 13.7% over GPF2 variety. For carotenoid content of leaves at 60 and 90 DAS, all treatments were at par, and the effect of treatments on varieties was non-significant (Fig. 3b). However, dual-inoculant treatments increased carotenoid content by 1.1 fold over the mono-inoculant treatments.

3.3.3 Symbiotic nitrogen fixation

The number of nodules (NN) at 60 and 90 DAS was significantly affected by all mono-inoculant treatments except *Mesorhizobium* sp. strain MR17 in the test chickpea varieties (Table 5). At 60 DAS, a significantly high number of nodules were observed in mono-inoculant treatments as compared to un-inoculated control. The highest number of nodules was registered with RB-1 + MR2 (35 NN plant⁻¹) over un-inoculated control. Also, an extra abundant number of nodules were obtained with recommended RB-1 + MR33 (13.8%) and RB-1 + MR2 (12.9%) over MR33 and MR2 alone treatments, respectively. Further, at 90 DAS, a significantly high number of nodules were observed with recommended strain MR33 (21.9%) and MR2 (14.6%) alone treatments over un-inoculated control. Among dual-inoculant treatments, the highest number of nodules were significantly recorded with recommended RB-1 + MR33 (36.6%) whereas the least was recorded with RB-1 + MR17 (14.6%) over un-inoculated control. Moreover, a significant increase was observed with recommended RB-1 + MR33 (12.0%), RB-1 + MR2 (12.8%) and RB-1 + MR17 (9.3%) over MR33, MR2 and MR17 alone treatments, respectively. Also, PBG7 Variety significantly increased the nodule number over GPF2 variety at 60 DAS (14.3%) and 90 DAS (20.5%).

Table 5
Effect of potential pesticide tolerant strains of *Mesorhizobium* sp. and rhizobacterium RB-1 on nodulation

Treatments	Number of nodules (NN plant ⁻¹)			Dry weight of nodules (mg plant ⁻¹)			Leghaemoglobin content (mg g ⁻¹ fresh weight of nodules)		
	60 DAS (90 DAS)			60 DAS (90 DAS)			60 DAS (90 DAS)		
	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean
Control	27 ± 3 (45 ± 5)	23 ± 3 (36 ± 5)	25 ± 2a (36 ± 4a)	136 ± 11.9 (186 ± 20.0)	126 ± 10.4 (161 ± 19.6)	131 ± 7.7a (174 ± 13.9a)	2.03 ± 0.13 (3.01 ± 0.18)	1.93 ± 0.14 (2.93 ± 0.15)	1.98 ± 0.09a (2.97 ± 0.11a)
MR33	31 ± 4 (55 ± 6)	26 ± 4 (45 ± 5)	29 ± 3b (45 ± 4b)	170 ± 14.5 (225 ± 17.8)	135 ± 8.5 (184 ± 20.4)	152 ± 9.6b (205 ± 11.8b)	2.38 ± 0.18 (3.60 ± 0.19)	2.24 ± 0.15 (3.22 ± 0.20)	2.31 ± 0.11b (3.41 ± 0.15a)
MR2	33 ± 3 (51 ± 6)	29 ± 3 (44 ± 4)	31 ± 2bc (44 ± 4bc)	161 ± 16.5 (221 ± 15.4)	134 ± 5.6 (171 ± 14.4)	147 ± 9.2bc (196 ± 15.9bc)	2.31 ± 0.17 (3.51 ± 0.19)	2.15 ± 0.13 (3.21 ± 0.13)	2.23 ± 0.10bc (3.36 ± 0.12a)
MR17	29 ± 3 (48 ± 5)	23 ± 4 (37 ± 6)	26 ± 2a (37 ± 4a)	152 ± 15.8 (204 ± 25.3)	116 ± 7.6 (178 ± 14.9)	134 ± 9.9a (191 ± 13.5cd)	2.10 ± 0.17 (3.44 ± 0.17)	1.99 ± 0.11 (3.21 ± 0.16)	2.04 ± 0.10a (3.33 ± 0.12a)
RB-1 + MR33 (Recommended)	35 ± 4 (61 ± 8)	31 ± 3 (51 ± 6)	33 ± 3d (51 ± 5d)	185 ± 19.2 (245 ± 22.1)	161 ± 12.0 (216 ± 17.5)	173 ± 11.4d (230 ± 14.2e)	2.66 ± 0.19 (3.94 ± 0.18)	2.42 ± 0.17 (3.73 ± 0.20)	2.54 ± 0.12d (3.83 ± 0.13a)
RB-1 + MR2	37 ± 5 (58 ± 5)	34 ± 4 (48 ± 5)	35 ± 3e (48 ± 4bce)	178 ± 17.4 (237 ± 23.2)	154 ± 11.3 (211 ± 16.6)	166 ± 10.5de (224 ± 14.2ef)	2.58 ± 0.17 (3.83 ± 0.20)	2.29 ± 0.17 (3.71 ± 0.21)	2.43 ± 0.13de (3.77 ± 0.14)
RB-1 + MR17	34 ± 4 (51 ± 6)	31 ± 4 (44 ± 6)	33 ± 3d (44 ± 4f)	178 ± 12.3 (223 ± 27.2)	140 ± 11.8 (200 ± 11.2)	159 ± 9.9ef (211 ± 14.5g)	2.36 ± 0.14 (3.75 ± 0.21)	2.25 ± 0.14 (3.52 ± 0.16)	2.31 ± 0.09ef (3.64 ± 0.13)
	32 ± 4* (53 ± 6*)	28 ± 4 (44 ± 5)		166 ± 15.4* (220 ± 21.6*)	138 ± 9.6 (188 ± 16.4)		2.34 ± 0.16* (3.58 ± 0.19)	2.18 ± 0.14 (3.36 ± 0.18)	
Variety (V)	1.15 (2.31)			5.9 (5.5)			0.77 (0.51)		
Treatment (T)	2.15 (4.33)			11 (10.3)			0.14 (0.96)		
V×T	NS (NS)			NS (NS)			NS (0.13)		

