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Therapeutic Applications and Biological Activities of Secondary Metabolites Isolated from *Pseudomonas* species

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Abstract

Soil bacteria are the necessary part of the soil ecosystem and they are involved in organic matter mineralization, biogeochemical cycles of nitrogen and carbon, and their distribution can be changed by litter quality. Soil samples were acquired from different locations of district Peshawar. To isolate *Pseudomonas* species serial dilution of the samples were performed by using Nutrient agar medium. Each dilution was added to sterile plates aseptically and the plates were incubated at 37°C for 24 hours followed by sub-culturing. A combination of technique, including colony morphological evaluation, gram staining and biochemical testing were used to identify isolated bacterial colonies after incubation. The antimicrobial activity of Pseudomonas species were subjected to primary screening against various test organisms (*Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*). During the antibacterial activity at 75µl concentrations of secondary metabolites, 11mm, 13mm and 19mm zones were made against *S. aureus*, *Klebsiella*, and *E. coli* respectively. At 100 µl concentration secondary metabolites showed 15mm,17mm and 24mm zones against *S. aureus, Klebsiella* spp. and *E. coli* respectively. The secondary metabolites of *pseudomonas* spp were tested for its phytotoxic activity against Lemna minor. (Metabolites did not show any phytotoxic activity). Insecticidal activity of the metabolites was performed on Black Garden ant (*Rhyzopertha dominica* and *Tribolium castaneum*). A total of 50 ants were used in this test. It was concluded from our study that *Pseudomonas spp* isolated from different locations of district Peshawar has antagonistic ability to inhibit growth of different pathogenic bacteria.

KEYWORDS
Pseudomonas species*;* secondary metabolites; *Klebsiella; E. coli* and *S. aureus.*

1.0 INTRODUCTION

Soil is generally known as the outer loose material of the earth crust, regarded as a three-phase system usually consist of solids, liquids and gases which are dispersed forming a heterogeneous matrix The soil environment constantly varies with locality and their constituents are not the same in all soil [1]. Soil bacteria are the necessary part of the soil ecosystem and they are involved in organic matter mineralization, biogeochemical cycles of nitrogen and carbon, and their distribution can be changed by litter quality [2], and soil properties as well as precipitation and temperature of different climatic conditions [3].

Soil and roots host a wide diversity of microbial communities, playing important roles in decomposition of organic matter, nutrient flow and

cycling and plant health. Humans often treat soil like dirt and pollution, but the fact is that the soil is serving as the nutrient base and is the most valuable habitat on this planet, and also functions as a gigantic bioreactor for degradation of pollutants as it sustains all life forms in the ecosystem and is very necessary for the existence of human life [4].

Microorganisms isolated from the soil provide most of the antibiotics which have adequately improved human health in the 20th century. In spite of history, studies are still in progress for the discovery of new antibiotics. So far, the bacteria have been considered as the most promising resource of antibiotics in the past and will surely remain a necessary resource of innovative natural products in the future as well. It has been observed that 80% of the total world's antibiotics are mostly produced from microorganisms [5].

Antibiotic resistance in bacteria is a major problem and is increasing day by day. Thus, urgent need to search the source of new antibiotics to control bacterial resistance. During last few years lot of work has been done that has observed the production of new antibiotics from various microbes. Soil fungi and bacteria have showed noteworthy and a vital role in the discovery of antibiotic. Soil depending upon environmental condition like type of vegetation, nutrient availability soil texture, presence of moisture in soil and cover the numbers and species of microbes differ. The number of microorganisms varies according to the environmental conditions [6].

Antibiotics have been used in human medicine and livestock welfare. However, misuse of antimicrobials in any field result in the development of antibiotic-resistant pathogenic microbes that affect both human and animal health. From the last few decades, use of antimicrobials in poultry production has been increased tremendously. Apart from using for therapeutic purposes, antibiotics are being used as growth promoters and prophylaxis [6]. Many studies provide evidence of spreading resistant bacterial strains from food animals to humans in the USA, European countries, and Denmark. Commonly known MDR pathogens *are E. coli, A. baumannii, Klebsiella, Salmonella*. and *P. aeruginosa*. Transmission of *P. aeruginosa* resistant to amoxicillin, Augmentin, tetracycline, cotrimoxazole, and chloramphenicol from poultry environment to humans was reported in Nigeria [6].

