Development of Nitrogen Fixing Bioinoculants Resistant to Inhibitors in Castor

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Abstract

In this investigation, effect of nitrogen fixing bioinoculants resistant to inhibitors in castor was studied on castor crop. Twenty-nine free living diazotrophic bacteria were isolated from different rhizospheric soils of castor crop. These isolates were tested under in vitro conditions for the ability to tolerate different concentrations of inhibitors present in castor seeds and characterized for indole acetic acid (IAA) production, ammonia excretion, nitrate reductase (NR) activity and siderophore production. Isolate CR5 and CR10 showed highest resistance to inhibitors. Maximum IAA production (13.99 μg ml^{-1}) and ammonia excretion (6.38 μg ml^{-1}) were shown by isolate CR5. Highest NR activity was detected in isolate CR5 (150.92 μg nitrite ml^{-1}) followed by isolate CR10 (128.01 μg nitrite ml^{-1}). Twenty isolates could produce siderophores.

Based on above characters two isolates (CR5 and CR10) were selected for further studies on castor crop under pot house conditions. Organic carbon remained constant while total nitrogen (N) and total phosphorous (P) contents of the soil enhanced considerably. Maximum plant height (48.8 cms), shoot fresh and dry weight (17.0 and 5.1 g respectively) and root fresh and dry weight (6.0 and 1.9 g respectively) were obtained with inoculation of isolate CR5 along with recommended dose of fertilizer (RDF). Highest survival count was also obtained in the treatment having isolate CR5 along with RDF.

Keywords: Inhibitors, Ammonia, IAA, Siderophore, Growth parameters, Soil properties.

Introduction

Castor *(Ricinus communis* L) is a significant non consumable oilseed crop and developed far and wide because its commercial significance. Its oil is utilized for developing surfactants, oils, fungistats, coatings, beautifying agents, pharmaceuticals and numerous different items. Castor bean has been broadly acknowledged as a horticultural answer for all subtropical and tropical areas that address the requirement for crops with low input costs and simultaneously give customary cultivating a feasible income from current non beneficial terrains.[12] It is a simple crop that does not require much attention during its growth. It can be used as a bioenergy crop in marginal or degraded lands and helps in improvement of soil properties by preventing nutrient leaching and soil erosion.

The green revolution had increased agricultural production on a large scale in India, but sustainability is still a concern. The use of chemical fertilizers will not only result in further loss of soil well-being but also potential outcomes of water pollution and determined weight on the financial framework.

The high production cost and environmental pollution brought about by the application of these fertilizers make it important to use other forms of fertilizers especially biofertilizers. Biofertilizers are good alternative for the productivity as well as sustainability of the food chain on global level. Microbial inoculants or biofertilizers play important role in providing nourishment to the crops through required nutrients and many other mechanisms.[30]

The free living diazotrophic bacteria present in rhizosphere like *Azotobacter, Bacillus, Clostridium, Beijerinckia* and *Klebsiella* help in promotion of plant growth mainly by nitrogen fixation.[42] They can also be involved in production of growth hormones and vitamins, solubilization of nutrients and plant protection, which are due to secretion of antibiotic compounds or competition for resources.[50] Additionally, these microorganisms may induce systemic resistance and protect plants against the pathogens.[37]

Application of biofertilizers to castor can improve plant growth and yield by improving soil fertility. These microorganisms increase the availability of different nutrients for the plants and save nitrogen (N) fertilizers.[2,14] Moreover, significant improvement in growth and yield of castor was reported when biofertilizers were used in combination with organic fertilizers.[35] Similarly, Kumar and Kanjana[23] concluded that the application of specific bacterial strains can enhance nutrients availability by accelerating the mineralization processes of organic matter in soil which in turn encourages the vegetative growth and yields of castor.

Castor is a crop with great utility in different industries as well as agricultural sectors as it is a source of many useful compounds in addition to oil. However, the presence of toxic components in different parts of the plant presents a major limitation in its use in fields and factories.[24] The castor bean contains many compounds that are poisonous to human beings, animals, plants[40] and microorganisms.[17,29] The major toxic protein ricin has been used as a biological weapon as a single milligram of it can kill an adult human.

