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Study of allelopathic interaction of alkaloid extracts of *Peganum harmala* plant and effects of bacterial population density

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Abstract
This study was conducted to find out the allelopathic interactions of the alkaloid extract of *Peganum harmala* on the number of bacteria present in the soil and the water of plant irrigation by preparing different concentrations of the alkaloid extract 10, 50, 250 and 500,and compared with the control treatment, variation was found in the number of bacteria present in the soil and irrigation water at different concentrations in each stage of plant growth.

Keyword: allelopathic interactions, alkaloid extract,*Peganum harmala*, Soil bacteria

Introduction

MICROBIAL population

Some studies have shown that one-third of plant photosynthesis is released into the soil in the form of root secretions, which can affect the microbial community in the rhizosphere Soil microorganisms play an important role in improving the physical and chemical properties of the soil, regulating the soil microbial community and its diversity, and maintaining soil quality and fertility. (Razani*et al* 2016), where allelopathy as a new weapon for species invasion has gained a prominent place in the field of invasive biology (Inderjit*etal* 2008).chemicals produced by plants affect plants the chemical substances produced by plants affect neighboring plants and soil microorganisms, ultimately changing the structure of plant communities (Kalisz *et al* 2020)

Microorganisms, as one of the most essential and active parts of the soil ecosystem, have a prominent role in promoting soil nutrient cycling and preserving system stability, and are critical for the continued operation of soil micro ecological state (Emery *et al*., 2019; Nazaries*et al.*, 2021). Furthermore, the soil microbial population can offer critical nutrients for crop growth and drive crop development through a variety of ways (Yang *et al*., 2019).Bioorganic fertilizer is a combination of organic fertilizers and probiotic bacteria that can stimulate a variety of soil microbes. It is also becoming more important in improving agricultural output, restoring soil fertility, and preventing soil pathogens (Huang *et al*., 2014; Schoebitz*et al*., 2014; Liu *et al*., 2016).Currently, scientists focus more and more on the allelopathic interaction between plants and the soil of their roots, but there are still few relevant studies. Studies have shown that the allelochemicals released by the roots of plants play avital role in the intractions between soil microorganisms (Vishwakarma*et al* 2017). Allelopathic substances released by plant roots can affect the microbial community in the rhizosphere soil, and the soil microbial community can also affect the allelopathy between plants and soil to a certain extent (Lu,.2017).

Soil enzyme activity is a sensitive indicator of soil environmental change; it is frequently related to soil physical and chemical properties, reflects the direction and intensity of soil biochemical processes, and has a significant impact on soil physical and chemical properties, fertility, and biological conditions. As a result, it is frequently employed as a

key index for assessing soil environmental quality (Guo *et al.*, 2020) Scholars are becoming more interested in the relationship between soil microbes and soil enzymes (Bain *et al.*, 2020).

Soil environmental parameters such as altitude, pH, organic matter, total nitrogen, alkali-hydrolyzable nitrogen, available potassium, and available phosphorus content have been linked to the composition and diversity of soil microbial communities(Batista *et al* ,.2020) which in turn impact soil enzyme activity(Peng andWang.,2016) each soil enzyme and environmental factor has a unique relationship with the S. chamaejasme rhizosphere soil microbial population. Jin investigated the bacterial population in the rhizosphere and root of S. chamaejasme in the Qinghai-Xizang Plateau and discovered that soil phosphorus, pH, latitude, altitude, and potassium were all positively associated to the bacterial community in the rhizosphere soil (Jin *et al.*, 2018).

It is still unknown how soil enzyme activities, environmental factor, and allelochemicals combine to influence the soil microbial population in the rhizosphere. Allelochemicals generated by plants during invasion may play a crucial role in habitat expansion competition. Allelochemicals generated by S. chamaejasme may be the primary mechanism for its invasion of the process altering the soil microbial community structure in the rhizosphere. The dynamics of cover crop- and microorganism-derived weed suppression will be determined by the rate of allelochemical release, the characteristics of the allelochemicals, and the activities of allelochemical-degrading and seedling-infecting microorganisms. These elements interact to generate a window of weed suppression potential. Residue-induced suppression of a sensitive plant occurs only when the plant's sensitivity period and the window of suppression potential overlap in time (Kruidhof et al. 2009).

Microbial activity may shorten this window if microbe-allelochemical interactions are antagonistic, but it may lengthen this window if these interactions are synergistic. We investigate the weed-suppressive ability of various soluble and insoluble fractions of cover crop residues. The total allelochemical potential of a cover crop residue is a combination of water-soluble phytotoxins released by residues prior to decomposition, insoluble phytotoxins released by microorganisms during decomposition, and the subsequent microbial transformation of these phytotoxins (Barnes and Putnam 1986).

Microbes can deactivate water soluble allelochemicals produced shortly after cover crop residue integration (Jilani*et al.* 2008), but they can also convert safe plant-derived molecules to more harmful ones (Williamson *et al.* 1992). Microbes play critical roles in the release of extra allelochemicals bound up in the refractory fractions of cover crop residues (Barnes *et al.* 1987). Because these insoluble allelochemicals can account for a large portion of cover crop residue's overall allelopathic potential (Harper and Lynch 1982), microorganisms may slowly release residue-derived allelochemicals, prolonging the efficacy of a cover crop. Given that agricultural soils are not sterile, it is critical to comprehend how microbial activity influences the allelochemical potential of cover crop residues (Inderjit 2005).

