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Investigation of the biodegradability of pendimethalin by *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Escherichia coli*

Zeinab Avarseji*, Fakhtak Taliei, Ebrahim Gholamalipuor Alamdari, Masoumeh Hoseini

University of Gonbad Kavous, Plant Production Department, Gonbad Kavous, Iran

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ABSTRACT

Pendimethalin is a persistent herbicide. It is the third most widely used selective herbicide applied in soil that negatively affects humans and the environment. The current experiment assessed the ability of three bacterial species to degrade this herbicide. Pendimethalin was added to flasks in a 125 mg/L concentration and 10^7 CFU.mL^{-1} of *Bacillus subtilis, Pseudomonas fluorescens*, and *Escherichia coli* were added separately to the mineral salts medium media (MSM) and stored on a rotary shaker. The bacterial cell number, wet biomass, and chemical oxygen demand (COD) were determined after seven days. The concentration of pendimethalin residue was then determined using high-performance liquid chromatography (HPLC). A completely randomized design (CRD) with three replicates was used to arrange the experimental units, except for HPLC with only one replicate. The experimental results showed that all three bacterial growths rose after seven days post-inoculation in the pendimethalin modified media. A comparison of the growth kinetics of bacteria in the herbicide modified media and the control showed that the bacteria grew faster in the presence of the herbicide. The reduction in the COD parameter occurred in all the tested bacteria, but the highest COD removal efficiency (85%) was observed with *B. subtilis*. The highest biological degradation of pendimethalin compared to the control occurred in the *B. subtilis* inoculated media (78%), which also produce the most cell density. Based on the HPLC results, all three bacterial species were capable of biodegrading pendimethalin herbicide, with *B. subtilis* as the most effective bacterium, followed by *E. coli* and *P. fluorescens*.

1. Introduction

Pendimethalin is a pre-emergence herbicide from the dinitroaniline family that is applied extensively nowadays and selectively controls specific broadleaf and a large number of grassy weeds in some crops [1]. It is also the third broadly utilized herbicide after glyphosate and parquet; it is the most commonly applied selective herbicide globally [2], initially registered as a herbicide in the United

*Corresponding author. Tel: +98911371036
E-mail: avarseji@gonbad.ac.ir
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States in 1972 [3]. The primary mode of action of pendimethalin is explained as inhibition of cell division and elongation that is accountable for chromosome dissociation and cell wall generation [4]. Pendimethalin is one of the persistent herbicides in the soil, with a complex chemical structure that can be firmly adsorbed by the soil. It has a 60-day half-life under tropical circumstances [5]. As a result of low water solubility (0.33 mg/L) and high soil organic carbon-water partitioning coefficient (Koc=17581), pendimethalin has a low possibility for leaching [6]. Based on the United States Environmental Protection Agency (USEPA) report, pendimethalin is categorized as a persevering bio-accumulative herbicide [7] that biomagnifies up to 70,000 times their original dose [8]. It is also extremely poisonous to marine and terrestrial invertebrates [9] and promotes thyroid follicular cell adenomas in rats [10]. It contains dinitroanilines that could produce carcinogenic nitrosamines [7], a possible reason for being categorized as a human carcinogen [10].Pendimethalin usually degrades in the environment in a biotic and abiotic manner, including volatilization, photo-degradation, and biodegradation [5]. However, its microbial degradation is an effective approach in the soil. A microorganism's degradation mechanisms consist of hydrolysis, oxidation, dealkylation, and reduction [11]. Furthermore, the metabolic pathways of many microorganisms with the potential to degrade pendimethalin have been identified [12]. In other words, microorganisms can utilize a range of xenobiotic compounds, including herbicides, for their growth and then mineralize and detoxify them [13]. Bioremediation is an accepted method for promoting the cleanup rate of polluted soil and water. Frequent exposure to a pesticide may evolve new abilities for soil microorganisms to break down such a chemical [14]. For example, it was reported that *Pseudomonas fluorescens* degraded cadmium and hexavalent chromium to about 75.9%, and 61.0% during the 24 hours after treatment, respectively [15]. *Azotobacter chroococcum* degraded 55% of pendimethalin with a concentration of 25 mg/L during 20 days [16]. Also, there is some evidence of pendimethalin being broken down by some microorganisms, including *Fusarium oxysporum*, *Paecilomyces variotii* [17], and *Bacillus circulans* [18]. Based on the results of Pinto et al. [12], the concentration of 25 mg/kg pendimethalin could be degraded by *Lecanicillium saksenae* up to 99.5% ten days after incubation. Still, there is little information about the pendimethalin biodegradation processes like biochemical, physiological, and genetic mechanisms. There are various reports about microorganisms capable of biodegrading herbicides. *Bacillus subtilis* is a Gram-positive, rod-shaped bacterium that forms heat-resistant spores used to study physiology and metabolism [19]. Furthermore, due to its highly efficient protein secretion system and adaptable metabolism, it has been widely used as a cell factory for microbial production of chemicals, enzymes, and antimicrobial materials for agriculture and medicine. It is commonly found in the soil and is nonpathogenic [19]. And some reports indicate that *B. subtilis* s-15 could be used to alleviate contamination from glyphosate-containing herbicides, increasing the microbial functional diversity in glyphosate-contaminated soils and thus enhancing the bioremediation of glyphosate-contaminated soils [20]. *Escherichia coli* belong to the Enterobacteriaceae family of the γ-(*Greek gamma*) Proteobacteria and include a large number of strains that differ in pathogenic potential. The results showed that this bacterium was able to tolerate high doses of the herbicide and completely degrade mesotrione after 3 h of exposure, as determined by HPLC [21]. *Pseudomonas fluorescens* are unicellular rods with a long straight or curved axis but not helical, motility by one or more polar flagella, Gram-negative, non-spores former, stalks, or sheaths [22]. The study by Moneke et al. [23] showed that *P. fluorescens* and *Acetobacter* sp. exhibited a high capacity for efficiently degrading glyphosate under the environmental conditions studied. Thus, the organisms could be exploited for the biodegradation of glyphosate and should be studied for their ability to degrade other organophosphates. Since the dinitroaniline herbicide like pendimethalin are extensively applied in field crops for weed control in Iran, they could last for a long time in the soil; therefore, repeated use of this herbicide would interfere with human and environmental health and crop rotation. Thus,

