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Phytophthora nicotianae Breda de Haan (syn *P. parasitica* Dastur) is an important broad-host range pathogen of brinjal (*Solanum melongena* L.). Currently available cultural practices and chemical control measures are often limited, ineffective and hazardous to human health and environment. On the other hand, biological control with microbial agents can be a safer, environment-friendly disease management alternative. In this study, treatment with bacterial agent *Pseudomonas aeruginosa* KUCd1 strain- a competent colonizer of the rhizosphere and rhizoplane of brinjal plants, could significantly improve plant growth as well as reduce *Phytophthora* infection in brinjal under *in vivo* conditions. It also induced systemic resistance against *Phytophthora nicotianae* and elicitated rapid defence response (as evident from the several fold increased activity of various defense-related enzymes; PAL, POD, PPO and CAT) protecting the plant from pathogen ingress and hence protection from disease. The present study thus explores its potential for use as biocontrol agent against *P. nicotianae* or as biofertilzer or both in future which requires further investigation.

Key words: Brinjal, PGPB, Biocontrol, *Phytophthora*

INTRODUCTION

Cultivated eggplant or aubergine (*Solanum melongena* L.) also known as brinjal in India, is one of its centres of diversity. It is grown worldwide for its edible fruits and is one of the most important vegetables in Asian diets. In India, West Bengal is the largest producer of brinjal contributing to 22.06 % share and it is the second most important crop in the state after potato (DRCSC report, 2006). However, its production and quality in this region have been often limited by plant diseases such as Phytophthora blight and damping off caused by *Phytophthora nicotianae* Breda de Haan (syn *P. parasitica* Dastur).

P. nicotianae is an important soilborne, broad host range pathogen affecting over 255 genera in 90 families and is considered to be one of the major threat affecting most of the vegetable and fruit crops worldwide (Cline *et al*, 2008) and specially so in Eastern parts of India (Guha Roy *et al*, 2009; Guha Roy and Grunwald, 2014). The management of most of these diseases relies on modifications in cultural practices and judicious use of fungicides. The fact that fungicides against *Phytophthora* alone represent 25% of the total annual fungicide market is perhaps an indicator of the extent of dam-

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age caused and dependence on chemicals to control these diseases. However their routine use is neither economically practicable nor environmentally suitable. Moreover, fungicide resistance within pathogen populations and environmental recalcitrance is increasingly shifting the choice and need towards biological control with microbes derived from natural sources such as soil, water, plants, and other organisms. These bicontrol agents when applied into the roots or rhizosphere cause direct antibiosis or show suppressive efficacy through ISR or plant growth promotion. A large number of enzymes have been associated with ISR including phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) (Silva *et al*, 2004; Nakkeeran *et al*, 2006; Sharma *et al*, 2007a) Here we present data suggesting that *P. aeruginosa* KUCd1 could improve plant growth as well as reduce *Phytophthora* infection in brinjal under *in vivo* conditions.

MATERIALS AND METHODS

Culture maintenance

The pathogen *P. nicotianae* isolate P10986, originally isolated from diseased tissue of brinjal was maintained on V_8JA medium (Guha Roy et al., 2006). The information on host, accession number, morphological and genetic characterization and maintenance of the test isolate has been mentioned elsewhere (Guha Roy *et al,* 2006, 2007, 2009). The isolation and identification of bacterial agent KUCd1 as *Pseudomonas aeruginosa* following biochemical tests and 16S rDNA sequence analysis and its maintenance on nutrient agar (Himedia, India) has also been described in our earlier publication (Sinha and Mukherjee, 2008).

Plant material

The seeds of brinjal (*Solanum melongena* L. local var. Purple round type) used for *in vivo* pot experiments were purchased from local nursery, Kalyani, Nadia, West Bengal. Before sowing, the seeds were surface sterilized with 1% sodium hypochlorite solution for 2 min, washed three times with sterilized distilled water (SDW), and blotted dry on sterile paper towels.

