Electronic Supporting Information

Unraveling the potential of pesticide-tolerant *Pseudomonas* sp. augmenting biological and physiological attributes of *Vigna radiata* (L.) under pesticide stress

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<u>Supplementary Methods</u>

Assessment of pesticide-induced morphological changes in bacterial strain using scanning electron microscopy (SEM)

Pesticide induced cellular damage/distortion in molecularly characterized bacterial strains was observed under SEM (JSM 6510 LV, JEOL, Japan) by growing bacterial strains in NB medium treated with fungicides: kitazin (2400 μgmL^{-1}), hexaconazole (1500 μg mL^{-1}) and metalaxyl at 28±2°C for 24 h. The culture grown in pesticide free medium served as control. After incubation, cultures were centrifuged at 12,000 rpm for 10 min. and pellet was suspended in 1× PBS and the cell pellets were washed again three times with 1× PBS and pre-fixed (4°C) with 2.5% glutaraldehyde for overnight. The cells were recovered by centrifugation at 10000 rpm for 5 min. and pellet was again washed with same buffer. After three successive washing, the fixed specimens were dehydrated in a graded series (30, 50, 70, 90 and 100%) of ethanol for 5 min. each. After this, cell pellets were centrifuged and re-suspended in PBS. Five millilitres of bacterial suspensions were smeared on cover slip and dried. The specimens were mounted and analyzed under the SEM to record changes in bacterial structures, if any, and images were recorded.

Determination cellular permeability of Pseudomonas sp. strain PGR-11 under CLSM

Alterations in the membrane integrity and bacterial mortality were assessed by fluorescence microscopy as previously used by Shahid and Khan (2018). For this, bacterial strains were grown in NB medium treated with different rates of each pesticide and incubated. To the 200 µL of pesticide treated and untreated (control) bacterial suspensions, 10 µL of acridine orange (AO: 15 μg mL^{-1} prepared in PBS) and 10 µL of propidium iodide (PI: 50 μgmL^{-1} prepared in PBS) were added. Bacterial suspensions stained both with AO and PI dyes was incubated at room temperature for 10 min. and centrifuged (at 5000 rpm) for 10 min. The supernatants were discarded to remove the unbound dyes while cell pellet was resuspended in 500 µL of PBS. The experimental setup was maintained in the dark conditions to avoid photobleaching of dyes. Images were recorded in CLSM (LSM-780, Confocal microscope, Zeiss, Germany). The samples were then observed for PI stained dead cells with an excitation/emission maxima of 493/636 nm for PI.

Inoculation impact of PGR-11 strain on photosynthetic pigments in V. radiata

Photosynthetic pigments (Chl a, Chl b, total chlorophyll and carotenoid content) in fresh foliage of was measured according to the method of Arnon (1949). The pigments were extracted from fresh leaves by macerating in 80% acetone. Absorption of chlorophyll and carotenoid content in the extract was determined using UV visible spectrophotometer (UV-2450, Shimadzu, Tokyo, Japan). The total photosynthetic pigments (Chl a, Chl b and total chlorophyll) was calculated as:

$$\text{mg chl. a/g tissue} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times V/1000 \times W$$

$$\text{mg chl. b/g tissue} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times V/1000 \times W$$

$$\text{mg total chl./g tissue} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times V/1000 \times W$$

Carotenoid content was determined by the formula as suggested by Krik and Allen (1965):

$$\text{Carotenoids (mg/g tissue)} = (A_{480}) + 0.114\,(A_{663}) - 0.638\,(A_{645})$$

Where, A_λ = absorbance at specific wavelength λ (nm); V= final volume of chlorophyll extracted in 80% acetone, and W= fresh weight of tissue extract.

Leaf Exchange parameters

The gas exchange parameters of strain PGR-11 inoculated and pesticide-treated foliage were also evaluated. Stomatal conductance (gs), rate of transpiration, internal CO_2 concentration (Ci), net photosynthetic rate (PN), and vapor pressure deficit (kPa) were measured using a Li-COR 6400 portable photosynthesis system (Li-COR, Lincoln, NE, USA). Net photosynthetic rate (A_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were all determined at ambient CO_2 concentration, temperature of 25–28 °C, 50±5 % relative humidity, and a photosynthetic photon flux density (PPFD) of 1000 µmol m^{-2} s^{-1} to record each measurement. Photosynthetic area was approximated as half the area of the cylindrical branches, as only the upper half received the unilateral illumination in the leaf chamber.

Seed Attributes

For protein estimation, 500 mg of seeds were soaked in phosphate buffer (pH =7.4) and extracted in 3 mL of 50 mM phosphate buffer (pH =7.8) containing 1mM EDTA and 2% w/v polyvinylpyrrolidone (PVP). The extract was spun at 5742 *g* for 10 min. at 4°C and supernatant was used for protein analysis. A- 0.2 mL aliquot was taken from the sample extract and the volume was made up to 1.0 mL. To it, 4.5 mL of copper solution was added and was allowed to stand for 10 min. Then, 0.5 mL of Folin's reagent was added to each tube and incubated for 30 min. for colour development. Absorbance of blue colour was read at 660 nm on a UV-Vis spectrophotometer. The protein concentration in the supernatant was determined using a calibration curve of BSA as a standard (Lowry et al., 1951).

