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THE EFFECT OF PESTICIDES USED IN CONVENTIONAL AND ORGANIC FARMING ON THE MICROBIAL FLORA

Hikmet Katırcıoğlu[1], Sema Çetin[2], Dürdane Kaya[2] and Semra Mirici[1]
[1]Gazi University, Faculty of Education, Department of Biology Education, Teknikokullar-Ankara, Turkey
[2]Kırıkkale University, Faculty of Arts and Sciences, Department of Biology, Yahşihan-Kırıkkkale, Turkey
E-Mail: hturk@gazi.edu.tr

ABSTRACT

In this study, Basudin 60 EM, Cupravit OB 21 and Dursban 4 of synthetic pesticides widely used in agriculture, bordeaux mixture of natural pesticides and Componion of biologic pesticides was investigated the effect on the bacteria isolated from microbial flora. In this context it was aimed to compare the effect of pesticides used in conventional and organic farming on microbial flora. The changes of metabolic activity was demonstrated according tothe effects of pesticides in the microbial flora. The leaf and soil samples obtained elaborately from the same station, *Bacillus cereus* DY6, *Micrococcus yunnanensis* DT1 and *Bacillus tequilensis* DT2 were selected as indicator strains. The changes in antibiotic susceptibility and generation time as metabolic activity of these microorganisms and genetic modifications that creates on indicator strain were investigated. As a result, it was determined that pesticides used in applications of organic farming changed metabolic activities of the bacteriaas much as conventional applications. It has been found that especially bordeaux mixture as a natural pesticide used in Turkey causes genetic modifications.

Keywords: synthetic pesticides, natural pesticides, biologic pesticides, microbial flora, metabolic activity.

1. INTRODUCTION

Pesticides are synthetic or biological products that are used to push away or destroy such as pathogenic microorganisms, insect, rodent, weeds, fungi which cause damage to agricultural products and loss product during production, consumption and storage of nutrients and regulate plant growth (Ataman, R, Petek, 2007 and Haktanır, K., Arcak, S., 1998). While the use of pesticides improve production in agriculture, on the other hand, unconscious and incorrect usage of pesticides are problem on bothhuman and environment health. Pesticides or conversion products can remain in foods, soil-water and air when pesticides used over recommended doses, in case mixed more than one drug is not required orfollowedenough time between the last application and the harvest period. In human fed with foods that contain high-dose pesticide residues and other organisms in the environment may be acute or chronic poisoning, the quality and aroma changes may occur especially in some products (Heming, J.C., Davis, A. C., Robinson, W. B., 1954).

For the first time it was understood impact of pesticides on living organisms by the presence of organic chlorinated pesticide residues in the human body in 1948-1951. It was determined while some pesticides are not toxic, some of them carcinogenic, impress the nervous system and even cause the mutation (Delen, N., Durmuşoğlu, E., Güncan, A., Güngör, N., Turgut, C., Burçak, A., 2005). Organized producers and consumers in order to eliminate negativity have begun to prefer agricultural methods and products which do not affect human health and produced products which do not destroy nature. Biyopesticides used in this context are known as substances that low toxic for non-target beneficial organisms, quickly crumble, non-mutagenic, selective display and cause minimal damage in the ecosystem (Raizada, R.B., Srivastava, M.K., Kaushal, R.A. ve Singh, R.P., 2001).

Today in the world, usage of pesticide and the results of this usage are always on the spotlight. Because, it is available that the use of intense pesticides in conventional agriculture, the use of controlled pesticides in good agricultural practice (GAP) and the use of natural pesticides in organic farming. It should be expanded the use of natural and biopesticides that are known to be less harmful, but considering even this biomaterials can be toxic compounds, such studies should be given more weight. Pesticides can affect microbial population by changing metabolic and physiological activity directly or indirectly. There is a lot of research on the role of pesticides used in agricultural applications in the ecosystem. However, detailed studies on the microbial flora did not reveal.

In this study, it was aimed to determine the effects of natural and biopesticides which are thought to be harmless, as well as synthetic pesticides which are considered to have adverse effects to the ecosystemon generation time and metabolic activity of bacteria.