Bacterial inoculum

Sixteen hours grown bacterial culture of rifampicin resistant KUCd1 was transferred into nutrient broth (HiMedia, India) and incubated in a shaking incubator (160 rpm) at 28 ÚC for 24 h. The bacterial cells were harvested and centrifuged at 10000 rev min^{-1} for 20 min, washed twice and re-suspended in SDW. The final cell viable count was adjusted to ~1×10^8 cfu/ml before using as bacterial inoculant in the pot experiments. A rifampicin resistant (150 µg/ml) spontaneous mutants of KUCd1 (Sinha and Mukherjee, 2008) was used for root colonization studies.

Pathogen inoculum

Zoospore production was induced and isolated as described by Ristaino (1990) from V_8JA maintained P10986 culture. The final concentration was adjusted to ~ 2×10^4 zoospores/ml and was applied immediately within 30 min of calibration.

Experimental set up

Plant growth and disease assessment studies for brinjal plants were performed twice during winter (January) and wet season (July) in order to investigate the seasonal influence on disease incidence and plant growth in the polyhouse. The experiment comprised of four treatments; viz., T_1 – Water (control); T_2 – KUCd1; T_3– *P. nicotianae* (P10986); T_4 – KUCd1 colonized plants challenged with P10986. Surface sterelized seeds of brinjal were either soaked in SDW (T_1 and T_3) or in a KUCd1 cell suspension (~1×10^8 cfu/ ml, T_2 and T_4) for 24 h for bacterization followed by air drying. Seeds were sown in seedling trays and after two weeks the seedlings were transplanted into pots (38 cm diameter) containing potting mixture of solarised garden soil, farmyard manure and sand (3:2:1). Fifty millilitre of KUCd1 cell suspension (T_2 and T_4), prepared as described in section 2.3, or SDW (T_1 and T_3) was applied by soil drench at the base of the stem of each brinjal seedling immediately after transplanting. Two weeks after transplantation, the zoospore suspensions (T_3 and T_4) or SDW (T_1 and T_2) were injected at the rate of 5ml/per plant into 4 holes (1 cm diameter X 1cm deep) around each plant. Four seedlings were transplanted in each pot. There were four pots/ replication and 4 replications per treatment.The plants were covered with polythene bags for 24 h, continuously watered for 4-5 days to prevent drying of the soil and then watered as needed.

Plant growth and disease assessment

Brinjal plants (4 weeks post inoculation) were uprooted, washed repeatedly in SDW to remove any adhering soil particles, and were blotted dry. The root and shoot fresh weight (g^{-1}plant) was recorded. These were then kept in the hot-air oven for 7 days at 60 ÚC for complete desiccation, and the dry weight (g^{-1}plant) was noted. The per cent mortality was calculated 30 days after pathogen inoculation.

Enzyme assay

The leaf samples were collected at 24 h, 48 h, 72 h, and 96 h post pathogen inoculation, washed with SDW and dried gently. The frozen leaf samples were homogenized in chilled 0.1 mol/L sodium phosphate buffer pH 7.0 with sterilized quartz sand and centrifuged at 15 000 rev min^{-1} for 20 min at 4 ÚC. The supernatant was stored at –80 ÚC. Protein concentrations of the samples were determined following Bradford's method (1976). Phenylalanine ammonia lyase (PAL) activity was assessed following the method as described by Zucker (1965) by measuring the amount of cinnamic acid produced at 290 nm and is expressed as U mg^{-1} protein. Peroxidase (POD) activity was determined following the method of Urbanek *et al,* (1991) and is expressed as µmol/L H_2O_2 min^{-1} (1 unit) g^{-1} protein taking into consideration that 4 mol/L of H_2O_2 are reduced to produce 1 mol/L of tetraguaiacol. Polyphenol oxidase (PPO) activity was determined following standard method (Mukherjee and Ghosh, 1975) and is expressed as increase in absorbance at 420 nm min^{-1} mg^{-1} protein. Catalase (CAT) activity was estimated following the method as described by Anand *et al,* (2007) and was expressed as $unit^{-1}$ mg^{-1} protein.

