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Research Article

EFFECT OF FUNGICIDE ON THE PLANT GROWTH PROMOTING TRAITS OF *RHIZOBIUM*, ISOLATED FROM GROUNDNUT FIELD

D. John Milton*., Chandrasekaran Swaminathan ., Rajendiran Krishnan and Jayabalan Jayaprakash

Department of Microbiology, St. Joseph's College of Arts and Science (Autonomous), Cuddalore 607 001, Tamil Nadu, India

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ABSTRACT

Emergence of resistance among the beneficial microbes against agro-chemicals is a key factor which enables the microbe to function in the presence of toxic agrochemicals like pesticide, fungicide, etc. In the present study, four *Rhizobium* isolates were isolated from groundnut field. The resistant profile of the isolates against the fungicide carbendazim were tested and the maximum tolerance up to 11,000 ppm were associated with all the four isolates. All the four isolates were also positive for the production of IAA, ammonia and HCN both in the presence and absence of carbendazim. In the present study, no isolates were able to solubilize P, and Zn.

INTRODUCTION

Groundnut is also called as peanut, an important legume crop cultivated around 100 countries. It is used for oil, food, and feed for animals. In India about 7.5 million hectares are used for the cultivation of groundnut and the oil extracted from groundnut is the major oil consumed for cooking. Foliar fungal diseases may pose serious threats to food security. Management of fungal disease involves both chemical and physical approaches including the manipulation of fugal resistance and use of synthetic fungicides. A fungicide is a specific type of pesticide that control fungal diseases by specifically inhibition or killing the pathogenic fungi.

Ground nut is being severely affected by many fungal diseases includes Tikka leaf spot, early leaf spot, late leaf spot, rust, root rot, *etc*. To control the fungal diseases, the farmers are started to apply fungicides specifically to control fungi for last two decades. The continuous application of fungicides results in the accumulation of fungicides in edible parts like seeds and causes severe effects on consumers including neurological problems and cancer. The continuous application of fungicides also results in the development of resistance among the rhizobacterial communities. Emergence of fungicide resistance among microbial communities enable them to survive in the presence of fungicide and helpful in the case of beneficial soilborne microbes to continue its beneficial activities like nitrogen fixation even in the soils with concentrated fungicide.

MATERIALS AND METHODS

Isolation of Rhizobium

Root nodules were collected from groundnut filed which was frequently exposed to the fungicide carbendazim. The nodules were thoroughly washed in tap water then immersed in 0.1 % mercury chloride for surface sterilization followed by repeated washing with distilled water to remove the traces of mercury chloride. The sterilized nodules were cut, crushed and was serially diluted. Then, 1 mL of serially diluted samples from 10^{-4} concentration was transferred to sterile petriplates and evenly distributed throughout the plates and sterile unsolidified YEMA medium was poured and it was allowed to solidify for the selective isolation of *Rhizobium*.

Morphological and Biochemical characterization of Rhizobium isolates

For cell shape, arrangement and motility, Gram staining and hanging drop technique were performed and the results were noted . Biochemical properties *viz.*,indole production, Methyl red, voges proskauer, urease production, Citrate utilization, TSI, oxidase and catalase were tested and the results were noted

Screening of Rhizobium isolates against different concentration of fungicide carbendazim

Corresponding author:* **Dr. D. John Milton
Department of Microbiology, St. Joseph's College of Arts and Science (Autonomous), Cuddalore 607 001, Tamil Nadu, India

To test pesticide resistant level for all the four isolates against the fungicide carbendazim, different concentration in ppm from 10, 000 ppm to 19,000 ppm were prepared and amended with nutrient agar and the cultures were streaked over the plates. The plates were incubated and the growth of the isolate in the plates containing fungicide indicated its resistance against that concentration of fungicide carbendazim

Effect of fungicide carbendazim on the plant growth promoting activities of Rhizobium isolates

a)Screening of *Rhizobium* for IAA production

Estimation of Indole acetic acid production was determined by the method of Gorden and Paleg (1957). The fresh bacterial cultures were inoculated in 10 ml of nutrient broth containing L-tryptophan (5 µg/ml) and incubated at room temperature for 5 to 7 days. The media were prepared with fungicide carbendazim at the rate of 1000 ppm and without carbendazim to compare the effect of fungicide on IAA production. After incubation, broth cultures were centrifuged at 3000 rpm for 5 minutes. Supernatant was taken and two drops of phosphoric acid was added followed by addition of Salkowaski reagent (50 ml of 35 % perchloric acid and 1 ml of 0.5 M $FeCl_3$). The absorbance of reaction mixture was measured at 530 nm after incubation in dark for 30 min.

b)Screening of *Rhizobium* isolates for ammonia production (Cappuccino and Sherman, 1992)

