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To The Study Of Isolation And Identification Of Plant Growth Promoting Pseudomonas Fluorescens Antagonist Effect For Controlling Plant Disease

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Abstract

Pseudomonas fluorescens was isolated from the rhizosphere of Roorkee-grown Okra. Only five isolates were chosen for further research based on their morphological, biochemical, and physiological characteristics. All five isolates were identified as Pseudomonas fluorescens from bergey's manual for the determination of bacteriology. Catalase, urease, amylase and citrate utilisation test all positive in all of the isolates. PFTT4 was identified to be a likely strain for all Plant Growth Promoting exercises like age of IAA, HCN, Ammonia, and phosphate solubilization subsequent to being assessed for their plant development advancing properties. Further, In vitro explores uncovered that PFTT4 diminished the development of phytopathogens like Fusarium solani and extraordinarily further developed seed germination just as all development boundaries like shoot and root length. Furthermore, Pseudomonas sp. PFTT4's plant growth-promoting and antifungal activities put forward to it could be there used because of bio-inoculant agents for Abelmoschus Esculentus.

Keywords :- Abiotic, antagonist, fungal infection, growth promoting rhizobacteria, pseudomonads, symbiotic.

Introduction

The rhizosphere (the area of soil around and encompassing the plant root) is critical for plant physical condition. Pseudomonas is an essential rhizosphere compound, and specific strain has been shown to improve plant physical condition in different types of crops, together with Lady finger. Pseudomonads are being studied extensively over the world to see if they can be used as crop protectants and soil health maintainers

(Aarab, et. al., 2015). They are the most metabolically and functionally flexible of the bacterial groups found in the rhizosphere. Plant growth can be aided, hampered, or harmed by interactions between soil microbes and plants in the rhizosphere. For plant-microbe interactions, the plant rhizosphere is an essential soil ecological habitat. Contingent upon the sort of microorganisms, soil supplement status, protection framework, and soil climate, colonisation through a scope of microbes in and around the roots can result in symbiotic, associative, naturalstic or parasitic relationship inside the plant (Almaghrabi, 2013). Okra (Abelmoschus Esculentus), a popular vegetable in many nations, has global economic and nutritional significance. This vegetable crop is widely produced and uses for economically purposes on the planet (Saravanan, 2003).

Pseudomonads are the world's generally assorted and naturally applicable bacterial gathering. Pseudomonas strains secrete many of chemicals like gibberellins, solubilize phosphate, cytokinins, auxins, they generate HCN, siderophores, and lytic enzymes stimulate plant development and are hence referred to be plant growth promoting rhizobacteria Abdel-Monaim, M.F. (2010). All non-pathogenic rhizobacteria, and a few separates of Pseudomonas aeruginosa, Pseudomonas aureofaciens, Pseudomonas fluorescens, and Pseudomonas putida smothered soilborne microorganisms by creating optional metabolites, for example anti-toxins, protease, HCN, and siderophore (Wahyudi, 2011).

Vegetable developing spaces of the world, Fusarium spp. And Rhizoctonia solani are significant soilborne contagious contaminations of both nursery and field created Ladyfingers, causing cataclysmic sicknesses, for example, root spoils and shrink, and at last diminished yield creation and quality. Chemical fungicides are mostly used to combat these infections. However, the widespread use of these chemicals poses a threat to the environment as well as human health.

As a result, alternate techniques to plant disease control should be highlighted. Plant growth-promoting rhizobacteria with biocontrol capacity could be a viable option. Pseudomonads have been shown to prevent pathogens that cause disease in Okra, according to several researchers (Patten, 2002). The goal of this study was to seclude, distinguish, and assess the development advancing and adversarial impacts of a few Pseudomonas fluorescens effect on Okra plants in Roorkee, India.