Root colonization bioassay

Plants were uprooted at 60 days after sowing (DAS) for rhizosphere and rhizoplane bacterial population study. The excised roots from these uprooted plants were vigorously shaken to collect the adhering rhizosphere soil particles, These particles were then mixed thoroughly on a sterile filter paper and 10 g of this mix was put into a 250 ml Erlenmeyer flask containing 100 ml of SDW. In order to estimate the rhizoplane bacterial population, the root segments were cut into small pieces, mixed randomly and 10 g of this mix was washed three times in SDW and homogenised with sterile mortar and pestle. These crushed roots were placed in a 250 ml Erlenmeyer flask filled with 100 ml of sterile water. The flasks were shaken at 160 rpm at 28 ºC for 30 min. The suspension was dilution plated on NA medium supplemented with 150 µg/ml rifampicin and cultured at 28 °C for 48 h.

Statistical analysis

The data were statistically analyzed using the SPSS software, version 17. Analysis of variance (ANOVA) was determined using general linear model and mean values were compared by Duncan's multiple range test (DMRT) at 5% probability. The standard error of mean (SEM) for each treatment was calculated.

RESULTS AND DISCUSSION

Plant growth assessment

In the seasonal trials, bacterized sets (T_2 and T_4) of brinjal plants demonstrated significantly higher values for all the measured growth parameters compared to non-bacterized sets (T_1 and T_3) . The results further indicated that irrespective of the seasonal variation, T_2 plants, accounted for the maximum increase in growth parameters compared to all other treatments. In T_4 plants although the per cent increase in growth parameters was lower compared to that recorded in T_2, it still registered significantly higher values compared to T_1 (control) and T_3 in the test plant (Table 1).

During winter trial, the increase in different growth parameters of T_2 plants over control was noted to be maximum for root length (32.2 %) followed by shoot dry weight (27.5 %), root dry weight (25 %), shoot length (20 %), root fresh weight (17.7 %) and shoot fresh weight (12.7 %). In T_4 plants this increase in growth parameter over control was reduced compared to T_2 plants and the maximum increase over control was observed in case of root length (22 %) followed by shoot dry weight (19.1 %), root fresh weight (10.3 %), shoot length (10.2 %), root dry weight (6.3 %) and shoot fresh weight (2.4 %).

Similarly in the wet trial, in case of T_2 plants the increases in growth parameters were 35.3 % in root dry weight, 35 % in root length, 24.6 % in dry

Table 1 : Effect of promising antagonistic isolate KUCd1 on growth performance of brinjal under artificial infestation of pot soils with P10986 in net house, 4 weeks post inoculation, during winter and wet trials.

Treatment+	Growth parameters* assessed during winter trial						Growth parameters* assessed during wet trial					
	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Sho ot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Control (T_1)	9.08 b (± 0.23)	0.68 c (±0.01)	0.16 b (± 0.01)	18.8 b (± 0.97)	19.88 b (± 0.85)	3.46 c (± 0.07)	10.5 b (± 0.29)	0.78 b (± 0.01)	0.17 c (± 0.01)	21.2 b (± 0.79)	21 c (± 0.44)	3.9 c (± 0.07)
KUCd1 (T_2)	12 a (± 0.39)	0.8 a (± 0.01)	0.2 a (± 0.01)	22.5 a (± 0.79)	22.41 a (± 0.39)	4.41 a (± 0.12)	14.2 a (± 0.71)	0.96 a (± 0.01)	0.23 a (± 0.01)	24.5 a (± 0.71)	23.9 a (± 0.14)	4.86 a (± 0.08)
P10986 (T_3)	6.83 c (± 0.24)	0.52 d (± 0.01)	0.12 c (± 0.01)	16.3 c (± 0.67)	16.66 c (± 0.62)	2.75 d (± 0.08)	8.92 c (± 0.31)	0.58 c (± 0.01)	0.13 d (± 0.01)	17.6 c (± 0.79)	20.7 c (± 0.13)	3.02 d (± 0.04)
KUCd1+P10986 (T_4)	11.1 a (± 0.57)	0.75 b (± 0.02)	0.17 b (± 0.01)	20.7 ab (± 0.4)	20.36 b (± 0.86)	4.12 b (± 0.05)	13 a (± 0.44)	0.86 b (± 0.02)	0.19 b (± 0.01)	22.5 ab (± 0.62)	22.7 b (± 0.41)	4.48 b (± 0.11)