Values presented are means ± SE: (*)=significant difference between mean values of two varieties, (NS) = non-significant, (SE) = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P≤0.05 according to Tukey's least significance difference (LSD).

Nodule biomass indicates the development of nodules. At 60 DAS, the dry weight of nodules was significantly affected by the mono-inoculant treatments apart from MR17 (Table 5 and Plates 6a-d). The highest dry weight of nodules was recorded with recommended RB-1 + MR33 (32.1%) and the least with RB-1 + MR17 (21.4%) over the un-inoculated control. Moreover, there was a significant enhancement in dry weight of nodules with recommended RB-1 + MR33 (13.8%), RB-1 + MR2 (12.9%) and RB-1 + MR17 (18.7%) over MR33, MR2 and MR17 alone treatments. At 90 DAS, significant variation was found among all treatments. Recommended RB-1 + MR33 (32.2%) showed the highest increase in the dry weight of nodules over the control while it was at par with RB-1 + MR2 (28.7%). The dry weight of nodules further significantly enhanced with RB-1 + MR33 over MR33 (12.2%) followed by over RB-1 + MR2 (14.3%) over MR2 alone treatment, respectively (Plate 6 d and 6e). Variety PBG7 performed significantly better over GPF2 variety at 60 (7.9%) and 90 DAS (17.0%).

Leghaemoglobin content was also significantly affected by all the treatments except with *Mesorhizobium* sp. MR17 alone over un-inoculated control (Table 5). However, strain MR2 (16.7%) was at par with the recommended strain MR33 (12.6%) for increasing the leghaemoglobin content. Recommended RB-1 + MR33 (28.3%) recorded the highest leghaemoglobin content whereas it was at par with MR33 alone treatment. However, RB-1 + MR2 significantly increased the leghaemoglobin content by 8.9% over MR2 alone treatment. At 90 DAS, the maximum amount of leghaemoglobin was obtained with recommended strain MR33 which further increased on dual inoculation with RB-1 as compared to un-inoculated control. However, recommended RB-1 + MR33 and RB-1 + MR2 were at par with MR33 and MR2 alone treatments, respectively. Nodulation parameters implied that the response of MR2 and recommended *Mesorhizobium* strain MR33 in the enhancement of SNF was better over the strain MR17. However, dual-inoculant treatments provided an added benefit in overall plant's performance.

