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RESEARCH ARTICLE

INCREMENT IN GROWTH PARAMETERS OF *ABRUS PRECATORIUS* BY PGPR DOES NOT CONFER INCREMENT IN ANTIMICROBIAL ACTIVITY

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ABSTRACT

Plant growth promotion using microorganisms is recently been studied extensively in order to compensate nutrient deficiencies and combat environmental hazards caused by chemicals. Apart from growth promotion microorganisms are also shown to affect the plant physiology, affecting tolerance to stress conditions, increase in active ingredients (colour, smell etc.,) etc. Pharmacological studies on various medicinal plants, including antibacterial, anti helmenthic, anticancer etc., are well documented. But the effect of microorganisms in increment of pharmacological effect in plant is studied at remote. In the present paper an attempt was made to study the impact of PGPR on increment of antimicrobial activities of different plant parts in *Abrus precatorious.* In general phytochemical properties of Abrus *precatorious* was found improved when treated with PGPR but antimicrobial activity was not significantly high compared to non treated plants.

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INTRODUCTION

Abrus precatorius (also called as gunja) is a popular medicinal plant of family-fabaceae. It is a slender, perennial climber, best known for its seeds, which are toxic because of the presence of abrin (Ross, 2003; Nadikarni, 1976; Warrierpk *et al*., 1993; Ranade, 1994). *Abrus precatorius* is an important plant in Ayurvedic preparations (Sudipta *et al*., 2012), which has many pharmacological affects/uses such as anti fertility (Zia-Ul-Haque *et al*., 1983), anti inflammatory (Anam, 2001) bronchodilatory (Kishor *et al*., 2012), antifungal (Dhawan *et al*., 1977) anti arithmetic, anti nociceptive, anti pyretic (Sudaroli and Chatterjee, 2007) anti allergic(Taur and Patil, 2011) anti oxidant, anti proliferative (Gul *et al*., 2013), anti cancer (Anbu *et al*., 2011), anti helimintic , analgesic(Sumeet gupta *et al.,* 2013), anti spasmodic. (Anant solanki and Maitreyi zaveri, 2012) anti viral, anti diabetic, anti chronicnephritis (Narendra and Bapodra, 2014) and anti spermatogenesis (Sarwat jahan *et al*., 2009). Different plant parts were reported to show good anti microbial activity against *E. coli*, *S. aureus*, *K. aerogenes*, and *S. typhimurium* (Adelowotan *et al*., 2008). Phytochemical and bio chemical investigations of *Abrus precatorious* i.e., presence of alkaloids, flavanoids, saponins, glycoside, tannins and triterpenoids is referred in many published works (Ghosal and Dutta, 1971). Recently, considerable information is generated where micro organisms called plant growth promoting rhizo-microorganisms (PGPR) were shown to improve plant health and growth by the virtue of PGPR traits (IAA, GA, HCN, Siderophore, β1, 3 glucanase, protease production etc.,). These PGPR were however studied at remote for effecting plant physiological aspects. In the present study an experiment was conducted to verify if plant growth promoting microorganisms (PGPR) were able to effect the anti microbial properties and other bio chemical properties in Abrus plant.

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MATERIALS AND METHODS

Bacterial cultures

One hundered and twelve unknown bacteria were isolated from rhizosphere soils pertaining to *Abrus precatorius* growing in Palamuru University and Wanaparthy of Mahabubnagar, India. Among them three bacterial cultures were identified as PGP rhizobacteria based on plant growth experiments conducted under standard controlled conditions using sterile soil as a substrate (temp 28 ± 2^0 C, 65% relative humidity). They were identified preliminarily as fluorescent Pseudomonas species (Ap-1) non fluorescent Pseudomonas species (Ap-2) and Bacillus species (Ap-3) depending on conventional cultural characters and biochemical reactions. Bacterial isolates used for antimicrobial activity namely *E.coli* (MTCC1089), *Salmonella typhimurium* (MTCC3224),

Klebsiella aerogenes (NCIM2282) and *Staphylococcus aureus* (MTCC737) were obtained from MTCC and NCIM, India.

Evaluation of PGPR traits

The selected three bacterial cultures Ap-1, Ap-2 and Ap-3 were qualitatively evaluated by standard methods for presence of PGPR traits (Praveen kumar *et al*., 2012). Detection of Indole Acetic Acid (IAA) Production of indole acetic acid in culture medium by bacterial isolate was detected by method given by Brick *et al*. (1991). Mineral salts media amended with 1% L-tryptophan was inoculated with bacterial culture and incubated. The culture filtrate (2ml) was mixed with Ortho Phosphoric acid (few drops) and Salkowski reagent (2ml). Presence of indole acetic acid is indicated by development of pink colour.