Materials & Method

Isolation of Pseudomonas fluorescens from the rhizospheric soil

Okra seedlings were tenderly eliminated from different fields in Roorkee these all transported toward the laboratory into sterilized plasticbags. Until further processing, each one trial be kept into the refrigerator on 4°-5° C (showed in Table- 1). Okra rhizospheric dirt was removed and dried by air. 1 gramme of soil was weighed, and successive dilutions were performed before plating on King's B plates and incubating for 24-36 hours at 28°C. Pure cultures were kept for further use lying on slant on NAM (Nutrient Agar Medium) at 4°-5° C temperature.

Gram staining for identification

Take a clean slide, prepare thin smear of old culture be created and heat fixed. For about 1 minute, a couple of

drop of precious crystal violet reagent put on the smear. Using running tap water, I washed the slide. I used Gram's iodine to flood the smear and let it sit for 2 minutes. Drop by drop, ethyl alcohol (95%) was used to decolorize the stain. For 2-4 minutes, I poured a few drops of safranine. Wash slide in faucet water and mount it in oil emulsion or glycerine prior to taking a gander at it under a magnifying lens (Microscope).

CT (Catalase test)

A drop of hydrogen peroxide (30%) was applied to the test culture on a clean slide, and the reported to result of bubbles was seen.

IP (Indole Production) Test

After autoclaving, each bacterial culture was injected separately in test tubes containing 5ml tryptone broth. One test tube was preserved when a be in charge of, with no bacterial culture inoculated. 1 ml of Kovac's reagent was added to each tube, including the control, after 48 hours of incubation. After a 10-15 minute break, the tubes were gently moistened. To allowing the reagent to rise to the top, the tubes were allowed to stand.

MRVP (Methyle Red and Voges-Proskauer) Test

Before autoclaving, the MRVP broth prepared and 6ml of it was poured in each test tube. Four test tubes were kept as controls, and the bacterial culture was put into all of the others. The test were incubated at 28°-32°C for 24-48 hours. 05 dropes of MR indicator were added to each test tube. Including the control and the colour change was observed. In the same way, for incubated test tubes and the control, 8-10 drops of Voges-Proskauer-I reagent and 2 drops of Voges-Proskauer- II reagent were used. The colour change in the test tubes was observed and compared to the control.

CU (Citrate Utilization) Test

Simmon’s citrate agar slants were formed and the bacterial culture was streaked on them. For 36-48 hours, the tubes were incubated at 28°-32°C. It was noticed that the slants' color changed.

Urease Production Test

Sticking an inoculating loop into the butt (bottom of the tube) and streaking the slants in a wavy pattern were used to inoculate urea agar slants. The results were seen after 24 hours of incubation at 27°-28° C. The tubes were placed in front of a control.

Starch Hydrolysis Test

Inoculated culture were placed on starch agar plates and incubated at 28°-30°C for 48 hours. Plates with healthy bacteria were soaked with iodine solution for 30 seconds using a dropper. The surplus iodine solution was poured out. The result shown formation of clear and clean zones around each isolate's line of growth, for example: - a change in medium color.

CFT (Carbohydrate fermentation test)

First we took the tubes of broth that had been sweetened with 4 different colors sweeteners (0.5% of each for example dextrose, mannitol, lactose, and sucrose) one durham tubes has been submerged in each tube. The test culture tubes were cultured for 24-26 hours at 28°-30°C. Signs of acid or gas generation, such as a change in color or the creation of bubbles, were examined for in the tubes.

IAA (Indole acetic acid) Production

Mansoor's description of IAA manufacturing was confirmed (2007). L-Tryptophan (0.1 g/l) was injected into bacterial colonies in nutrient broth. At 4°C, exponentially growing cultures were centrifuged for 15 minutes at 10000 rpm. Two drops of Salkowski reagent were added to the supernatant (2 ml) (1 ml of 0.5 M FeCl3 in 50 ml of 35 percent HClO4). The appearance of pink color confirmed the presence of IAA.