3.3.4 Antioxidant enzyme activities

All the treatments significantly increased the SOD and CAT activities over the un-inoculated control (Figs. 4a and 4b). The highest increase in SOD activity was recorded with *Mesorhizobium* sp. strain MR2 (1.3 fold) and the lowest with *Mesorhizobium* sp. strain MR17 (1.2 fold) over un-inoculated control. However, SOD activity further enhanced by 1.5 fold with dual-inoculant treatment RB-1 + MR2 followed by a 1.4 fold increase with recommended RB-1 + MR33 over un-inoculated control. In congruence with SOD activity, significant enhancement in CAT activity was recorded with dual-inoculant treatments viz RB-1 + MR2 and recommended RB-1 + MR33 (1.08 fold each) over *Mesorhizobium* alone treatments viz MR2 and MR33. PBG7 variety demonstrated significantly high SOD and CAT activities (1.2 and 1.1 fold, respectively) over GPF2. These results showed that antioxidant enzyme activities were affected more significantly by the *Mesorhizobium* sp. strain MR2 as compared to recommended MR33 in mono-and dual-inoculant treatments. This presented strain MR2 as a potential candidate for improvement of plant growth and productivity in chickpea under field conditions. This can be explored as an alternative to the recommended MR33 strain in the formulation of biofertilizers in chickpea.

3.3.5 Soil health

In terms of dehydrogenase activity (Fig. 5a), all treatments differed significantly with un-inoculated control except MR17. However, the recommended strain MR33 (16.1%) and strain MR2 (15.7%) performed at par for improving soil health. The highest effect of dual-inoculant treatments on soil dehydrogenase activity was found with RB-1 + MR2 (33.1%) followed by recommended RB-1 + MR33 (29.7%) while the least was recorded with RB-1 + MR17 (14.2%). Further, recommended RB-1 + MR33 (11.7%) and RB-1 + MR2 (15.0%) significantly increased soil dehydrogenase activity over MR33 and MR2 alone treatments, respectively. Variety PBG7 performed significantly better by 16.3% over variety GPF2. On the contrary, the strain MR2 performed at par with the recommended strain MR33 for enhancement in soil urease activity at 90 DAS (Fig. 5b). Also, the highest soil urease activity was witnessed with dual-inoculant treatment of strain MR2 with RB-1 (448µg) followed by recommended RB-1 + MR33 (440µg) over un-inoculated control (361µg) while the least was recorded with RB-1 + MR17 (426µg). Further, RB-1 + MR2 and recommended RB-1 + MR33 significantly increased the urease activity by 7.4% and 5.3% over MR2 and MR33 alone treatments, respectively. PBG7 variety recorded significantly high urease activity (2.5%) over GPF2 variety. This also indicated that the strain MR2 affected the soil enzyme activities closely followed by recommended strain MR33 with improvement on dual inoculation with RB-1.

3.3.6 Total protein content of seeds, yield attributing traits and grain yield

In terms of the number of pods, significant differences existed among all the treatments except MR17 compared to the un-inoculated control (Table 6). All the dual-inoculant treatments were at par with each other, however, an extra abundant number of pods were registered with RB-1 + MR2 (17.5%) and recommended RB-1 + MR33 (12.7%) over viz. MR2 and MR33 alone treatments, respectively. Mono-inoculant treatments with the recommended strain MR33 was at par with the *Mesorhizobium* sp. strain MR2. The performance of PBG7 variety for the number of pods was significantly better (10.3%) over GPF2 variety.

Table 6

Effect of potential pesticide tolerant strains of *Mesorhizobium* sp. and rhizobacterium RB-1 on yield attributing traits and grain yield

Treatments	Number of Pods plant ⁻¹			Number of Seeds pod ⁻¹			Hundred seed weight (g)			Grain yield (Kg ha ⁻¹)		
	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean	PBG7	GPF2	Overall Mean
Control	36 ± 2.2	33 ± 2.1	35 ± 1.5a	1.63 ± 0.14	1.65 ± 0.11	1.64 ± 0.09a	13.8 ± 0.83	12.8 ± 0.26	13.3 ± 0.45a	1657 ± 51.70	1557 ± 40.25	1607 ± 36.34a
MR33	43 ± 3.4	38 ± 2.4	40 ± 2.1bc	1.99 ± 0.15	1.83 ± 0.17	1.91 ± 0.11b	15.1 ± 0.77	13.4 ± 0.24	14.3 ± 0.46b	1792 ± 42.63	1652 ± 34.76	1722 ± 35.30c
MR2	43 ± 3.8	38 ± 1.9	40 ± 2.2bc	1.91 ± 0.18	1.77 ± 0.10	1.84 ± 0.10bc	15.7 ± 0.79	14.0 ± 0.24	14.9 ± 0.47c	1784 ± 39.37	1639 ± 33.52	1711 ± 34.55cd
MR17	39 ± 3.7	36 ± 2.6	37 ± 2.2b	1.79 ± 0.13	1.72 ± 0.13	1.75 ± 0.09bd	14.7 ± 0.68	13.1 ± 0.30	13.9 ± 0.43ab	1655 ± 58.01	1621 ± 36.27	1638 ± 34.19ab
RB-1 + MR33 (Recommended)	48 ± 3.0	42 ± 1.8	45 ± 1.9d	2.14 ± 0.16	1.91 ± 0.14	2.02 ± 0.11e	15.7 ± 0.82	13.4 ± 0.27	14.6 ± 0.54bd	1874 ± 29.01	1711 ± 38.87	1792 ± 33.43f
RB-1 + MR2	50 ± 3.1	44 ± 2.1	47 ± 2.0de	2.20 ± 0.16	2.04 ± 0.15	2.12 ± 0.11ef	16.1 ± 0.83	14.5 ± 0.36	15.3 ± 0.50ce	1864 ± 29.23	1691 ± 38.00	1778 ± 36.16fg
RB-1 + MR17	44 ± 4.2	41 ± 3.0	42 ± 2.5df	2.04 ± 0.17	1.90 ± 0.13	1.97 ± 0.10eg	15.4 ± 0.73	13.8 ± 0.15	14.6 ± 0.43d	1754 ± 30.91	1669 ± 32.05	1711 ± 26.05e
Mean	43 ± 3.3*	39 ± 2.3		1.96 ± 0.16	1.83 ± 0.13		15.2 ± 0.78*	13.6 ± 0.26	14.4 ±	1769 ± 40.12*	1648 ± 36.25	
Interactions:	1.72			0.53			0.3			21.2		
Variety (V)	3.22			0.10			0.6			39.7		
Treatment (T)	NS			NS			NS			56.1		
V×T												