+ Plants treated with KUCd1 were challenge inoculated with *P.nicotianae* (P10986). Untreated plants served as negative control when exposed to water treatment while plants exposed to pathogen served as positive control. *Data are the means (± SEM, standard error of means) of each growth parameters from two separate experiments. Means followed by different letter in a column are significantly different according to Duncan's multiple range test (DMRT) at $p \leq 0.05$.

weight of shoot, 23.1 % in fresh weight of root, 15.7 % in shoot length and 13.8 % in fresh weight of shoot compared to control. In T_4 plants this increase was 23.8 % for root length, 14.9 % for shoot dry weight, 11.8 % for root dry weight, 10.3 % for root fresh weight, 8.2 % for shoot fresh weight and 6.3 % for shoot length. The percentage increase in all the growth parameters registered higher values in wet trials excepting root fresh weight and shoot length which registered slightly higher values in winter trial.

Evaluation of efficacy of biocontrol agent in reducing Phytophthora infection under controlled conditions

The disease control efficiency of KUCd1 for root rot disease of brinjal was calculated 30 days post inoculation with pathogen. Brinjal plants (T_3) challenge inoculated with P10986 resulted in 100% mortality of plants. No plant mortality was observed in either T_1 or T_2 sets. The bacterized plants challenge inoculated with pathogen (T_4) showed reduced mortality rate compared to non-bacterized pathogen inoculated (T_3) sets. KUCd1 reduced mortality in T_4 sets of brinjal by 68.75 % (Table 2).

Induction of defense-related enzymes

PAL, POD, PPO and CAT activity was measured in root, stem and leaves of brinjal plants exposed to different treatments during 24 – 96 h post payhogen inoculation assay period. In all the cases, the challenge inoculated bacterized (T_4) plants showed significantly higher levels of enzyme activity compared to all other treatments (T_2, T_1 and T_3).

Phenylalanine ammonia lyase (PAL; EC 4.3.1.5) activity

The bacterized plants (T_2 and T_4) showed significantly higher levels of PAL activity compared to non bacterized (T_1 and T_3) plants (Fig. 1A, B, C). Maximum increases in PAL activity was noted in the leaves of T_4 plants. In comparison to control the increases in PAL activity ranged between 124.5 % to 136.7 % in T_4 plants, 48.6 % to 57.9 % in T_3 plants and 86.4 % to 104.2 % in T_2 plants (Fig. 1C) whereas, in roots (Fig. 1A) during the same study period the increases in PAL activity ranged between 61 % to 112.9 % in T_4 plants, 27.4 % to 60.9 % in T_3 plants and 41.8 % to 73.8 % in T_2 plants.

PAL showed least levels of induction in stem (Fig. 1B) compared to all other plant part. Here the increases in PAL activity compared to control varied between 34.4 % to 90.3 % in T_4 plants, 7.6 % to 49.8 % in T_3 plants and 22.9 % to 69.5 % in T_2.

Peroxidase (POD; EC 1.11.1.7) activity

Similar to PAL activity, the bacterized (T_2 and T_4)

Table 2 : Effect of *Pseudomonas aeruginosa* KUCd1 treatment on mortality rate of brinjal plants 30 days after challenge inoculation with *Phytophthora nicotianae* (P10986)

Treatment*	Total number of plants	Number of dead plants	Mortality (%)	Control efficiency (%)
T_1	32	0	0	
T_2	32	0	0	
T_3	32	32	100	
T_4	32	10	31.25	68.75

*The plants were exposed to four treatments (a) T_1 are the control plants that were neither bacterized with KUCd1 nor was it exposed to pathogen P10986; (b) T_2- plants bacterized with *Pseudomonas aeruginosa* strain KUCd1; T_3 – are the plants challenge inoculated with P10986 without bacterization and (T_4) are the KUCd1 bacterized plants that were challenge inoculated with pathogen *P.nicotianae* (P10986).