Phosphate solubilization

The capability of isolates to solubilize phosphate be tested using Pikovskaya's agar plates. The plates were checked used for the appearance of clearing zones surrounding the colonies after 4 days of incubation at 281°C (appropriate to solubilization of inert phosphate by produce macrobiotic acid by microorganisms).

Zinc solubilization

Zinc solubilization by microbes was carried out according to Sayyed's method (2005). The bacteria were identified using Tris-minimal media plates containing zinc phosphate and a pH indicator called bromophenol blue. Appropriate in the direction of the solubilization of non-living Zinc by bacteria, inoculate plates be incubate at 28°C for concerning a week and the apparent region approximately the colony was recorded.

HCN Production

Modified approach was used to determine HCN production (2010). Isolates' exponentially growing cultures (108 cells/ ml) were streaked on solid agar plates supplemented with 4.4 g glycine/ I, filter paper soaked in 0.5% picric acid in 1% Na2CO3 added to the upper lid of the plates at the same time. Para film was used to seal the plates. After 48-72 hours of incubation at 281°C, the hue changed from yellow to light brown, sensible brown or strong brown, indicating possible HCN production.

Ammonia Production

A bacterial isolate will be tested in peptone water to see if it can generate ammonia. 48-hour-old cultures were incubated in 08ml peptone water and incubated at 28°-30°C for 70-72 hours. On the culture broth over the slide, 0.5 mL Nessler's reagent was applied. A yellow to brown precipitation emerged within a few minutes, indicating moderate to high ammonia production (Ramette, 2003).

Antagonistic Activities

The development of secluded strains contrary to the parasitic infection Fusarium solani was measured using a

dual culture approach (MTCC 3871). Agar blocks (5mm in diameter) were inserted in the centre of the assay plate from the margin of a 5 day old fungal pathogen culture. One loopful (24-hour-old) isolated strain culture founded 02 cm. away from the pathogen. Plates were incubated for 3-7 days at 281°C. The hindrance zone was determined utilizing the recipe: Inhibition zone (rate) = 100C-T/C, where C addresses spiral development in charge and T addresses outspread development in double culture (Yazdani, 2009).

Bacterization of Seed

Bacterial strains (PFTT1-PFTT5) were refined in supplement stock for 48 hours in a shaker at 281°C. At 4°C, the cultures were centrifuged for 15 minutes at 8000 rpm. To achieve a final population density of 1 108 cells/ml, the way of life supernatants were disposed of and the pellets were washed and resuspended in sterile refined water. Independently, bacterial cell suspensions were joined with 1% CMC answers for structure slurry, which was then covered on the outer layer of seeds. Okra seeds covered with a 1% CMC slurry were utilized as a manage (Patten, 2002).

Germination of Seed

For the pot examine, sterile nursery soil was utilized. The dirt was ground into fine particles prior to being sanitized in a 160°C broiler for 2 hours. Okra seeds were gathered from the Roorkee neighborhood market. We picked solid seeds that were comparative in structure and size. In sets of three, bacterized seeds were planted iin the pots. Seeds treate with just 1% CMC were utilized as a benchmark grouping. As necessary, the pots be watered. The following were the treatments: T1; Pseudomonas spp. bacterized seeds PFTT1. T2; Pseudomonas spp. bacterized seeds PFTT2. T3; Pseudomonas spp. bacterized seeds PFTT3. T4; Pseudomonas spp. bacterized seeds PFTT4. T5; Pseudomonas spp. bacterized seeds PFTT5. Up to 21 DAS, root weight, shoot length, shoot weight, root length, and germination percentage be considered.

Result

Isolation of Pseudomonas fluorescens

Rhizobacteria samples secluded from Okra using King's medium and a serial dilution approach. On the basis of early investigation, total 5 isolates (PFTT1, PFTT2, PFTT3, PFTT4, and PFTT5) were examined.

