Screening of herbicidal agent from soil microbiota as a prospective biocontrol alternative

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Abstract: **On a global scale, the issue is population growth and rising food consumption. As a result, chemical fertilizers and chemical herbicides are being introduced into the soil to meet the population demands of growing countries such as India and Pakistan. They not only destroy soil microflora and fauna, but they also cause pollution, water pollution, and health issues, as well as increase production costs. As a result, there is an urgent need to find a substitute for these dangerous compounds. Thus, the greatest alternative method is biological control, sometimes known as 'biocontrol', the use of microorganisms to limit pathogen development and kill weeds and reduce disease effects. There are some organic farming but the problem is the control of weeds. The use of the biocontrol agent *Pseudomonas* sp. in the suppression of these weeds as well as the increase of plant development has proven significant. Even the result can be observed sooner than any other chemical product. Thus, the introduction of a bacterial suspension solution into the soil zone where it could suppress weed plants would be intriguing. The experiment for biocontrol cum enhancer has been experimented on a weed called *Cyperus rotundas* for herbicide control and the experiment for enhancer is experimented on rice plants. During the test period within 3 days, the herbs died and the rice plants showed decent growth as compared to the control plant.**

Keywords**: Biocontrol, *Pseudomonas* sp., enhancer, *Cyperus rotundas*, herbicide, rice plants.**

1. INTRODUCTION:

Weeds are a nuisance in many land uses. The prevalence of herbicide-tolerant weeds and bans on the use of cosmetic pesticides have provided a strong impetus for the development of new weed control strategies. The use of bacteria, fungi, and viruses to achieve this goal has gained increasing attention over the last 30 years. The advantages of this strategy include reduced environmental impact, improved target specificity, reduced development costs compared to conventional herbicides, and identification of novel herbicide mechanisms.

The word 'biocontrol' or ' biological control' was first referred to as the direct or indirect participation of an organism in reducing pathogen development and reducing disease. Plant diseases are the leading source of crop loss globally [1]. Biocontrol is the prevention of one organism's defined actions by one or more additional species, frequently portrayed as natural enemies. In biocontrol, live creatures or organic materials are utilised to mitigate the harm that destructive organisms (pests, weeds, and microbiological infections) do. They produce harvests that are free of any pests and are mostly utilised to reduce pest inhabitants [2]. The use of biocontrol agents increases the probability of disease resistance while reducing the need of pesticides. Bacterial strains used as biocontrol agents are largely from the *Agrobacterium*, *Bacillus*, and *Pseudomonas* genera. These bacterial biocontrol agents promote plant development by suppressing minor or large phytopathogens and producing plant growth-promoting compounds such as gibberellins and auxins. They are a rich source of plant growth promoters, including microbial consortiums, macronutrients, micronutrients, and immune boosters. Generally, they are used to treat seeds/seedlings and improve the decomposition of organic matter, thereby enriching the soil and increasing plant vigor.

Many bacteria have also been investigated as potential biological weed control agents. Among these, *Pseudomonas fluorescens* and *Xanthomonas campestris* have received the most attention [3]. *Pseudomonas* sp. are effective plant-growth-promoting microorganism (PGPM) biocontrol agents for a variety of phytopathogens and pests. Plant Growth Promoting Microorganisms are mainly free-living bacteria that colonies in the rhizosphere and benefit crop plants. Bacteria of the rhizosphere (rhizobacteria) include symbiotic rhizobium, mycorrhiza, actinomycetes, and free-living bacteria, sustain their life in the narrow region of soil here roots are being influenced by various functions. Plant phytohormone production promotes seedling emergence, iron chelation, antibiotic production, improved nutrient absorption, and seedling emergence. Siderophores are iron-chelating substances released by bacteria on or near the roots that influence the development of rhizobacteria in the plant. Aside from its extensive use in agricultural industries. As mentioned above, *P. fluorescens* has received much attention as a bioherbicide cum growth-promoting agent [4]. Strains of *Pseudomonas* sp. were able to trigger plant growth through different traits like nitrogen fixation, phosphate solubilization, production of organic acids, and Indole Acetic Acid (IAA) [5]. The genus *Pseudomonas* sp. is the most numerous among the catalogued genera of gram-negative bacteria [6]. They are aerobic, non-spore-forming, which are straight or slightly curved about 0.5-1.0 µm by 1.5-5.0 µm and are motile with one or more flagella, ubiquitous in agricultural soils and are well adapted to grow in the rhizosphere. It appears that they retain a very strict aerobic respiratory metabolism with oxygen [7]. Hence, in this original research work, we have studied the inhibitory effects of *Pseudomonas* sp. on the weeds (*Cyperus rotundas*) in rice plants, as a biocontrol agent. For a century, biological control (or biocontrol) has been used to refer to practically all pests biological control (or biocontrol) has been used to refer to practically all pest kinds [8]. For instance, rodents [9], weeds, mosquitoes [10], and insect pests and diseases of crops [11]. The actions of protective antagonists in the food chain, such as in food and animal feed processing [12], as well as crucial medical treatments for humans[13], are also governed by the principles of biological control, even though

these disciplines use different sets of terminology. For a lengthy period, bacteria have been added to soil, seeds, roots, and other plant structures to aid in the growth and development of plants. Especially in India, rice is a crop that is important to the economy as a whole. Diseases brought on by fungus, bacteria, and viruses reduce rice yield by 5% [14]. The use of biocontrol plant growth-promoting rhizobacteria (PGPR), which are capable of inhibiting or avoiding phytopathogen damage, is one of the growing research areas for the control of various phytopathogenic agents [15]. The collection of PGPR strains that specifically improve seed emergence, plant height, and yield of numerous crops includes strains from the genera Pseudomonas and Rhizobium[16]. Pseudomonas spp. often produces antibiotics as part of their biocontrol strategy to reduce fungal infections[17]. According to Das et al. [18], PGPR may be applied more widely in agriculture for biocontrol of plant diseases and biofertilization.