Table 3 : Rhizosphere and rhizoplane colonization[a] of *Pseudomonas aeruginosa* strain KUCd1 in brinjal plants grown in pot soils at 45 DAS

Treatments[d]	Rhizosphere[c]	Rhizoplane[c]
T_1	NCF	NCF
T_2	7.72 (± 0.02)	7.65 (± 0.03)
T_3	NCF	NCF
T_4	6.52 (± 0.03)	6.45 (± 0.06)

[a]Data are the means of three replications (± SEM, standard error of means). [b]Log number of CFU g^{-1} of rhizosphere soil. [c]Log number of CFU g^{-1} of fresh root. [d] Plants were exposed to T_1 – Water (control); T_2 – KUCd1; T_3– *P. nicotianae* (P10986); T_4 – KUCd1 colonized plants challenged with P10986; NCF = No colonies found

plants showed significantly higher POD activity compared to non bacterized (T_1 and T_3) plants during the 24 – 96 h post inoculation period (Fig.1D, E, F). POD activity increased rapidly in first 48 h following pathogen (P10986) inoculation in bacterized plants and continued further increase in the 96 h post inoculation period. The percentage increase in POD activity was highest in the leaves of T_4 plant showing 2.4 fold increases in POD activity compared to control (T_1) plants after 96 h. However, there was 44.8 % to 144.8 % increase in POD activity in the leaves of T_4 plants, 24.1 % to 46.4 % in T_2 plants and 34.5 % to 65.5 % in T_3 plants compared to control during 24 h - 96 h assay period (Fig.1F).

In roots, the T_4 plants demonstrated an increase in POD in the range of 105.6 % to 189.5 % while in T_3 it was in the range of 5.6 % to 73.7 % and in T_2 it was in the range of 33.3 % to 131.6 % (Fig. 1D). Statistical analysis revealed that initially the induction of POD in T_3 was not significant compared to T_1 (control) in the 24 h post inoculation period but later in the 48 – 96 h post inoculation period it was a significantly different from all other treatments.

In stem, there was no significant difference in the 24 h post inoculation period between the treatments T_1 and T_3. The increase in POD activity in T_4 plant was between 2.1 fold to 3.1 fold compared to control (T_1). The increase in POD activity was however in the range of 23.5 % to 123.5 % in T_2 plants and 5.9 % to 100 % in T_3 plants (Fig. 1E).

Polyphenol oxidase (PPO; EC 1. 14.18. 1) activity

Here too bacterized plants (T_2 and T_4) showed significantly higher levels of PPO compared to non bacterized plants (T_1 and T_3). However, the levels of PPO in the initial 24 h post inoculation period in the bacterized T_2 and T_4 plants were not significantly different among themselves (Fig. 2A, B, C). The leaves of T_4 plants demonstrated maximum increase in PPO activity during the entire study period of 24 - 96 h in the range of 36.1 % to 125 % while the recorded increase in T_3 plants was in the range of 11.1 % to 66.7 % and 27.8 % to 77.8 % in T_2 plants compared to (Fig. 2C).

In stem (Fig. 2B) during the 24 – 96 h post inoculation assay period the increase in PPO activity was in the range of 46.2 % to 90 % in T_4 plants, 2.6 % to 40 % in T_3 plants and 28.2 % to 57.5 % in T_2 plants. While in root (Fig. 2A) this increase in PPO activity showed least levels of induction compared to all other plant part and varied between 28 % to 66 % in T_4 plants, 6 % to 18.9 % in T_3 plants and 22 % to 45.3 % in T_2 plants.

Catalase (CAT; EC 1.11.1.6) activity

Catalase activity increased rapidly in the first 48 h following pathogen inoculation and gradually decreased in both bacterized (T_4) and non-bacterized (T_3) plants (Fig. 2D, E, F). The bacterized plants (T_4) upon challenge inoculation showed significant difference in catalase induction compared to non bacterized (T_1 and T_3) plants during the 24 – 96 h assay period. In roots (Fig. 2D), the T_4 plants demonstrated a maximum increase in catalase activity to 82 % (48 h) and then decline in the activity to 42.4 % (96 h) while in T_3 it showed maximum increase to 73.8 % (48 h) and then decline in activity to 36.7 % (96 h) compared to control. In T_2 plants the catalase activity showed gradual decline in activity from 21.7 % (24 h) to 13.6 % (96 h) compared to control.