Several studies indicated that the extracts of the *Lycium barbarum* plant had anti-microbial activity, as it was found that the ethanol and methanol extracts of the leaves of the *Lycium barbarum* plant were effective against pathogenic bacteria Klebsiella pneumonia, *Escherichia coli* and *Shigella shinga*, and the methanol extract showed strong activity against bacterial infections. As (Al-Askary and Malih,2021).The antibacterial effects of aqueous garlic extract and Crude Juice against Gram-positive and Gram-negative bacterial isolates, from burn unit were studied. The bacteria isolates were including *Staphylococcus spp, Serratiaspp. ,Pseudomonas spp, Enterobacter spp, Acinetobacter. Spp.* and *Ochrobactrumanthropi*.The results of the study of garlic extract both types of aqueous

extract and crude juice in the degree of impact inhibition as results showed that Crude Juice had a greater effect than aqueous extract effect(Azeez., 2015).

2-Material and methods

2-1 Isolation and identification of bacterial species from soil and irrigation water

2-1-1Count Bacteria in soil and water

Bacteria present in the soil and irrigation water were isolated using a series of dilutions for both soil and water, whereby 1 g of soil was added to 9 ml of distilled water in a test tube, shaken well, and then 1 ml of soil suspension was transferred to test tubes containing 9 ml of distilled water. Water 1 ml of irrigation water was taken and added to a test tube containing 9 ml of distilled water then a series of dilutions were made from 10^{-1} to 10^{-10}. It was shaken well and three dilutions10^{-4}, 10^{-5} ,10^{-6} were used. 1 ml was taken from each dilution and added to Petri dishes with a diameter of 9 cm containing a sterile nutrient agar medium before pouring, the dishes were shaken several times to ensure the homogeneity of the medium, and the dishes were incubated at a temperature of 28℃ for a period of 24 hours. After that, the bacterial colonies were counted using a colony counting device, (Rabeendran*et al*., 1998).

3-Result

3-1 Detection number of bacteria in the soil

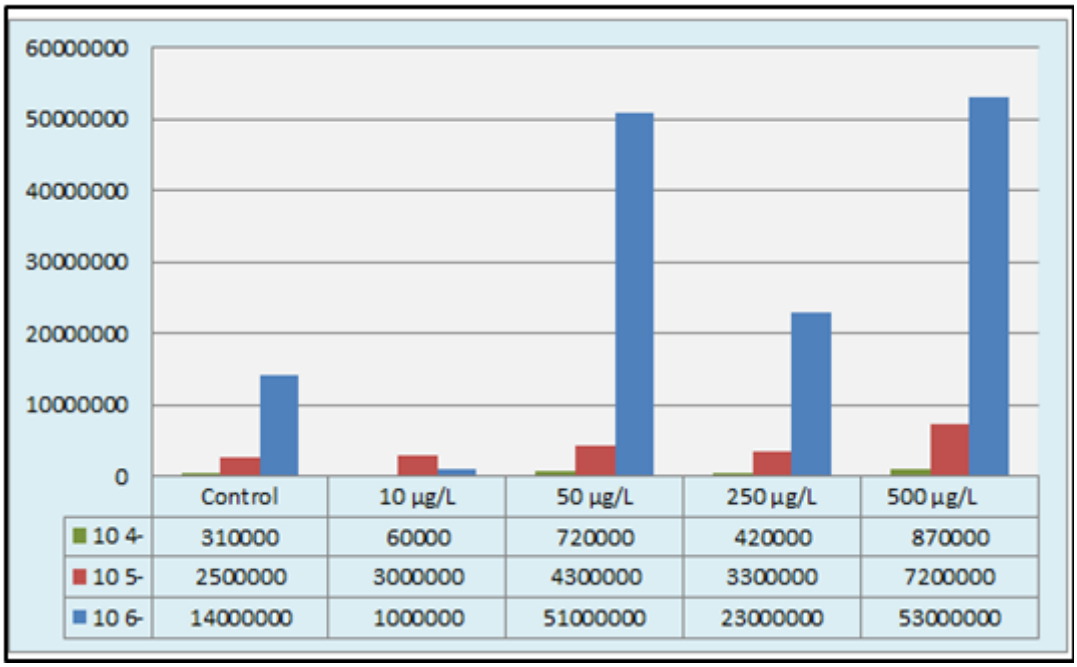
3-1-1 First stage: Detection of bacteria numbers before and after seed germination.

Before planting the seeds in the soil, the soil under study was examined to detect the number of bacteria before adding the alkaloid extract. After planting the seeds in the soil, the alkaloid extract was added at concentrations of 10, 50, 250, and 500. The numbers of bacteria present in the soil were detected after adding the alkaloid extract. It was found decrease in the number of bacteria in concentration 10 compared to the control treatment. At a concentration of 50,250,500 we notice an increase in the number of bacteria compared to the control treatment, when comparing the concentrations with each other, we notice an increase in the number of bacteria in concentration 50 and 500 compared to with the rest of the concentrations. We note from table (3-1) that there are significant differences in dilution 10^{-4} for all concentrations, We also notice that there are significant differences in dilution 10^{-5} in all concentrations, We also notice that there are significant differences in the 10^{-6} dilution in all concentrations as we notice in Figure (3-1)

Table (3-1) Detection of bacteria numbers before and after seed germination.