Pseudomonas fluorescens strains are antibiotic producers which having a wide spectrum of antimicrobial activity against pathogenic bacteria such as *Salmonella typhimurium, Proteus vulgaris, Candida albicans, Bacillus subtilis* and *Escherichia coli* [7]. Studies also showed that *Pseudomonas spp.* could also suppress 92% of sclerotia germination of Sclerotium rolfsii. Also, it is found that 92% of stem rot disease intensity could be suppressed when soil is inoculated with Pseudomonas fluorescens. The antagonistic effect of *pseudomonas spp* could also decrease the disease severity of some plant pathogens such as Fusarium wilt on shallot [8].

Pseudomonas species are resistance to a different variety of antibiotics including quinolones, and aminoglycosides and β-Lactamase [8]. As many as 3,800 biologically active microbial metabolites have been isolated from different bacterial species. *Pseudomonas* species produces 795 bioactive substances, including 610 antibiotics and 185 substances with bioactive properties other than

antibiotic activity. More bioactive substances have been isolated from terrestrial *Pseudomonas aeruginosa* (*P. aeruginosa)* than from marine *P. aeruginosa*, and there are a vast number of terrestrial *Pseudomonas* species that are well known as producers of bioactive substances [9].

Pseudomonas strains are ideal biocontrol agents for inhibiting crop diseases. Many *Pseudomonades* are capable of producing antimicrobial compounds, like phenazines, pyrrolnitrin, phenazine-1-carboxamide,phenazine-1-carboxylic acid, 2,4-diacetylphloroglucinol, rhamnolipids, butyrolactones, N-butylbenzene sulfonamide and caralicin. In addition, *Pseudomonas* produce chitinases and proteases. Their production is mainly induced by the biomass of fungal pathogens and their cell walls [10].

Secondary metabolites that are the object of natural product chemistry studies. The wide structural variability of these compounds has attracted the curiosity of chemists and their biological activities have inspired the pharmaceutical industry to search for lead structures in microbial extracts. Screening of microbial extracts reveals the large structural diversity of natural compounds with broad biological activities, such as antimicrobial, antiviral, immunosuppressive, and antitumor activities, that enable the bacterium to survive in its natural environment [11].

The present research study was mainly focused on exploration of *P. aeruginosa* for maximum production of metabolites against multidrug resistant bacteria. In the current scenario, antibiotic resistance phenomena are so high to the existing antibiotics available in the market. Therefore, there is a need to introduce new antibiotic producing microbial species that produce multidimensional secondary metabolites which was effective against bacterial pathogenic strains.

2.0 MATERIALS AND METHODS

2.1 Samples Collection and Processing

Soil samples were acquired from different locations of district Peshawar. Surface level soil samples were collected in polyethylene bags and were processed following standard procedure [12].

2.2 Isolation and Identification of *P. aeruginosa*

Nutrient agar medium was used to isolate *Pseudomonas* species. Serial dilution of the samples were performed. Each dilution was added to sterile plate using sterile pipette under aseptic conditions and the plates were incubated at 37°C for 24 hours. To isolate *Pseudomonas* species sub-culturing on nutrient agar medium was carried out. A combination of technique, including colony morphological evaluation, gram staining and biochemical testing (citrate test, indole and methyl red test) were used to identify isolated bacterial colonies after incubation. Clinical Laboratory Standards Institute (CLSI, 2020) guidelines were followed to ensure accuracy and reliability in the laboratory practices [13].

2.3 Antimicrobial activity of *Pseudomonas* species

The antimicrobial activity of *Pseudomonas* species was subjected to primary screening against various test organisms (*E. coli, Staphylococcus aureus* and *Klebsiella pneumoniae*) from Microbiology lab, Abasyn University Peshawar using the cross-streak method on Muller-Hinton Agar (MHA). For the

determination of antimicrobial activities, a modified cross-streak method (MCSM) was used. For this purpose, MHA plates were prepared which were inoculated with bacterial isolates by a single streak in the center of the Petri dish which were incubated for 48hrs at 37°C. This long-term incubation was given to the active microorganism to provide enough time to them to produce the antibacterial substance, which was, in turn, diffuse into the medium. In the cross-streak method, a specific distance was kept between the test pathogens streak i.e. 1 cm; while the length and width of the streak line were 3 cm and 0.5 cm, which were streaked perpendicularly to the streak of antibacterial growth, whereas, the length and width of antibacterial streak should be 7 cm and 0.5 cm. These plates were then incubated for 48hrs in order to determine that do the test organisms made any growth towards the antibacterial streak, or whether they remain static, or whether some kind of lysis of the test pathogens occurred [14].