investigating the microbial degradation for eliminating this herbicide is necessary. This experiment was done to assess the capability of three bacterial species, namely *B. subtilis, P. fluorescens,* and *E. coli,* to degrade pendimethalin in the laboratory.

2. Materials and methods

2.1. Bacterial isolates, chemicals and medium

Three bacterial isolates were prepared from the collection of the Iranian Biological Resource Center (IBRC): *Escherichia coli* (IBRC-M 10871), *Bacillus subtilis* (IBRC-M 10742), and *Pseudomonas fluorescens* (IBRC-M 10752. The *B. subtilis* and *P. fluorescence* are deemed risk group 1, so they do not cause disease in healthy adult humans; *E. coli* is considered risk group 2 and is unlikely to be a significant risk to laboratory workers or the environment [24]. Pendimethalin EC (Emulsifiable Concentrate) 33% and pendimethalin chromatography analytical standard were obtained from Aria Shimi (Tehran, Iran) and Merck (Germany), respectively. The Nutrient Agar (NA) and mineral salts were purchased from Merck (Germany). All high-performance liquid chromatography (HPLC)-grade chemicals were purchased from Sigma- Aldrich (Germany).

2.2. Growth of the isolates in the pendimethalin-medium

The bacterial isolates were pre-cultured on a nutrient agar medium. Then, the isolates were re-cultured on a Mineral Salt Media (MSM) agar media enriched by pendimethalin. Pendimethalin was used in the media as a carbon source. The culture media was prepared following the method defined by Álvaro et al. [18]. And 10.48 g KH_2PO_4, 9.28 g K_2HPO_4, 6.08 g (NH_4) SO_4, 1.248 g $MgSO_4$.7H2O, 0.368 g NaCl, 0.368 g $CaCl_2$.2H_2O, 0.132 g $FeCl_2$.6H2O, and 15 g agar were added to one liter of distilled water. After filtering and adjusting the pH to 7.0, the medium was sterilized by autoclaving for 15 min at 15 psi. The sterile pendimethalin was prepared using a microbiological filter (0.22 µm), and its proper concentration was added to the sterilized MSM medium before bacterial inoculations.