Herbicides are one type of pesticide that is used in agricultural areas to combat noxious weeds, which are mostly to blame for lower crop output [19]. However, indiscriminate use of herbicides in various production systems above predetermined threshold levels poses a major hazard to both plants [20] and rhizospheric organisms and related biotic processes [21]. The dosage rates, activity, and persistence of these compounds in soils affect how herbicide application affects soil microflora and plants [19].

P. fluorescens has drawn a lot of interest as a biological weed control agent. This species comes in a variety of strains, some of which are helpful to plants [22], while others are harmful. Three strains of *P. fluorescens* have been the focus of studies into the suppressive effects of this organism, and each of them has been found to inhibit plant growth and/or germination by producing extracellular metabolites [23].

2. METHODOLOGY:

Study area:

Purba Bardhhaman district extends from 23°53′ N to 22°56′ N Latitude and 88°25′ E to 87°56′ E Longitude. Lying within Burdwan Division, the district is bounded on the north by Birbhum and Murshidabad, on the east by Nadia, on the south by Hooghly and Bankura, and the west by Paschim Bardhaman districts. It is mainly a flat alluvial plain area. This alluvial soil is formed of alluvium brought down by the Ajay, Damodar, Bhagirathi, and many other rivers. This district is mainly known for agricultural occupation. This experiment is carried out on the soil of Kshirgram (village of Mongalkote CD block) and Karui (village of Katwa II CD block), of the community development block (CD block), which constitutes an administrative division in the Katwa subdivision of the Purba Bardhaman district. The primary crop farmed in the Bardhaman district is rice, with a large portion of it being sold to adjacent states and nations while also providing for the area's needs on a daily basis. Bardhaman is well-known as the "Granary of West Bengal" since rice farming and trading make up the majority of its economy. The three primary rice varieties—the Aus, or autumn rice, the Aman, or winter rice, and the Boro, or summer rice can be distinguished by their unique traits.

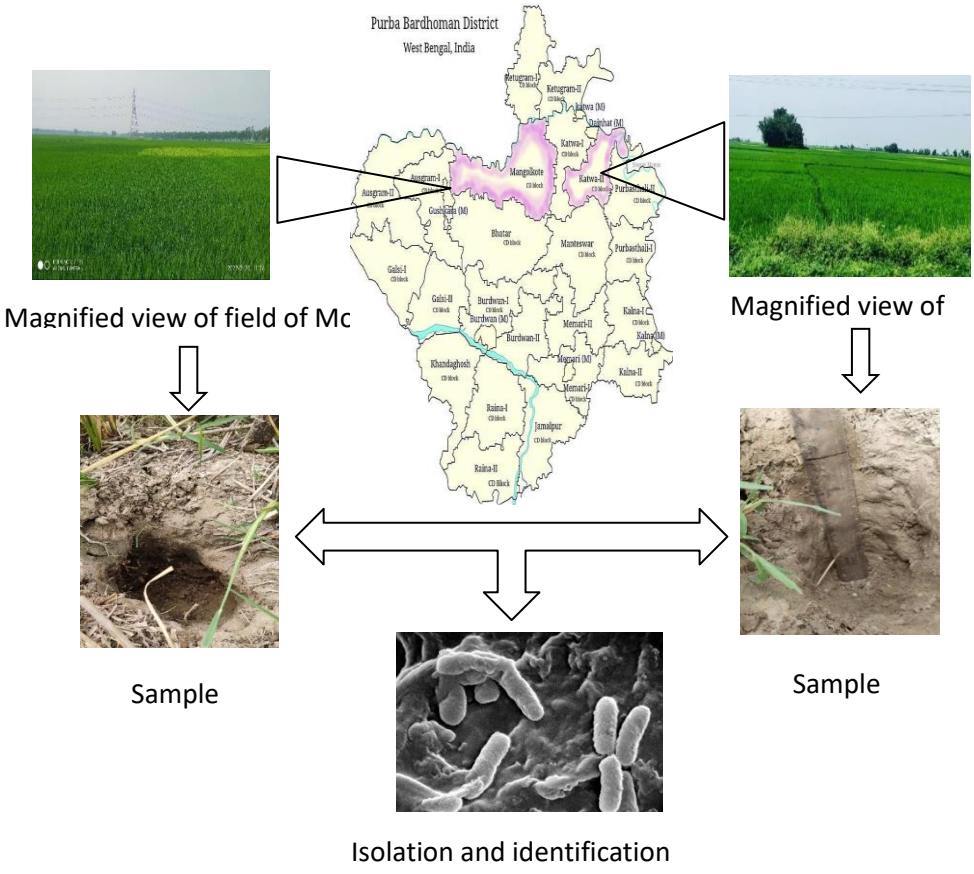

Fig 1: Isolation of *Pseudomonas* sp. from the agro-field of Bardhaman district

a. **Sample collection**:

The soil samples were collected from the Kshirgram (23.5141°N 88.0273°E.), Karui (23.521194°N 88.093417°E), and Putsuri (23.44°N 88.17°E) of Purba Bardhhaman. About 15gm of the samples were collected from different agricultural fields in different containers. The samples were cleaned by hand and the unwanted particles were handpicked and discarded.

b. **Isolation of bacteria**:

The method proposed by Vlassak et al. was performed to isolate Pseudomonas sp. In this process, a conical flask containing 90 ml of saline was filled with 10g of soil from each soil sample. The sample was stirred on a vortex for 15 minutes while successive soil suspension dilutions were made. Then there were serially diluted from 10^{-1} to 10^{-6} for Pseudomonas species. The plates were incubated for a day at 30 ±1°C for the growth of Pseudomonas colonies on KB plates which is the selective media (composition Protease peptone 20gm/LGlycerol 10gm/LK2HPO4 1.5gm/LMagnesium sulphate 1.5gm/Land agar 15g/l), and the colonies were manually enumerated and documented.

c. Identification of Bacterial Isolates

Morphological Characterization:

The isolated colonies were checked for their purity and then studied for colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures by observing under a microscope. [24]

Pigment test:

The test tubes containing KB broth and plate containing sterilized Kings B agar medium were filled with bacterial strain PR25. isolate, cultured for five days, and monitored.

Biochemical tests and fermentation tests:

The reason behind performing biochemical tests is that it depends on the assumption that each type of bacterium responds differently and offers different kinds of positive or negative results due to their distinct metabolic properties. The identification of bacteria has been transformed by molecular microbiology approaches. There are different biochemical tests performed [25] and the protocols are followed which are briefly described below.

Methyl Red Test

Sterilized MR broth (composition Buffered peptone 7gm/LDextrose 5gm/LDipotassium phosphate 5g/l) tubes were inoculated with the test culture and incubated at 28±2 °C for 48h. After incubation five drops of methyl red indicator were added [24]. The test culture was injected into sterilized glucose phosphate broth tubes and incubated at 282 °C for 48 hours. After incubation, five drops of methyl red indicator were gently mixed into each tube.

For the test, red colour generation was considered positive, while yellow colour creation was considered negative. The result is present in the result section.

Voges Prausker's Test

In the pre-sterilized MR broth tubes, test cultures were inoculated and incubated at 37 °C for 48h. After incubation ten drops of Baritt's reagent A were added and gently shaken followed by the addition of 10 drops of Baritt's reagent B [24].

The presence of red suggests that the test bacteria manufacture acetoin from pyruvate and uses the butanediol route, indicating that it is VP+. The absence of red colour formation suggests that the test bacteria do not make acetoin from pyruvate and do not use the butanediol route, indicating that they are VP-. The result is present in the result section.

Indole Production

Sterilized SIM agar (composition Casein peptone 20 gm/LMeat peptone 6 gm/LSodium thiosulfate 0.3 gm/LFerric ammonium citrate 0.2 gm/Land agar 15 g/l) slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ±2 °C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing the production of pink to red colour were recorded as positive whereas no colour change even after the addition of the appropriate reagent suggests a negative for indole production.[24]. The principle behind it is that Indole is produced via reductive deamination of tryptophan through the intermediary molecule indole pyruvic acid. Tryptophanase catalyses the deamination process, which removes the amine (-NH2) group from the tryptophan molecule. The process produces indole, pyruvic acid, ammonium (NH4+), and energy as by-products [25]. As a coenzyme, pyridoxal phosphate is required. When indole is mixed with Kovac's Reagent (amyl alcohol containing hydrochloric acid and p-dimethylamino benzaldehyde), the solution changes from yellow to cherry red. Because amyl alcohol is not water soluble, the red colouration will settle to the top of the soup in the form of an oily film. The result is present in the result section.

Starch Hydrolysis

Sterile starch agar (composition Peptic digest of animal tissue 5 gm/LSodium chloride 5 gm/LYeast extract 1.5 gm/L Beef extract 1.5 gm/L Starch, soluble 2 gm/Land agar 15 gm/L) plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28±2 °C for 24- 48 hrs. After incubation, the plates were flooded with iodine solution for 5 mins. Here, Iodine combines with starch to produce a dark brown colour. As a result, starch hydrolysis will generate a clean zone around bacterial growth. Since some bacteria can break down starch. The result is present in the result section [25].

Catalase test

This test was performed to concentrate on the presence of catalase catalysts in bacterial provinces. Loopful bacterial colonies were taken on glass slides and one drop of H_2O_2 was added. The presence of the catalase enzyme was indicated by the gas bubble's appearance means the test is positive whereas no bubble formation means a negative test [26]. The result is present in the result section.