2.4 Secondary metabolites Extraction

The shake flask fermentation method was used for the production of secondary metabolites. The Broth culture was used as a production medium. Then the medium was incubated in an orbital shaking incubator using 150 rpm at 35°C for 24 to 72 hrs. After an incubation period of 14 days, secondary metabolite extraction was carried out. About 200-500 µL of 40% hydrochloric acid (HCL) were added to the bacterial culture. After that, the culture was blended using a blender. An equal volume of ethyl acetate was added and the culture were mixed for 40 min. This culture slurry was filtered through cheese cloth. The filtered medium was added to a separated funnel and allowed to stand for 10min that were resulted in two layers. The organic layer containing metabolites of interests were separated from the aqueous phase and were washed with 2M brine solution to purify further. To remove remaining traces of water from the organic layer, anhydrous sodium sulphate (Na^2SO4) were added to it. Finally, the isolated metabolites were then concentrated by rotary evaporator at 45°C and 150 rpm (Revolutions per minute) [12].

2.5 Bioactivities

2.5.1 Antibacterial activity

Identified MDR bacterial species (*Klebsiella, E. coli* and *S. aureus*) were collected from Microbiology Research Laboratory (MRL) of Abasyn University Peshawar. The secondary metabolites of *P. aeruginosa* were screened against these bacterial strains. The agar well diffusion method was used for the screening of Antibacterial Activities [12].

2.5.2 Phytotoxic Activity

The Metabolites of *P. aeruginosa* was evaluated for phytotoxic activity against *Lemna minor plant.* The E-medium were prepared by using all components in appropriate amount and were autoclaved at 121°C temperature at 15 Psi pressure. Dimethyl sulfoxide was used to prepare a stock solution (1mg/mL). From this stock solution 10, 100 and 1000 µg/mL concentrations were used [12].

2.5.2.1 Procedure:

A stock solution of the test sample was prepared 30 mg/ 1.5mL using Dimethyl sulfoxide (DMSO). Different concentrations (i.e., 10, 100 and 1000 µg/ml) were prepared from this stock solution. 20 mL medium

was added to each sterilized plate and after proper examination ten healthy plants of *Lemna minor* plants were transferred to plates containing the medium. It was made sure that each plant had a rosette of three healthy fronds. The plates were marked as 10, 100 and 1000 and the desired dose concentration was added to each plate. One plate was taken as a control to which only the medium was added. The plates were kept in a growth chamber and the temperature was set at 30 C^0 and humidity was maintained at 60 by placing a beaker of sterilized water in the growth chamber and a photoperiod of 12 hours for seven days. DMSO was used as negative control and parquet as a positive control. On the eighth day, the fronds were visually examined and percent growth inhibition was calculated using the following formula [12].

$$\text{Growth inhibition (\%)} = \frac{\text{No of fronds in sample}}{\text{No of fronds in Control}} \text{ X } 100$$

2.5.3 Insecticidal Activity

The metabolites of *P. aeruginosa* were tested for insecticidal activity. Filter paper was adjusted to the size of petri dishes and was positioned in the petri dish. The activity was performed at concentrations rate of 10, 100 and 1000 µg. A total of 10 healthy insects (*Rhyzopertha dominica* and *Tribolium castaneum*) Black Garden ant was introduced in each plate separately and the result were observed after 24, 48 and 72 hours.

2.5.4 Statistical Analysis

Data were shown in graphs and tables by using Microsoft excel and ANOVA chi squares. Categorical parameters were reported as the number and percentage. The data were analyzed using SPSS software version 22.0 [15].

3.0 RESULTS AND DISCUSSION

3.1 Isolation of *Pseudomonas* species

A total 10 Soil samples were collected from different locations of district Peshawar. Nutrient agar medium was used to isolate *Pseudomonas* species. Serial dilution of the samples was performed. To isolate *Pseudomonas* species sub-culturing on nutrient agar medium was carried out as shown in Figure 1.