The isolated *Rhizobium* bacteria were tested for their capacity to produce ammonia in peptone water. Fresh cultures of bacteria was inoculated in 10 ml of peptone water in tubes and incubated for 48 to 72 hrs at room temperature. The media were prepared with fungicide at the rate of 1000 ppm and without fungicide to compare the effect of fungicide on ammonia production After 72 hrs, Nessler's reagent (0.5 ml) was added to bacterial suspension. Development of brown to yellow color indicated ammonia production

c)**Screening of the *Rhizobium* isolates for HCN production**

HCN (Cyanogen) production was determined by modified method of Bakker and Schippers (1987). Cultures of strains were streaked on solid agar plates supplemented with 4.4 g glycine litre^{-1}, with simultaneous addition of filter paper soaked in 0.5 % picric acid in 1% Na_2CO_3 on the streaked region along with the un-inoculated control. The media were prepared with fungicide at the rate of 1000 ppm and without fungicide to compare the effect of fungicide carbendazim on HCN production They were incubated at room temperature. Development of color from yellow to light brown, moderate brown or strong brown indicated the HCN production

d)Screening of the *Rhizobium* isolates for phosphate solubilisation

All the *Rhizobium* isolates were screened for their efficiency in phosphate solubilization on Apatide's medium. *Rhizobium* isolates, isolated from root nodule of groundnut spot inoculated on Apatide medium plate and incubated at 37°C for 4-5 days. The media were prepared with carbendazim at the rate of 1000 ppm and without carbendazim to compare the effect of fungicide on phosphate solubilization. Phosphate solubilization is observed by forming clear zone around the bacterial colony

e)Screening of the *Rhizobium* isolates for Zinc solubilization

All the for *Rhizobium* isolates were screened for their efficiency in Zinc solubilization on nutrient agar amended with insoluble zinc source (ZnO). *Rhizobium* isolates, isolated from root nodule of groundnut spot inoculated on the medium and the plates were incubated at 37°C for 4-5 days. The media were prepared with fungicide at the rate of 1000 ppm and without fungicide to compare the effect of fungicide on Zn solubilization. Zinc solubilization is observed by forming clear zone around the bacterial colony

RESULTS AND DISCUSSION

Four *Rhizobium* isolates RF_1, RF_2, RF_3, and RF_4 were isolated and the morphological and biochemical characterization of the isolates were given in the table-1. This was accordance with the findings of Bhattacharyya and Jha (2012), who found that most of the Rhizobacteria belonging to those found extracellularly are gram negative rods while the lower proportion being Gram positive rods, cocci , pleomorphic. In contrast, many gram positive rods have been isolated previously, from soil by many researchers (Kaymak *et al.,* 2008; Karpagam and Nagalakshmi, 2014). In a study conducted by Baudoin *et al.* (2002), they found that bacilli are common in soil, whereas spirilli and cocci are very uncommon in natural environments

The tolerance level of fungicide carbendazim was tested and the results were given in the table- 2. The results showed that all the four isolates were able to grow upto 11,000 ppm concentration of fungicide carbendazim. Further increase in fungicide retard the growth of all the four isolates. The variation in tolerance to pesticide by rhizobacteria could probably be due to the fact that rhizobacteria adopt different strategies to overcome the toxic effects of pesticides including fungicides and such mechanisms included biodegradation (Yang and Lee, 2008) and enzymatic hydrolysis (Dumas *et al.,* 1989; Herman *et al.,* 2005).

All the isolates showed significant quantities of IAA production with and without fungicide carbendazim, while the maximum production was recorded by the isolate RF_4 in both in the presence of fungicide and the absence of fungicide, remaining isolates showed moderate level of IAA production (Fig.-1).

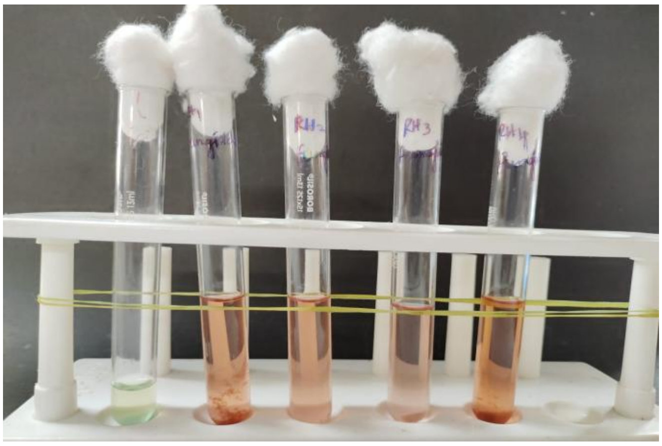

Fig.1 IAA production by *Rhizobium* (last one from left showed maximum IAA production by RF_4)