Dilution / concentration	10^{-4}	10^{-5}	10^{-6}
Control	310000	2500000	14000000
10µg/L	60000	300000	1000000
50µg/L	720000	4300000	51000000
250µg/L	420000	3300000	23000000
500µg/L	870000	7200000	53000000
LSD	3588.09	4588.09	4788.09
P-Value	0.000	0.002	0.000

P-Value $\leq$ 0.05



Figur (3-1)bacteria numbers before and after seed germination in the soil

3-1-2 the second stage is detection of bacteria when the stem buds leaf.

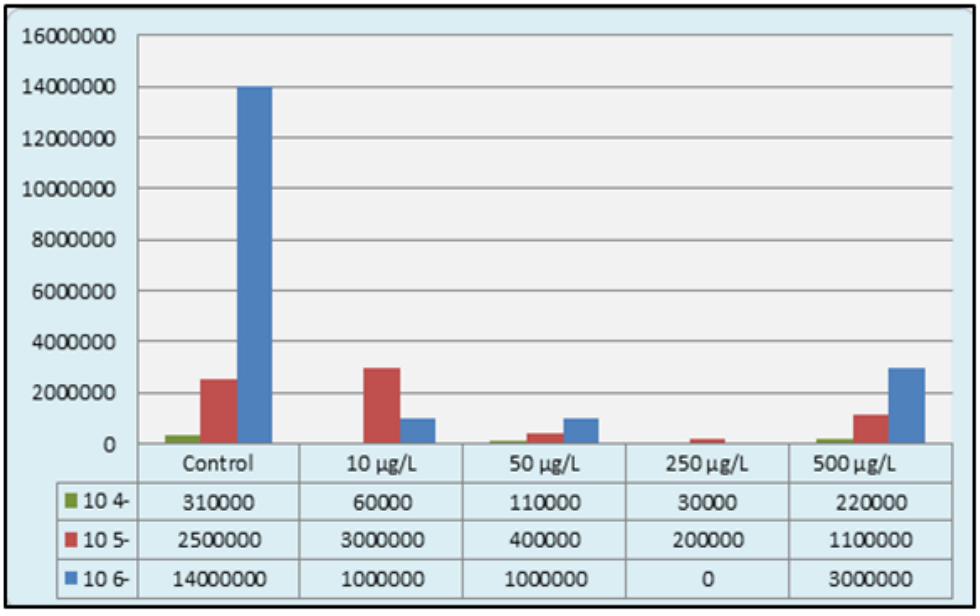
At this stage, after the completion of seed growth and the emergence of stem buds, and after adding the alkaloid extract to the soil, the number of bacteria present in the soil was detected. It was found decrease in the number of bacteria in concentration 10,

50,250and500compared to the control treatment. when comparing the concentrations with each other, we notice an increase in the number of bacteria in concentration 500 compared to with the rest of the concentrations . We note from table (4-15) that there are significant differences in dilution 10^{-4} for all concentrations, notice that there are significant differences in dilution 10^{-5} in all concentrations, We also notice that there are significant differences in the 10^{-6} dilution in all concentrations as we notice in Figure (4-17)

Table (3-2) detection of bacteria when the stem buds appear.

Dilution / concentration	10^{-4}	10^{-5}	10^{-6}
Control	310000	2500000	14000000
10 µg/L	60000	300000	1000000
50 µg/L	110000	400000	1000000
250 µg/L	30000	200000	0
500 µg/L	220000	1100000	3000000
Lsd	3466.33	4766.87	999.59 4
p-value	0.000	0.000	0.000

P-Value ≤ 0.05



Figur (3-2) numbers of bacteria when the stem buds appear

3-1-3 the third stage detection number of bacteria after the completion of stem growth.

After completing the growth of the plant stem at this stage, the numbers of bacteria in the soil are detected after adding the alkaloid extract the numbers of bacteria present in the soil

were detected after adding the alkaloid extract. It was found increase in the number of bacteria in concentration 10, 50,250and500 compared to the control treatment, when comparing the concentrations with each other, we notice an increase in the number of bacteria in concentration 50 compared to with the rest of the concentrations. We note from table (3-3) that there are significant differences in dilution 10^{-4} for all concentrations, We also notice that there are significant differences in dilution 10^{-5} in all concentrations, We also notice that there are significant differences in the 10^{-6} dilution in all concentrations as we notice in Figure (3-3)

Table (3-3) detection number of bacteria after the completion of stem growth

Dilution / Concentration	10^{-4}	10^{-5}	10^{-6}
Control	310000	2500000	14000000
10 µg/L	1310000	11100000	91000000
50 µg/L	1420000	12100000	101000000
250 µg/L	1210000	9200000	73000000
500 µg/L	1360000	11100000	92000000
Lsd	6332.89	7849.99	9893.34
p-value	0.000	0.005	0.000