2.3. Study of pendimethal in microbial degradation

For the biodegradation studies, the MSM broth medium (without agar) was prepared as explained. The medium was then dispersed in 50 mL quantities and autoclaved. A total of 21 Erlenmeyer flasks were used. Then, 125 mg/L pendimethalin sterilized by membrane filtration was added to the medium, as the carbon source, before bacterial inoculation.

3. Results and discussion

The experiment was carried out using a liquid MSM medium amended with pendimethalin. It was inoculated with three different species of bacteria, namely *B. subtilis, P. fluorescens,* and *E. coli,* and monitored using bacterial cell density, bacterial fresh weight biomass, COD, and HPLC analysis.

3.1. Cell density

The comparative growth response of the three bacteria in the presence of pendimethalin showed that there were no significant differences between bacterial cell density at the beginning of the experiment (Analysis of variance is not shown) with the count of 0.56×10^6, 0.36×10^6, and 0.32×10^6 $CFU.mL^{-1}$ for *B. subtilis*, *P. fluorescens,* and *E. coli,* respectively. But seven days later, the bacterial cell density showed significant differences (*LSD= 0.263, P≤0.01*). The analysis of the population showed a rising in microbial growth after seven days of inoculation in all three bacteria. The increasing rate for *B. subtilis, P. fluorescens,* and *E. coli* cell density was 97.2, 97.05, and 88.4%, respectively (Figure 1-a). When comparing the growth kinetics of bacteria in the herbicide modified media with the control (without herbicide) in the 7th day after inoculation, it is clear from Figure 1(b) that the bacteria grow faster and to a higher number of cells when the herbicide is present in the media and the maximum count is 12.9×106 $CFU.mL^{-1}$ recorded from media enriched by *B. subtilis*. According to Figure 1(b), the rate of cell density increase for *E. coli* is higher than other bacteria and is equal to 70.73%, while this rate is 63.28% and 45.12% for *B. subtilis* and *P. fluorescens*, respectively (Figure 1(b)). On the other hand, the growth in the control treatment was very slow; the maximum cell count (4.7×106) was achieved after seven days for *B. subtilis*. And this result is an indication of the

adaption of the microorganisms to this type of herbicide; once they are adapted to that concentration of the herbicide, they grow very well. Moreover, the study of Mu'azu et al. [27] revealed that the bacterial strains were able to grow and consume pendimethalin as a sole carbon source, making them a mechanism for bioremediation based on their results. The *Bacillus* sp. and bacterial consortium showed a considerable growth pattern of OD 0.424 and OD 0.481, respectively. Their results indicated that bacterial population growth increased as the herbicides remained longer in the soil. According to the results of Elsayed and El-Nady [28], pendimethalin treatment soils affected the microbial population growing in such a way that *Pseudomonas putida* showed an overall increase in cell density. Their results indicated that soil bioremediation by *P. putida* and compost was considered to be a successful method for detoxification of pendimethalin in soil.

3.2. Fresh weight biomass

In order to illustrate the association between bacterial biomass yield and pendimethalin degradation, batch experiments were conducted for three bacterial species. The growth of bacterial biomass in the presence of pendimethalin indicated that bacteria could grow fast in shaken flask conditions and at the end of the experiment; a significant difference (LSD= 0.014, P ≤0.01) was observed between the studied bacteria in terms of microbial biomass (data not shown). The relatively higher value in bacterial biomass with the amount of 0.073 g was obtained in *B. subtilis,* followed by *P. fluorescens* (0.0544g); the minimum biomass was obtained from *E. coli* (0.036 g). However, interestingly, a high amount of increasing rate was observed with all three bacteria after seven days of culture in pendimethalin contaminated media compared to the control, with the rate of 75.5% for *B. subtilis* and 66% and 66.2% for *P. fluorescens* and *E. coli*, respectively (Figure 2). It was further noted that the growth of bacteria was comparatively less but continued in control. The observed growth may be due to the availability of nutrients and favorable conditions. It is acknowledged that the consumption of herbicides as energy sources could result in the high biomass of bacteria in pendimethalin amended media.