Values presented are means ± SE: (*)=significant difference between mean values of two varieties, (NS) = non-significant, (SE) = standard error. Means followed by dissimilar letter(s) in a column are significantly different from each other at P≤0.05 according to Tukey's least significance difference (LSD)

The number of seeds per pod also showed significant differences for all the treatments, however, interaction between variety and treatment was non-significant (Table 6). In mono-inoculant treatments, the highest improvement in the number of pods was recorded with strain MR2 (16.5%) while strain MR17 (6.7%) displayed the least increase over un-inoculated control. Further, among dual-inoculant treatments, enhancement in the number of pods was highest with RB-1 + MR2 (29.3%) followed by recommended RB-1 + MR33 (23.2%) and RB-1 + MR17 (20.1%) over un-inoculated control. The number of seeds further enhanced by 10.9% with RB-1 + MR2 over MR2 alone followed by 8.0% with recommended RB-1 + MR33 over MR33 alone. Means of varieties (PBG7 and GPF2) were at par for the number of pods.

All the treatments showed significant variation for the hundred seed weight except MR17 alone treatment (Table 6). However, strain MR2 (12.0%) and the recommended strain MR33 (6.8%) were at par with each other for the improvement of seed weight as compared to un-inoculated control. The highest seed weight was observed with RB-1 + MR2 (15.0%) followed by recommended RB-1 + MR33 (9.8%) and RB-1 + MR17 (9.8%) over un-inoculated control. However, all the dual-inoculant treatments were at par with individual *Mesorhizobium* alone treatments for improvement of the seed weight. There was a significant effect of PBG7 variety on the enhancement of seed weight by 11.8% over GPF2 variety. Protein content of seeds did not show any significant difference with the microbial treatments and the varieties. Among mono-inoculant treatments, the highest protein content was observed with MR33 (20.2%) followed by MR2 (20.0%) and MR17 (19.7%) as compared to un-inoculated control (18.9%). While, among dual-inoculant treatments, the maximum protein content

was recorded with RB-1 + MR2 (21.4%) followed by recommended RB-1 + MR33 (21.1%) and RB-1 + MR17 (20.5%). An additional increase in protein content of seed was noticed with dual-inoculants viz. RB-1 + MR2 and recommended RB-1 + MR33 by 1.04 fold each over MR2 and MR33 alone treatments, respectively. For grain yield, a significant difference was observed with all the mono-inoculant treatments except MR17 (Table 6) while recommended MR33 (7.2%) was at par with MR2 (6.5%) over un-inoculated control. However, in dual-inoculant treatments, significantly high grain yield was registered with recommended RB-1 + MR33 (11.5%) followed by RB-1 + MR2 (10.6%) and least was noticed with RB-1 + MR17 (6.5%) over un-inoculated control. In corroboration with growth and symbiotic parameters, dual-inoculant treatments; recommended RB-1 + MR33 (4.1%) and RB-1 + MR2 (3.8%) significantly increased the grain yield over *Mesorhizobium* alone treatments viz MR33 and MR2, respectively. Grain yield in PBG7 variety was significantly affected (7.3%) by the microbial inoculant treatments over GPF2 variety.