P-Value ≤0.05

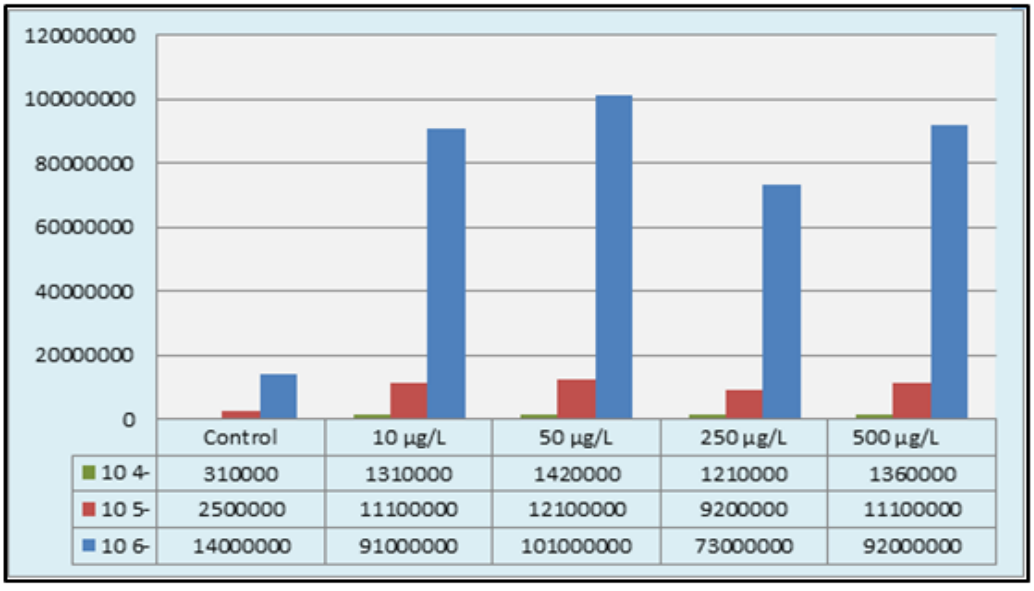


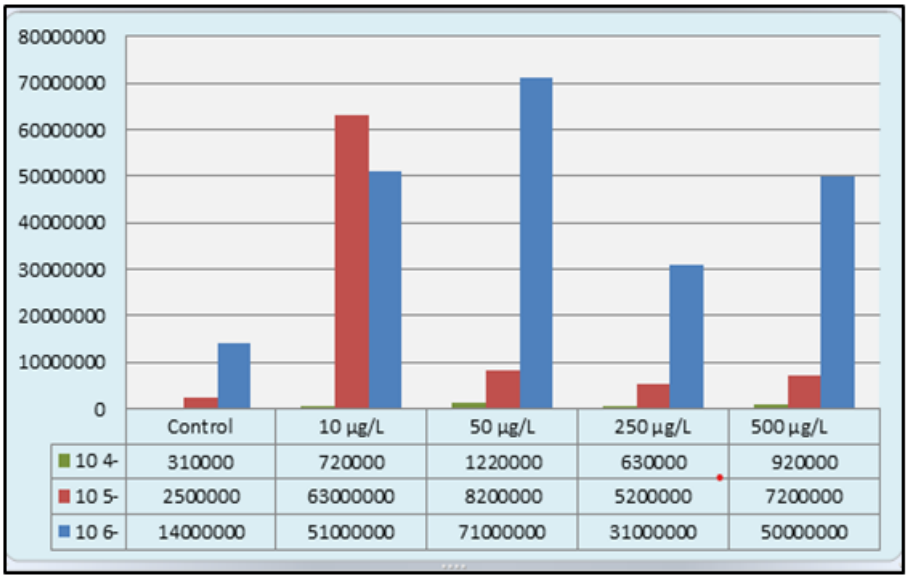
Figure (3-3) number of bacteria after the completion of stem growth

3-1-4 the fourth stage: Detection of the number of bacteria after the complete maturity of the leaves.
After the complete maturity of the leaves of the plant, the number of bacteria present in the soil was detected after adding the alkaloid extract at this stage, the numbers of bacteria present in the soil were detected after adding the alkaloid extract. It was found increase in the number of bacteria in concentration 10, 50,250and500 compared to the control treatment. When comparing the concentrations with each other, we notice an increase in the number of bacteria in concentration 50 and 500. We note from table (3-4) that there are significant differences in dilution 10^{-4} for all concentrations, notice that there are significant differences in dilution 10^{-5} in all concentrations, notice that there are significant differences in the 10^{-6} dilution in all concentrations as we notice in Figure (3-4)

Table (3-4) Detection of the number of bacteria after the complete maturity of the leaves.

dilution / concentration	10-4	10^{-5}	10^{-6}
Control	310000	2500000	14000000
10µg/L	720000	6300000	51000000
50µg/L	1220000	8200000	71000000
250µg/L	630000	5200000	31000000
500µg/L	920000	7200000	50000000
LSD	8922.67	8600.00	102000.00
P-Value	0.000	0.000	0.000

P-Value ≤ 0.05



Figur (3-4) the number of bacteria after the complete maturity of the leaves.

3-2-Detection of the numbers of bacteria present in the irrigation water.