Similar trend was observed in both stem (Fig. 2E) and leaves (Fig. 2F) of T_4 and T_3 plants where there was a significant increase in catalase activity over control reaching maximum at 48 h followed by a gradual decrease in activity. At 48 h, this increase in activity over control in the stem was 73.8 % in T_4 plants and 64.4 % in T_3 plants while in leaves it was 59 % in T_4 plants and 29.4 % in T_3 plants.

Root colonization bioassay

The data on root colonization bioassay (Table 3) indicates KUCd1 to be a good colonizer of rhizosphere and rhizoplane of pretreated brinjal plants both in the absence (T_2) or presence of pathogen P10986 (T_4). The lower population size of KUCd1 in T_4 plants compared to T_2 plants probably suggests for direct/localized competition between KUCd1and *Phytophthora nicotianae* P10986 for colonization/infection site. No bacterial colonies of KUCd1 could be isolated from the rhizosphere and rhizoplane of non- bacterized T_1 (without pathogen inoculation) and T_3 (pathogen inoculated) plants. .

The results of the present study clearly indicate that KUCd1 is an aggressive root colonizer. It improved plant growth in brinjal and also had a protective effect against *P. nicotianae* under *in vivo* controlled conditions. Additionally, it is a producer of HCN, siderophore; and showed resistance to heavy metal toxicity such as cadmium, nickel, etc. (Sinha and Mukherjee (2008). In order to be an effective biological control agent, efficient colonization of the rhizosphere is a pre-requisite for FLPs (Haas and Defago, 2005). Here KUCd1 fulfilled the criteria and the population remained above critical values in both rhizosphere and rhizoplane of brinjal plants. The relative population of KUCd1 in the P10986 challenge inoculated bacterized sets were lower than that in non-challenged bacterized sets suggesting greater competition in the former. Bacterization thus improved plant growth through better root colonization by KUCd1 and therefore can

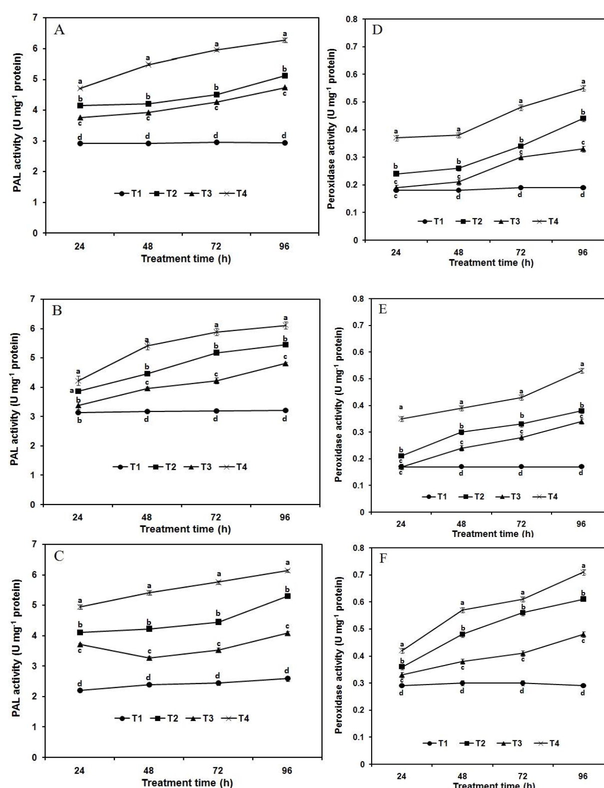

Fig. 1 : Effect of KUCd1 on induction of phenylalanine ammonia lyase (PAL) in root (A), stem (B), leaves (C); and peroxidase in root (D), stem (E), leaves (F) of brinjal plants during the experimental period of 24 h to 96 h post *Phytophthora nicotianae* P10986 inoculation. The four treatments are represented by T1-control (non-inoculated and non-bacterized), T2- KUCd1 treated, T3-P10986 treated and T4- KUCd1+ P10986 treated. Means followed by the same letter are not significantly different between treatments according to Duncan's multiple range test (DMRT) at $p \le 0.05$. Bars represent (±) SE of mean.

be easily correlated with growth promoting biocontrol properties of the test organism. These findings are in agreement with earlier reports where rhizosphere colonization with different strains of *P. aeruginosa* significantly improved plant health and promoted growth as evidenced by increases in seedling emergence, vigour, and yield (Khan, 2006; Adesemoye *et al,* 2008); reduction in disease severity, improved seedling stand, vigour and plant health in bacterized Chilli (Sharma *et al,*