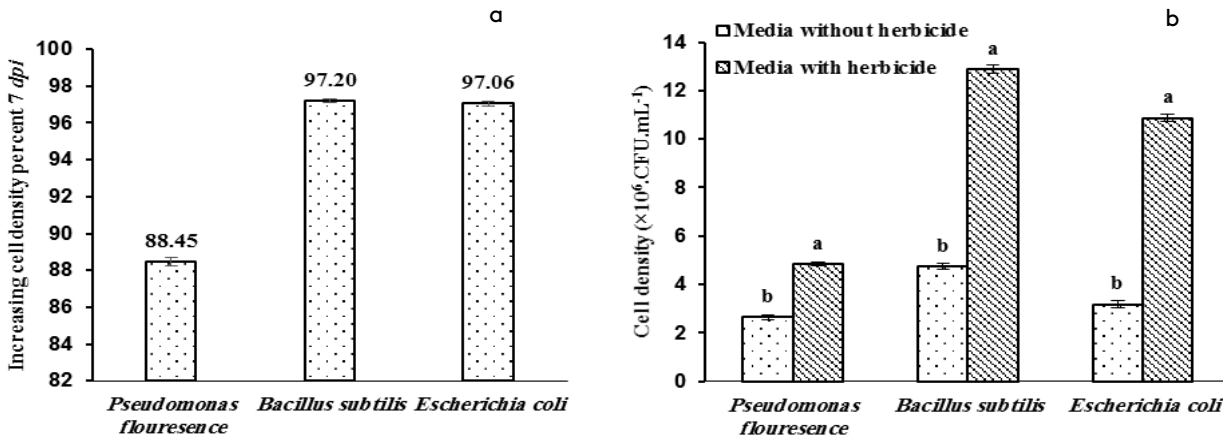

Fig. 1. a. Increasing percentage of bacterial cell density in MSM media supplemented with pendimethalin from day one to day seven and b. Growth of different bacteria in media with and without pendimethalin at 7 dpi (For each bacteria, growth in herbicide modified media was compared to its control separately using t-student, P≤0.01).

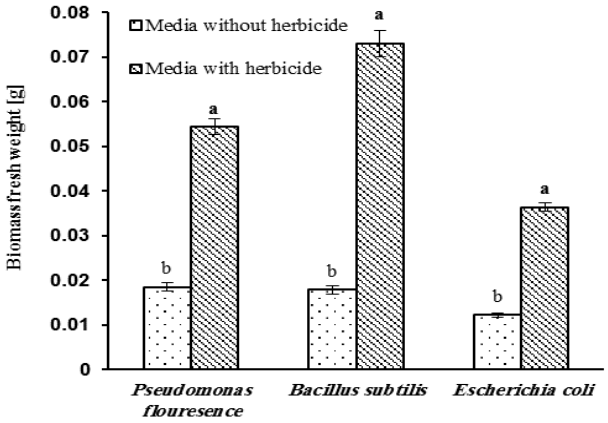

Fig. 2. Fresh weight biomass of *B. subtilis, P. fluorescens* and *E. coli* in MSM media contained pendimethalin compared to the control, 7 dpi. (For each bacteria, growth in herbicide modified media was compared to its control separately using t-student, P<0.01).

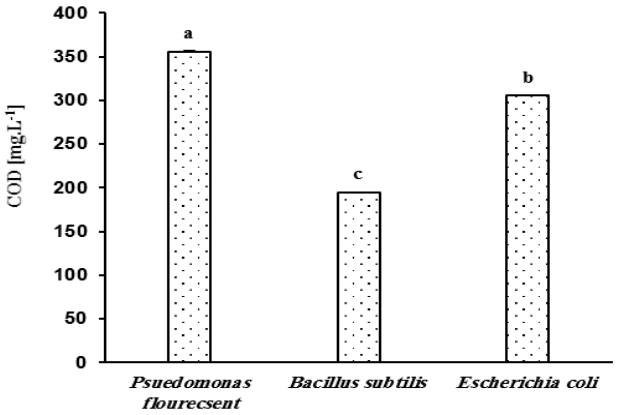

Fig. 3. COD reduction of pendimethalin with *B. subtilis, P. fluorescens,* and *E. coli* at seven dpi (LSD= 35.13, P≤0.01).