3.2 Microscopy and Biochemical Test Result

For the identification of bacterial isolates, different biochemical tests were performed like catalase, oxidase, coagulase, TSI, Urease and Indole tests following standard procedure.

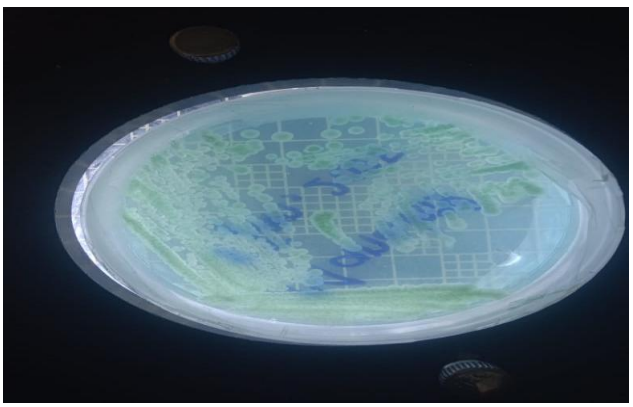


Figure 1 *Pseudomonas spp* growth on Nutrient agar media

3.3 Secondary Metabolites Production and Extraction

After incubating the fungus in Nutrient broth for 10 days, the secondary metabolites produced in the flasks were isolated through ethyl acetate. The rotary evaporator was used to separate the Ethyl acetate from metabolites and the metabolites produced were weighed and their values were recorded as 0.9564.

3.4 Antibacterial activity of secondary metabolites

In the current study 75 and 100µl concentration of secondary metabolites were used. The antibacterial potential of secondary metabolites was checked against *Klebsiella*, *E. coli* and *S. aureus*. At 75 µl concentrations showed 11mm.zone against *S. aureus* and 13mm zone against *Klebsiella* and and 19mm zone. against *E. coli*. At 100 µl concentration secondary metabolites showed 15mm.zone against *S. aureus*, 17mm zone against *Klebsiella spp.* and 24mm zone against *E. coli.* The Positive control was ciprofloxacin. DMSO was used as negative control. Result are shown in Fig.2 and Table 1.

Table 1: Antibacterial activity of secondary metabolites

S. No	Isolates	secondary metabolites Zone of inhibition (mm)		
		75 µl	**100 µl**	**+ve control**
1.	*E. coli*	19±0.58	24±0.42	26±0.56
2.	*Klebsiella spp.*	13±0.56	17±0.52	22±0.56
3.	*S. aureus*	11±0.47	15±0.52	19±0.56

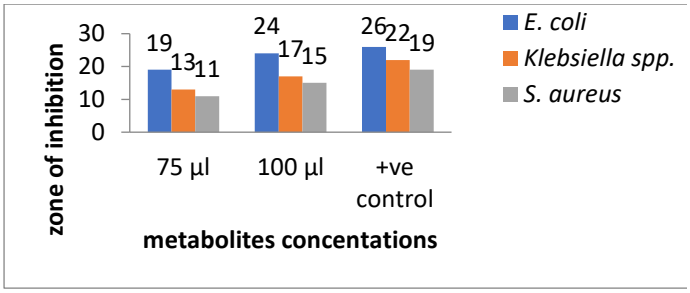


Figure 2 Antibacterial activities of secondary metabolites isolated from *pseudomonas spp.*

3.5 Phytotoxic Analysis

The secondary metabolites of *pseudomonas spp*. were tested for its phytotoxic activity against *Lemna minor*. Metabolites did not show any phytotoxic activity

3.6 Insecticidal activity of *Rhyzopertha dominica*

Insecticidal activity of the metabolites was performed on Black Garden ant (*Rhyzopertha dominica*) in which ethanol was used as positive control while methanol as negative control. A total of 50 ants were used in this test (10 for negative control, 10 for Positive control while 30 for different concentration of metabolites). The results have been shown in the table 2.