It is mainly found in seeds, however, in lower concentrations, it is present throughout the plant. In addition to it castor bean also synthesizes several secondary metabolites including steroids, saponins, alkaloids, flavonoids, tannins, phenols, phytates, oxalates and glycosides in different parts of the plant which inhibits microbial growth.[18]

Different leaf extracts of castor showed significant antibacterial activity against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (NCTC 6571).[19] The seeds contain glycosides of stearic, dihydroxystearic, ricinoleic and isoricinoleic acids, lipases and ricinine. These inhibitors are responsible for antimicrobial activity of castor seed extracts against bacteria and fungi.[31,38] Some of these inhibitors are quite stable in soil and affect soil microbial community which in turn affect soil health and fertility. It was found that fungal and bacterial populations declined in soils cultivated with castor.[52] However, there are certain microorganisms which can withstand castor inhibitors and effectively degrade them.[48,52] This raises the possibility of developing biofertilizers for castor crop which can survive at high concentrations of its inhibitors.

Till now, there are no reports on biofertilizers of castor which are resistant to its inhibitors. Since castor is an important commercial crop, there is a strong need to develop biofertilizers which can withstand its inhibitors and may also degrade them so that the soil health can be retained in the castor grown fields and plant growth can be promoted more efficiently. Therefore, the present study aims at isolation, characterization and application of nitrogen fixing bioinoculants resistant to inhibitors, as biofertilizer for castor crop.

Material and Methods

Soil sample collection, isolation and characterization of bacterial isolates: The soil samples were collected from the rhizosphere of castor crop from different districts (such as Mahendergarh, Rewari and Hisar) of Haryana state (India). Soil was used for isolation of free living diazotrophs by enrichment culture technique using Jensen's nitrogen free medium (JM). Soil isolates were identified based on morphological characteristics. The various isolates were tested for their growth on JM plates containing different concentrations of inhibitors extracted from seeds[24] viz. 1, 3, 5 and 7%. Isolates were characterized for IAA production[44], ammonia excretion[8], NR activity[26] and siderophore production.[43] Two bacterial isolates (isolate CR5 and CR10) were selected based on above characteristics for further studies under pot house conditions.

Experimental site and investigation design: The study was conducted at CCS HAU, Hisar (Haryana) under pot house conditions. Basic physico-chemical properties of experimental soil are detailed in table 1. *Azotobacter chroococcum* Mac-27 is a commercial inoculant used as reference strain. Twelve treatments were established using completely randomized design in 36 pots: T1 (no chemical fertilizer), T2 (RDF), T3 (75% RDF), T4 (isolate CR5), T5 (RDF + isolate CR5), T6 (75% RDF + isolate CR5), T7 (isolate CR10), T8 (RDF + isolate CR10), T9 (75% RDF + isolate CR10), T10 (*Azotobacter chroococcum* Mac-27), T11 (RDF + *Azotobacter chroococcum* Mac-27) and T12 (75% RDF + *Azotobacter chroococcum* Mac-27). Each treatment had three replications.

Recommended dose of fertilizer for castor crop is $N_{80}P_{60}K_{30}S_{30}$ (Kg ha^{-1}) as per package and practices of CCS HAU, Hisar. Recommended doses of nitrogen in the form of urea, phosphorus in the form of sodium dihydrogen phosphate (NaH_2PO_4), potassium in the form of potassium chloride (KCl) and sulfur in the form of magnesium sulfate ($MgSO_4.7H_20$) were applied as per the treatments.

Table 1
Physicochemical properties of the soil at the start of experiment

Parameter	Value / Type
Texture	Sandy loam
Electrical Conductivity	0.22 (dS/m)
pH	7.12
Organic Carbon	0.34 (%)
Total N	380 (Kg ha^{-1})
Total P	234 (Kg ha^{-1})
Diazotrophic count	5.85 log no. cfu/g soil)

Inoculation of castor seeds with bioinoculants: The seeds of castor (DCH 177) were surface sterilized with 0.1% $HgCl_2$ for 3 minutes followed by 5-6 successive washings with sterile distilled water. These seeds were first treated with fully grown (10^8 ml^{-1}) selected bacterial isolates (CR5 and CR10) and *A. chroococcum* Mac-27 and sown in pots containing 5 kg unsterilized soil according to the treatments. Five seeds were sown initially and one plant per pot was maintained after germination.

Determination of microbial count: Rhizospheric soil samples collected from castor grown pots were used for microbiological analysis and diazotrophic bacterial count was determined at different stages of plant growth i.e. 15, 30, 45, 60, 75 and 90 days by dilution plate count method. The soil was serially diluted and hundred µl of each sample from various dilutions (10^{-3}, 10^{-4} and 10^{-5}) were spread over Jensen's nitrogen free medium plates. The plates were incubated for 3-5 days at 28±2ºC and colonies appeared were counted. The counts were calculated on per g soil basis using formula:

No. of cfu (colony forming units) x dilution factor/ volume taken (ml)

Analysis of soil chemical properties and plant growth parameters: Organic carbon, total nitrogen (N) and phosphorus (P) in soil were determined before sowing and

at 90 days after sowing (DAS) by the methods of Kalembassa and Jenkinson[21], Kjeldahl's method[7] and John[20] respectively. Plants were uprooted at 90 DAS and observations on plant height, shoot weight (fresh and dry) and root weight (fresh and dry) were recorded. Root and shoot dry weights were recorded after drying the samples in an oven at 65°C till constant weight was observed.