4. Discussion

The effect of pesticides on legume-rhizobia symbiosis depends on rhizobial species, type, and the concentration of pesticide (Kumar et al. 2010). Results from our *in-vitro* screening experiment also revealed appreciable differences among strains of *Mesorhizobium* sp. for tolerance to different pesticides and doses with significantly high tolerance shown in herbicides viz. pendimethalin and ready-mix followed by fungicide (carbendazim) and insecticide (chlorpyrifos). Higher tolerance with pre-emergence herbicide (pendimethalin) in our investigation might be due to the decomposition of complex N-containing pendimethalin molecule which might have been used as a proteinaceous source providing both C and N to the microbes (Cycon and Pitrowska-seget 2016). A similar explanation might be attributed to tolerance with pre-plant herbicide ready-mix, which is a combination of pendimethalin + imazethapyr. As per the observations of Drouin et al. (2010), herbicide imazethapyr was not found to have any inhibitory effect on all the *Rhizobium* strains under study. However, in alignment with our study, a significant reduction in the number of rhizobia was recorded with fungicides (Captan and thiram) by Dunfield et al. (2000). Also in another study, a significant reduction in rhizobia was observed in combined seed treatment of chickpea with fungicide (Captan) and insecticides (Endosulfan and Chlorpyrifos) (Kunal and Sharma 2012). However, in our experiment, the highest inhibitory effect towards the growth of strains of *Mesorhizobium* sp. was presented by the insecticide (chlorpyrifos) as compared to other pesticides. Further, an overall decline in growth index with double doses of different pesticides might be due to their toxic effect mainly on the moderately and poorly tolerant strains of *Mesorhizobium* sp.

The rhizosphere is a zone of intense molecular interactions due to deposition of the root exudates, that supports higher microbial growth than the surrounding soil known as the “rhizosphere effect” (Badri et al. 2009). The influence of bacteria in the rhizosphere is largely due to the production of auxin phytohormones (indolic compounds) which depends on L-tryptophan as the main precursor (Moreira et al. 2016). Etesami et al. (2015) indicated the importance of IAA in nodulation events as it helps to circumvent the plant defence system for successful host colonization. Therefore, IAA production becomes one of the important parameters for assessing the effectiveness of rhizobia in plant growth promotion and nodulation. It has been reported by Raut et al. (2017) that IAA production varies from bacteria to bacteria and pesticide to pesticide. Further, Kulandaivel and Nagarajan (2014) confirmed that the strains of *Pseudomonas* and *Azospirillum* produced IAA for up to 1.25% concentration of pesticide endosulfan while *Klebsiella* strain inhibited the IAA production. Similar variations in the IAA production by *Mesorhizobium* strains with different pesticides in our study might be attributed to inhibition or change in IAA producing metabolic pathways by the pesticides. Moreover, a significant increase in the IAA production with tryptophan supplementation in all the treatments of *Mesorhizobium* strains with pesticides as well as control treatment without pesticides was attributed to the utilization of tryptophan as the precursor for IAA biosynthesis. Further, on an overall basis, among different pesticides, a significant increase in the IAA production with pendimethalin treatment as compared to the control might be attributed to the utilization of pendimethalin as a substrate for the production of energy used for the synthesis of IAA. However, a significant reduction in the IAA production with the ready-mix, followed by chlorpyrifos and carbendazim as compared to the control could be due to the imposition of stress or toxic effect of pesticides on biosynthetic machinery of *Mesorhizobium* strains for production of important biomolecules like IAA. Such a variation of IAA in *Mesorhizobium* strains with different pesticides is well supported by the study of Drouin et al. (2010), who noticed different effects of various classes of pesticides (insecticides, herbicides and fungicides) on rhizobia.

Iron (Fe) is an essential micronutrient for plants and microorganisms, involved in various important biological processes, such as photosynthesis, respiration, chlorophyll biosynthesis and SNF. Bacteria can overcome the nutritional Fe limitation by using chelating agents called siderophores that form a complex with Fe^{3+} which may be absorbed by plant species for partial Fe nutrition. Competition for iron acquisition among rhizobia determines successful persistence in the rhizosphere leading to efficient host colonization (Rainey et al. 2016). For siderophore production, under pesticide stress in our study, pesticide tolerant *Mesorhizobium* sp. strains MR2 was observed to be more promising followed by recommended strain MR33 and strain MR17. Our study is in confirmation with the work of Ahemad and Khan (2012) who reported siderophores-production by *Mesorhizobium* sp. strain MRC4 on CAS agar medium amended