Table 2: Insecticidal activity of secondary metabolites against *Rhyzopertha dominica*

Positive control (Ethanol) Ants that died out of 10 ants.	**Concentration** (secondary metabolites)	**No. of ants that died out of 10.**	**Death (%)**
3	50%	0	0
	75%	3	30
	100%	4	40

3.7 Insecticidal activity of *Tribolium castaneum*

Insecticidal activity of the metabolites was performed on Black Garden ant (*Tribolium castaneum*) in which ethanol was used as positive control while methanol as negative control. A total of 50 ants were used in this test (10 for negative control, 10 for Positive control while 30 for different concentration of metabolites). The results have been shown in the table 3.

Table 3. Insecticidal activity of crude metabolites of *Pseudomonas spp.*

Positive control (Ethanol) Ants that died out of 10 ants.	**Concentration** (secondary metabolites)	**No. of ants that died out of 10.**	**Death (%)**
3	50%	0	0
	75%	4	40
	100%	5	50

3.8 Combinatorial Activity of metabolites

Synergistic Effect of Antibiotics and metabolites Synergistic effect was determined using Kirby–Bauer disk diffusion method. During study it was observed that coated Ceftriaxone showed highest activity of (33.3±0.9mm), followed by Ciprofloxacin (30±0.5mm), against *E. coli* Antibacterial activity against *S. aureus* c revealed that maximum activity was shown by Ceftriaxone (31.2±0.6mm) followed by Ciprofloxacin (28.6±1.1mm). Antibacterial activity against *K. pneuminia* revealed that maximum activity was shown by Ceftriaxone (21.2±0.6mm) results as shown in table 4.

Table 4 Combinatorial Activity Antibiotics with metabolites

S. No	**Antibiotics with Metabolites**	***E. coli* F. (Zone of inhibition)**	***S.aureus* (Zone of inhibition)**	*K. Pneumonia* L. **(Zone of inhibition)**
1	Ampicilli	19±1.1mm	19±0.5m	14±0.5mm
2	Ciproflox	29±0.5mm	28.6±1.1	18.6±1.1mm
3	Co-	25±0.5mm	25±0.8m	15±0.8mm
4	Chloramp	26±0.5mm	24±0.5m	14±0.5mm
5	Ceftriaxo	33.3±0.9mm	31±0.5m	21±0.5mm

3.9 DISCUSSION

In the current study A total 10 Soil samples were collected from different locations of district Peshawar. Nutrient agar medium was used to isolate *Pseudomonas* species. Serial dilution of the samples was performed. To isolate *Pseudomonas* species sub-culturing on nutrient agar medium was carried out. For the identification of bacterial isolates, different biochemical tests were performed like catalase, oxidase, coagulase, TSI, Urease and Indole tests following standard procedure.

In the previous study it is evaluated that secondary metabolite of microorganisms, have recently gained much attention for their potential application as biologically active antimicrobial compounds. The present study was performed in order to investigate the production of a biologically active phenazine compound by a locally isolated strain of *P. aeruginosa*. Thirty different strains were isolated from soil and water on a selective medium. The production ability of the studied strain was then improved by studying the effect of different physiological and growth conditions. The results showed that the maximum output of phenazine compounds was obtained when the isolated strain was cultivated in mineral salt medium (MS), under shaking conditions of 100 rpm, for five days at 35°C. The secondary metabolites produced were finally extracted using ethyl acetate and subjected to TLC for compounds separation. The selected band exhibited potent antimicrobial, antioxidant and anticancer activities[16].

In the current research study, the antibacterial potential of secondary metabolites were checked against *Klebsiella, E. coli* and *S. aureus*. At 75 µl concentrations showed 11mm.zone against *S. aureus* and 13mm zone against *Klebsiella* and 19mm zone against *E. coli*. At 100 µl concentration secondary metabolites showed 15mm.zone against *S. aureus,* 17mm zone against

Klebsiella spp. and 24mm zone against *E. coli.* The Positive control was ciprofloxacin. While in the other study various *in vitro* screening approaches were used to test the antimicrobial activity of the isolated actinobacteria to test against different bacterial strains including; *p. aeruginosa, S. hemolytic, Escherichia coli, S. typhi*, *Enterobacter spp.* and *S. aureus*. *S. thermolilacinus* strain exhibiting significant broad-spectrum antagonistic activities against MDR pathogens that could serve as a candidate for novel drug discovery[17].