Oxidase test

Strip strips of Whatman’s No. 1 filter paper were soaked in a freshly prepared 1% solution of tetramethyl-phenylene-diamine dihydrochloride. After draining for about 30 seconds, the strips were freeze-dried and stored in a dark bottle tightly sealed with a screw cap. A strip should be removed, laid in a Petri dish, and sprayed with diluted water to use. Using a platinum loop, the colony that is to be tested shall be lifted and smeared in an area of moisture.[26]

Positive reactions are indicated by an intense deep purple colour that appears within 5–10 seconds, and negative reactions by the absence of colour or colouration that occurs more than 60 seconds after the first stimulus. The result is present in the result section.

Gelatine liquefaction:

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatine deep tubes and incubated for 24 h at 28°C. After that, the tubes were kept in a refrigerator at 4ºC for 30 minutes. The isolates showing the liquefied gelatine had been taken to be positive and those that resulted in solidification of glucose at freezing were considered negative for this test [27].

Casein Hydrolysis Test:

Sterile skim milk agar (composition Skim Milk Powder 28 gm/LTryptone 5 gm/LYeast Extract 2.5 gm/LDextrose 1gm/Land agar 15 gm/L) plates were inoculated by *Pseudomonas* sp. in either a straight line or in a zig-zag pattern. The plate was then incubated at 25-37 °C. The milk agar plate cultures were examined for the presence of a clear area or zone of proteolysis, surrounding the growth area of each bacteria colony.

Some microbes can break down casein protein by creating a proteolytic exoenzyme known as proteinase (caseinase). Milk agar is utilized in the lab to show such an action. Following the inoculation and incubation of the agar plate cultures, organisms secreting proteases will show a zone of proteolysis, which is visible as a clear region surrounding the bacterial growth. This reduction of opacity is a result of a positive hydrolytic reaction that produces soluble, non-colloidal amino acids.[25]

In the absence of protease activity, the medium surrounds the organism's growth. remains opaque, a negative effect.

Urea test:

It is an important diagnostic test for distinguishing Proteus members from gram-negative pathogens. Urease is produced in large quantities by Proteus vulgaris. Growing the test organisms on urea broth or agar medium with the pH indicator phenol red (pH 6.8) is used to perform the urease test. During incubation, urease-producing bacteria create ammonia, which elevates the pH of the medium/broth. As the pH rises, the hue of the phenol red changes from yellow (pH 6.8) to red or deep pink (cerise). Failure to acquire a deep pink colour due to a lack of ammonia production indicates a lack of urease generation by the bacteria.[27].

Carbohydrate fermentation test:

The carbohydrate fermentation principle asserts that the action of an organism on a carbohydrate substrate causes acidification of the medium, which can be measured using a pH indicator dye. A fermentation medium comprises a baseline medium containing a single carbohydrate for fermentation (dextrose, sucrose, mannitol, etc.). However, the medium incorporates a variety of pH indicators. In addition to a pH indicator for detecting acid generation from fermentation. Different organisms' carbohydrate fermentation processes can be used to distinguish between bacterial groups or species. Each test tube containing different carbohydrate were inoculated with bacteria. They were incubated at 35°C for 24 to 48 hrs.[28] As a result, if the colour changes to red colour at neutral pH:

Red to pink: due to the production of the base.

Red to yellow: due to the production of acid.

3. RESULTS AND DISCUSSIONS:

The microbial population is found in agricultural rhizospheric soils, primarily rice, and mustard. The highest population of *Pseudomonas* sp. was identified in the Bardhaman district's rice rhizospheric soils. Though the bacterial strain that were being isolated in king's B agar are followed through biochemical tests. The observations are explained by tabular form as well as in pictorial views.

Microscopic view:

The bacterial colonies appear as reddish/pink rods under the microscope. As they are unable to retain the principal stain (crystal violet), they are Gram-negative bacteria. So, the bacteria strain (PR25) can be suggested to *Pseudomonas* sp., though further biochemical tests were done for confirmation[28].

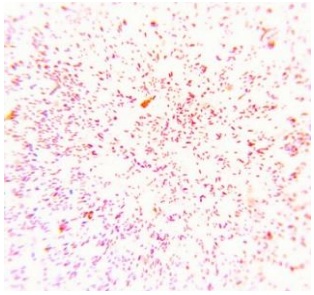

Fig 2: Gram-stained bacterial strain (PR25) under 100X magnification (oil emulsion).

Pigment test:

The plates containing sterilized Kings B agar medium were filled with bacterial strain (PR25). Isolate then cultured for five days, and monitored. The colony produces an additional pigment which is blue to green in color.