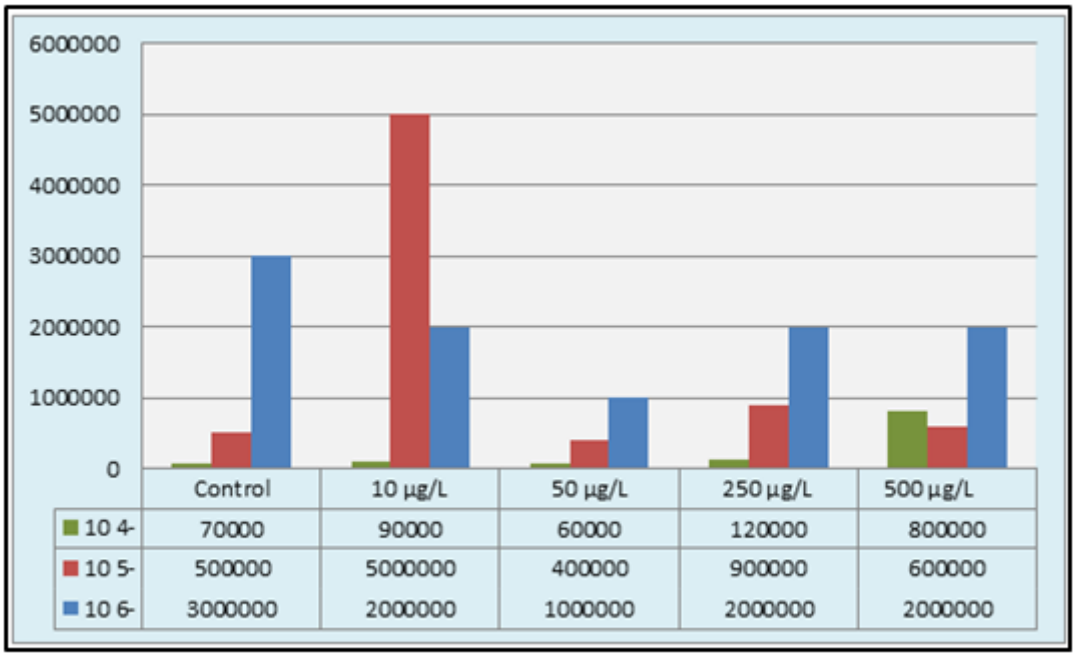
3-2--1 First stage: Detection of bacteria numbers in water before and after seed germination.

Before planting the seeds in the soil, the irrigation water under study was examined to detect the number of bacteria before adding the alkaloid extract. The numbers of bacteria present in the irrigation water were detected after adding the alkaloid extract. It was found increase in the number of bacteria in concentration 10 compared to the control treatment at a concentration of 50 notice decrease in the number of bacteria compared to the control treatment ,at the concentration of 250 notice an increase in the number of bacteria compared to the control treatment, a t concentration 500 notice an increase in the number of bacteria compared to the control treatment, when comparing the concentrations with each other notice an increase in the number of bacteria in concentration 10 compared to with the rest of the concentrations . The table (3-5) is showing there are significant differences in dilution 10^{-4} for all concentrations, also notice that there are significant differences in dilution 10^{-5} in all concentrations, showing that there are no significant differences in the dilution 10^{-6} in all concentrations as we notice in Figure (3-5).

Table (3-5) Detection of bacteria numbers in water before and after seed germination.

Dilution / concentration	10^{-4}	10^{-5}	10^{-6}
Control	70000	500000	3000000
10 µg/L	90000	500000	2000000
50 µg/L	60000	400000	1000000
250 µg/L	120000	900000	2000000
500 µg/L	80000	600000	2000000
Lsd	4368.12	4423.22	5450000.13
p-value	0.000	0.004	0.481

P-Value ≤ 0.05



Figur (3-5) bacteria numbers in the water before and after seed germination

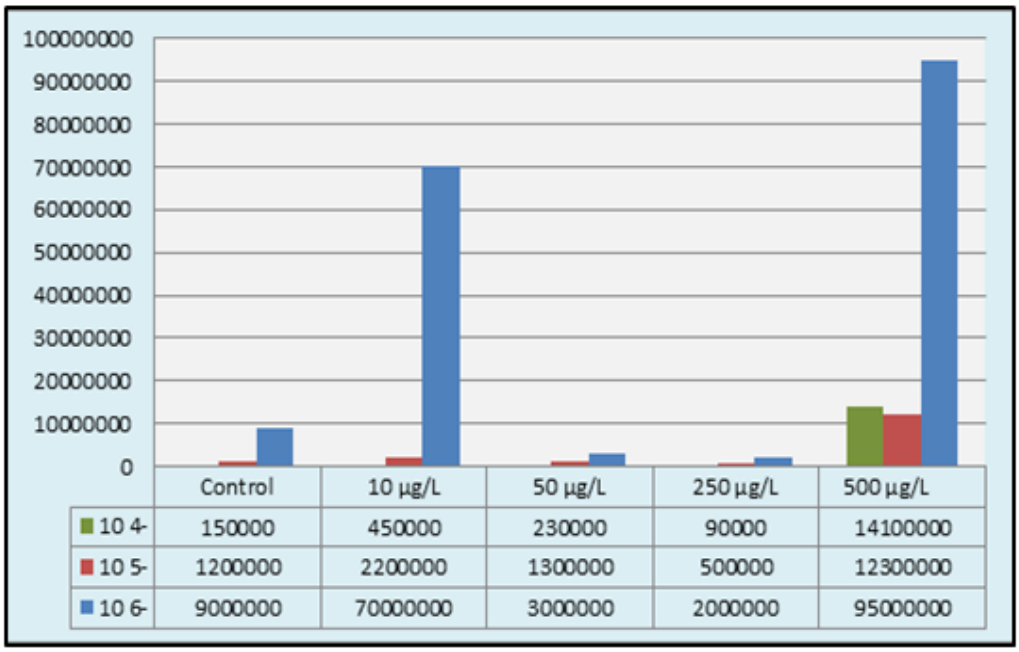
3-2-2- The second stage is detection numbers of bacteria in water when the stem buds appear.