Supplementary Tables

Table S1: Details of Fungicides used the in present study

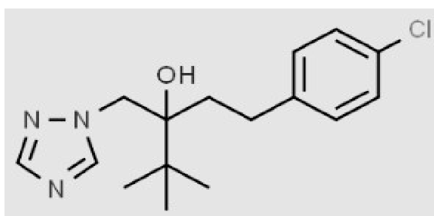
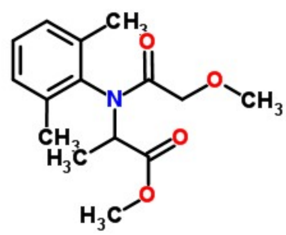
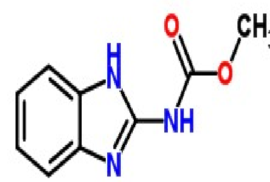
Characteristics	Fungicide used		
	Tebuconazole (TBZL)	**Metalaxyl (MTXL)**	**Carbendazim (CBZM)**
Common name	Tebuconazole	Metalaxyl	Carbendazim
Chemical name	(RS)- 1-(4-Chlorophenyl)- 4,4-dimethyl-3-(1H, 1,2,4-triazol-1-ylmethyl)pentan- 3-ol	methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate	methyl benzimidazol-2-ylcarbamate
Chemical family	Triazole	Anilide	Benzimidazole
Grade	Commercial (48% EC)	Commercial (35% w/w)	Commercial (50% w/w)
Trade name	Folicur	Ridomil, Subdue, Apron,	Bendaco
Appearance	transparent liquid	Fine white powder	White, crystalline solid
Molecular weight	307.82 g/mol	279.33 g/mol	191.187 g/mol
Empirical formula	$C_{16}H_{22}ClN_3O$	$C_{15}H_{21}NO_4$	C9H9N3O2
Solubility	Water/DMSO	Water/DMSO	Water/DMSO
Chemical structure			

Table S2. The chlorophyll fluorescence parameters used in this study

Parameters	formula	Description
Fv/Fm	(Fm − F0)/Fm	Maximum quantum yield of PSII photochemistry measured in the dark-adapted state
Y(PSII)	(F0m − Fs)/F 0m	Effective quantum yield of photochemical energy conversion in PSII

Table S3: Screening of PGPR strain for plant growth promoting attributes

PGPR strains	Indole-3-acetic acid production (µg/mL)					ACC deaminase (µM α-ketobutyrate mg^{-1} protein h^{-1})	Siderophore	NH_3	HCN
	0*	50*	100*	200*	400*				
PGR-1	12.4	19.3	23.4	26.7	43.2	11.2	++	++	+
PGR-2	7.8	14.3	26.7	35.8	52.4	9.3	+	+	+
PGR-3	6.9	12.4	18.6	28.9	43.8	15.7	+	+	+
PGR-4	11.4	18.9	25.7	33.1	47.8	23.6	++	+	+
PGR-5	14.5	22.5	29.6	39.0	52.3	12.6	++	+	+
PGR-6	8.6	11.7	22.5	32.1	45.0	16.7	+	+	+
PGR-7	7.4	13.2	20.6	30.2	38.9	15.3	+	+	-
PGR-8	13.2	18.9	34.6	38.6	48.1	18.9	+	++	-
PGR-9	18.9	23.4	32.1	40.2	54.0	24.7	+	++	+
PGR-10	9.4	16.9	28.9	37.9	49.3	19.3	+	++	+
PGR-11	22.1	32.4	43.6	57.8	82.9	34.6	+++	++	+
PGR-12	12.0	20.0	28.0	34.0	54.6	23.0	+	++	-
PGR-13	17.2	26.8	38.7	48.9	58.3	12.0	++	+	-
PGR-14	8.6	17.0	27.0	37.9	51.0	9.4	++	+	+
PGR-15	7.0	15.0	23.4	33.1	48.3	18.0	++	+	-

Each value is the mean of three replicates (*n* = 3). ACC = 1-amino cyclopropane 1-carboxylate deaminase, NH_3 = ammonia and HCN = hydrogen cyanide.

Table S4: Pesticides tolerance

PGPR strains	Pesticide tolerance (μg mL^{-1})		
	Carbendazim	**Metalaxyl**	**Tebuconazole**
PGR-1	400	800	400
PGR-2	400	400	400
PGR-3	200	400	200
PGR-4	100	200	100
PGR-5	50	200	50
PGR-6	200	800	800
PGR-7	400	1200	800
PGR-8	200	200	800
PGR-9	10	400	1200
PGR-10	50	100	1200
PGR-11	800	1200	1600
PGR-12	200	200	1200
PGR-13	400	1000	800
PGR-14	200	200	800
PGR-15	200	400	400

Each value is the mean of three replicates (n = 3).