In the current study insecticidal activity of the metabolites was performed on Black Garden ant (*Rhyzopertha dominica*) in which ethanol was used as positive control while methanol as negative control. A total of 50 ants were used in this test (10 for negative control, 10 for Positive control while 30 for different concentration of metabolites). Insecticidal activity of the metabolites was performed on Black Garden ant (*Tribolium castaneum*) in which ethanol was used as positive control while methanol as negative control. A total of 50 ants were used in this test (10 for negative control, 10 for Positive control while 30 for different concentration of metabolites).

In the previous study researchers identified an insecticidal biosurfactant produced by *Pseudomonas protegens* F6 (F6) and examined its use for aphid control. The growth of F6 was accompanied by increased aphid mortality and decreased water surface tension. Bioassay-guided chromatography coupled with instrumental analyses, nuclear magnetic resonance (NMR), and time-of-flight mass spectrometer (TOF MS) identified orfamide A as a major metabolite that showed insecticidal activity against green peach aphid (*Myzus persicae*). This is the first report of orfamide A as an insecticidal metabolite against *Myzus persicae* [18].

In the other study researchers inoculated phytotoxic activity plants with *P. stutzeri*, the expression of these genes was further enhanced. In conclusion, *P. stutzeri* inoculation had a positive effect on the growth and antioxidant system of *L. minor* treated with NP as it enhanced RGR, increased chlorophyll and carotenoid contents, and decreased Cu content and oxidative stress. These findings suggested that *P. stutzeri* has the potential to promote aquatic plant growth and counteract the negative impacts of NP on these plants. While in the present study the secondary metabolites of *pseudomonas spp.* were tested for its phytotoxic activity against *Lemna minor*. Metabolites did not show any phytotoxic activity[19].

4.0 CONCLUSION

It is concluded from current study that the isolated bacteria showed excellent growth on Nutrient Broth media at the temperature range of 35 °C to 37°C. Against all the three MDR bacterial species e.g. *Klebsiella, E. coli* and *S. aureus,* the EtOAc extract of *Pseudomonas spp.* exhibited inhibition. Insecticidal activity assay of crude EtOAc extract was determine. Crude EtOAc extract exhibited significant activity at 100microlitr concentration. The secondary metabolites of *pseudomonas spp.* were tested for its phytotoxic activity against *Lemna minor*. Metabolites did not show any phytotoxic activity. Insecticidal activity of the metabolites was performed on Black Garden ant (*Rhyzopertha dominica*) in which ethanol was used as positive control while methanol as negative control. A total of 50 ants were used in this test (10 for negative control, 10 for Positive control while 30 for different concentration of metabolites). It was concluded from our study that bacteria isolated (*Pseudomonas spp*) from different locations of district

Peshawar has antagonistic ability to inhibit growth of different pathogenic bacteria.

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CONFLICT OF INTEREST
The authors declare no conflict of interest

REFERENCES

[1]Geatz, G. W., Needelman, B. A., Weil, R. R., & Megonigal, J. P. (2013). Nutrient Availability and Soil Organic Matter Decomposition Response to Prescribed Burns in Mid-Atlantic Brackish Tidal Marshes. *Soil Science Society of America Journal*, *77*(5), 1852-1864.

[2]Oh, S., Lu, N., Kim, Y. K., Lee, S. J., & Lee, S. R. (2012). Relationship between the soil-water characteristic curve and the suction stress characteristic curve: Experimental evidence from residual soils. *Journal of Geotechnical and Geoenvironmental Engineering*, *138*(1), 47-57.

[3]Nielsen, U. N., Osler, G. H., Campbell, C. D., Neilson, R., Burslem, D. F., & Van der Wal, R. (2010). The enigma of soil animal species diversity revisited: the role of small-scale heterogeneity. *PLoS One*, *5*(7), e11567.

[4]Nannipieri, P., Trasar-Cepeda, C., & Dick, R. P. (2018). Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biology and Fertility of Soils*, *54*, 11-19.

[5]Jiao, F., Wen, Z. M., & An, S. S. (2013, December). Soil water storage capacity under chronosequence of revegetation in Yanhe watershed on the Loess Plateau, China. In *SpringerPlus* (Vol. 2, No. 1, pp. 1-5)

[6]Deveau, A., Gross, H., Palin, B., Mehnaz, S., Schnepf, M., Leblond, P., ... & Aigle, B. (2016). Role of secondary metabolites in the interaction between Pseudomonas fluorescens and soil microorganisms under iron-limited conditions. *FEMS microbiology ecology*, *92*(8), fiw107.