At this stage, after the completion of seed growth and the emergence of stem buds and after adding the alkaloid extract the number of bacteria present in the water was detected. It was found decrease in the number of bacteria in concentration 10 compared to the control treatment, at a concentration of 50, we notice increase in the number of bacteria compared to the control treatment at the concentration of 250 notice an decrease in the number of bacteria compared to the control treatment a t concentration 500 notice an increase in the number of bacteria compared to the control treatment , when comparing the concentrations with each other, we notice an increase in the number of bacteria in concentration 500,10 compared to with the rest of the concentrations The table (3-6) is showing that significant differences in dilution 10^{-4},10^{-5},10^{-6} for all concentrations as showing Figure (3-6).

Table (3-6) detection numbers of bacteria in water when the stem buds appear

dilution / concentration	10^{-4}	10^{-5}	10^{-6}
Control	150000	1200000	9000000
10 µg/L	450000	2200000	70000000
50 µg/L	230000	1300000	3000000
250 µg/L	90000	500000	2000000
500 µg/L	1410000	12300000	95000000
Lsd	34100.03	4478.79	6748.90
p-value	0.000	0.000	0.000

P-Value ≤ 0.05



Figur (4-23) numbers of bacteria in water when the stem buds appear.

3-2--3 The third stage detection number of bacteria after the completion of stem growth.

After completing the growth of the plant stem at this stage increase in the number of bacteria in concentration 10 compared to the control treatment, at a concentration of 50 notice an decrease in the number of bacteria compared to the control treatment, at the concentration of 250 notice an increase in the number of bacteria compared to the control treatment, a t concentration 500 notice an decrease in the number of bacteria compared to the control treatment when comparing the concentrations with each other we notice an increase in the number of bacteria in concentration 10 compared to with the rest of the concentrations.

The table (3-7) is showing that significant differences in dilution $10^{-4,}$ $10^{-5}$for all concentration and showing that no significantdifferences in the 10^{-6} dilution in all concentrations as showing in Figure (3-7)

Table (3-7) detection number of bacteria after the completion of stem growth

Dilution / concentration	10^{-4}	10^{-5}	10^{-6}
Control	60000	400000	2000000
10 µg/L	80000	300000	10000000
50 µg/L	30000	200000	1000000
250 µg/L	80000	500000	2000000
500 µg/L	500000	300000	1000000
Lsd	3467.78	2300.00	43000000
p-value	0.007	0.038	0.223