Table 2
Colony morphology of various bacterial isolates

No. of Bacterial isolates		Colony Characteristics	Gram Reaction
9	(CR28, CR1, CR4, CR11, CR14, CR15, CR23, CR26, CR29)	Large, watery, round, raised	-
7	(CR5, CR7, CR10, CR6, CR16, CR24, CR27)	Large, round, opaque, raised	-
7	(CR20, CR22, CR3, CR12, CR17, CR21, CR25)	Medium, round, watery	-
6	(CR18, CR2, CR8, CR9, CR13, CR19)	Small, round, raised, opaque	-

Table 3
Growth of bacterial isolates on Jensen's nitrogen free medium supplemented with inhibitors in castor

Isolate no.	Percentage Inhibitors			
	1	**3**	**5**	**7**
CR1	++	±	-	-
CR2	++	+	-	-
CR3	+	±	-	-
CR4	++	+	±	-
CR5	+++	+++	+++	++
CR6	-	-	-	-
CR7	+++	+++	++	±
CR8	+	-	-	-
CR9	++	+	-	-
CR10	+++	+++	+++	++
CR11	++	+	-	-
CR12	++	+	-	-
CR13	+	±	-	-
CR14	++	++	±	-
CR15	++	+	-	-
CR16	-	-	-	-
CR17	++	++	±	-
CR18	+++	++	++	+
CR19	+	-	-	-
CR20	+++	++	++	±
CR21	++	+	-	-
CR22	+++	++	++	+
CR23	+	±	-	-
CR24	+	-	-	-
CR25	++	+	-	-
CR26	++	++	±	-
CR27	-	-	-	-
CR28	+++	++	++	±
CR29	-	-	-	-

+++ = Excellent ++ = Very good + = Good ± = Poor – = Negative

Results

Isolation, morphological characterization and screening of bacterial isolates for resistance to inhibitors in castor: A total of 29 bacterial isolates were obtained from rhizospheric soils of castor from different locations. Ability to grow on Jensen's nitrogen-free medium is accepted as preliminary criterion for the isolation of potential free-living nitrogen fixers from soil. Preliminary description of bacterial isolates was done based on morphological characters viz. size, shape, gram staining and pigmentation on JM plates. Colonial variation was observed among 29 isolates as shown in table 2. All isolates were found to be gram negative.

All the bacterial isolates were tested for resistance to inhibitors in castor by incorporating different concentrations of inhibitors viz. 1, 3, 5, 7% in JM plates. A decrease in growth of the isolates was observed with increase in concentration of inhibitors. Maximum growth was observed at 1% concentration of inhibitors. At 7% concentration, isolate CR5 and CR10 showed excellent growth followed by isolate CR18 and CR22 (Table 3). However, isolates CR6, CR16, CR27 and CR29 were unable to grow at any concentration of inhibitors.

Plant growth promoting traits: All soil isolates were found to produce IAA. Maximum IAA production was observed by isolate CR5 (13.99 µg ml^{-1}) (Table 4). Among all the isolates, only 17 were capable of excreting ammonia under shaking conditions. Isolate CR5 was found to be the highest ammonia excretor (6.38 µg ml^{-1}) (Table 4). Nitrate reductase activity was shown by 23 isolates. Highest activity was shown by CR5 (150.92 µg nitrite ml^{-1}) followed by CR10 (128.01 µg nitrite ml^{-1}) (Table 4). Out of 29 soil isolates, 20 were found to be capable of producing siderophores (Table 4).