2007a,b; Siddique and Meon, 2009) and tomato plants compared to untreated plants when grown in pathogen-infested soil (Sharma *et al,* 2007b). *P. aeruginosa* strains such as 7NSK2 (Buysens *et al,* 1996), PNA1 (Anjaiah *et al,* 2003), NJ-15 (Bano and Mussarat, 2003), PUPa3 (SunishKumar *et al,* 2005) and have been reported to have biocontrol activity against phytopathogens. In a recent report significant enhancement in plant growth abitily and biocontrol activity was observed in plants inoculated with FP6 isolate of *P. aeruginosa* (Bakthavatchalu *et al,* 2012). with *P. aeruginosa* strain RM-3 (Minaxi and Saxena, 2010) and with PGPR2 (Illakkiam *et al,* 2014).

Pre-treatment with KUCd1 also elicited resistance in brinjal plants by induction of host defense-related enzymes PAL, POD, PPO and catalase before inoculation with pathogen (P10986) which showed further increase in enzymatic activity post pathogen inoculation. The result obtained are in agreement with those of Siddique and Meon (2009)

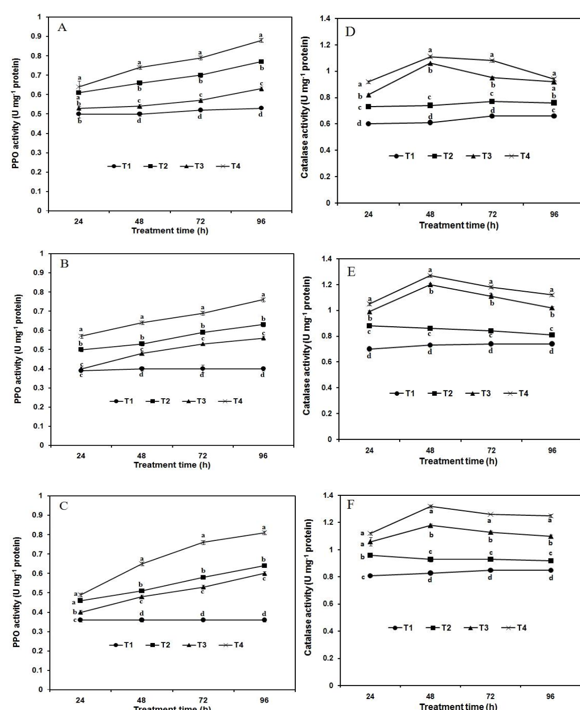

Fig. 2 : Effect of bacterial agent *P. aeruginosa* KUCd1 on induction of polyphenol oxidase (PPO) in root (A), stem (B), leaves (C); and catalase in root (D), stem (E), leaves (F) of brinjal plants during the experimental period of 24 h to 96 h post *Phytophthora nicotianae* P10986 inoculation. The four treatments are represented by T1-control (non-inoculated and non-bacterized), T2- KUCd1 treated, T3- P10986 treated and T4- KUCd1+ P10986 treated. Means followed by the same letter are not significantly different between treatments according to Duncan's multiple range test (DMRT) at $p \leq 0.05$. Bars represent (±) SE of mean.

who observed similar increase in activities of defense-related enzymes in chilli plants whose seeds were bacterized with *P.aeruginosa* UPMP3 and UPMB3.

The possible defense mechanisms includes physical (lignin) and chemical (quinones) barriers arising from the enhanced enzyme activities associated with ISR that fortifies plant cell wall strength while altering host physiology and metabolic responses. Induction of POD and PAL and accumulation of lignin and phenolic compounds to reinforce plant cell walls, have been correlated with disease resistance in a number of plant–pathogen interactions. PAL plays a key role in phenyl propanoid pathway, lignin being one of its major product. On the other hand PPO catalysed conversion of endogenous phenolic pool to quinones triggers a complex series of non-enzymatic secondary reactions that directly or indirectly affect the ability of plant to resist disease. Increases in PAL and its relation to resistance to pathogen infection in plant tissues or triggering of ISR pathways in bean roots have been demonstrated by earlier workers (De Meyer and Höfte, 1997; Van Wees *et al,* 1999). The high POD and PPO activities detected in treatments are linked to lignifications and generation of hydrogen peroxides that inhibit pathogens directly or generate other free radicals with antimicrobial effects. In the present study too, bacterial treatments resulted in significant increases in PAL, POD and PPO levels in brinjal plants suggesting a similar triggering of ISR and hence the resistance.