3.3. Chemical oxygen demand (COD)

The reduction rates of *pendimethalin* solution on the COD parameter by *B. subtilis*, *P. fluorescence,* and *E. coli* are presented in Figure 3, which reveals different results depending on the bacterial species in the medium. COD is a circuitous assessment of the amount of organic matter. According to the results, the initial COD, which was calculated as 1290.7, 1286.3, and 1262.3 mg/L for *B. subtilis*, *E. coli,* and *P. fluorescent*, respectively, decreased 71-85% seven days after inoculation. It seemed that the COD-removal efficiency differed as the bacteria varied in the liquid medium. The highest COD removal efficiency was recorded from *B. subtilis*, decreasing the COD from 1290.7 mg/L to 194.7 mg/L. In the same period, *E. coli* and *P. fluorescent* showed a 76.3% and 71.8% reduction rate, respectively. *B. subtilis* achieved 85% removal in the COD parameter in seven days, so the higher COD removal efficiency showed the higher degradation of the herbicide.

According to Erguven et al. [29], the COD removal efficiency of trifluralin biodegradation by *Bacillus muralis, Micrococcus luteus,* and *Micrococcus yunnanensis* was 91%, *Clostridium tetani was 86%,* and *Bacillus simplex* was recorded as 86%. For the trifluralin degradation experiment, the COD removal efficiency reached 69% with bacteria mix and the ratio with a mix of fungal culture was 67% for the trifluralin during the same period.

3.4. HPLC analysis

The analysis of pendimethalin utilization by pendimethalin-degrading bacterial isolates in MSM containing pendimethalin (125 mg/L) was determined based on the herbicides reduction on the standard curve. According to the results, the peak extracted in 44:27-44:50 minutes is related to the concentration of pendimethalin herbicide (Table 1, Figure 4).

Table 1. HPLC analysis of pendimethalin concentration seven days after inoculation by *P. fluorescence, E. coli, and B. subtilis*.

	Retention Time	Area	Concentration (mg/L)
control	44.30	3,408,591	113.78
P. fluorescens	44.271	2,438,629	82.01
E. coli	44.50	1,080,564	37.72
B. subtilis	44.217	689,124	24.93

Measuring the amount of pendimethalin herbicide in the control sample (without bacteria) showed that the concentration of pendimethalin herbicide was 113 mg/L, which degraded about 9% of 125 mg/L pendimethalin after seven days. However, its concentration in the inoculated samples with *P. fluorescens*, *E. coli,* and *B. subtilis* were respectively 82.01, 37.72, and 24.93 mg/L after seven days (Table 1). The HPLC results revealed a drastic reduction of herbicide in all samples containing the bacteria compared to control. By degrading 78% of pendimethalin concentration, *B. subtilis* showed a significantly higher rate of pendimethalin

metabolism (Figure 5). The results summarized in Table 1 show that the reduction percent of herbicide concentration in *E. coli* and *P. fluorescens* is 66.8% and 27.9%, respectively. *B. subtilis* showed the highest rate of pendimethalin metabolism that proves the capability of this bacteria as a good agent of biodegradation of pendimethalin contaminated sites. Mu'azu et al. [27] also reported that the analysis of pendimethalin utilization by HPLC revealed a higher reduction of pendimethalin by *Bacillus* sp. and *Pseudomonas* sp., demonstrating the ability of *Bacillus* sp., *Pseudomonas* sp., and bacterial consortium as a good agent of bioremediation of herbicide contaminated sites.

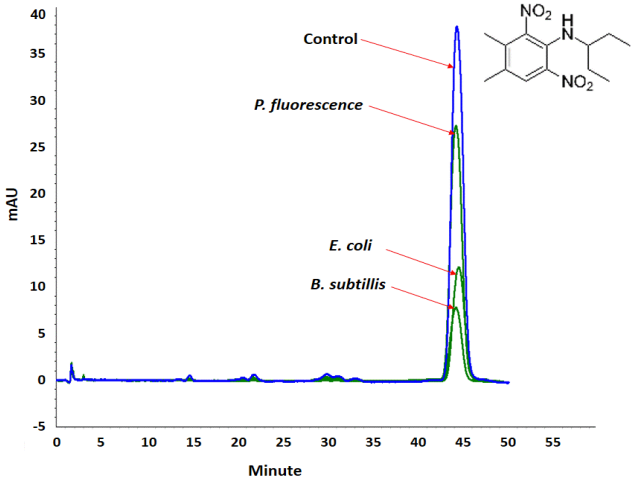

Fig. 4. HPLC overlay chromatogram of control treatment (blue) and B. subtilis, E. coli and P. *fluorescens* (green) of pendimethalin used in 125 *mg/L* after seven days.