P-Value ≤ 0.05

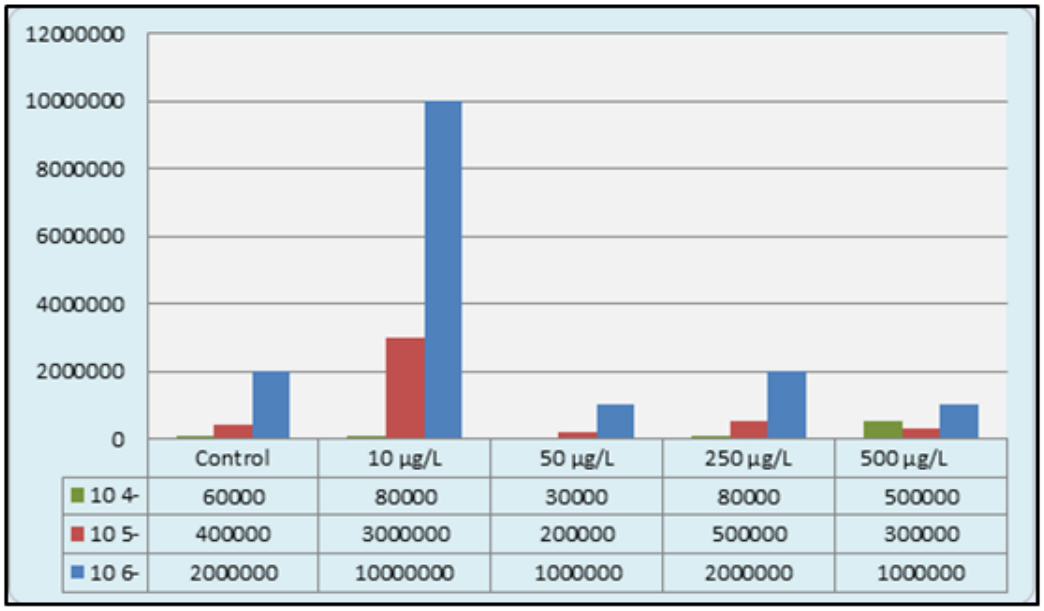


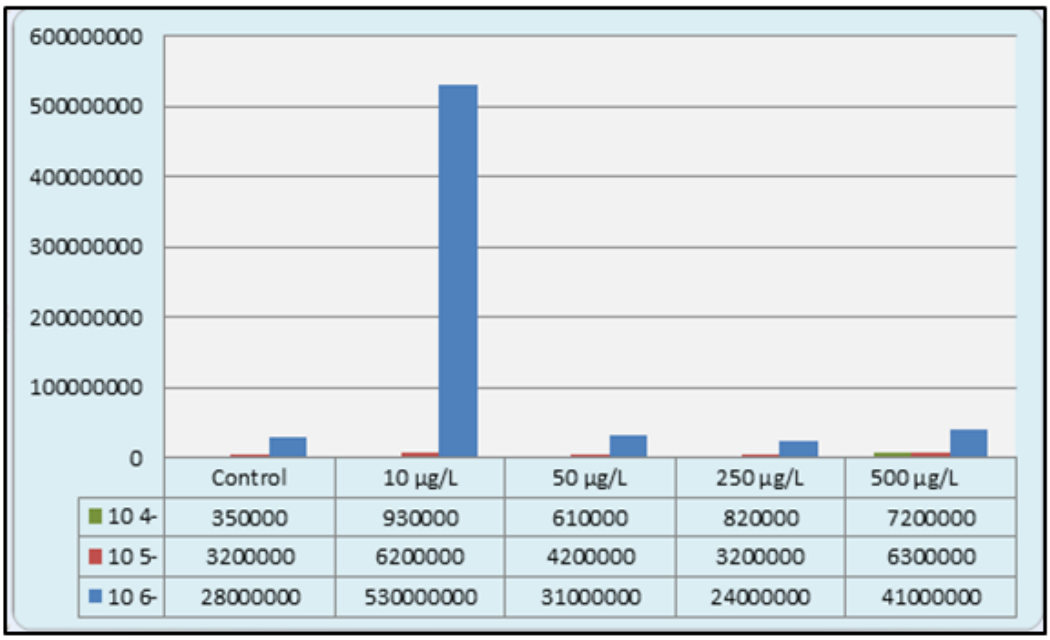
Figure (3-7) number of bacteria in the water after the completion of stem growth

3-2- 4 -the fourth stage: Detection of the number of bacteria after the complete maturity of the leaves.
After the complete maturity of the leaves of the plant, the number of bacteria present in the water was detected after adding the alkaloid extract at this stage, It was found increase in the number of bacteria in concentration 10,50,250and 500 compared to the control treatment. when comparing the concentrations with each other we notice an increase in the number of bacteria in concentration 10. The table (3-8) is showing that significant differences in dilution 10^{-4}, 10^{-5}, and 10-6 for all concentrations as showing in Figure (3-8).

Table (3-8) Detection of the number of bacteria after the complete maturity of the leaves.

Dilution / concentration	10^{-4}	10^{-5}	10^{-6}
Control	350000	3200000	28000000
10 µg/L	930000	6200000	530000000
50 µg/L	610000	4200000	31000000
250 µg/L	820000	3200000	24000000
500 µg/L	7200000	6300000	41000000
Lsd	2378.22	9489.77	84241.33
p-value	0.000	0.000	0.000

P-Value ≤ 0.05



	Control	10 µg/L	50 µg/L	250 µg/L	500 µg/L
10 4-	350000	930000	610000	820000	7200000
10 5-	3200000	6200000	4200000	3200000	6300000
10 6-	28000000	530000000	31000000	24000000	41000000

Figure (3-8) the number of bacteria in the water after the complete maturity of the leaves.

Discussion

4-1-1- First stage: Detection of bacteria numbers before and after Seed germination.

The results of the current study are shown an increase in the number of bacteria at concentration 50, concentration 500 and 250 compared to the control treatment and a decrease in the number of bacteria at concentration 10 compared to the control treatment, This study agrees with the findings(Al-Arousi and Wasfi 1974) It explains the rise of microbes in the roots of the bean plant more than that of barley, in addition to the fact that the thickness of the peg root is more than fibrous, and the presence of adventitious roots with an increase in the initial root area, but the effect of the root circumference also appeared on the soil microbes. The comparison is attributed to the fact that the rootstock of the barley plant grows in large numbers of long, thin roots. It gives ample space to absorb water and essential nutrients in large quantities. And the microorganisms grow densely on these roots compared to the tap roots, the microorganisms spread clearly on the root hairs (Na *et al*., 2018)

5--1-2-The second stage: detection of bacteria when the stem buds appear.

