Biocontrol Properties and Functional Characterization of Rice Rhizobacterium *Pseudomonas* sp. VSMKU4036

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Abstract

A total of 30 fluorescent pseudomonads (FPs) were showed significant antagonistic activity against different fungal phytopathogens with different level of the zone of inhibition (ZOI) for *Rhizoctonia solani* (5mm-34mm), *Macrophomina phaseolina* (9mm-37mm), *Sclerotium rolfsii* (4mm-36mm), *Helminthusporium solani* (5mm-27mm), *Fusarium oxysporum* (2mm-25mm) and *Fusarium oxysporum* RACE (4mm-31mm) compared to control. The maximum growth of our selected isolate VSMKU4036 was observed in King'B Broth (KBB), pH 7.0 and at 37°C. The VSMKU4036 isolate has been recognized as *Pseudomonas* sp, based on the morphological, biological, and different functional characteristics. Antagonistic rhizobacterium *Pseudomonas* sp VSMKU4036 produced antimicrobial traits, such as plant growth promotion and various functional characters like siderophores, hydrogen cyanide (HCN), phosphate solubilization, indole acetic acid (IAA), biofilms formation, protease, gelatinase, amylase, and pectinase. Our superior biocontrol isolate VSMKU4036 was high resistance to tetracycline, streptomycin and nalidixic acid, however, it was sensitive to ampicillin and rifamycin. *Pseudomonas* sp VSMKU4036 showed maximum resistance to cadmium, nickel chloride, copper sulphate, magnesium sulphate, zinc chloride and ferric chloride where as highly sensitive to mercuric chloride, and selenium dioxide compared to control.

Keywords: *Pseudomonas* sp, biocontrol, functional characters, resistance, sensitivity

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INTRODUCTION

In recent scenario, the tremendous increase of population and industries are given a huge task to the environment and agriculture sectors by the way terrestrial and agricultural land has been polluted by industrial and human waste. Due to the accumulation of toxic heavy metals and continuous application of antibiotic residues have drastically inhibited the growth of the microbial population. Besides, about 90% of microbial beneficial character would be declined due to toxic heavy metals and chemical fertilizer deposits. Moreover, the soil is an ordinary source for all the plants, animals, and microorganisms because they depend on soil directly or indirectly for their survival. Rhizobacteria are responsible for promoting plant growth and protecting plants from various natural and artificial proceedings and also for the nutrient cycling in the soil (Liu et al., 2013). The intense use of chemical fertilizers and pesticides is causing significant threats to the atmosphere and to community health (Riah et al., 2014). The continuous usage of these chemicals’ fertilizers discharged the heavy metals through irrigation for a long duration in the agricultural field, suppose if keep on doing this practice in the field of agriculture, the soils will become impotent. Further, through this practice non-biodegradable materials enter into our food chain. Hence, most of the agriculturally important crops were attained the risk of all life forms (Lal et al., 2013).

The development of threshold chemicals is affecting microbial diversity and reduce soil fertility. It will create a negative impact on human health (Huang et al., 2009). Numerous Industries together with electroplating, chemical manufacturing, paper making, printing and dyeing, hardware and leather industries discharge their untreated effluents along with different heavy metals toward soil and water bodies. Hence, an alternative measure is urgently needed for reducing heavy metals. The use of bioherbicides, biopesticides, bioinsecticides, and bio fungicides, etc., is the alternative to the agrochemicals for the protection of the environment, plants and all kinds of herbivorous animals and rhizosphere microbes from the health defects. Therefore, promoting, this practice must be prime research to identify the potential biocontrol agents (Baishya and Sarma, 2014).

The rhizosphere directly influences the microbial substance with interrelationship to promote the richness of the population (Kloepper et al., 1999; Antoun and Kloepper, 2001). Free-living rhizobacteria are associated with the rhizospheric region to form symbiotic relationships with plants, the plant secrets specific organic compounds that are used for competition for nutrients and solid attachment sites, this type of mechanism unlighted to determine the symbiotic relationships (Singh et al., 2013).