Generally, the degradation of the soil pollutants could be sped up by applying exogenous degrading bacteria [31]. Based on the results of this study, all three bacteria were able to efficiently reduce the amount of herbicide in the medium within seven days. The measurement of cell density, biomass, and COD in all three bacteria confirmed the results obtained from HPLC. So, the increase in bacterial density caused the increase in pendimethalin biodegradation, and the population growth rate in samples containing herbicides compared to those without showed a significant increase. Based on the results of HPLC, the highest biological degradation of pendimethalin compared to the control treatment occurred in the *B. subtilis* (78%) media, which also produced the most cell density, followed by *E. coli* (66.8%) and *P. fluorescens* (27.9%). It should be noted that besides the efficiency of *B. subtilis,* the ability of *E. coli* to degrade pendimethalin was also good enough for use as a biological treatment to eliminate environmental pollutants. In the present investigation, an efficient pendimethalin-degrading bacteria was recognized as *B. subtilis* from the genus *Bacillus,* broadly scattered in nature and popular for their capabilities to break down large numbers of pollutants. According to the experimental results, *B. subtilis* could degrade 80% of 125 mg/L pendimethalin within 7 *dpi* compared to the first day of inoculation and 78% degradation compared to the control at 7 *dpi*. According to other pendimethalin degrading species earlier reported, *Lecanicillium saksenae* broke down 99.5% of pendimethalin with a concentration of 25 mg/kg during 10 days [12], *Azotobacter chroococcum* degraded 55% pendimethalin within 20 days [16], and *Clavispora lusitaniae* degraded 74% of 200 mg/L pendimethalin within eight days [11]. The successful degradation of herbicides by applying bacteria and fungi has been reported for many compounds, including trifluralin [29], mesotrion [31], imazapyr by *Streptomyces* sp. [32], and glyphosate by *Pseudomonas* sp., *Arthrobacter atrocyaneus,* and *Flavobacterium* sp [3]. Hou et al. [10] reported the biodegradation of pendimethalin and endosulfan by *B. safensis*, *B. subtilis,* and *B. cereus,* which was investigated in MSM media. The half-life of pendimethalin was reduced up to 6.9 days by applying *B. cereus*. Furthermore, Abdel–Moteleb and Hassan [10] showed pendimethalin degradation by *B. megaterium* with 5.6 days in a treated mineral liquid medium, which was a significant reduction when compared to the reported half-lives (30-90 days) of pendimethalin in soil [33]. *Bacillus spp.* are reported as degraders of many organic compounds such as petroleum products [34]. The results of Megadi et al. [18]also showed that the *Bacillus* species are capable of degrading a large number of aromatic compounds in the environment. Based on this investigation, *E. coli* had a degradation ability of 66.8% of pendimethalin, which makes it the second potent bacteria after *B. subtilis* in the biodegradation of pendimethalin at the current study which belongs to the Enterobacteriaceae family and is a Gram-negative facultative anaerobic non-spore-forming

motile rod bacteria [34]. Although *E. coli* has long been characterized as a well-known organism, its potential for consuming aromatic compounds as a carbon and energy source is not completely known [35]. The current understanding of the biodegradation ability of *E. coli* affirms that this bacterium could catabolize aromatic compounds and provide common aerobic degradation paths close to the ones in relevant environmental bacteria like those of the genus *Pseudomonas,* including *Pseudomonas aeruginosa*. Besides, just as different *Pseudomonas* strains have specific aromatic substrates for growth, intraspecies variation concerning the ability to mineralize different aromatic compounds have also been identified in *E. coli* [35].

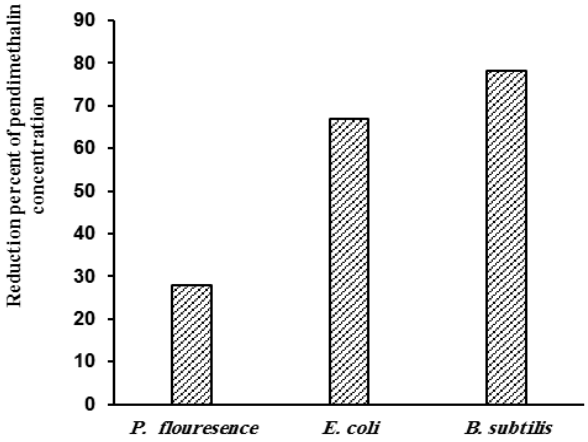

Fig. 5. Pendimethalin reduction percent by *P. fluorescens, E. coli* and *B. subtilis* in MSM media compared to control at 7 *dpi*.