Identification of Gibberellic acid

Identification of Gibberllic acid produced by bacterial isolate was done following the method given by Holbrook *et al*., (1961). The presence of gibberellic acid produced was evaluated by reacting the culture supernatant with 30% HCl which was read at 254 nm using UV-Visible spectrophotometer (Elico, India), and compared with standard Gibberellic acid (sigma).

Sideophore detection and quantifications

Sideophore production was detected by presence of orange hallows around the bacterial colony on CAS agar plates (Schwyn and Neilands, 1987) after 72hr growth.

Hydrogen cyanide (HCN) detection:

Detection of HCN was carried out in a medium amended with L-Glycine following the method given by Bakker and Schippers (1987), change in colours of Whatmann No.1 filter paper (soaked in picric acid and Na_2CO_3) from yellow to reddish brown when exposed to gases liberated from culture medium was considered positive for HCN .

Detection of ammonia production

All the bacterial isolates were grown in peptone water medium. The tubes were inoculated with 100 micro liters of 24 hours grown culture and incubated at $28\underline{+}2$ ^{0}C for 72 hours. Ammonia accumulation in the media was detected by an addition of 0.5 ml of Nessler's reagent to each tube. Appearance of faint yellow to brownish colour indicates production of ammonia (Dye, 1962).

Detection of 'P' solubilization

All the test isolates were spot inoculated on to Pikovskaya's agar plates containing insoluble tri-calcium phosphate (Pikovskaya, 1948).They were incubated for 10days in room temperature ($28 \underline{+} 2^oC$) and observed for presence of clear zones around the colonies indicating positive for 'P' solubilization.

Detection of Chitinase and Protease

Test for the detection of chitinase was carried out as per the method of Hirano and Nagao (1988). Petri plates containing nutrient agar (sterile) amended with 0.1% colloidal chitin (for protease N.A+1% skimmed milk powder) were used for screening. Fresh cultures of bacterial isolates were spot inoculated on to media and incubated for 4 days (for chitinase) and 48hours (for protease), clear zone around the bacterial colony indicate chitinase or protease production.

Plant growth promotion studies

Abrus precatorious seeds were procured from Palamuru University campus, India. Seeds were initially subjected to physical treatment by rubbing on rough surface and later kept in concentration H_2SO_4 for half an hour in order to break the dormancy because of hard seed coat. Treated seeds were surface sterilized with 1% sodium hypoclorite for 5 min and washed with sterile distilled water for five to ten times. A short term plant experiment was conducted in plastic pots of 2 kg capacity filled with 1800 grams sterile soil (autoclaved for 3 days consecutively at 121 ^{0}C) according to the method given by Ali *et al*. (2009) the soil used for pot experiment contained (pH 7.4) 200.4, 20.0 and 190.46 kg / hector of available N, P, K respectively. Sterile seed of *Abrus precatorious* were bacterized with Ap-1, Ap-2, and Ap-3 bacterial strains at the rate of 2 X 10^6 cfu / gm employing talc-cmc as carrier material. An uninoculated control devoid of any bacteria was maintained. Each treatment comprised of 6 replicates with 10 seeds per pot, thinned down to 2 seed / pot after germination, experiment was continued for 120 days, later plants were carefully excavated analyzed for root-shoot lengths, dry mass, leaf area (measured by LI3100 Lincoln Nebraska USA leaf area meter) total chlorophyll (measured by Minolta spad chlorophyll meter 502 and expressed in spade units) and net photosynthetic rate in the morning using photosynthesis system (LI-COR 6400 Nebraska USA).

Plant material and crude (aqueous) extract preparation

Plant material of *Abrus precatorious* (120 days old) from plant growth experiment treated with Ap-1, Ap-2, Ap-3 and control were used for preparing different extracts. Root and Shoot parts (leaves & stem) obtained from different treatment were dried at ambient temperature (55-60^{0}C) for 5-9 days. The dried plant material were reduced to fine powder using an electric blender. The powdered material (150 gram) each was packed in Soxhlet apparatus and extracted exhaustively using methanol and petroleum ether. The solvents were evaporated in a hot air oven at 45^{0}C until the concentrated extracts of methanol and petroleum ether is obtained. Aqueous (crude) extracts 100 mg/ml of different plant materials were obtained by dissolving in distilled water. Insoluble extracts were solubilized by 10% solution of dimethylsulphoxide (DMSO) in water. Phyto chemical screening (in aqueous extracts) (Kapoor *et al*., 1969; Harbourn, 1984; Odebiyi and Sofowora, 1978; Kokate *et al*., 2012).