Microorganisms are adapting themselves when they are exposed to polluted environments at different absorptions of heavy metals then microbes will become highly resistant to metals. Among different trace metals, an inorganic fertilizer phosphate becomes a major natural resource (Lu et al., 1992; Saberi and Hassan, 2014). Since, most of the antagonistic bacteria are solubilizing Phosphate, through which, microbes were stimulating the plant development. Naturally, soil-borne bacteria obtained, the potential for produce growth hormones, siderophores, antibiotics, symbiotic N₂ fixation, hydrolytic enzyme production, and other nutrients secretion for protection of plants from pathogenic microbes (Bharathi et al., 2004; Ahmad et al., 2006; Egamberdiyeva, 2007). Due to biocontrol and growth-promoting capacity for control of pathogens with diverse mechanisms (Shanmugaiah et al., 2010), fluorescent pseudomonades (FPs) have been attracting much attention worldwide in recent days.

Almost all species of pseudomonades can produce a growth substance like auxin, cytokinins, siderophore, Hydrogen Cyanide (HCN), production of antibiotics against multiple drug-resistant bacteria (MDR), antifungal and antibacterial effect of plant pathogens (Alstrom and Burns, 1989; Bauer et al., 1996; Glick et al., 1998; Lata, 2003). An antagonistic potential of toxic mineral resistant rhizobacterium is most important for plant health. Hence, this present research work focused with different objectives (i) Antagonistic activity of FPs towards different diseases causing soil-borne fungus (ii) screening for functional characters such as siderophore, HCN, IAA, biofilm, phosphate solubilization and hydrolytic enzyme production (iii) to evaluate the selected isolate VSMKU3046.
for resistance and sensitivity to various heavy metals and different antibiotics.

MATERIALS AND METHODS
Fluorescent pseudomonad’s (FPs)
A total of 30 FPs was isolated from rice rhizosphere, Southern districts of Tamil Nadu, India. All FPs were preserved in King’s B agar medium (King et al., 1954) and 30% glycerol stock at 80°C for further research work.

Fungal pathogens
The phyto pathogens were obtained from our laboratory collection and that have been maintained in a particular medium with a controlled environment. Further fungal strains in a specialized setting with the specific medium and maintenance were elaborately stated in our previous report (Nithya et al., 2019).

FPs against plant fungal pathogens
FPs were tested towards soil borne disease causing pathogens such as R. solani, S. rolfsii, M. phaseolina, H. solani, F. oxysporum and F. oxysporum. RACE by dual plate method with three replications (Shanmugaiah et al., 2010). One-day old FPs grown on King’s B agar was streaked at the edge of the Petri plate, 3 cm from the mycelial disk, and the disks were kept for three days at 28±2°C. Based on the superior antagonistic potential activity isolate VSMKU4036 was selected for additional studies.

Biochemical analysis for selected isolate VSMKU4036
The isolate VSMKU4036 was defined based on the features of morphological character, color development, and cluster appearances. Best owing to Bergey’s Manual of Determinative Bacteriology (Williams et al., 1994), most of the biochemical tests were performed.

Growth of Pseudomonas sp VSMKU4036 in different media
The isolate VSMKU4036 was defined based on three nutrient solutions like King’s B broth (KBB), Nutrient broth (NB) and Luria Bertani broth (LBB) was used to govern the best growth and cultural circumstances for the supreme growth. Approximately 100 ml of separate medium was fitted with a conical decanter in 250 ml after sterilization 1 ml of preculture (Pseudomonas sp VSMKU4036) was inoculated in each flask. Culture was introduced separately in each medium and that was kept on in environmental shaker at 28±2°C for 200 rpm up to 24 hrs. We quantified growth at 600 nm by spectrophotometer.

Growth of VSMKU4036 at various pH and temperature
Pseudomonas sp VSMKU4036 progress was optimized at various temperatures (25°C to 45°C) and pH (3–10). The growth of the isolate VSMKU4036 was raised in an environmental shaker at 200 rpm and the OD was read at 600 nm (Shanmugaiah et al., 2010). The growth was measured in King’ B broth up to 24 hrs compared to control.

Qualitative assay for IAA
Pseudomonas sp VSMKU4036 was grown in King’s B broth, added with 0.1mg/ml of Tryptophan in 250ml conical flask and incubated up to 48 hrs at 28 ± 2°C. The bacterial culture was collected by centrifugation at 10,000 rpm for 10 min. Cells free culture filtrate was assorted with 2 ml of Salkowski’s combination (2% 0.5 FeCl3 in 35% HClO4 solution) and incubate in a shady condition for about 30 min. The establishment of pink color specifies the creation of IAA.