4. Conclusions

The study results indicated the strong potential of microorganisms for degrading pendimethalin in a mineral salt medium. *B. subtilis* effectively biodegraded and consumed the pendimethalin as a sole source of carbon in the MSM media. However, *E. coli* also had an acceptable degradation rate; *P. fluorescence* showed the weakest efficiency in pendimethalin degradation. The use of such microorganisms in removing herbicides from polluted environments through biodegradation techniques is promising and deserves more investigation.

References

[1] Li, L., Wei, D., Wei, G., Du, Y. (2013). Transformation of cefazolin during chlorination process: products, mechanism and genotoxicity assessment. *Journal of hazardous materials*, *262*, 48-54.

[2] Tomlin, C. D. (2009). *The pesticide manual: a world compendium* (No. Ed. 15). British Crop Production Council.

[3] Singh, B., Singh, K. (2016). Microbial degradation of herbicides. *Critical reviews in microbiology*, *42*(2), 245-261.

[4] Ma, J., Xu, L., Wang, S., Zheng, R., Jin, S., Huang, S., Huang, Y. (2002). Toxicity of 40 herbicides to the green alga Chlorella vulgaris. *Ecotoxicology and environmental safety*, *51*(2), 128-132.

[5] Mohan, S. V., Krishna, M. R., Muralikrishna, P., Shailaja, S., Sarma, P. N. (2007). Solid phase bioremediation of pendimethalin in contaminated soil and evaluation of leaching potential. *Bioresource technology*, *98*(15), 2905-2910.

[6] Kočárek, M., Artikov, H., Vořišek, K., & Borůvka, L. (2016). Pendimethalin degradation in soil and its interaction with soil microorganisms. *Soil and water research*, *11*(4), 213-219.

[7] USEPA. Persistent bioaccumulative toxic (PBT) chemicals. (1999). United States Environment Protection Agency, Final rule, Fed. Regist., *64*, 5866658753.

[8] Ritter, L., Solomon, K. R., Forget, J., Stemeroff, M., O'Leary, C. (1995). Persistent organic pollutants: an assessment report on: DDT, aldrin, dieldrin, endrin, chlordane, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls, dioxins and furans. *December international programme on chemical safety (IPCS) within the framework of the inter-organization programme for the sound management of chemicals (IOMC)*.

[9] Kamrin, M. A. (1997). *Pesticide profiles: toxicity, environmental impact, and fate*. CRC press.

[10] Abdelbagi, A. O., Hammad, A. M. A., Elsheikh, E. A. E., Elsaid, O. E., Hur, J. H. (2017). Biodegradation of endosulfan and pendimethalin by three strains of bacteria isolated from pesticides-polluted soils in the

Sudan. *Applied biological chemistry*, *60*(3), 287-297.

[11] Han, Y., Tang, Z., Bao, H., Wu, D., Deng, X., Guo, G., Dai, B. (2019). Degradation of pendimethalin by the yeast YC2 and determination of its two main metabolites. *RSC Advances*, *9*(1), 491-497.

[12] Pinto, A. P., Serrano, C., Pires, T., Mestrinho, E., Dias, L., Teixeira, D. M., Caldeira, A. T. (2012). Degradation of terbuthylazine, difenoconazole and pendimethalin pesticides by selected fungi cultures. *Science of the total environment*, *435*, 402-410.

[13] Belal, E. B., Zidan, N. A., Mahmoud, H. A., Eissa, F. I. (2008). Bioremediation of pesticides – contaminated soils. *Journal of agricultural research,* 2008, *34*, 588 – 608

[14] Vidali, M. (2001). Bioremediation. an overview. *Pure and applied chemistry*, *73*(7), 1163-1172.

[15] Khan, M. W. A., Ahmad, M. (2006). Detoxification and bioremediation potential of a Pseudomonas fluorescens isolate against the major Indian water pollutants. *Journal of environmental science and health, Part A*, *41*(4), 659-674.

[16] Kole, R. K., Saha, J., Pal, S., Chaudhuri, S., Chowdhury, A. (1994). Bacterial degradation of the herbicide pendimethalin and activity evaluation of its metabolites. *Bulletin of environmental and contamination and toxicology*, *52*, 779-786.