with insecticides (fipronil and pyriproxyfen). Cell wall degrading enzymes like the protease and cellulase are produced by rhizobia which play a role in the degradation of the plant cell wall for infection thread formation and also in antagonism against pathogenic fungi. In congruence with our findings, protease production has been reported in rhizobia strains by Oliveira et al. (2010). Also, Robledo et al. (2012) purified a cell-bound bacterial cellulase (CelC₂) enzyme from *Rhizobium leguminosarum* bv. trifolii nodulating the clover. In contrast to these studies, we observed positive siderophore production also in presence of herbicides (pendimethalin and ready-mix). Further, we analyzed the effect of mono-inoculation of pesticide tolerant strains of *Mesorhizobium* sp. and their dual inoculation with the recommended culture of PGP strain rhizobacterium RB-1 along with recommended application of pesticides with *in-vitro* seed germination assay. Several studies have demonstrated a significant enhancement in seed germination and seedling vigour in chickpea on co-inoculation with *Rhizobium* and PGP bacteria (Abdel Fattah 2015; Verma et al. 2013; Kaur et al. 2015). As per our findings also, significant improvement in germination and vigor index was noticed with potential pesticide tolerant strains of *Mesorhizobium* sp. alone as compared to the un-inoculated control that significantly improved with individual dual inoculation treatments.

Plant height indicates the growth index of the crop. Production of PGP substances like IAA affects many physiological activities of plants including cell enlargement, cell division, root initiation, growth rate, phototropism, geotropisms, and apical dominance and also the symbiotic rhizobium-legume interaction. Liu et al. (2017) reported that effective IAA producing isolates can stimulate plant growth due to their early colonizing ability to circumvent the plant defence mechanisms. This is well in line with the present study, where mono-inoculant treatments with strains of *Mesorhizobium* sp. resulted in increased plant height, dry weight of shoot and root over un-inoculated control with a synergistic effect in dual-inoculant treatments. Pesticides produce negative effects on crop physiology—especially on photosynthesis—leading to a potential decrease in both the growth and the yield of crops as demonstrated by Giménez-Moolhuyzen et al. (2020). However, in our study significant enhancement in plant growth was noticed with all the treatments despite the application of pesticides as compared to the un-inoculated control (with recommended pesticides application alone). This was correlated to the production of IAA by the strains of *Mesorhizobium* sp. under pesticide stress in our *in-vitro* experiment. Our results are in corroboration with Ahemad and Khan (2012) who attributed the improvement in plant growth parameters and productivity in chickpea to IAA production in pesticide tolerant *Mesorhizobium* sp. strain MRC4. It has been confirmed by Kannan et al. (2015) that there is a positive interaction between root colonization, nutrient uptake and growth promotion of plants. In line with these finding, our results for the increased chlorophyll content with dual-inoculant treatments of *Mesorhizobium* strains with RB-1 are related to efficient uptake of nutrients such as Mg (an important part of chlorophyll molecule) and N and Fe (play role in photosynthesis and enzymes involved in chlorophyll synthesis). Nodulation is an important trait for effective SNF in leguminous plants, however, pesticides have been reported to trigger root hair deformations and inhibit symbiosis (Fox et al. 2007). In the present investigation, efficient nodulation caused by pesticide tolerant strains of *Mesorhizobium* sp. might be caused by counteraction of phytotoxic effects of pesticides by their biodegradation or enzymatic hydrolysis (Martani et al. 2011). Our results are in agreement with Ahemad and Khan (2011) for improvement of nodulation parameters (nodule number and nodule dry weight) with herbicide-tolerant *Bradyrhizobium* strain MRM6 with PGP traits as compared to un-inoculated plants in greengram. Moreover, in a previous study, Gonzalez et al. (1996) showed that herbicide imazethpyr did not have any direct influence on *Rhizobium* spp. and decline in pea nodulation was attributed to the effect on plant growth rather than on rhizobia. Similarly in our study, the intrinsic tolerance of strains of *Mesorhizobium* sp. towards pesticides ameliorated the harmful effects of pesticides and substantial production of PGP substances by rhizobacterium RB-1 further improved the plant growth and development by creating more infection sites. This accounted for the significant increase in nodulation with dual inoculation treatments. In line with this, Etesami et al. (2015) established that IAA production by bacteria in root nodules activates H⁺-ATPase required for energy production in nodules. Further, Ghosh et al. (2015) correlated the production of IAA and other PGP substances by *Rhizobium undicola* to the plant-microbe interaction and nodule function in *Neptunia oleracea*. These findings are in close corroboration to our results on improved nodule biomass and N fixation with multifunctional pesticide tolerant strains of *Mesorhizobium* sp. as compared to the un-inoculated control. Synthesis of O₂ scavenging leghaemoglobin is an index of efficient SNF in legumes as it maintains high levels of ATP (Diez-Mendez et al. 2015). As per our results, higher nodulation and N fixation with dual-inoculant treatments might have increased the nodule occupancy leading to increased leghaemoglobin content over mono-inoculant treatments. Moreover, multifunctional traits of potential pesticide tolerant *Mesorhizobium* strains and PGP strain RB-1 *viz.* siderophores mediated uptake of iron (a constituent of leghaemoglobin protein) might have contributed to the increased leghaemoglobin content in dual-inoculant treatments (Gonzalez et al. 2016). Our results are well supported by Korir et al. (2017) where co-inoculation of *Rhizobium* and *Paenibacillus polymyxa* enhanced leghaemoglobin concentration, nitrogenase activity, and N fixation efficiency in common bean.