Gram staining and morphological characteristics

Isolate colonies form be described as spherical and yellow green in color. Gram negative and rod shaped isolates were discovered in every case.

Biochemical Characterization

The whole of the disengages (PFTT1, PFTT2, PFTT3, PFTT4, and PFTT5) were observed to be positive for urease, citrate usage, catalase creations and starch hydrolysis however negative for indole creation and MRVP creation (Table- 1).

Indole acetic acid Production

IAA production was discovered in all five Pseudomonas spp. isolates (PFTT1, PFTT2, PFTT3, PFTT4, and PFTT5). The deepest pink colour produced by PFTT5 indicated the highest Indole acetic acid production (Table 02).

Phosphate solublization and Zinc Solubilization

On Pikovskaya's agar plate, all of the confines are fit to make clear corona around the spot vaccination. Phosphate solubilization ability was demonstrated in such clearing zones around the bacteria (Table- 2). Because there were no halo zones around the colonies, not any of the isolate was capable to solubilization of zinc (Table- 2).

HCN and Ammonia Production

Aside from PFTT3, the whole disengage of Pseudomonas fluorescens framed HCN, as shown through a change in channel paper tone. As shown by the serious earthy colored shade of the pass through a channel paper, PFTT4 delivered the most HCN (Table 02). In peptone stock, all of the disengage produced alkali by creating yellowish earthy colored accelerates (Table-2 and Fig 01).

Table- 1: Morphological and biochemical characterization of Pseudomonas fluorescens

parameter	isolate				
	PFTT1	PFTT2	PFTT3	PFTT4	PFTT5
Shape	Coccus	Coccus	Rod	Rod	Rod
Colonies	Circle	Circle	Circle	Circle	Circle
Colony Growth	Slow growth	Fast growth	Slow growth	Fast growth	Fast growth
Colonies growth	Light greenish	Yellowish green	Yellowish green	Yellowish green	Light green
Gram staining	+	+	+	+	+
MR test	+	-	+	+	+
VP test	-	+	-	-	-
Citrate	-	-	-	-	-

utilization test					
Urease test	+	+	-	+	+
Starch hydrolysis test	-	-	+	-	-
Catalase test	+	+	-	+	+
Indole test	-	-	+	-	-
Carbohydrate fermentation					
Mannitol	+	+	+	+	+
Dextrose	-	+	-	-	+
Lactose	+	+	+	+	+
Sucrose	-	+	-	-	+

Table- 2: Plant growth promoting activities of Pseudomonas fluorescens Abelmoschus Esculentus

Isolations	IAA	Zinc Solubilization	Phosphate solubilization	Ammonia Production	HCN production	Antagonist
PFTT1	+	++	-	-	+	-
PFTT2	++	+	-	++	+++	+
PFT	++	+	-	+	+	-

T3						
PFT T4	+	++	-	-	+	-
PFT T5	++	+	-	++	+++	+

Abberevations:-

A-, IAA POSITIVE; IAA NEGATIVE, +, B - , +, PHOSPHATE SOLUBILIZATION POSITIVE, -, PHOSPHATE SOLUBILIZATION NEGATIVE, +, LITTLE RADIANCES<0.5 CM WIDE ENCOMPASSING PROVINCES; -, ABSENCE OF CORONA DEVELOPMENT; ++, MEDIUM CORONAS> 0.5 CM WIDE ENCOMPASSING SETTLEMENTS; +++, enormous radiances >1.0 CM WIDE ENCOMPASSING STATES;