Siderophore Production
Iron chelating agent siderophore creation by VSMKU4036 was observed by the technique of Schwyn and Neilands (1987). Biocontrol isolate VSMKU4036 was developed for 48 hrs in King’s B medium (King et al., 1954). King’s B medium inoculated with 2 mol l-1 pasteurized filter FeSO4·7H2O was used as a control aid. The culture was centrifuged for 10 min at 10,000 rpm, and 1 ml of cell-free suspension was combined with 1 ml of chrome-azurol S solution (Himedia, India). After 15 min, the medium color switched from blue to reddish-brown. Reddish-brown color development was indicated the production of siderophores by our selected isolate Pseudomonas sp VSMKU4036.

Production of Hydrogen cyanide (HCN)
The Lorck method (1948) estimated the output of HCN by VSMKU4036 isolate. Biocontrol isolate VSMKU4036 was developed for 48 hrs in King’s B medium (King et al., 1954). King’s B medium inoculated with 2 mol l-1 pasteurized filter FeSO4·7H2O was used as a control aid. The culture was centrifuged for 10 min at 10,000 rpm, and 1 ml of cell-free suspension was combined with 1 ml of chrome-azurol S solution (Himedia, India). After 15 min, the medium color switched from blue to reddish-brown. Reddish-brown color development was indicated the production of siderophores by our selected isolate Pseudomonas sp VSMKU4036.

Qualitative assay for Phosphate solubilization and Hydrolytic enzymes
Our fresh culture Pseudomonas spVSMKU4036 was spotted on the medium Pikovskaya (Pikovskaya, 1948) (pH 6.8) and hatched up to 2 days at 28°C. The clear zone was
observed around selected isolate VSMKU4036. The production of hydrolytic enzymes like chitinase, cellulase, gelatinase, protease, amylase and pectinase by our isolate VSMKU4036 in a nutrient agar medium, added with 1% individual substrates.

**Assay for Biofilm formation by Pseudomonas sp. VSMKU4036**

The selected isolate *Pseudomonas* sp. VSMKU4036 was inoculated in a glass tube containing King’s B nutrient solution hatched at 37°C in static condition up to two days. *Pseudomonas fluorescence* PF5 and *Pseudomonas fluorescence* CHAO were used as a positive control. After the incubation period, the culture was drained from the culture tube. The culture tubes were washed thoroughly with sterilized distilled water, followed by 1% of crystal violet solution was added to the attached cells in test tubes, and kept for 10 to 15 min at room temperature (Merritt et al., 2011). The culture tubes were rinsed with distilled water again to remove unwanted traces and the biomass of attached cells (biofilm) was quantified by solubilization of dye in 5 ml of 95% ethanol (Konstanze et al., 2019). The absorbance was measured at 600 nm.

**Heavy metal resistance/sensitivity test of VSMKU4036**

The resistance/sensitivity of isolated VSMKU4036 was tested with commercially available heavy metals like Lead acetate, Cadmium sulphate, Nickel chloride, Selenium dioxide, Zinc chloride, Ferric chloride, Copper sulphate, Mercurl chloride and Magnesium sulphate with different concentrations by well diffusion method. The 24h old culture of VSMKU4036 was swabbed on the nutrient agar (NA) and completely allowed to dry up to 10 min before making the wells. About 9 mm wells were made in NA by the sterile cork borer. After that, the above-mentioned heavy metals with various concentration (1, 2, 4, 6, 8 and 10 mM/ml) were introduced into the wells. The plates were hatched up to 24 hrs at 30°C till bacterial growth was detected. The diameter (mm) of each zone was measured and noted at the end of gestation. The sensitivity and resistance profiles were verified based on the diameter of the clearance of the zone and the assessment according to the standard chart.

**RESULTS AND DISCUSSION**

**Antifungal activity of isolate VSMKU4036**

Among 30 FPs, 27 isolates of FPs were shown the different spectrum of biocontrol activity towards various fungal plant pathogens. Of which an isolate was showed superior antagonistic activity against various fungal phytopathogens designated as VSMKU4036. The minimum antagonistic activity was observed against *R. solani*. However, the prominent antagonistic activity was observed against *M. phaseolina* from 9 mm to 38 mm compared to control (Table 1). Fluorescent pseudomonades are predominantly obtained from the various rhizosphere such as rice, sugarcane, groundnut and banana (Charulatha et al., 2013). Beneficial microbes like *Pseudomonas* spp, *Bacillus* spp, *Streptomyces* sp and *Tichoderma* spp are continuously giving strong antagonistic potential, further antagonistic microbe would be developed as a bio-inoculant for plant growth and plant protection (Shanmugaiah et al., 2008; Shanmugaiah et al., 2015; Harikrishnan et al., 2016; Vaishnavi et al., 2019). Among different PGPR, fluorescent pseudomonads are significant groups of microbes in agricultural crop protection.
Hence, the efficacy of FPs for the promotion of plant growth and the scope for biocontrol established by various researchers (Shanmugaiah et al., 2006; Zhou et al., 2012; Mohammed et al., 2020).