Application of agrochemicals like pesticides induce a significant amount of abiotic stress resulting in the production of reactive oxygen species (ROS) that leads to the disruption of cellular activities. Superoxide dismutase enzyme (SOD) converts superoxide anion free radical (O₂⁻) to H₂O₂. On the contrary, catalase enzyme (CAT) plays a role in converting H₂O₂ to oxygen and water. In our study, mono-

inoculant treatments in chickpea with pesticide tolerant strains of *Mesorhizobium* sp. presented significantly high SOD activity against ROS formed in response to the environmental stresses generated under field conditions. This further confirmed their robustness for environmentally stressed conditions correlating with their intrinsic tolerance towards pesticides. Further, a significant increase in SOD activity with dual-inoculant treatments in our study is in congruence with Fatnassi et al. (2015) who reported enhanced SOD activity with combined inoculation of *Rhizobium* + PGPR as compared to the control in *Vicia faba*. Similarly, an increase in SOD and CAT activities has been reported in the co-inoculation of *Piriformospora indica*, *Pseudomonas* sp. and *Mesorhizobium cicer* over single inoculation with *P.indica* and *Mesorhizobium* alone in chickpea (Mansotra et al. 2015). In addition to this, Dong et al. (2014) observed that in petroleum contaminated soils, the co-inoculation of PGPR *Serratia marcescens* + AMF *Glomus intraradices* significantly improved the SOD activity over un-inoculated control. However, an overall decrease in catalase as compared to SOD activity in our study was attributed to more consumption of the catalase enzyme to detoxify ROS (Fatnassi et al. 2015). Soil enzymes viz urease and dehydrogenase are important indicators of soil microbial activity and play a role in nutrient (N and H) recycling. Our findings for the significant increase in soil enzyme activities with pesticide tolerant strains of *Mesorhizobium* sp. is well supported by the work of Siczek and Lipiec (2016) where high soil urease and dehydrogenase activity in faba bean with *Rhizobium* over control was attributed to changes in microbial community structure and enzymatic processes in response to rhizobia. Corresponding to other growth and SNF parameters in our study, a significant increase in soil enzyme activities with all dual-inoculant treatments might be attributed to a higher root system which led to the accumulation of root exudates in the rhizosphere attracting higher microbial populations for mediating the enzyme activities. Batista et al. (2016) postulated that facilitated nutrient uptake by PGP bacteria along with phosphate solubilization (that provides P for ATP) and siderophores mediated uptake of iron (that acts as e⁻ carrier in nitrogen fixation reaction carried out by nitrogenase enzyme), enhanced the fixed N that was incorporated as proteins. Similarly, in our study, an increase in protein content with dual-inoculant treatments might be attributed to the increased availability of fixed N that was further explained by facilitated uptake of micronutrients such as Mo acting as a cofactor in nitrogenase enzyme. This is further correlated to IAA production, which helps in nutrient uptake with the proliferation of roots (Haris and Ahmad 2017). Similar reports on co-inoculation with *Rhizobium* and PGP bacteria have shown improvement in seed protein and N content in soybean (Hungria et al. 2015; Masciarelli 2014) and chickpea (Verma et al. 2013). Results on plant growth and SNF, are well in line with our study on the significant enhancement in the number of pods with dual-inoculant treatments that was attributed to an increase in the number of branches with PGP rhizobacterium RB-1. In addition to this, improved photosynthetic rates due to improvement in nutrient and water absorption in dual inoculation treatments resulted in higher nutrient assimilation in grain leading to increased seed weight and grain yield in our experiment. Similarly, a positive correlation between IAA production and grain yield was suggested by Verma et al. (2013) in chickpea. Our results are further supported by a pot experiment done by Ahmad et al (2016) where single inoculations of greengram significantly increased grain yield with *Bradyrhizobium* sp. and with *Azotobacter* sp. alone over control which further improved on dual inoculation (*Bradyrhizobium* + *Azotobacter*). Improvement in growth parameters, yield attributing traits and grain yield in different chickpea genotypes might be the result of differential interaction of variety with microbial inoculants which could be further attributed to variation in rhizospheric community structure in response to root exudates (Nasr Esfahania et al. 2016).