[7]Raaijmakers, J. M., & Weller, D. M. (1998). Natural plant protection by 2, 4-diacetylphloroglucinol-producing Pseudomonas spp. in take-all decline soils. *Molecular Plant-Microbe Interactions*, *11*(2), 144-152.

[8]Schito, A. M., & Alfei, S. (2020). Antibacterial activity of non-cytotoxic, amino acid-modified polycationic dendrimers against *Pseudomonas aeruginosa* and other non-fermenting gram-negative bacteria. *Polymers*, *12*(8), 1818.

[9]Munhoz, L. D., Fonteque, J. P., Santos, I. M. O., Navarro, M. O. P., Simionato, A. S., Goya, E. T., ... & Andrade, G. (2017). Control of bacterial stem rot on tomato by extracellular bioactive compounds produced by *Pseudomonas aeruginosa* LV strain. *Cogent Food & Agriculture*, *3*(1), 1282592.

[10]Yahia, M., Mohamed, M., Othman, M., Mostafa, D., Gomaa, M., Fahmy, M., ... & Abu-Hussien, S. (2020). Isolation and identification of antibiotic producing *Pseudomonas fluorescens* NBRC-14160 from Delta Soil in Egypt. *Arab Universities Journal of Agricultural Sciences*, *28*(3), 797-808.

[11]Sansinenea, E., & Ortiz, A. (2011). Secondary metabolites of soil *Bacillus spp*. *Biotechnology letters*, *33*, 1523-1538.

[12]Idrees, M., Ahmad, B., Ahmad, K., Bashir, S., Ali, A., & Aman, K. (2018). Anti-tuberculous, phytotoxic and insecticidal activities of secondary metabolites obtained from *Aspergillus* and *Penicillium* species isolated from soil. *Journal of Pakistan Medical Association*, *68*(1024).

[13]Ema, F. A., Shanta, R. N., Rahman, M. Z., Islam, M. A., & Khatun, M. M. (2022). Isolation, identification and antibiogram studies of *Escherichia coli* from ready-to-eat foods in

Mymensingh, Bangladesh. *Veterinary World*, *15*(6), 1497

[14]Joshi, S. R., Banerjee, S., Bhattacharjee, K., Lyngwi, N. A., Koijam, K., Khaund, P., & Nongkhlaw, F. M. (2015). Northeast Microbial Database: a web-based databank of culturable soil microbes from North East India. Current Science, 1702-1706

[15]Allen, P., Bennett, K., & Heritage, B. (2014). *SPSS statistics version 22: A practical guide*. Cengage Learning Australia.

[16]El-Masry, H. M., Atwa, N. A., El-Beih, A., Agwa, M., Khafagi, I. K., Mansour, S. R., & Eldiwany, A. I. (2023). Phenazine-Producing Pseudomonas aeruginosa OQ158909: A Promising Candidate for Biological Activity and Therapeutic Applications. *Egyptian Journal of Chemistry*, *66*(11), 281-303.

[17]Talpur, M. K. A., Qazi, M. A., Phulpoto, A. H., Maitlo, M. A., Phulpoto, I. A., Syed, F. H., ... & Kanhar, N. A. (2020). Bioprospecting actinobacterial diversity antagonistic to multidrug-resistant bacteria from untapped soil resources of Kotdiji, Pakistan. *Biologia*, *75*, 129-138.

[18]Jang, J. Y., Yang, S. Y., Kim, Y. C., Lee, C. W., Park, M. S., Kim, J. C., & Kim, I. S. (2013). Identification of orfamide A as an insecticidal metabolite produced by *Pseudomonas protegens* F6. *Journal of agricultural and food chemistry*, *61*(28), 6786-6791.

[19]Gulmez, O., Aksakal, O., Baris, O., & Bayram, E. (2023). *Pseudomonas stutzeri* improves the tolerance of Lemna minor to Cu (OH) 2 nanopesticide by regulating the uptake of copper, antioxidant defense mechanisms, and the expression of metacaspase-1, chlorophyllase, and stress-responsive genes. *Plant Physiology and Biochemistry*, *203*, 108002.