Saponins The Powdered plant material (300 mg) was boiled with 5 ml water for 2 min; the mixture was cooled and mixed

vigorously and left for 3 min. The formation of frothing indicates the presence of saponins.

Tannins

One ml of aqueous extract (300 mg ml^{-1}) was added to 2 ml of sodium chloride (2%), filtered and mixed with 5 ml 1% (w/v) gelatin solution. Precipitation indicates the presence of tannins.

Triterpenes

The powdered plant material (300 mg) was mixed with 5 ml chloroform and warmed at 80°C for 30 min, few drops of concentrated sulfuric acid was added and mixed well. The appearance of red color indicates the presence of triterpenes.

Alkaloids

Each plant sample (0.5g) was separately stirred with 1% hydrochloric acid (HCl) on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with two drops of Mayer's reagent. Turbidity in the filtrate on addition of Mayer's reagent was regarded as the evidence for the presence of alkaloids in the extract.

Flavonoids

Each plant sample (300mg) was boiled with 5ml water for 2 min. To this few drops of NaOH solution was added. Appearance of intense yellow colour which turns colourless up on addition of few drops dilute acid indicates presence of flavanoids.

Glycosides

Coarsely powered plant material (1g) was boiled with 1.0 ml of Sulfuric acid in a test tube. It was filtered while hot and then cooled; to this equi-volume chloroform was added. The chloroform layer of the mixture was separated and to it 10 ml of ammonia was added. The presence of reddish brown precipitate in the filtrate was taken as positive for glycosides

Antimicrobial Activity

Approximately 10ml of triptric soy agar (TSA) was poured in to sterile Petri plates to make TSA agar base plates. Exponential bacterial culture (24 hr activated) in broth were inoculated (0.2m) on to the plates and evenly spread with a sterile cotton bud. The paper discs for the anti microbial test were prepared by taking 1ml aliquots each of different plant extract in separate eppendorf tubes. Sterile paper disc (of whatman paper -5.00mm diameter) were dipped in the extracts for 1 hr and later dried in an oven at 45^0C in order to evaporate different solvents. Disc were properly placed on to the surface of inoculated TSA plate, properly labelled and incubated (at $36\underline{+}2^0$C for 48 hr). Presence of inhibition zones of at least 5mm diameter around the discs was taken as positive.

RESULTS

A total of 112 different isolates with varying colony morphologies were obtained from rhizospheres of wild *Abrus precatorius*. These isolates were further evaluated for their

Table 1. PGPR traits of three bacterial isolates from rhizosphere of *Abrus precatorius*

S.No	Isolate	IAA	Gibberellic Acid	Siderophore	HCN	Ammonia	Protease	β-1,3 glucanase	Chitinase	P' solubilization
1	Ap-1	+	--	+	+	+	+	--	--	+
2	Ap-2	+	+	--	--	+	+	--	--	--
3	Ap-3	--	--	--	--	+	--	+	+	+

+= positive; - = negative

Table 2. Effect of three PGP bacteria on plant parameters of *A. precatorius* after 120 days after sowing

S.No	Isolate	Root length (cm $Plant^{-1}$)	Shoot length (cm $Plant^{-1}$)	Dry Mass (mg $Plant^{-1}$)	Leaf Area (cm^2)	Total Chlorophyll (spad reading)	Photosynthetic activity (m mol^{-2} S^{-1})
1	Control	34.26^a	68.46	16.14	15.75^a	14	14.21^a
2	Ap-1	52.21	90.41	18.46^b	17.2^b	28^a	15.36^b
3	Ap-2	40.27^b	87.62^a	17.21^a	16.66^a	29^a	15.22^b
4	Ap-3	38.46^{ab}	86.12^a	17.42^{ab}	17.17^b	16	14.06^a

Values are the means of six replicates significant at $p < 0.01$ (Two way ANOVA)
Values super scribed by same alphabet in a column are not significantly different according to Fishers L.S.D test ($p < 0.05$)

Table 3. Phytochemical constituents in crude extract of *A.precatorius*

		Alkaloids		Flavanoids		Saponins		Glycosides		Tanins		Triterpenoids	
S.No	Isolate	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
1	Control	+	+	++	+	+	++	--	++	--	+	+	+
2	Ap-1	++	+	+	+	+	++	--	++	--	++	++	--
3	Ap-2	++	--	+	+	+	+	+	++	--	+	--	--
4	Ap-3	--	--	+	--	+	--	-	+	--	+	--	--

+= average, ++= Good, -= Absent

Table 4. Antimicrobial activity of different plant extacts of *A.precatorius* treated with PGPR