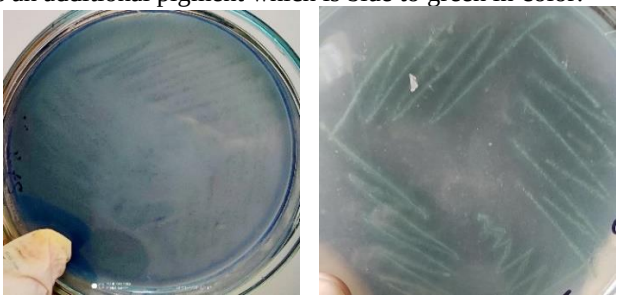

Fig 3: Bluish-green color pigments of the bacterial strain (PR25)

Biochemical test:
According to Bergey's Manual for Determination of Bacteriology, certain biochemical tests were carried out to identify of bacterial strain (PR25). in tabular form:
Table no. 1: Table containing results of biochemical tests of PR25 bacterial strain

Characteristics/ Biochemical tests	**Bacteria strain (PR25)**
Gram staining	Negative
Shape	Rods
Spore (sporing/ non-sporing)	Non-sporing
Methyl Red Test	Negative
Voges Prausker's Test	Negative
Indole Production	Negative
Starch Hydrolysis	Negative
Catalase test	Positive
Gelatin Hydrolysis Test	Positive
Casein Hydrolysis Test	Positive
Urea test	Positive
Dextrose	Negative
Mannitol	Negative
Sucrose	Negative

Observation and results that are being observed are described in brief:

1.1. Methyl Red Test:

After adding methyl red of about 0.1gm in MR broth, a pale-yellow colour appeared on the surface region. This means the result is negative.

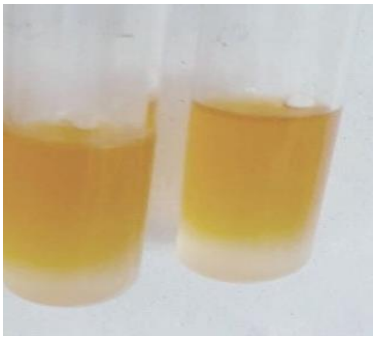

Fig 4: Yellow colour appeared, lack of red colour appearance in test tube.

1.2. Voges Prausker's Test:

After adding 6 drops of 5% alpha-naphthol, and 2 drops of 40% potassium hydroxide in VP broth, a copper colour appeared on the surface region. This means the result is negative.

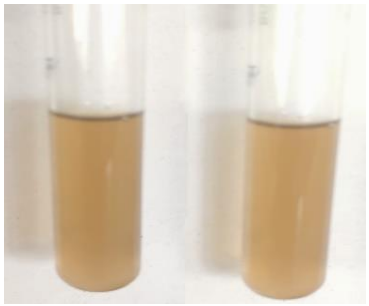

Fig 5: Copper colour appeared, lack of pink-red colour

1.3. Indole Production:

After adding 5 drops (0.5ml) of Kovac's reagent to the Tryptone broth, no colour changes happened and a yellow or slightly cloudy formation took place on the surface. This means the result is negative.

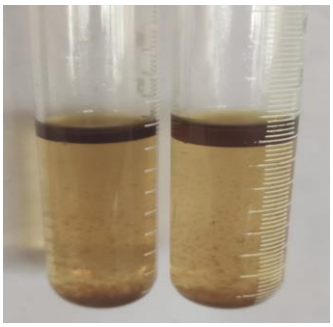

Fig 6: No colour change after adding Kovac's reagent

1.4. Starch Hydrolysis:

After 24 hrs of incubation of starch agar culture plate, 3-4 drops of 10% Iodine solution were added on the edges of the colony. After 10-15 mins no clear zone was observed around the growth thus it is a sign of the presence of starch but it is not hydrolyzed as well as the organism has not evolved any extracellular enzyme.

Fig 7: Blue color colony for lack of enzyme hydrolysis

1.5. Catalase test:

After adding a small colony by looping in 3% H_2O_2 on a slide, copious bubble formation occurred. This means it is a positive test.

Fig 8: Bubble formation on adding loopful PR25 colonies in H_2O_2

Gelatine hydrolysis:

The test tube which contains the bacterial culture had liquified the gelatine but the test tube which was the control solidified. Thus, the test is positive.

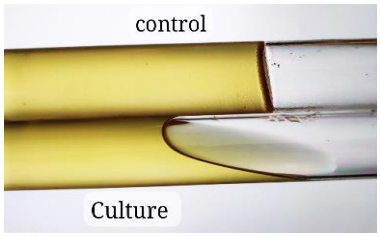

Fig 9: Total liquefaction of the inoculated test tube

1.6. Casein Hydrolysis Test:

After incubation, the casein agar plate had a clear transparent zone around the area where the colonies were growing. This means that outside of their cells, bacteria can produce enzymes (e.g. caseinase) that degrade the milk protein casein (which means a positive result).

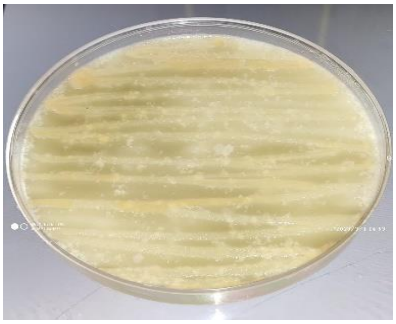

Fig 10: Clear zones around the colonies on the petri plate.

1.7. Urea test:

In 15 minutes to 1 day, the urea media produced an intense magenta to brilliant pink color. This means that urea had been hydrolysed to produce ammonia therefore the result is positive.