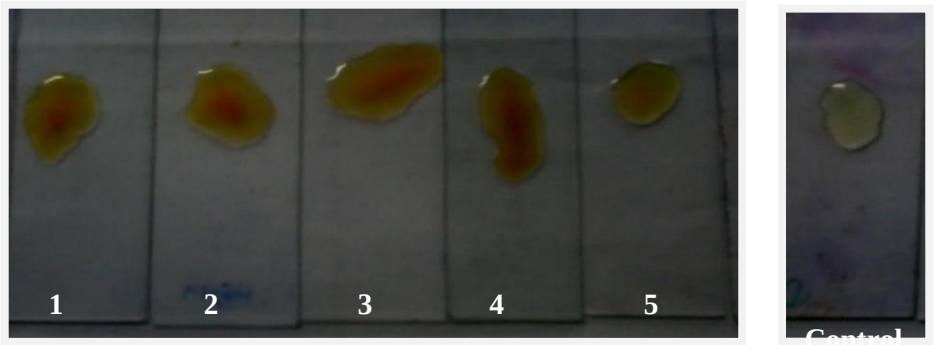


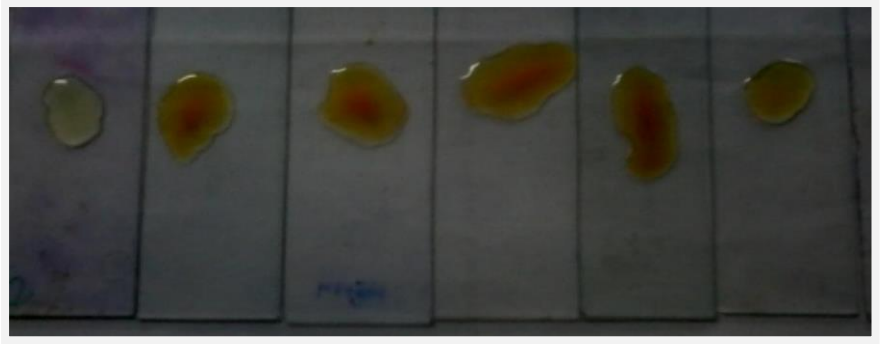
Fig.- 1. Plant growth promoting activities of Pseudomonas fluorescens

Antagonist activity

The whole of the Pseudomonas disconnects be tried for Fusarium solani opposing action. The development of test microorganisms on PDA plates at 28°C was stifled by Pseudomonas spp. PFTT4. The term of hatching compares to an expansion in parasitic restraint. Following 7 days of hatching, Pseudomonas spp. PFTT4 had the option to obstruct 42% of Fusarium solani, as per the normal outspread development restraint rate (Table 02).

Pot Trial Studies

Pseudomonas spp. disconnects were utilized to bacterize okra seeds of uniform shape and size (PFTT1 to PFTT5). Following 22 days of planting in pots, seeds closed up with the aforementioned microbial inoculants showed initiated vegetative qualities. Pseudomonas sp. PFTT4 had the best seed germination, shoot, and root length. Shoot new and dry weight, just as root new and dry weight, showed comparative expansion propensities. The medicines with Pseudomonas spp. PFTT1, PFTT4, and PFTT5 delivered the best number of plants. Pseudomonas sp. PFTT4 bacterized okra seeds brought about an extensive expansion in seed germination rate (82.2%), trailed by PFTT5 (75.8%). Seed germination was 40% in the control treatment (Table