**Identification of selected isolate VSMKU4036**

Selected isolate VSMKU4036 is gram’s negative, motile, rod shape, catalase oxidase-positive and optimum temperature is 37°C. Further, based on the morphology, fluorescent pigment production, physiological and various biochemical analyses, the selected isolate was identified as *Pseudomonas* sp (Table 2).

FPs can produce pyocyanin pigments, which indicates, the production of phenazine. Phenazine pigments contain reactive nitrogen intermediate (RNI), RNI is important for antimicrobial activity. Hence, the presence of pyocyanine pigments indicates the selected isolate comes under the genus *Pseudomonas*. Some of the biochemical assays facilitate for identification of *Pseudomonas* spp up to the genus level, such as catalase, oxidase, citrate utilization, and mannitol reactions are positive compared to control (Charulatha et al., 2013).

**Optimum growth of VSMKU4036**

The maximum growth of selected isolate *Pseudomonas* sp VSMKU4036 was observed in King’s B Broth with a pH 7.0 at 37 °C compared to other media, pH, temperature, and control (Fig. 1, 2 & 3).

Numerous biotic and abiotic factors play a significant role in the development of microorganism both in lab and field conditions. For example, aeration is needed for aerobic bacterial and become a restrictive influence for the progress of *Pseudomonas* sp. The unexpected shape of the growth curve would be developed due to the depletion of oxygen and changes in metabolism. Most of the microbial growth, nitrate as an alternate electron acceptor and can grow an aerobically (Palleroni, 1991). Further, different carbon, nitrogen, and metals sources could be enhanced the production of microbial substances or products (Shanmugaiah et al., 2008).

**Biocontrol mechanism by *Pseudomonas* sp VSMKU4036**

Numerous biocontrol principles like Antibiosis, parasitism, induced resistance, and nutrient competition were identified as diverse mechanisms for biological control of various plant diseases (Harikrishnan et al., 2016). The remarkable bio-inoculants are giving good results under controlled conditions (Timmusk et al., 2017). Basic understanding of molecular action that leads to the advancement of bio-regulation of

<table>
<thead>
<tr>
<th>Table 1. Antagonistic activity of <em>Pseudomonas</em> sp. against plant fungal pathogens</th>
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<tbody>
<tr>
<td>No. Plant pathogenic fungus</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>4</td>
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<td>5</td>
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<td>6</td>
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<tr>
<th>Table 2. Biochemical analysis of <em>Pseudomonas</em> sp VSMKU4036</th>
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<tbody>
<tr>
<td>Morphology, biochemical and functional characters</td>
</tr>
<tr>
<td>Gram’s staining</td>
</tr>
<tr>
<td>Shape</td>
</tr>
<tr>
<td>Catalase</td>
</tr>
<tr>
<td>Oxidase</td>
</tr>
<tr>
<td>Blood Agar growth</td>
</tr>
<tr>
<td>Indole</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Mannitol</td>
</tr>
<tr>
<td>Methyl Red</td>
</tr>
<tr>
<td>VogesProskeur</td>
</tr>
<tr>
<td>SimmonCitrate Agar</td>
</tr>
<tr>
<td>Temperature</td>
</tr>
</tbody>
</table>
biological control agents (BCAs) is very important for the application of an actual antagonistic effect in agrarian fields.