Table 4
Characterization of bacterial isolates for plant growth promoting traits

Isolate no.	Ammonia excretion (ug ml^{-1})	IAA production (ug ml^{-1})	NR activity (µg nitrite ml^{-1})	Siderophore Production
CR1	1.19	2.86	121.23	+
CR2	1.57	1.95	103.34	-
CR3	-	2.50	76.98	+
CR4	-	7.80	-	+
CR5	6.38	13.99	150.92	+
CR6	1.03	2.68	45.55	+
CR7	1.19	2.23	126.19	+
CR8	-	2.80	-	-
CR9	3.50	8.41	64.78	+
CR10	1.29	4.94	128.01	+
CR11	-	12.00	-	+
CR12	3.18	6.42	34.12	+
CR13	1.12	7.80	-	-
CR14	-	2.26	44.56	-
CR15	-	10.43	-	+
CR16	3.23	3.85	109.43	+
CR17	4.82	3.70	111.34	+
CR18	-	1.95	55.56	+
CR19	-	11.30	50.30	+
CR20	-	3.31	80.75	+
CR21	1.02	9.95	112.57	-
CR22	-	3.46	122.00	+
CR23	3.60	4.52	-	-
CR24	1.04	8.62	86.44	+
CR25	2.25	7.47	65.89	+
CR26	-	7.89	23.56	-
CR27	1.57	3.28	54.77	-
CR28	-	4.16	49.69	-
CR29	4.77	5.27	124.34	+

+ = Detected - = Not Detected

To see the full effect as biofertilizers on castor crop, two bacterial isolates (CR5 and CR10) were selected for pot house studies based on resistance to inhibitors in castor and plant growth promoting traits.

Survival and establishment of inoculated bacterial isolates: Studies on survival count of inoculated isolates i.e. CR5, CR10 and *Azotobacter chroococcum* Mac-27 showed significant difference in all the treatments. The viable count in all the treatments increased up to 60 DAS except the treatments having isolate CR5, in which increase in count was observed up to 75 DAS. Maximum count was observed in the rhizosphere of plants inoculated with isolate CR5 along with RDF i.e. treatment T5 (6.74 log no. cfu/g soil) followed by isolate CR5 along with 75% RDF i.e. treatment T6 (6.67 log no. cfu/g soil) at 75 DAS. However, a decrease in bacterial count was observed at 90 DAS in all the fertilization treatments (Table 5).

Effect of bacterial isolates on soil chemical properties and plant growth parameters: Organic carbon, total nitrogen and total phosphorus were determined in soil before sowing and at 90 DAS. No significant difference was observed in different treatments with respect to organic carbon. It was 0.34% in all treatments except the treatments having isolate CR5 along with RDF and *A. chroococcum* Mac-27 along with RDF (0.35%) at 90 DAS. Total N and total P contents of soil were found to increase in the treatments having chemical fertilizers at 90 DAS.

Maximum total N was observed in the treatment having isolate CR5 along with RDF and isolate CR10 along with RDF (385 Kg ha^{-1}). Highest total P was observed in the treatment having *A. chroococcum* Mac-27 along with RDF (252 Kg ha^{-1}) (Table 6).

Both isolates considerably increased plant height, root, shoot fresh and dry weight as compared to their respective controls and reference strain *Azotobacter chroococcum* Mac-27. However, isolate CR5 was found to promote plant growth more efficiently.

Table 5
Diazotrophic viable count in rhizosphere of castor under pot house conditions (log no. cfu/g soil)

Treatments	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
T1	5.90	6.04	6.08	6.15	6.08	6.00
T2	6.04	6.18	6.23	6.28	6.26	6.20
T3	5.95	6.08	6.11	6.18	6.11	6.08
T4	6.11	6.26	6.32	6.43	6.43	6.41
T5	6.28	6.46	6.60	6.72	6.74	6.73
T6	6.23	6.41	6.57	6.66	6.67	6.65
T7	6.04	6.20	6.28	6.36	6.34	6.32
T8	6.20	6.40	6.54	6.64	6.64	6.63
T9	6.15	6.32	6.43	6.51	6.51	6.49
T10	6.00	6.11	6.20	6.26	6.23	6.18
T11	6.11	6.28	6.34	6.38	6.36	6.34
T12	6.08	6.23	6.32	6.34	6.32	6.28
Critical difference at 5%	0.10	0.09	0.05	0.05	0.06	0.06