Table S5: Morphological and biochemical characterization of *Pseudomonas* sp. PGR-11

Characteristics	***Pseudomonas* sp. PGR-11**
Morphology	
Gram reaction	Negative rod
Colony character	Round, creamish, opaque, yellow-green
Biochemical reactions	
Citrate utilization	positive
Indole	negative
Methyl red	positive
Nitrate reduction	positive
Oxidase	positive
Voges-Proskauer	negative
Catalase	positive
Carbohydrate utilization	
Dextrose	positive
Lactose	positive
Mannitol	positive
Sucrose	positive
Hydrolysis	
Starch	positive
Gelatin	positive
Tolerance to	
CBZM	800 $\mu g\ mL^{-1}$
TBZL	1600 $\mu g\ mL^{-1}$
MTXL	1200 $\mu g\ mL^{-1}$

Supplementary Figures

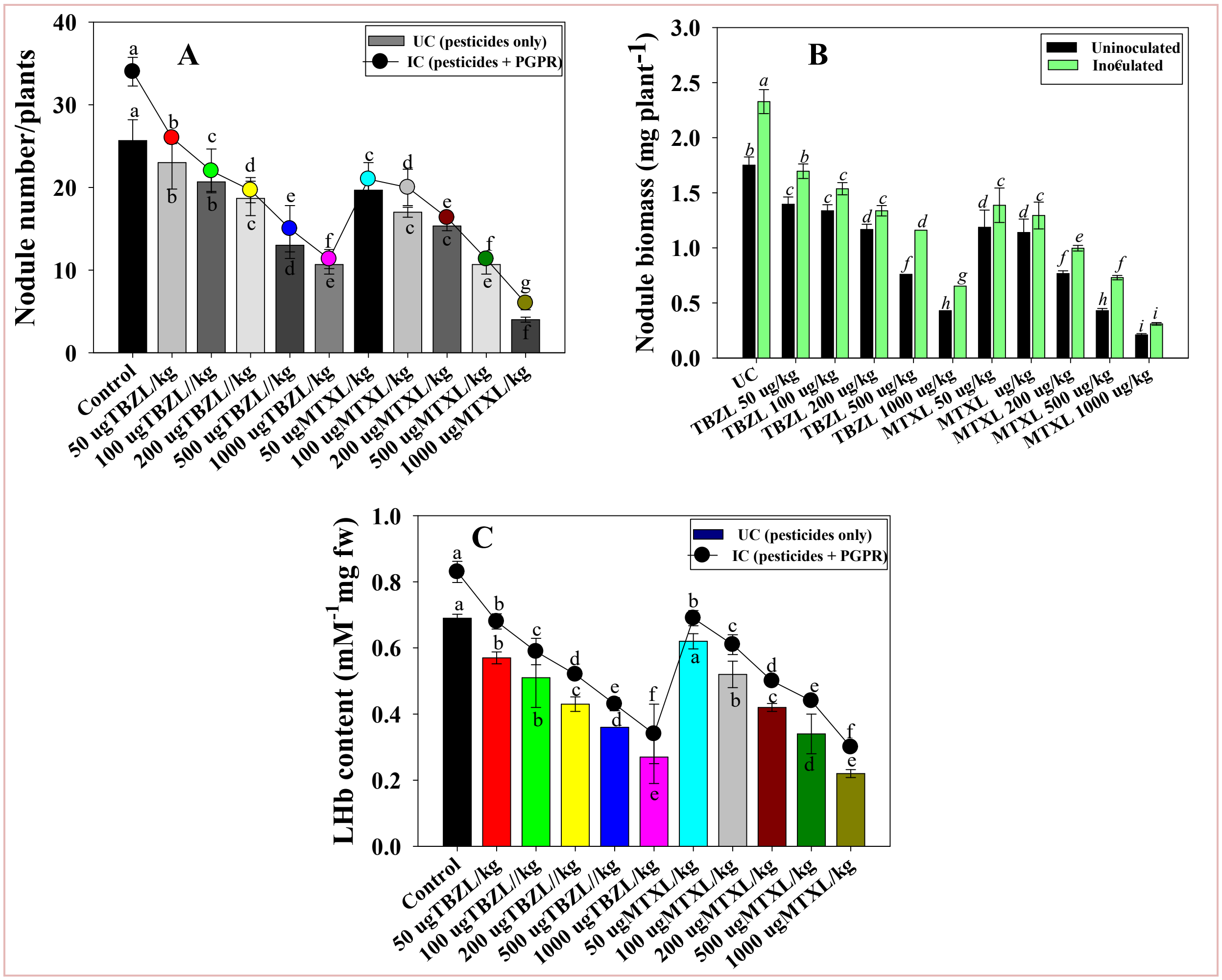


Figure S1: Impact of *Pseudomonas* sp. strain PGR-11 on nodule number (A) nodule biomass (B) and LHb content extracted from root nodules detached from *V. radiata* raised in soil treated with increasing concentrations of TBZL and MTXL. Each value is a mean of three independent replicates. Bar and line diagrams represent the mean values of three replicates (n = 3) of three plants/pot. Mean values followed by different letters are significantly different ($p \leq 0.05$) as determined by the DMRT test. Vertical and scattered bars represent means± SDs (n = 3). Here, TBZL = tebuconazole and MTXL= metalaxyl.