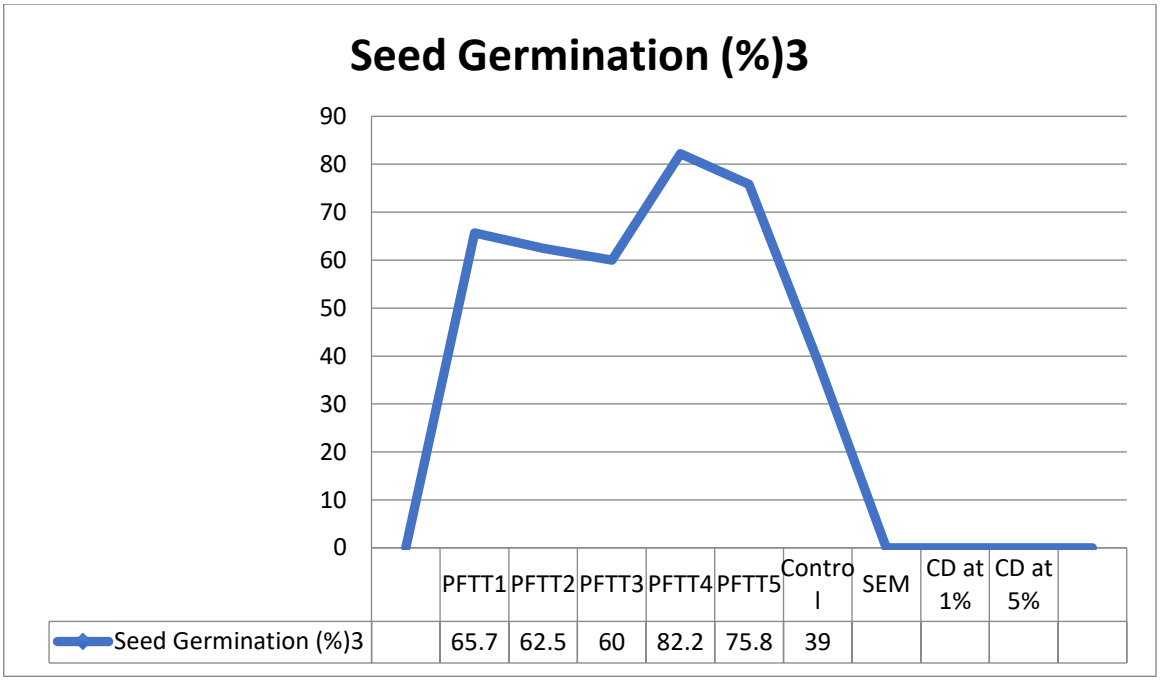
03). In contrast with the control, each of the measurements were upgraded and were huge at 1% and additionally 5%.

Table 03: Pseudomonas fluorescens effect on seed germination and vegetative growth of Okra

Isolates	Seed Germination (%)	Root length (cm)	Shoot length (cm)	Root weight (g)		Shoot weight (g)	
				Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
PFTT 1	65.7	3.567*	4.80**	0.0210*	0.1**	0.232**	0.10*
PFTT 2	62.5	4.325	3.465**	0.016**	0.012**	0.215^{ns}	0.90^{ns}
PFTT 3	60.00	3.68**	3.44**	0.112^{ns}	0.006^{ns}	0.186^{ns}	0.103*
PFTT 4	82.2	5.896**	4.322**	0..32*	0.012**	0.432*	0.117*

							*
PFTT 5	75.8	4.100^{ns}	3.400 **	0.018^{ns}	0.007*	0.240^{ns}	0.2200*
Control	39.0	55.7	2.57*	4.70**	0.210*	0.01**	0.22**
SEM		52.5	4.25	2.45**	0.16**	0.011**	0.25^{ns}
CD at 1%		060.0	3.867^{ns}	3.444**	0.0112^{ns}	0.006^{ns}	0.186^{ns}
CD at 5%		65.7	3.567*	4.25**	0.21*	0.01**	0.23**

Graph 01: Graph scale of Pseudomonas fluorescens effect on seed germination and vegetative growth of Okra



Abbreviations :-

SEM = STANDARD BLUNDER MEAN

CD = CRITICAL DIFFERENCES

VALUES ARE MEAN OF 3 ARBITRARILY CHOSE PLANTS FROM EACH SET, ** HUGE AT 01%, * HUGE AT 5% WHEN CONTRASTED WITH CONTROL, NS = NON-HUGE WHEN CONSTRASTED WITH CONTROL, CONTROL (NON-FACTORIZED SEEDS).