2. LITERATURE REVIEW

%14 - 80 of agricultural pesticides is applied to soil (D.N. Denizeri, 2001). The 75% of pesticide used in the United States is in the agricultural area (Şevken, S., 2009). The most widely used pesticides are synthetic used in our study and classified as a synthetic, natural and biopesticide. When analyzing the studies, it was seen that the studies of effects on organisms focuses on synthetic pesticides. (R. Bilaloğlu, 1982; M. Çelik, 2003; Pandey, R. M., 2008; Aydemir, N., 2008; Bolle, P., Mastrangelo, S., Tucci, S. Paola., Evandri, M. G., 2004;] Koca, S., 2008; G. Kara, 1998; R. Kırımlı, 2007; Gill. S. A., Shaukat. S. S., 2000; Özörgücü, B., Türkan, Oğuz, G., Gönüz, A., Acar, O., 1995).

Several studies have been conducted to determine the effects of pesticides on microorganisms and studies contain the data obtained from the numeric change in microflora at the result of the application in soil. Odeyeni and colleagues (1977) investigated the impact of fungicid (Phygon, Spergon ve Thram) on Rhizobium strains form small nodule on peas and detected to reproduce sensitive Rhizobium strains in environment has more fungicid concentration (Odeyeni, O. ve M. Alexander, 1977). It has been shown synthetic pesticide used against blue mold disease in tobacco reduce the number of soil microfungus (Özörgücü, B., Tort, N., Gönüz, A., 1991). However, Diğrak and colleagues (1996) and Kaçkar and colleagues (1998) have reported the number of total microorganism has not changed in the soil applied synthetic pesticides (Dığrak, M., Kırbağ, S., Özçelik, S., 1996 and Dığrak, M., Kaçar, N., Sönmez, A., 1998).

It is stated there is 2.5 million bacteria, 400.000 fungi, 50.000 algae and 30.000 protozoa in a gram of fertile agricultural soil (Yıldırım, E., 2008). The species to be found in large numbers in the soil and comprise 90% of bacteria population are *Pseudomonas sp, Arthrobacter sp, Clostridium sp, Achromabacter sp, Bacillus sp, Micrococcus sp* ve *Flavobacterium sp*. (Nesime Cebel, 2011).

While one part disappears due to evaporation and dispersion, the other part remains on the plant and surface of the soil during pesticides implementation by spraying. Usually the leaves of plants are devoid of the microorganisms when they ocur first. However, there are different microorganisms on the leaf surface gradually and continue their life. Leaf surface microflora is influenced by many factors such as the host type, leaf structure, maturity status and vegetation density. The total number of microorganism depends on atmospheric conditions such as temperature and humidity (Fokkema, N. J., 1983).

3. MATERIALS AND METHODS

Isolation and identification of bacteria from microbial flora

Leaf and soil samples used in the study non-applied the agricultural pesticide is provided from 2 station (Station 1: Demirısık Village, Station 2: Kuyuluk Region) specified as an agricultural landin Mersin. In designated stations the soilcollected from 5 cm depth of selected parcels as 100/100 cm and leaves collected from trees in the region were brought to the laboratory under sterile conditions. Prepared 10 gram dilue soil and leaf samples were incubated in plates containing the nutrient agar and enrichment media. The isolations were carried out from the colonies formed as a result of incubation. The different colonies were inoculated onto nutrient agar medium were incubated at 30°C for 24 hours. Bacterial isolates stocked with 30% sterile glycerol in sterile eppendorf. All isolates were stored at -20 °C.In this way total 50 bacteria isolation was carried out from soil and leaf samples collected from agricultural land. Gram staining, spore staining, colony morphology and mobility have been investigated for determination of morphological, physiological and biochemical properties of isolated pure cultures (Temiz, A., 2008).3 isolates the most easily breeding and obtained intensive biomass were selected for further identification can be made. Primarily DNA isolation and sequence analysis are reviewed from samples given as pure for molecular identification. According to the base sequence analysis of the 16S rRNA gene region replicated with 16S rDNA PCR method was conducted in Gazi University Life Sciences Application and Research Center.