In this present study, we selected isolate VSMKU4036 was produced hydrolytic enzymes like protease, amylase, gelatinase, and pectinase, however, the isolate VSMKU4036 did not produce chitinase and cellulase (Table 3). Hydrolytic enzymes like protease, gelatinase, amylase, pectinase, chitinase and cellulase play an important role in altering the cell walls of fungi. Because, these enzyme, which could be involved in the control of different plant diseases like Verticillium wilt of cotton, common blight of bean and rice diseases (Yang et al., 2008; Shanmugaiah et al., 2008; Zheng et al., 2011). Further, glucanase, siderophores, hydrogen cyanide, and volatile substance production showed the direct antagonistic consequence and for the nutrition competition, by the way, FPs are effectively controlling the sheath blight of rice and 

Our isolate VSMKU4036 produced various biocontrol traits like solubilization of phosphate, IAA, iron-chelating agents siderophore, HCN, and

![Figure 1](image1.png)

**Fig. 1.** Growth of *Pseudomonas* sp VSMKU4036 at different medium

![Figure 2](image2.png)

**Fig. 2.** Growth of *Pseudomonas* VSMKU4036 at various temperatures
biofilm formation compared to control (Table 3). Phosphate (P) solubilization is one of the most essential elements for plants survival and it is understood that P-solubilizing microbes are increasing inorganic phosphate by allowing the mineralization of impenetrable phosphate content to a soluble state via excreting organic acids by antagonistic FPs (Gyaneshwar et al., 2002; Buch et al., 2008). FPs can produce up to 50 % of the total rhizosphere microbial inhabitants and other soil microbes solubilize phosphates (Sharma et al., 2013).

The prime plant regulating substances such as IAA, phytohormones are stimulating and increasing plant biomass. Rhizobacteria (PGPR) is producing plant growth substances for the development of root and stem system (Vacheron et al., 2013). Similarly, FPs are concealing iron-starvation substance molecules to boost the Fe nutrition status in the field of agricultural sector (Ahemad and Kibret, 2014). Iron healing agents are recognized and produced immune system for the suppression of bacterial and fungal pathogens by the competition of iron attainment (Aznar and Dellagi, 2015). The volatile molecules are produced by plant obtained rhizobacterium, especially FPs against various plant pathogens. Various rhizosphere isolates are known to produce cyanogenic toxic effects on different prokaryotic as well as eukaryotic pathogens (Cernava et al., 2015). Biofilm layers are formed in all parts of plants (stems, leaves, plant rhizosphere, soil particles and organic compost) by rhizobacterial community producing exopolysaccharides (Prigent-Combaret et al., 2012).

In recent days research has been explored that antagonistic bacteria could form a bio-plastic layer and adhesion on most of the plants as well as rhizosphere. Due to the formation of biofilm by rhizobacteria more antimicrobial substance could be encouraged by sugar substance are called root exudates (Zhang et al., 2015; Zhou et al., 2016). The previous studies concentrated on the mechanisms of biocontrol and were linked to the possessions of the developed biofilm by antimicrobial material excretion.

**Antibiotic resistance/sensitivity**

Among five commercially available antibiotics, our isolate VSMKU4036 was high resistance to tetracycline, moderate resistance to streptomycin and nalidixic acid, however less resistant to ampicillin. Despite this, our isolate is highly sensitive to rifampicin compared to control (Table 4). According to our literature survey, *Pseudomonas stutzeri* ST6 found several resistant to heavy metals and antibiotics (Barbieri et al., 1989).

In agreement with our result, the majority of the bacteria are highly resistant to antibiotics, because of the rigorous usage of antibiotics together with agriculture and medicine (Levy, 1998). Moreover, in the Indian ecosystem predominantly antibiotic-resistant microbes were present in the agriculture and water bodies (Malik et al., 2008; Ansari et al., 2008). Based on the resistance and sensitivity, we could find out the stability and viability of antagonistic microbes in the rhizosphere of different agriculture crops. In general, the antibiotic resistance genes were transferred through transconjugation, since plasmids are ubiquitous in *Pseudomonas* spp. As per our observation, an exploration of the viability of antagonistic microorganisms in the agriculture field was confirmed through the transmission of conjugative plasmids or gene disperses by horizontal transfer. It is also one of the key reasons why multiple antibiotic resistances evolve (Malik and Aleem, 2011). But, in addition, antibiotic development of microorganisms associated with the rhizosphere is widely accepted for growth-promotion and biocontrol capacity against pathogenic microbes (Glick et al., 2007; Shanmugaiah et al., 2010).

**Heavy metal resistance/sensitivity**

Our selected isolate *Pseudomonas* sp VSMKU4036 exhibited a different range of resistance and sensitivity towards heavy metals. Among nine different heavy metals, our isolate was maximum resistance to cadmium sulfate, magnesium sulfate, copper sulfate, zinc sulfate, ferric chloride, and nickel chloride and less resistance to selenium dioxide. In comparison, the extreme sensitivity to acetate and mercury chloride as compared to control was observed (Table 5).