Ammonia production and HCN production by the *Rhizobium* isolates was tested and the results were given in the Fig.- 2 & 3. All the isolates were able to develop brown color, but the dark brown color was developed by the isolate RF_4 followed by RF_1, which indicated the maximum production of ammonia over other isolates. For HCN production, all the isolates changed the filter paper color into brown when compared with control. The

Table1 Morphological and Biochemical characterization of the *Rhizobium* isolates

S.No	Isolate	Gram staining	Motility	Indole	MR	VP	Urease	Citrate	TSI	Oxidase	Catalase
1	RF_1	G (-) rod	Motile	-	+	+	+	+	A/A	+	+
2	RF_2	G (-) rod	Motile	-	+	+	+	+	A/A	+	+
3	RF_3	G (-) rod	Motile	-	+	+	+	+	A/A	+	+
4	RF_4	G (-) rod	Motile	-	+	+	+	+	A/A	+	+

Table 2 Tolerance level of *Rhizobium* isolates against carbendazim

S.No.	Concentration in ppm (Fungicide)	*Rhizobium* isolates			
		RF_1	RF_2	RF_3	RF_4
1	1000	+	+	+	+
2	2000	+	+	+	+
3	3000	+	+	+	+
4	4000	+	+	+	+
5	5000	+	+	+	+
6	6000	+	+	+	+
7	7000	+	+	+	+
8	8000	+	+	+	+
9	9000	+	+	+	+
10	10000	+	+	+	+
11	11000	+	+	+	+
12	12000	-	-	-	-
13	13000	-	-	-	-
14	14000	-	-	-	-

color change was excellent in the filter paper kept over the isolate RF_3. The results clearly evident that the fungicide affected all the plant growth promoting parameters. Similar finding was observed by Munees Ahemad and Mohammad Saghir Khan, 2011. In their study increased the concentration of fungicide decreased the activity of all the plant growth promoting activity including HCN, ammonia production except production of polysaccharides by *Mesorhizobium* sp. strain MRC4 isolated from pesticide contaminated soil.

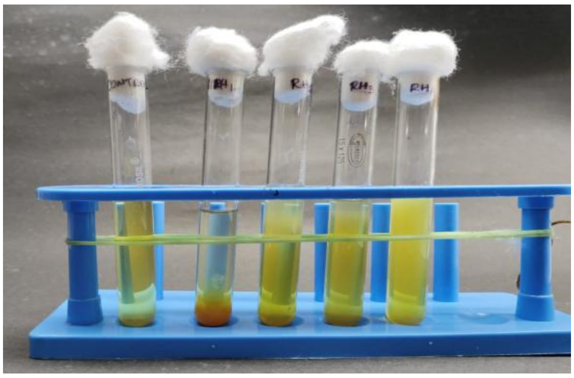

Fig.2 Ammonia production by the *Rhizobium* isolates.

The *Rhizobium* isolates isolated from this study showed no phosphate solubilization efficiency. Whereas, phosphate solubilization by *Rhizobium* was reported by Sijilmassi *et al.*, 2020. In their study the phosphate solubilization of *Rhizobium* was better even under drought stress condition. Only few researchers found that the *Rhizobium* could solubilize phospahte and Zinc (Sijilmassi *et al.*, 2020). Whereas many researchers found that many bacterial isolates isolated from rhizosphere showed better performance in such as *Pseudomonas* species like *P. fluorescens* (Manasa *et al.*, 2017), *A. chroococcum* (Chen *et al.*, 2018), *A. salinestris* (Chennappa *et al.*, 2018),*Bacillus* species *B. cepacia* (You *et al.*, 2020), *B. subtilis* (Ahmad *et al.*, 2018), *B. megaterium* (Wyciszkiewicz *et al.*, 2017), from different rhizosphere soils of various crops in conventional as well as pesticide contaminated environments.

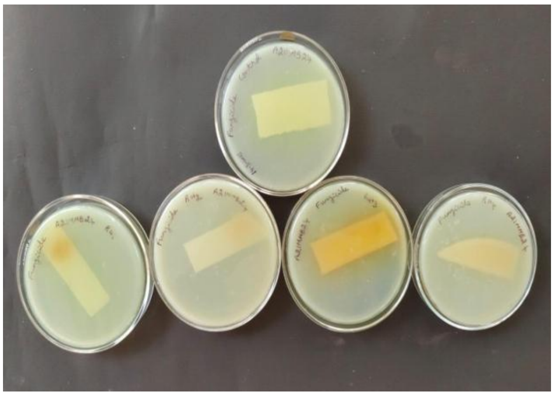

Fig.3 HCN production by *Rhizobium* isolates

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