5. Conclusions

Current agricultural practices in Punjab have witnessed indiscriminate use of pesticides (including herbicides, insecticides and fungicides) due to which ecological performance of bio-inoculants may be compromised resulting in a reduction in the yield and productivity of the legumes. In the present study, *in-vitro* developed bacterial strains for pesticide tolerance revealed three robust strains of *Mesorhizobium* sp. viz. MR2 and MR17 and recommended MR33 that showed the highest plant growth promotional activities. Although, under laboratory conditions, strains MR2 and MR17 performed significantly better over the recommended strain MR33 for PGP traits, yet under field investigation, *Mesorhizobium* sp. strain MR2 was at par with recommended strain MR33 for enhancement of plant growth, SNF and yield in chickpea over un-inoculated control. Further dual-inoculant treatments with recommended PGP strain RB-1 significantly enhanced all the parameters. The potential pesticide tolerant strains MR2 and MR33 can be further explored as compatible dual-inoculants with recommended RB-1 for chickpea under environmentally stressed conditions (pesticide application) at multiple locations. Therefore, screening for robust multifunctional pesticide tolerant *Mesorhizobium* might open new avenues for improving chickpea productivity in pesticide stressed soils of Punjab. Overall, these findings postulate that bio-augmentation of chickpea with multifunctional *Mesorhizobium* with improved pesticide tolerance might be helpful in the formulation of effective bio-inoculants consortia in establishing successful chickpea–*Mesorhizobium* symbiosis. However, concerted research and development are needed to integrate engineered microorganisms in the agro-ecosystem for sustainable agriculture.

Declarations

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

Figure 1

Growth index of strains of *Mesorhizobium* sp. with recommended pesticides:- pendimethalin (PD), ready mix (pendimethalin+imazethpyr) (RM); carbendazim (B); chlorpyrifos (CHR): *Dosage of pesticides:- single dose (1×), double dose (2×)

Figure 2

Indole acetic acid (IAA) production by pesticide tolerant strains of *Mesorhizobium* sp. with recommended doses of pesticides:- a pendimethalin (PD) b ready mix (imazethpyr+pendimethalin) (RM) c carbendazim (B) d chlorpyrifos (CHR) as compared to control treatments (C) in tryptophan's presence (Trp+) and absence (Trp-). *The values presented are Means $\pm$ SE. All the treatments means differed significantly with each other and also with pesticide and tryptophan treatments according to Tukey's least significance difference (LSD) at $P \leq 0.05$.

3a

3b

Figure 3

Effect of potential pesticide tolerant strains of *Mesorhizobium* sp. and rhizobacterium RB-1 on chlorophyll and carotenoid content
 *Values presented are overall means of PBG and GPF2 chickpea varieties $\pm$ SE (standard error). All the treatments means were at par with each other both at 60 and 90 DAS according to Tukey's least significance difference (LSD) at $P \leq 0.05$.

4a

4b

Figure 4

Effect of potential pesticide tolerant strains of *Mesorhizobium* sp. and rhizobacterium RB-1 on antioxidant enzymes activities in chickpea
 *Values presented are overall means of PBG and GPF2 chickpea varieties $\pm$ SE (standard error). Means followed by dissimilar letter(s) on error bars are significantly different from each other at $P \leq 0.05$ according to Tukey's least significance difference (LS

Figure 5

Effect of potential pesticide tolerant *Mesorhizobium* sp. strains and rhizobacterium strain RB-1 on soil enzyme activities in chickpea.

*Values presented are overall means of PBG and GPF2 chickpea varieties $\pm$ SE (standard error). Means followed by dissimilar letter(s) on error bars are significantly different from each other at $P \leq 0.05$ according to Tukey's least significance difference (LSD)

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