The earlier study was stated that, similar to our results most of the bacterial isolates showed high resistance to zinc and iron associated to
lead, in another way, some of the heavy metals like mercury and cadmium were the most toxic, thereby inhibiting the growth of rhizobacterial isolates even in lower concentration compared to control, because, if the concentration of heavy metals increase, the metal become representing a toxic effect on the development of rhizobacterial isolates (Mohan et al., 2016).

Continuous deposition of Cd in agriculture has resulted in various physiological, biochemical and plant absorption of mineral nutrients. In another way, cd accumulation could be disrupted the Calvin cycle and photosynthesis biochemical reactions (Mobin and Khan 2007). Since, the fluctuations in biochemical reaction in most of the plants, finally, the production level going to be very less compared to less heavy metal amended soils (Feng et al., 2010). Most of the previous studies showed that heavy metal resistance is expressed regularly by plasmid and transposon-positioned genes and is also transferable in situ microflora to native microflora (Malik and Jaiswal, 2000). Cadmium is not intricate in any helpful biotic processes, and it is recognized to disturb enzyme activities, impede DNA-mediated transformation in microorganisms. Further, this toxic metal is interfering with the symbiosis between microbes and plants, and increase plant disposition to attack of plant, fungal pathogens (Kabata-Pendias and Pendias, 2001).

Even, after a small yield of heavy metals such as Cd, Cd, and Mc was consumed by human and animals which enter into the food chain

### Table 3. Functional characters of *Pseudomonas* sp VSMKU4036

<table>
<thead>
<tr>
<th>Functional characters</th>
<th>Positive (+)/Negative (-)</th>
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<tbody>
<tr>
<td>Protease</td>
<td>+</td>
</tr>
<tr>
<td>Amylase</td>
<td>+</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>+</td>
</tr>
<tr>
<td>Pectinase</td>
<td>+</td>
</tr>
<tr>
<td>Cellulase</td>
<td>-</td>
</tr>
<tr>
<td>Chitinase</td>
<td>-</td>
</tr>
<tr>
<td>Phosphate solubilization</td>
<td>+</td>
</tr>
<tr>
<td>IAA</td>
<td>+</td>
</tr>
<tr>
<td>Siderophore</td>
<td>+</td>
</tr>
<tr>
<td>HCN</td>
<td>+</td>
</tr>
<tr>
<td>Biofilm formation</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 4. Evaluation of Antibiotic Resistance of *Pseudomonas* sp. VSMKU4036

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Antibiotics</th>
<th>Concentration (mM)</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tetracycline</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Ampicillin</td>
<td></td>
<td>-</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Rifampicin</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Streptomycin</td>
<td></td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>Nalidixic acid</td>
<td></td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

that can cause severe diseases like nerve and neuron relates diseases such as diabetic’s, cancer, paralysis, and eye blindness. Hence, biosorption, biodegradation, and biomineralization by the microorganisms is the only way for the alternative method to protect our ecosystem without any harmful effect.

**CONCLUSION**

Plant rhizospheric microorganism *Pseudomonas* sp. VSMKU4036 could be used as a microbial antagonist and can be used to remove pesticides, antibiotics deposits and deprivation of heavy metal from agricultural land. In addition, microorganisms of the rhizosphere may be developed as a current inoculant, a remarkable innovation and replacement for ecologically approachable plant disease control and increasing plant productivity.

**ACKNOWLEDGMENTS**

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

**AUTHORS’ CONTRIBUTIONS**

GV, carried out research and generate the data and drafted the manuscript. KN, PS, and MR worked for manuscript interpretation, NB, SG, and PTM outline and reviewed the manuscript and VS administered and revised the manuscript for improvisation. GS, PTM and VS read and approved the manuscript.

**FUNDING**


**ETHICS STATEMENT**

Not applicable.

**DATA AVAILABILITY**

All data were generated or analyzed during this study and included in the manuscript.

**REFERENCES**


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**Table 5. Heavy Metal Tolerance exhibited by *Pseudomonas* sp. VSMKU4036**

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>Concentration (mM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>-</td>
</tr>
<tr>
<td>Cadmium sulphate</td>
<td>++</td>
</tr>
<tr>
<td>Nickel chloride</td>
<td>++</td>
</tr>
<tr>
<td>Selenium dioxide</td>
<td>-</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>++</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>++</td>
</tr>
<tr>
<td>Mercuric chloride</td>
<td>-</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>++</td>
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