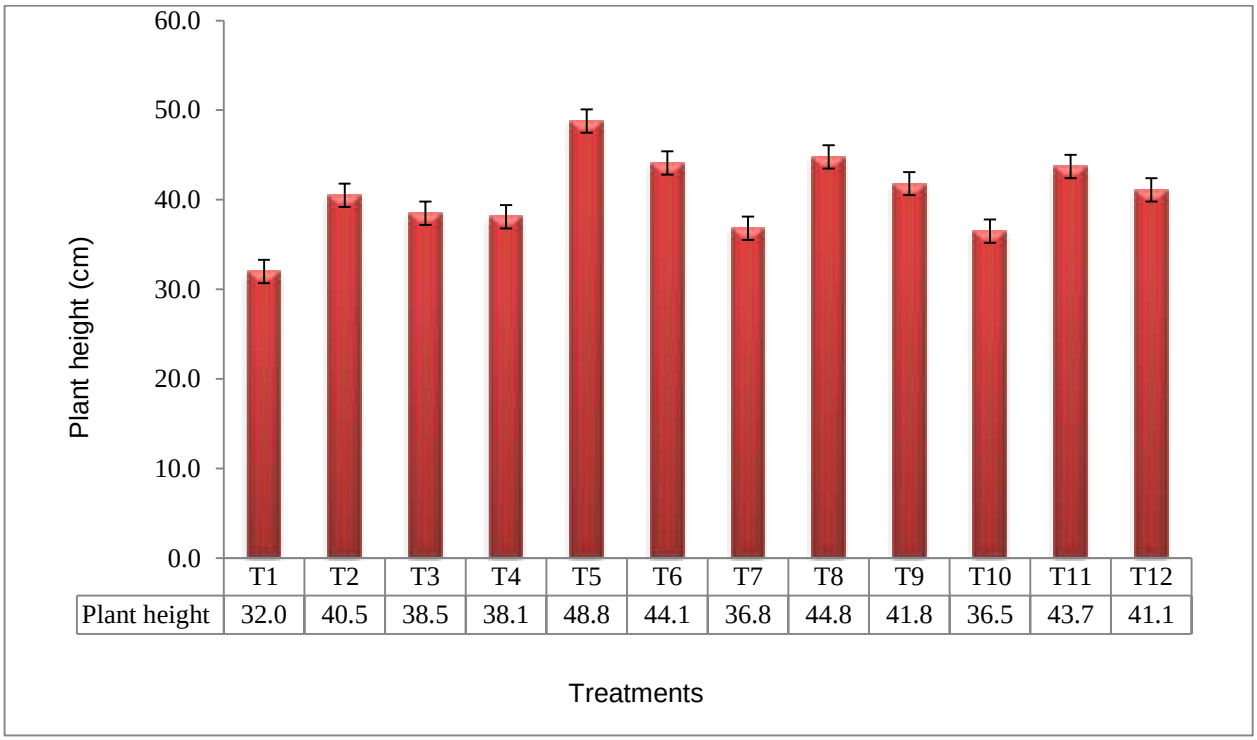
Table 6
Effect of different treatments on organic carbon, total N and total P under pot house conditions at 90 DAS

Treatments	Organic Carbon (%)	Total N (Kg ha^{-1})	Total P (Kg ha^{-1})
T1	0.34	377	232
T2	0.34	384	250
T3	0.34	382	244
T4	0.34	378	233
T5	0.35	385	251
T6	0.34	383	246
T7	0.34	377	232
T8	0.34	385	251
T9	0.34	382	244
T10	0.34	378	233
T11	0.35	384	252
T12	0.34	383	245
Mean	0.34	381	242
CD at 5%	N.S.	2.72	3.01

Maximum plant height was observed with inoculation of isolate CR5 along with RDF i.e. treatment T5 (48.8 cm) (Fig. 1). Highest shoot weight (fresh and dry) was observed with inoculation of isolate CR5 along with RDF i.e. treatment T5 (17.0 g and 5.1 g respectively) (Fig. 2). Highest root weight (fresh and dry) was observed with inoculation of isolate CR5 along with RDF i.e. treatment T5 which was 6.0 g and 1.9 g respectively (Fig. 3).

