

## Study the Effects of Chemical Pesticides on Soil Bacterial Community on Khat Agriculture in Dhala Governorate, Yemen

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### Abstract

Pesticides are one of the most hazardous groups of organic compounds polluting the environment. In the Khat production in Yemen, the applications of pesticides are a common practice. Therefore, this study was investigated the adverse effect of widely used pesticides on soil bacterial populations in Khat agriculture samples in Al-Hasein district, Dhala Governorate, Yemen. The pesticides used in studying area are identified. The number of bacterial colonies per gram of sample soil was determined by total plate count method and the isolated was identified according standard methods. The result was recoded eighty-eight (88) types of pesticides classified under 27 active ingredients in the studying area. Also, the present result showed that the average of bacterial colonies in sample collected from site treated with pesticides is much higher than the average of bacterial colonies in sample collected from site not treated with pesticides. Also, the number of bacteria found on soil sample affected by duration of pesticides used in studying area. The number of bacterial colonies in the area treated with pesticides for 20 years is less than area treated with pesticides for 5 years which indicated that the pesticides have significant effect on bacterial community in soil. It was found that the number of bacterial colonies tested sample was slightly affected by seasons change. Six of bacterial species have been identified in tested sample. From this study, it is evident that the pesticides which used for Khat tree play a great role in killing or reducing the agricultural soil organisms and harmful for Yemeni ecosystem. Therefore, a further investigation are needed to estimation their persistence of toxicity on soil.

**Key Words:** Bacterial colonies; Khat; Pesticides; Soil sample

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### Introduction

The fertility of soil depends not only on the textures of soil but also on the biological ability within it. The microbial diversity may have been changed following pesticide use, and such changes may affect soil fertility. Soil microorganisms therefore play important roles in the soil fertility. The use of pesticides to protect crops may alter the soil biological ability either by direct or indirect action, but the knowledge of soil microbial ability to degrade pesticides and the influence of pesticides on microbial diversity in soil is still limited [1].

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Continual widespread use and release of pesticides has become an everyday occurrence, resulting in environmental pollution. It has been estimated that only 0.1% of applied pesticides reaches the target pests and the remaining 99.9% accumulates in soils and affects directly or indirectly the microbial community and enzyme activities [2-5]. Microorganisms are essential components of soil ecosystem as they degrade soil organic matter and sustain biogeochemical transformations of elements [6]. As they respond rapidly to any changes in soil composition, soil microbial communities are regarded as bioindicators and used for the assessment of soil quality and for the prediction of soil degradation [7].

Some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms. Use of Phosphoric insecticides such as Dursban and Cardona led to the revitalization of soil microbes. While the other types of Phosphoric insecticides have shown an increase in the numbers of fungi and a decrease in the numbers of bacteria [8]. In experiments conducted to examine the impact of the Phosphoric insecticide Chloropyrifos. It was found that led to a change in the functions and composition of soil microorganisms [9]. Also, it was mentioned that the succession of pesticide use on a crop for many years leads to soil degradation and weakening of the economic attributes of the cultivated crop [10].

## Materials and Methods

### Study area

This study has been conducted on Al-Hasein district, Dhala Governorate, Yemen. This area where the cultivation of Khat tree is concentrated and the farmers are used the pesticides with an intensive and random way for the Khat productive for long times which may be changed on the soil microbial community in agriculture.

### Identification of pesticide used

The pesticides used in the studying area have been obtained by asking the farmers directly and recorded in logbook.

**Collection of soil samples:** Soil samples at 10–15 cm depth were collected from four areas located at Al-Hasein district, Dhale governorate, as listed in Table (1). In each area, two site were selected, one site (tested sample) is planted with Khat tree and used the pesticides for long time, while another site (control sample) is not used the pesticides and not planted with Khat tree. From each site in each area, two samples were collected and were immediately transferred to the laboratory. The samples were collected during summer and winter seasons.

No.	Area Name	Sample site	State of soil	Area code/symbol
1	Shaka	Tested sample	Planted with Khat 20 years ago	B1
		Control sample	Not planted with Khat	A1
2	Khubar	Tested sample	Planted with Khat 15 years ago	B2
		Control sample	Not planted with Khat	A2
3	Marfed	Tested sample	Planted with Khat 10 years ago	B3
		Control sample	Not planted with Khat	A3
4	Musaiter	Tested sample	Planted with Khat 5 years ago	B4
		Control sample	Not planted with Khat	A4

**Table 1:** The distribution of the areas of the study as well as their symbols.

**Determination of the number of bacteria of soil samples**

The experimental tests were carried out in the microbiology laboratory at the National Drug Authority in Khormaksar, Ministry of Health - Aden Governorate. One gram of each sample individually was diluted with 99 mL of sterile normal slain. The dilution solution was shaken strongly by hand for 15 min. Then, one mL of diluted solution was transferred into surface petri dishes 9 cm in diameter which contained 20 mL of a solidified of Nutrient Agar (Rapid Biotec, Uk) and incubated at 37°C for 3 days. The number of colony forming units (cfu) per ml in each plate was counted and multiplied by the dilution factor. The microbial content was taken as the average of duplicate determinations [12].

**Identification of isolated bacteria**

Identification of the isolated bacterial species was preliminarily performed by macroscopic examination of the microbial morphology of the pure cultures of isolated bacteria and other typical growth characteristics on non-selective, selective, and differential culture media and complemented with Gram staining [13].

**Results and Discussion**

Eighty-eight 88 pesticides were recoded under 27 active ingredients in the study area of Al-Hasein district in Dhale governorate in the studied sites (Shaka, Merfad, Musiater and Khubar areas). It has been found through the recording and enumerating processes that all studied sites are used the same pesticides mentioned as listed in Table (2).