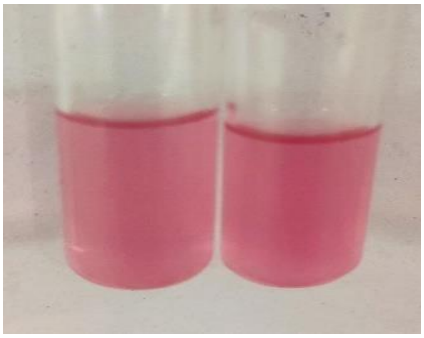

Fig 11: Pink colour formation in the inoculated test tube

1.8. Carbohydrate fermentation test:

After 24 to 48 hrs of incubation, the colour of fermented media in test tubes of maltose, sucrose, and dextrose changed into orange, and in test tubes of fructose and mannitol the colour changed to yellow, this means that maltose, sucrose, and dextrose were typically positive with the alkaline formation and in the test tube of fructose and mannitol were typically negative with the acid formation

Fig 12: inoculated test tubes of maltose, sucrose, and dextrose were typically positive on the other hand fructose and mannitol were negative.

Thus, by observing and analysing the results with the help of Bergey's Manual, it can be concluded that the isolated bacteria are *Pseudomonas* sp.

OD Value and optimization of bacterial growth curve:

OD values were mainly measured on the variation of protein compound that is peptone, variation of pH concentration that is in an acidic medium, in a neutral medium, and in an alkaline medium, and variation in temperature in incubation for 24 hours.

- Based on the variation of Peptone concentration:

Table no. 2: Table containing OD value based on different peptone concentrations.

PEPTONE CONC./10ML	CONTROL	OD VALUE 1	OD VALUE 2	OD VALUE 3	MEAN VALUE
0.0 gm	0.00	0.00	0.00	0.00	0.00
0.1 gm	0.00	0.065	0.07	0.05	0.06167

0.3 gm	0.00	0.11	0.15	0.09	0.11667
0.5 gm	0.00	0.11	0.16	0.09	0.12

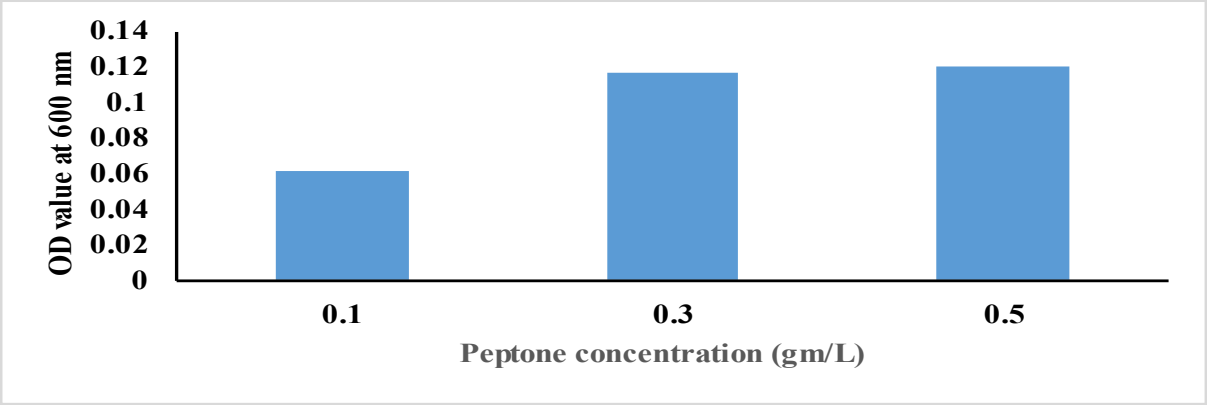

Fig 13: Curves of bacterial growth based on different peptone concentrations

- Based on the variation of pH concentration:

Table no. 3: Table containing OD value based on different pH concentrations.

PH CONC. /10ML	CONTROL	OD VALUE 1	OD VALUE 2	OD VALUE 3	MEAN
0	0	0	0	0	0
HCL (PH= 3)	0	0.45	0.48	0.4	0.443333
NEUTRAL (PH= 7)	0	0.392	0.396	0.389	0.392333
NAOH (PH=9)	0	0.379	0.381	0.375	0.378333

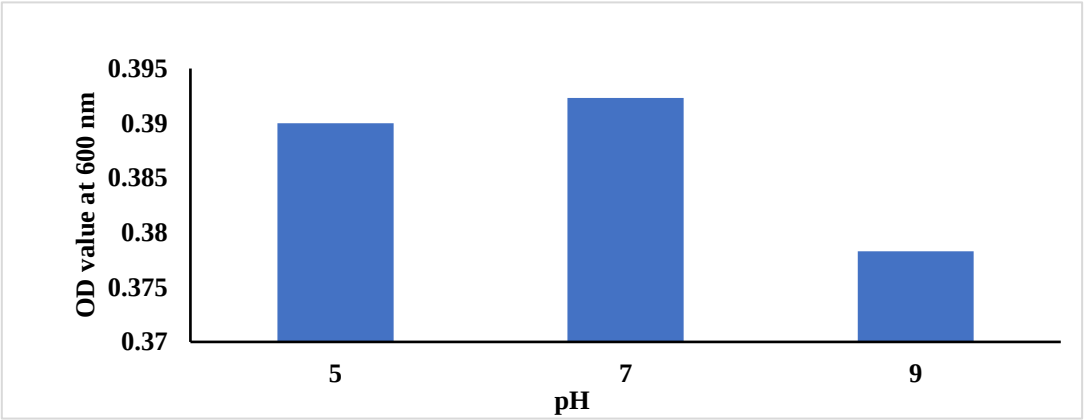

Fig 14: Curves of bacterial growth based on different pH concentrations

- Based on the variation of temperature:

Table no. 4: Table containing OD value based on different temperatures

Temperature/10ml	Control	OD value 1	OD value 2	OD value 3	Means
0 degree C	0.00	0.00	0.00	0.00	0.00
29 degree C	0.00	0.3	0.38	0.29	0.3233
37 degree C	0.00	0.38	0.4	0.35	0.3767
42 degree C	0.00	0.33	0.39	0.32	0.3467

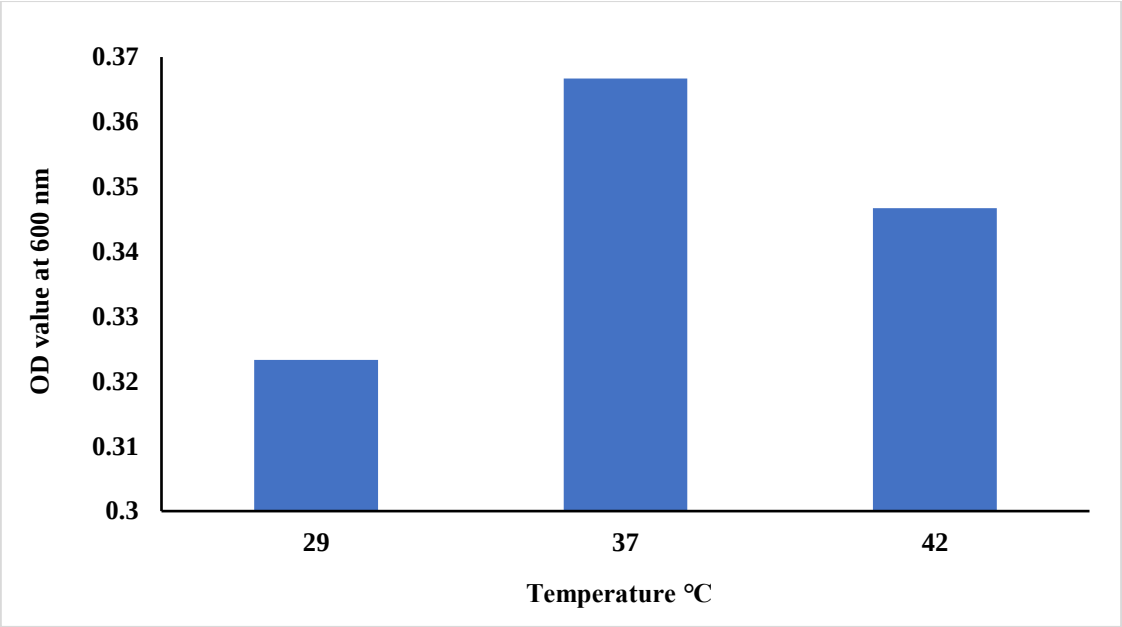

Fig 15: Curves of bacterial growth based on different temperatures.

Thus, by observing and analysing those values and graphs, it can be concluded that:

a. In the case of variable peptone concentration, 0.5 grams of peptone per 10 ml had shown maximum growth of bacteria.
b. In the case of variable pH concentration, acidic media per 10 ml had shown maximum bacteria growth, and neutral and alkaline media had the same bacterial growth.
c. In the case of variable temperature, 37℃ of 10 ml had shown maximum growth of bacteria than other lower and higher temperatures. this means for Pseudomonas 37℃ is the most suitable temperature for growth.

Experiment on weeds and rice plants:

Control:

In the control pot, there was a weed plant and a rice plant in normal condition. The pot was not treated with a solution. They were watered every day with (70ml). These unwanted plants grow alongside agricultural plants which are of little significance; instead, they consume the nutrients provided to crop plants, reducing their supply to the primary crop plants. Thus, the result also showed the same. The weed had grown healthier than the rice. After few days rice plats get weaker.

Table no. 5: Table contains growth measures of both the plants:

Measurement	*Cyperus rotundas* (plant A)	Rice (plant B)
Day 1 (1st April,23)	7 cm	15 cm
Day 2 (7th April,23)	7.4 cm	15.5 cm
Day 3 (15th April,23)	8.3 cm	16.2 cm
Day 4 (19th April,23)	9 cm	16.8 cm
Day 5 (26th April,23)	9.7 cm	17 cm
Day 6 (2nd May,23)	10cm	17.3 cm
Day 7 (8th April,23)	10.4 cm	17.3 cm

Fig 16: Plants condition on day 1

Fig 17: Plants condition on day 7.

Experiment on test Plant:

The pot contains only *Cyperus rotundas* weeds. Those plants were treated with media containing *Pseudomonas* sp. bacteria whose concentration was 0.01% (V/V). The treatment was provided with a 70ml treatment solution on alternative days starting from 1st April,23 to 10th April,23. After observing for 7 days no changes had occurred. So, the concentration was a little higher from 0.1% to 0.2% and then the previous procedure was followed again from 12th April,23 to 22nd April,23. While the testing period, the progression of drying or killing those weeds was very slow. Therefore, the concentration was again increased by a little higher from 0.2% to 0.3% and then the previous procedure was followed again from 24th April,23 to 1st May,23. The expected result had been observed after applying 4 doses.