[17] Singh, S. B., Kulshrestha, G. (1991). Microbial degradation of pendimethalin. *Journal of environmental science and health*, *26*, 309-321.

[18] Megadi, V. B. Tallur, P. N. Hoskeri, R. S. Mulla, S. I. & Ninnekar, H. Z. (2010). Biodegradation of pendimethalin by *Bacillus circulans*. *Indian journal of biotechnology, 9*, 173- 177.

[19] Su, Y., Liu, C., Fang, H., Zhang, D. (2020). Bacillus subtilis: a universal cell factory for industry, agriculture, biomaterials and medicine. *Microbial cell factories*, *19*(1), 1-12.

[20] Yu, X. M., Yu, T., Yin, G. H., Dong. Q. L., An, M., Wang, H. R. Ai, C. X. (2015). Glyphosate biodegradation and potential soil bioremediation by *Bacillus subtilis* strain Bs-15. *Genetic and modular research*, 14(4), 14717-30

[21] Olchanheski, L. R., Dourado, M. N., Beltrame, F. L., Zielinski, A. A., Demiate, I. M., Pileggi, S. A., Pileggi, M. (2014). Mechanisms of tolerance and high degradation capacity of the herbicide mesotrione by Escherichia coli strain DH5-α. *PloS one*, *9*(6), e99960.

[22] Kumar, H., Franzetti, L., Kaushal, A. Kumar D. (2019). *Pseudomonas fluorescens*: a potential food spoiler and challenges and advances in its detection. *Annals of microbiology*, *69*, 873-883.

[23] Moneke, A. N., Okpala, G. N. & Anyanwu, C. U. (2010). Biodegradation of glyphosate herbicide *in vitro* using bacterial isolates from four rice fields. *African journal of biotechnology*, *9*(26), 4067-4074.

[24] Iranian Biological Resource Center (IBRC) https://ibrc.ir/index.aspx?siteid.

[25] Hang, Y. D. (2017). Determination of oxygen demand. *In food analysis* (pp. 503-507). Springer, Cham.

[26] Shah, J., Mustaun Rasu, M. D., Shehzad, F. U. (2011). Quantification of pendimethalin in soil and garlic samples by microwave-assisted solvent extraction and HPLC method. *Environmental monitoring and assessment*, *175*(1-4), 103-8.

[27] Mu'azu, W. M. A., Okpanachi, I. Y., Faruq, U. A., Fatimah, T. (2018). Characterization and role of pendimethalin catabolizing bacteria isolated from agricultural soil in Bauchi, Bauchi state. GSC. *Biological and pharmaceutical science*, *5*(3), 12-19.

[28] Elsayed, B., El-Nady, M. F., (2013). Bioremediation of pendimethalin-contaminated soil. *African Journal of microbiology research*, *7*(21), 2574-2588.

[29] Erguven, G. O., Bayhan, H., Ikizoglu, B., Kanat, G., Nuhoglu, Y. (2016). The capacity of some newly bacteria and fungi for biodegradation of herbicide trifluralin under agiated culture media. *Cellular and molecular biology, 62*(6), 74-79.

[30] Zhang, H., Mu, W., Hou, Z., Wu, X., Zhao, W., Zhang, X., Pan, H., Zhang, S. (2012). Biodegradation of nicosulfuron by bacterium *Serratia marcescens* N80. *Journal of environmental science and health*, *47*, 153-160.

[31] Shelton, D. R., Khader, S., Karns, J. S., Pogell, B. M. (1996). Metabolism of twelve herbicides by Streptomyces. *Biodegradation, 7*, 129–136.

[32] Worthing, C. R., Hance, R. J. (1991). The Pesticide manual. 9t h Ed. *A world compendium. The British crop protection council. Surrey UK*, 763-764.

[33] Okerentugba, P. O., Ezeronye, O. U. (2003). Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *African journal biotechnology, 2*(9), 288-292.

[34] Desmarchelier, P., Fegan N. (2011). Pathogens in milk, E*scherichia coli*. Editor(s): John W. Fuquay, Encyclopedia of dairy sciences (Second edition), Academic press, 60-66.

[35] Díaz, E., Ferrández, A., Prieto, M. A., García, J. L. (2001). Biodegradation of aromatic compounds by Escherichia coli. *Microbiology and molecular biology reviews*, *65*(4), 523-569.