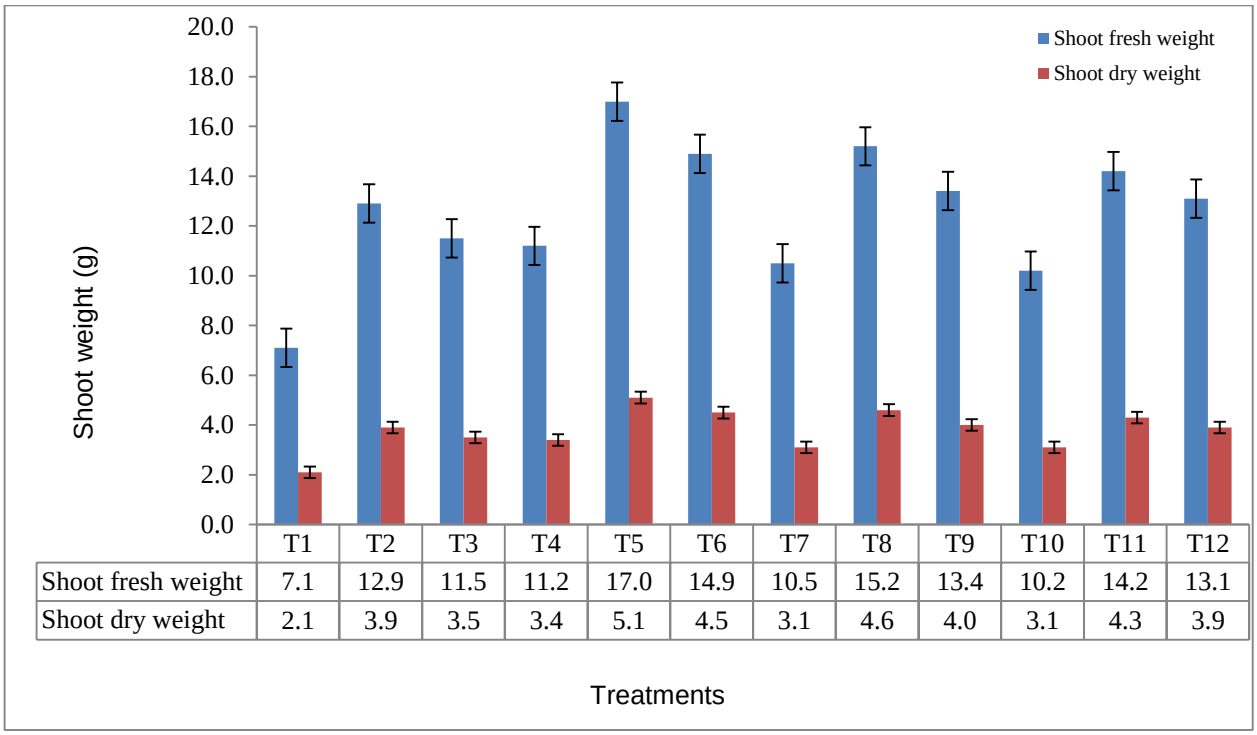
Discussion

Castor, a plant of family *Euphorbiaceae* (Cooke 1908), is grown worldwide because of its medicinal value and oil for industrial purposes. Castor contains several compounds like ricin, ricinine, *Ricinus communis* agglutinin and synthesizes many secondary metabolites in different parts of the plant which show antimicrobial effect against soil microorganisms which, in turn, affect soil health and fertility.[18]



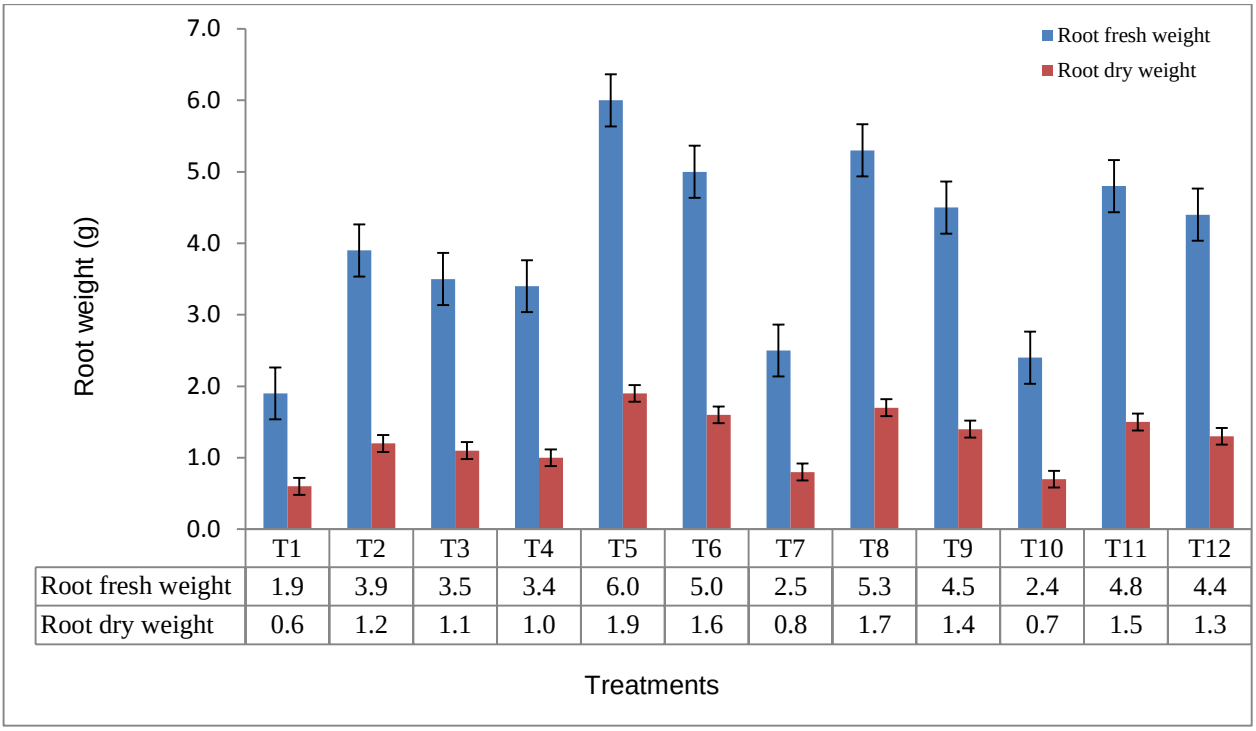
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Plant height	32.0	40.5	38.5	38.1	48.8	44.1	36.8	44.8	41.8	36.5	43.7	41.1