At this stage, the results showed a decrease in the number of bacteria compared to the control treatment. This result is consistent with some studies (Al-Taie.,2002 and Al-Mawla 2006) that showed that microorganisms growing in the root periphery can play a key role in antibiotic processes as the root periphery provides the first line of defense for roots against the effects of pathogenic microorganisms. The root perimeter is an area very rich in organic matter and full of bacterial activity due to the presence of plant roots, which in turn carry out absorption and storage processes in some plants, as they provide a suitable environment for the growth and existence of many plants. Beneficial and pathogenic microorganisms, that this presence creates a kind of competition between these organisms for nutrients, which, with their metabolites and enzymes, try to inhibit or destroy other organisms, by certain mechanisms (Cao *et al*., 2022).

4-1-3-The third stage: detection number of bacteria after the completion of stem growth.

At this stage, the results showed an increase in the number of bacteria compared to the control treatment, and this result is consistent with the findings of some studies Soil microbes are able to rapidly adapt to novel chemicals (Top and Springael 2003). For example hydrocarbon-degrading microbes increased gradually in soil contaminated by crude oil (Oudot*et al.*, 1989). Native soil microbes gradually acquired the ability to degrade xenobiotic compounds by adaptation after repeated application (Hole *et al*,.2001). (Lankau 2011) found that soil microbe communities showed increasing resistance to allelochemicals of A. petiolata over time in terms of taxa richness and community composition of bacteria, fungi and arbuscular mycorrhizal fungi. Soil pathogens also increased with increasing abundance or residence time of invasive plants (Dostal *et al.*, 2013; Flory & Clay 2013)

4-1-4-The fourth stage: Detection of the number of bacteria after the complete maturity of the leaves.

At this stage the results showed an increase in the number of bacteria compared to the control treatment, after the complete maturity of the leaves and this result is consistent with the findings of (Teresa and Gerald 1996). The plant provides a unique environment for microorganisms, and the contribution of the plant in this area is By supplying food through various root secretions such as sugars, amino acids and acids Organic matter from the decaying plant tissues and the dead tissues sloughed off from the roots, which is a source It is large for the organic matter on which non-autotrophic organisms feed, , which in turn affects the growth and reproduction Microbes and helps dissolve some insoluble mineral substances such as phosphorus and potassium compounds Calcium and its availability in the soil, which affects its fertility, as well as increasing the validity of the availability Minor elements such as: Cu, Mn, Zn, F (Wang *et al.*,2016)

4-2-Detection of the numbers of bacteria present in the irrigation water.

4-2-1-First stage: Detection of bacteria numbers in water before and after seed germination.

The results shown that the bacteria count increases at concentration 10, 250 and 500 compared to the control treatment, while the concentration 50 decreases the number of bacteria compared to the control treatment, This result is consistent with the findings of some studies(Uronen *et al.*, 2007) the release of dissolved organic material (DOM) due to plankton lysis in allelopathic Alexandrium tamarense treatments may have increased the amount of bioavailable carbon and supported the growth of bacteria. Similarly, mixed culture trials of Rhodomonas salina and Prymnesium parvum lead to a significant increase of DOC concentration after 30 min and an increase in bacterial biomass after to 12 h. Other studies(Fistarol *et al.*, 2004) have shown that the presence of allelopathic substances could eliminate up to 80 % of nanoflagellates over 3–4 days, and therefore contribute to a high bacterial abundance in algal filtrate treatments(Farooq *et al.*,2014)

4-2-2-The second stage: detection species of bacteria in the water when the stem buds appear.

The results at this stage, that the number of bacteria increased at the concentration 10 and 500 compared to the control treatment, while the concentration 50 and 250 decreased the numbers of bacteria compared to the control treatment, This result is in agreement with the work (Amanidaz*et al.*,2015). An increase in heterotrophic bacteria in a water body may be due to problems with treatment, effect of microbial growth in the flow system or the

presence of biofilm and it may also increase the risk of gastroenteritis (Ainsworth *et al*,. 2004) .

4-2-3-The third stage: detection number of bacteria after the completion of stem growth

The results of the current study showed increase in the number of bacteria in concentration 10,250 compared to the control treatment, at a concentration of 50, 500 notice an decrease in the number of bacteria compared to the control treatment, The effect of allelochemicals on bacterial abundance and production has been reported in a few studies (Fistarol *et al.*, 2004; Uronen *et al.*, 2007). In this study the effect of allelochemicals on bacterial composition in natural communities is reported for the first time. Studies on Alexandrium spp blooms revealed a high abundance of bacteria that were repeatedly found in association to phytoplankton blooms (Wichels*et al.*, 2004). Bacteria associated with diatom cultures revealed specific bacterial communities (Kaczmarska*et al.*, 2005)

4-2-4-the fourth stage: Detection of the number of bacteria after the complete maturity of the leaves.

The results of the current study showed increase in the number of bacteria in concentrations 10, 50,250 and500 compared to the control treatment, the effect of the extract is positive in all concentrations, this result is in agreement with the work (Amanidaz*et al.*, 2015). An increase in heterotrophic bacteria in a water body may be due to problems with treatment, effect of microbial growth in the flow system or the presence of biofilm and it may also increase the risk of gastroenteritis (Prevost *et al.*, 1989)

CONCLUSION

The alkaloid extract of the *Peganum harmala* contributed to a variation in the number of bacteria present in the water and soil at each stage of plant growthwhen compared with the control treatment

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