		E.coli						*S.typhimurium*						*K.aerogenes*						*S.aureus*					
		Root			Shoot			Root			Shoot			Root			Shoot			Root			Shoot		
S.No	Isolate	C	M	PE	C	M	PE	C	M	PE	C	M	PE	C	M	PE	C	M	PE	C	M	PE	C	M	PE
1	Control	16	ND	10	18	ND	ND	8	ND	ND	14	ND	16	4	ND	2	4	3	ND	10	16	13	8	ND	ND
2	Ap-1	15	6	8	14	ND	ND	6	ND	ND	12	ND	12	4	ND	ND	2	2	ND	8	12	13	6	ND	ND
3	Ap-2	16	ND	2	16	ND	ND	8	ND	ND	14	ND	15	3	ND	ND	3	3	ND	11	14	14	4	ND	ND
4	Ap-3	12	ND	5	12	ND	ND	6	ND	ND	13	ND	10	1	ND	1	NA	3	ND	10	12	8	1	ND	ND

ND=Not detected

ability to increase the plant growth under green house conditions. Among them three isolates (Ap-1, Ap-2, and Ap-3) were promising compared to rest of the isolates. These isolates were further compared to un inoculated control (Table 1) and it was observed that all the three isolates significantly improved ($p<0.05$) various plant growth parameters. Isolate Ap-1, showed increased root length (52.21 cm /plant) shoot length (90.41cm /plant) dry mass (80.46mg/plant), leaf area (17.2cm^2) significantly ($p<0.05$) compare to control, Ap-2 and Ap-3. However total chlorophyll (28) and photosynthetic activity (15.36 m mol^{-2} S^{-1}) were not significantly different. Increment in plant growth by the isolates Ap-1and Ap-3 comparatively was not significantly different in most of the plant growth parameters. However these were significantly higher compared to control ($p<0.05$). All the selected three isolates (Ap-1, Ap-2, and Ap-3) were further evaluated for different PGPR traits (Table 2). These three isolates varied in their ability to show positive for tested traits. All the three isolates were found positive for ammonia production. Siderophore and HCN production were observed only in isolate Ap-1. Similarly, 1, 3 glucanase and chitinase were observed in isolate Ap-3, gibberellic acid was observed only in Ap-2. Indole acetic acid and protease production were not shown by isolate Ap-3. These three isolates were further evaluated for their ability to increase different phyto chemical constituents in crude root and shoot extracts of *Abrus precatorius* (Table 3). In general it was observed that these increment was not much pronounced under the treatment of Ap-1, Ap-2, Ap-3 compared to control. The test for tannins in shoot, alkaloids and terpenoids in root gave strong reaction in Ap-1 treated plants. In Ap-2 treated plant, roots showed positive reaction for the presence of glycosides. However terpenoids in roots and shoot and alkaloids in shoot showed a negative reaction. Interestingly alkaloids and terpenoids in root and shoot and flavanoids in shoot were absent in Ap-3 treated plants. Anti microbial activity of root and shoot extracts (crude , methanol, pet ether) from *Abrus precatorious* treated with Ap-1, Ap-2, Ap-3 against four different bacteria (*E. coli, S .typhimurium, K. aerogenes and S. aureus*) is presented in Table 4. It can be observed that none of the PGPR isolates treated plant extracts showed increased antibacterial activity compared to control. The crude extracts were found inhibiting all the tested four bacterial species. But these were not high when compared to control. Some of the extracts showed no activity even though the crude extract showed minimum activity (Pet. ether extracts of roots against *K. aerogenes*). Interestingly methanol extracts of root treated with Ap-1, showed inhibition against *E.coli*.

DISCUSSION

Plant growth promotion by microorganisms is a recent area for exploration where several reports are available depicting increment in plant growth parameters of various plant species. Mere presence of more number of PGPR traits does not confer an isolate as efficient PGPR. Isolates with few PGPR traits were also observed to efficient PGPR in earlier reports. However very few reports are available showing PGPR induced change in plant physiological and phytochemical characters. Ali *et al*. (2009) reported PGPR induced secretion of special proteins in plants (hsps) subjected to heat and drought stress. Venkateswarlu *et al*. (2008) and Camprubi *et al*. (2013) reported increased azardirachtin and essential oil content in neem tress and rosemary plants treated with mycorrhiza respectively. In the present study, in contrast to above no significant increase in antibacterial activity was observed in plants treated with PGPR isolates. However, little improvement was observed in phytochemical quantities (in terms of reaction vigor). Usage of pet ether and methanol extracts may have missed some of the important bioactive ingredients of crude extracts. Further investigations including various other solvents and isolation of bioactive substance are yet to be studied.

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