Fig. 1: Effect of bacterial inoculants on plant height of castor bean at 90 DAS



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Shoot fresh weight	7.1	12.9	11.5	11.2	17.0	14.9	10.5	15.2	13.4	10.2	14.2	13.1
Shoot dry weight	2.1	3.9	3.5	3.4	5.1	4.5	3.1	4.6	4.0	3.1	4.3	3.9

Fig. 2: Effect of bacterial inoculants on shoot fresh and dry weight of castor bean at 90 DAS



	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Root fresh weight	1.9	3.9	3.5	3.4	6.0	5.0	2.5	5.3	4.5	2.4	4.8	4.4
Root dry weight	0.6	1.2	1.1	1.0	1.9	1.6	0.8	1.7	1.4	0.7	1.5	1.3

Fig. 3: Effect of bacterial inoculants on root fresh and dry weight of castor bean at 90 DAS

Biofertilizers which can withstand and may also degrade these inhibitors will not only help in maintaining soil fertility but will also promote plant growth. In the present study, attempts are made to isolate and characterize different bacterial isolates from castor rhizosphere resistant to its inhibitors and evaluate them for growth promotion of castor.

Out of 29 soil isolates, 25 were found to survive at 1% concentration of inhibitors and their number decreased with increase in concentration of inhibitors. This is because a number of toxic compounds are present in the seeds of castor which show significant antibacterial activity against many bacterial species.[1,16,22] Saponins and phenolics (present in roots and seeds of castor) showed significant antibacterial activity against *Klebsiella halize* and *Staphylococcus aureus* at increasing phytochemical concentrations.[15]

Isolate CR5 and CR10 showed excellent growth at 7% concentration of inhibitors. It shows high resistance of these isolates to inhibitors. Antimicrobial activity of methanolic extracts of castor seeds against some bacteria (*Staphylococcus aureus* ATCC15156, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and yeast (*Candida albicans*) was studied. All test organisms were inhibited by seed extracts but minimum inhibitory concentration (MIC) was different for different organisms (0.723 – 9 µg ml^{-1}).[39]

In the present investigation, all twenty-nine isolates produced IAA ranging from 1.95-13.99 µg ml^{-1}. IAA production is one of the important properties of rhizospheric bacteria contributing to plant growth.[47] There are many studies on increment of IAA production by bacteria when grown in tryptophan containing media.[41,51] Various researchers have reported that IAA production varies with species, strains and growth conditions.[32,36,46] Ammonia excretion was observed in seventeen isolates out of twenty-nine ranging from 1.02-6.38 µg ml^{-1}. Ammonia is the immediate product of nitrogen fixation in nitrogen fixing micro-organisms. It is directly taken up by the plants and used in various metabolic functions. Many diazotrophic bacteria capable of secreting ammonia enhance plant growth.[10]

In this study, twenty isolates out of twenty-nine could produce siderophores. Siderophores are iron chelating low molecular weight compounds produced by many bacteria i.e. *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Serratia*, *Bacillus*, *Enterobacter*, *Rhizobium* and *Klebsiella*[13,25] in large amount when conditions are iron limiting.[34] In a similar way, Tank et al[45] observed that *Pseudomonas sp.* isolated from the rhizosphere of chickpea plants produced siderophores in large amount.