The first dose containing treatment of 0.3% inoculated solution had been given on 24th April 23. On the next day (25th April,23) of the first dose, the weeds were getting degraded by forming yellow colour at the tip of the leaves. Then on the next day (26th April,23), second dose was given. The plants were degrading fast. On fifth day (28th April,23), a third dose was provided to the weeds. They were 70% dried. On the seventh day(30th April,23) fourth dose was provided. On the next day (1st May,23), those weeds were completely dried which means that they were being killed or degraded by the treatment that was provided.

Fig 18: Plants' condition on the 25th of April,23.

Fig 19: Plants condition on the the28th

Fig 20: Plants condition on 1st May,23 (8th day)

Experiment on rice plants and weeds

In the third experiment, the pot contains weed and rice plants. This experiment was done to observe that *Pseudomonas* sp, can kill weeds and enhance crop plant growth. So, these plants were given Pseudomonas sp. bacterium medium at a concentration of 0.02% (V/V). From the 18th to the 23rd of April, a 70ml treatment solution was administered on alternate days.

From the 3rd day, the weeds are getting degraded and dried on the other hand the rice plants had grown very well and healthier.

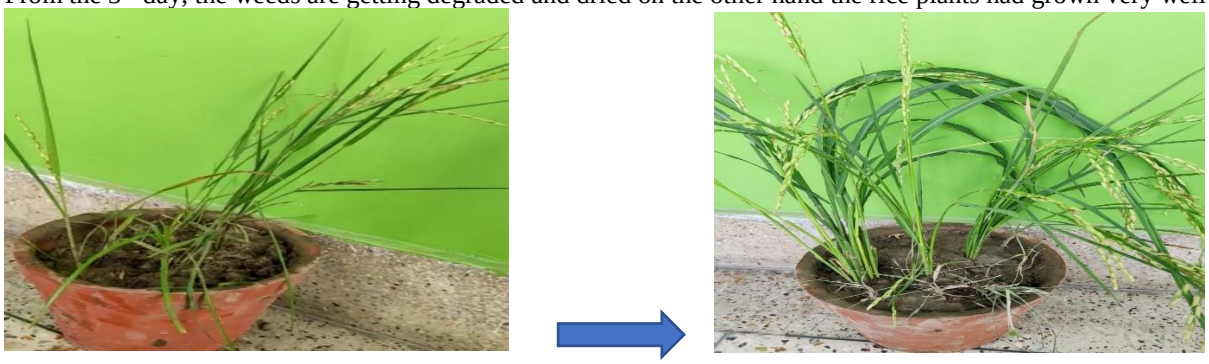

Fig 21: After adding the treatment for a few weeks

2. Conclusion:

Plant growth-promoting microorganisms (PGPM) are now one of the best alternatives to artificial fertilizers and pesticides for plant growth promotion and disease management. The exudates of plant roots interact with Pseudomonas species and aid in root colonization. *Pseudomonas* sp. has a beneficial influence on plant development and biological control in host plants. Pseudomonas

strains have also been widely used in the detoxification of some organic and inorganic water contaminants [4], as well as in the bioremediation of heavy metals and pesticides [6]. and it also has the ability to kill those unwanted plants the grow in crop fields which has no use in crops rather it uptake the nutrient from primary plant. *Pseudomonas* sp. can kill weeds by using quorum quenching, which is the process of interfering with the chemical signals of the weed's cells, preventing them from communicating with each other. This disrupts the ability of the weed to grow and spread, eventually resulting in its death. Pseudomonas bacteria also act as biocontrol agents by inhibiting the growth of other harmful pathogenic bacteria that cause plant diseases [11]. They produce antibiotics that are toxic to competing bacteria, which prevents the growth and spread of disease-causing bacteria. Environmental and consumer concerns have generated interest in the development of biocontrol agents as an alternative, environmentally friendly strategy to protect agricultural and horticultural crops from filo pathogens [15]. *Pseudomonas* sp. is one of the proven biological control agents. Many success stories by several scientists around the world describe various Pseudomonas strains that can significantly control various fungal, bacterial and nematode diseases such as cereals, horticultural crops and oilseeds.

3. Future aspect:

The extensive use of chemicals such as pesticides, pesticides and fertilizers has eroded the balance between the environment and the ecosystem. We rely too much on the use of chemicals in agriculture. Pesticides have the ability to be a quick solution to all our problems today, but they are creating serious problems for future generations. To maintain soil health and ecological balance, we must adapt to sustainable farming practices. To practice sustainable agriculture, the use of biocontrol agents for disease control and crop growth should be considered. Pseudomonas fluorescens is a PGPR useful for plant growth kills weeds and for the treatment of various plant diseases. It has been used in agriculture for decades as a biofertilizer and biocontrol agent. Future prospects include simple in situ propagation methods, longer shelf life, identification of potential biological regulator strains, and their inheritance, which plays an important role in plant defence against many biotic and abiotic organisms. Further insights into the up-and down-regulation of transcriptional, proteomics and transcriptional stressors may be included.

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