Catalase is a general stress enzyme. Our results show that the enzyme activity was high in KUCd1-pretreated brinjal plants challenged with P10986 while in plants inoculated with pathogen (P10986) alone it increased initially but then decreased subsequently. Similar findings were also reported in *P. fluorescens*-pre-treated cucumbers challenged with *Pseudoperenospora cubens* and *Erisiphe cichoracearum,* and *Brassica* species in the initial stages of infection by *Alternaria* which makedly dropped at later stages (Anand *et al,* 2007).

Therefore, this increase in peroxidase, polyphenol oxidase, catalase and phenylalanine ammonia lyase activities in tissues of brinjal plant can be interpreted as an inducible defense mechanism used for protection against pathogen invasion through lignin deposit at the plant cell wall and trigerring of ISR.

Microbial induced defense in plants may present differential level of efficiency in suppressing pathogen attack depending on the ability of the pathogen to avoid activated host defense. However, rhizobacteria isolate with ISR have increased chances of success in suppressing disease caused by a broad spectrum of pathogen than biological control agent with single biocontrol mechanism (Silva *et al,* 2004).

The success of KUCd1as a biocontrol agent may be attributed to the repertoire of arsenals that have been detected to have HCN and siderophore producing ability. Both HCN and high levels of siderophores (Sinha and Mukherjee, 2008) production by KUCd1 can play an important role in inhibition of phytopathogenic oomycetes by depriving them of this essential element since the siderophores from pathogen have lower affinity and the bacterial siderophore-iron complexes can be exploited by plants. Strains of *P. aeruginosa* has also been shown to induce systemic resistance, cause disease reduction and/or promote plant growth in bean, rice, tomato and chilli (De Meyer and Hofte, 1997; Audenaert *et al*, 2002; Buysens *et al*, 1996; Saikia *et al*, 1996; Sharma *et al*, 2007a,b).

HCN on the other hand inhibits the terminal cytochrome c oxidase in the respiratory chain and binds to metalloenzyme and has been reported to cause disease suppression due to its production (Ahmad *et al*, 2008, Hassanein, 2009; Lanteigne *et al*, 2012; Illakkiam *et al*, 2014). Also, the HCN producing *Pseudomonas* strain was found to show more pronounced beneficial effect on plant shoot dry mass than the non producers as reported by Goel *et al,* 2002. The antagonistic effect of *Pseudomonas* was thus thought to be due to the production of HCN and siderophore or synergistic interaction of these two or with other metabolites (Ahmad *et al*, 2008). KUCd1 being a producer of HCN might as well have used this trait to suppress plant disease in the various plant–pathogen interactions under study. Therefore, KUCd1 that happens to have many of these biocontrol traits was able to make use of one or more of these mechanisms to reduce the growth of the pathogenic *Phytophthora* sp. (Guha Roy, 2007) while inducing plant growth.

In conclusion, the present study demonstrated primarily the *P. nicotianae* antagonistic and plant growth promoting property under controlled *in vivo* conditions amongst the other multifunctional properties of *P. aeruginosa* strain KUCd1. The said bacterial strain had a broad antagonistic activity which helps in establishing and resisting against deleterious microorganisms occupying the microbial niche in the rhizosphere particularly the oomycetous plant-destroyer, *P. nicotianae*. Further siderophore production and antimicrobials released by the strain reflect their rhizospheric competitiveness that can be beneficially combined with plant protection and its PGPR traits to help enhance the plant growth. These together with resistance to cadmium toxicity and other heavy metal tolerance could prove to be an added advantage in able to survive in soil containing high concentration of these metal ions and still carry out its biocontrol and PGPR function while playing a significant role in bioremediation as well. However, field evaluation is necessary to determine its efficacy under natural ecosystem.

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