No.	Active ingredients	Common Name	No.	Active ingredients	Common Name		
1	Methidathion	Super acide	8	Triazophos	Novacron		
		Cobra Acide	9	Dimethoate	Perfekthion		
2	Abamectin	Super mectin	10	Lambda Cyhalothrin	Atromax		
		Cruise A.A			Lamix		
		Saimk for			Phoeinx		
		Zemectin			Lothrin		
		Slomectin			Mentec		
		Mixed super			Miral		
		Crawn	11	Acetamipride	Belarmose		
		AL-Maheeb			Cruise A.A		
		Shahin			Al-kaseh		
		Galex			Jagoar		
		Nadal			Mixed super		
		Soscam			Crawn		
		Cliper Rise			Miprid		
		Dungu			Devonad		
3	Kresoxim-methyl	Stroby			Tacross		
		Simks			Artebon		
		Agro Max			AL-EfreetF		
4	Pyridaben	Feron			Topride		
		Triedol			Euro		
		AL-Maheeb			Super Qasm		
5		Mas					AL-Shabah

	Enamectin Benzoate	Emak	12	Deltamethrin	Crystal
		Mores			Methrin
		Dungu			Delta yam
		Antrek	13	Carbendazim	Scan
Fenzole Score	Super Walter				
6	Difenoconazole	Agro Skar	14	Bifenthrin	Crystal
		Siko Amistar	15	Fendvalerate	Sred sun
		Antrek	16	Fipronil	AL-Ambrattor
		Imidacloprid	17	Phnpropatren	Crash
7	Imidacloprid	Faster	18	Chlorpyrophos	AL-Kashf
		Rahib Star	19	Bifenthrin	Agrotlstar
		Miral	20	---	Superphose AL-Awal
		AL-Fatak	21	Methomyl	Agrin ate
		AL-mosre Hiper	22	Amidacloprid	AL-qassam
		Kenador	23	Thiamethoxam	Engeo super
		Castro	24	Diniconazole	Wopro Role
		Afeedx	25	Penconazole	Topas
		Jastar	27	Methomyl	Agrinate
		Temolex			AL-Tofan
		Quaster	28	Azoxystroben	Quadris
		London			Siko Amistar

**Table 2:** The name of pesticides that used in studying area.

The irresponsible and unconscious use of pesticides is one of the most serious problems which face the world and result in an imbalance in the ecosystem components [14-17]. The continually of Khat cultivation in the same soil for long years will inevitably lead to the loss of the soil microorganisms and therefor the destruction of the soil itself fertility due to loss of essential substances. It was indicated that the chemical pesticides and soil-added chemicals affect soil microorganisms, and result in a change in the functions of organisms and affect their activity. Also, it leads to the accumulation of these substances in agricultural soil [10].

The effects of various pesticides on bacterial count are presented in Table (3). The average number of bacterial colonies obtained from the soil sample at the site B1 (treated with pesticides for 20 years) was 7.9 cells/g in the first season and 12.3 cells/g in the winter season, in comparison with the control site A1 which was based on rainfed agriculture only (i.e. rely on rainfall for water), without tampering with the soil by the farmers. The number of bacterial colonies was very high and gave an average of 124.7 cells/g in the summer season and 133.1 cells/g in the winter season. These findings are similar to the study of Nasser and Al-Daas, [18] indicated that when pesticides reach directly or indirectly to the soil, they affect the microorganisms and their activity.

It was reported that many types and species of microorganisms, including phosphate soluble bacteria, spread in the soil around the rhizosphere area near the roots of the plant and thus they are the most affected organisms by pesticides [19-21]. From the present result, it is clear that the number of bacteria count affected by use of pesticides. The bacterial colonies in soil sample collected for site B1 which treated with pesticides for 20 years are less than bacterial colonies in soil sample site B3 which treated with pesticides for 20

years. This is evident that the use of pesticides for many years leads to kill or decrease the number of harmless or beneficial microorganisms in the soil significantly and for varying periods of time as a result of adding pesticides to soil which in turn leads to loss its fertility.

These results indicate that the use of pesticides in an intensive manner led to soil degradation and a significant reduction in the number of bacteria compared with the control sites. In a similar study by Al-Moorman [22]. and Dickrell, [10] demonstrated that the consecutive use of a pesticide on a crop for many years leads to soil degradation, weakening and destruction of its organisms, and also leads to a multiplication of the economic characteristics of the cultivated crop. The current results showed that the average number of bacterial colonies in soil sample selected from control sites (B1 to B4) are much than average number of bacterial colonies in soil sample selected from tested sites (A1 to A4). Also, the various in results between two seasons due to the climate change in studying area and rain seasons (Tale 3).

No.	Area Name	Site tested	Average of bacterial count per gram (cells/g)	
			Summer Season	Winter Season
1	Shaka	Sample (B1)	7.9	12.3
		Control (A1)	124.7	133.1
2	Khubar	Sample (B2)	105.1	68.5
		Control (A2)	138.8	183.9
3	Marfed	Sample (B3)	153.9	152.6
		Control (A3)	247.4	249.2
4	Musaiter	Sample (B4)	339.0	317.0
		Control (A4)	736.0	625.0

**Table 3:** The number of bacterial colonies in soil samples.

The results of current study revealed that the most common bacteria identified in soil samples are *Micrococcus* sp., *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., *Actinomycetes* sp., and *Leuconostoc* sp.

### Conclusion

The effect of pesticides varied greatly with the type, rate, and time after application of pesticides. Efforts must be made to determine the proper type and dosage of pesticide for agricultural crops in order to prevent their adverse effect on the environment in general and on useful soil microorganisms in particular. This study revealed that the all pesticides used for Khat tree have generally led to a decrease in the number of bacteria in the soil. Thus, they have the potential to cause environmental pollution.

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