Nitrate reductase activity of all bacterial isolates was checked. Twenty-three isolates out of twenty-nine showed nitrate reductase activity ranging from 23.56 – 150.92 µg nitrite ml^{-1} (Table 7). Nitrate reduction can be assimilatory or dissimilatory and both processes are catalyzed by the microorganisms. In this phenomenon, nitrate is converted into ammonia and some other products by microorganisms.[10] The first step in this system is reduction of nitrate to nitrite and is catalyzed by nitrate reductase enzyme. Furina et al[11] found that *A. chroococcum* C8 and *A. indicum* 8 reduced nitrate to nitrite under anaerobic conditions and produced 32.5 and 767.5 mg N per mg dry weight respectively.

In this study, diazotrophic viable count in rhizosphere was determined in all the treatments at an interval of 15 days till 90 DAS. An increase in diazotrophic count was observed in all the treatments up to 60 DAS and later on a decrease in count was observed ranging from 6.00-6.73 log no. cfu/g soil at 90 DAS. It seems that under the positive influence of the root exudates, population of rhizobacteria might be increasing.[33] With the growth of the plant, however, the energy of the plant is directed towards the seed and fruit formation and there is decrease in root exudation.[27,28]

These changes perhaps may lead to a consequent decrease in the diazotrophic population during later stages of plant growth as observed in this study. Moreover, concentration of inhibitors of castor might increase in soil with time but microbes can tolerate these inhibitors only up to a certain limit, so this may also be the reason for decrease in population of diazotrophs in later stages of plant growth.

Present work is supported by Venkateswarlu et al[49] who reported decrease in populations of native rhizobia (*Bradyrhizobium* sp.) in 88 soil samples from 13 different legume growing fields when castor was cultivated in preceding year. Basinger et al[3] also observed negative effect of ricin on microbial activity. Highest count was observed in the rhizosphere of plants treated with isolate CR5 followed by isolate CR10 and in the treatments having isolate CR5, increase in count was observed up to 75 DAS. This might be due to resistance of these strains to inhibitors of castor present in soil. Similarly, *Actinomycetes* concentrations as high as 30,000/g of soil have been identified in castor field soils. Two bacterial genera, *Pseudomonas* and *Erwinia* were found to effectively degrade the toxic protein ricin in *in vitro* assays.[52]

Soil organic carbon is the most important component of the natural carbon cycle as it defines soil health and fertility. It provides nutrients for plants, improves texture, biological and physical properties of the soil and minimizes effect of toxic substances. In present investigation, under pot house conditions, organic carbon remained constant (0.34%) in most of the treatments at 90 DAS. The percentage of soil organic carbon can vary largely depending upon temperature, rainfall, soil nutrition and soil type, farming methods, microbial activity and climate change.[4]

In this study, total N and P increased in the treatments having chemical fertilizers ranging from 377-385 Kg ha^{-1} and 232-252 Kg ha^{-1} respectively at 90 DAS. Nitrogen and phosphorus are two major essential macronutrients required for growth and development of plants, therefore, they are commonly added as fertilizers to enhance crop yield. To achieve maximum crop production, nitrogenous and soluble phosphatic fertilizers are being used to such extent which is problematic for both environment as well as economy.[6]

Thus, the use of plant growth promoting rhizobacteria including N_2-fixing and phosphate solubilizing bacteria as biofertilizers is increasing day by day in India because of deficiency of soil N and P in a large part of cultivated soils.[5]

There was notable increase in all plant growth parameters including plant height, root and shoot weight (fresh and dry) at 90 days in all the treatments. Plant height ranged from 32.0-48.8 cm, fresh shoot weight from 7.1-17.0 g and dry shoot weight varied from 2.1-5.1g at 90 DAS. Fresh root weight ranged from 1.9-6.0 g and dry root weight varied from 0.6-1.9 g at 90 DAS. Maximum increase in plant parameters was observed with inoculation of isolate CR5 followed by isolate CR10. Bacterial isolate CR5 and CR10 established well and helped in overall functioning of plant machinery. This may result in the better growth of castor plants treated with selected isolates.

Application of *Azospirillum* sp. as reported by Hussein et al[14] to castor increased the plant height, seed yield, oil yield and the contents of crude protein, crude fiber and ash. Similarly, Aruna et al[2] observed that treatment with AM fungi increased the shoot and root length, total plant length, fresh and dry weight, number of leaves per plant and leaf area in castor bean.

Conclusion

This study demonstrated that nitrogen fixing bacteria isolated from rhizosphere of castor are not only resistant to its inhibitors but also promote plant growth by providing many plant growth promoting substances. Our results suggest that use of efficient strains from castor rhizosphere as biofertilizers for castor can be a good solution for dealing with problem of its inhibitors and maintaining soil fertility.

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