Discussion

Flow research depicts the starting exertion finished to isolate PGPR from the rhizospheric top soil of Roorkee-developed Okra. Due to the high supplement accessibility as root exudates, the rhizosphere gives an optimal environment to soil microorganisms. The number of inhabitants in organic entities that live in a given climate is special and is impacted by the physical and natural components that exist in that climate. Additionally, Patel (2015) utilized King's medium to extricate 10 fluorescent pseudomonad strains from assorted rhizospheric soil of yield plants like rice, maize and bazra. Kumar, et al., (2005) recognized 115 Pseudomonas fluorescnes isolates from the rhizosphere of soybean in Cirebon, Plumbon, Indonesia. All of the detaches tried positive for catalase, citrate use, urease age and starch hydrolysis yet negative for indole blend and MRVP.

IAA production was discovered in all of the isolates. In PBRI, Haridwar, India, Bacteria that produce Indole acetic acid have been shown to stimulate root elongation and plant growth (Kalita, 2015). The development of natural acids, for example, gluconic, acidic, lactic, fumaric, and succinic acids is connected to phosphate solubilization by bacterial separates (Blumer et al., 2000).

Natural corrosive amalgamation brings down soil pH, bringing about the age of H+, which replaces Ca 2+ and discharges HPO42-into the dirt arrangement. Pseudomonas and Bacillus were recognized as the essential

phosphate solubilizers by Ashrafuzzaman et al., (2009). Immunization utilizing phosphate solubilizing microbes further developed maize development and grain yield, cut compost uses, and brought down ozone depleting substance outflows, as per Mandal et al., (2009). As per Verma et al. (2010), 70% of their detaches been able in the direction of solubilize phosphate in the scope of 5.8 to 13.45 mg/100ml and and advance chickpea improvement. These investigations back up our decision.

Except for PFTT3, all detaches that twisted the shade of the channel paper from yellow to orange-brown were assigned HCN makers (Tilak, et al., 2005). Pseudomonas spp. produces HCN, which represses the development of phytopathogens, as per Saad, M.M. (2006). HCN is a wide range antibacterial atom occupied with organic control of root contaminations by various plant connected fluorescent pseudomonads, as indicated by León et al. (2015). In this examination, every one of the disconnected life forms was observed to be acceptable smelling salts makers. Also, Kaur et al. (2013) discovered smelling salts age in 95% of Bacillus secludes and 94.2 percent of Pseudomonas disconnects, which upholds our discoveries. The shading changes from brown to yellow tone were a positive test for alkali creation. (Patel, 2015).

PFTT4 was observed in the direction of suppress Fusarum solani growth (in vitro) in our investigation. Wahyudi et al. (2011) found that Pseudomonas spp. have antifungal efficacy against Fusarium solani. Mansoor et al. (2007) discovered that using P. aeruginosa and P. lilacinus alone or in combination effectively controlled F. solani. In a pot trial investigation, okra seed bacterized with Pseudomonas Fluorescnes PFTT5 showed critical expansion in shoot length, root length and dried shoot and root weight. PFTT4 and PFTT5 treated seeds germination rates were 83.3 percent and 76.7 percent, separately.

In the benchmark group, only 40% of seeds developed (uninoculated seeds). These discoveries are like those of Sayyed et al. (2005), who found that immunizing wheat seed with P. fluorescens NCIM 5096 expanded the pace of germination by 10%. Ashrafuzzaman et al. (2009) showed an increment in seed germination when rice seeds were pretreated with Plant growth promoting rhizobacteria detaches (Blumer, et. al., 2000). likewise asserted this Plant growth promoting rhizobacteria worked on nut development and seed rise.

Therefore, it's feasible to presume that Pseudomonas spp. bacterial strains will gather more consideration in the field of biofertilization and natural control due to their multifunctional capacities. Pseudomonas spp. (PFTT4), which have great plant development boosting characteristics like IAA age, phosphate solubilization, HCN creation, alkali creation, and biocontrol, could be utilized as bioinoculants for Okra and different harvests, as per the current review (Chen, W, et. al., 2015).

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