The antibacterial effect of pesticides used and determination of the MIC (Minimum Inhibition Concentration) values

As synthetic pesticides widely used in agriculture insecticide Basudin 60 EM (diazinon), fungicide Cupravit OB 21(copper oxychloride) and organophosphorus insecticide Dursban 4 (klorpirifos - ethyl), as a natural pesticide fungicide bordeaux mixture (98% copper sulfate II and calcium hydroxide) and as a biological pesticide Companion (Bacillus subtilis GB03)were used in this study. *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Bacillusspizenii* ATCC 6633, *Escherichia coli* KKU and *Salmonella sp.* KKU were used in the study as a test bacteria. Microdilutions were done for the determination of MIC concentrations of pesticides on these bacteria by using U based 96 well plates.100 µl nutrient broth, 50 µl bacterial culture to be tested and ranging from 10-100 µl pesticides have been added into the wells (Eom, S., Park, J., Yu, D., Choi, J., Choi, J., Lee, M., & Kim, Y., 2011 and Sasidharan, S., Darah, I., Noordin, M. K. M. J., 2010).

Although it is known the antibacterial properties of synthetic pesticides used in the study, it has not been obtained sufficient information about natural and biyopestisit as a fungicide on the market. Because of that, the effects of general inhibition tested for the purpose of the determination of the antibacterial properties of pesticides to be tested by using disc diffusion method. 2 doses as pesticide applications on the market have been used. These are; land dose, also taken into consideration data obtained after MIC tests (application dose on the label) and high dose (application dose used by farmers). *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Bacillusspizenii* ATCC 6633, KKU *Escherichia coli* and *Salmonella sp*. KKU were used in experiments as the test bacteria. Disc diffusion method was applied according to the Kirby-Bauer Disc Diffusion Test Protocol (Anonim, http://mikrobiyoloji.thsk.saglik.gov.tr, 2016).

The determination of the effect on the microbial flora of pesticides

It was considered impacts of pesticides on microbial flora; such as properties of the antibiotic susceptibilities, changes in generation time and genetic modifications.

The determination of antibiotic susceptibility

Several antibiotic susceptibility of activated (according to 0.5 Mac Farland) strains in medium with or without pesticides was determined according to the recommendations of "Clinical and Laboratory Standards Institute (CLSI, 2011) using Mueller-Hinton agar with disc diffusion method. In a sensitivity test, penicillin (10U), tetracycline (30 µg), ampicillin (10 µg), gentamicin (120 µg), erythromycin (15 µg) and vancomycin (30 µg) antibiotic discs were used. Zone diameters obtained as a result of the antibiotic susceptibility tests were evaluated according to CLSI NCCLS (2011) data.

The determination of generation number and time

Strain shows maximum change in antibiotic susceptibility was selected in order to determine the effect of pesticides on bacterial growth. This strain is *Bacillus tequilensis* DT2. *Bacillus tequilensis* DT2 strain was incubated in nutrient broth for 24 hours in 37 ^{0}C and control group was formed by obtaining culture in appropriate to 0.5 Mac Farland turbidity. The synthetic pesticide Cupravit (high dose), natural pesticide bordeaux mixture (land=MIC and high dose) and biyopestisit Companion (MIC dose), modified maximum antibiotic susceptibility of the bacteria tested in the trial groups, was applied to *Bacillus tequilensis* DT2 strain. The disseminated inoculation by taking 100 µl from 0., 2., 4., 6., 8., 10., 26., 28. 50. tubeon nutrient agar plates were incubated for 24 hours at 30 ^{0}C. The bacterial strains were caunted after 24 hours, generation number and generation time were calculated according to the following formula (Arda, M., 2000).

$$n = \frac{\log b - \log a}{\log 2} \qquad g = \frac{t \times \log 2}{\log b - \log a}$$

n = generation number
g = generation time
a = initial number of microorganism
b = number of microorganism at the end of the period
t = time

The determination of genetic modification

Bordeaux mixture which is detected maximum change in bacterial growth and antibiotic susceptibility placed in the detection of genetic modifications has chosen as an indicator pesticide. Activated *Bacillus cereus* DY6, *Micrococcus yunnanensis* DT1 and *Bacillus tequilensis* DT2 strains inoculated to the nutrient agar plates containing land dose (15 mg/ml) and high dose (240 mg/ml)of bordeaux mixture and incubated for 24 hours at 37 ^{0}C. Base sequences analysis of 16S rRNA gene of the strains treated and not treated with pesticide was conducted in Gazi University Life Sciences Application and Research Center. Accordingly, modifications have been identified by comparing the results obtained from strains treated and not treated with pesticides.

4. RESULTSAND DISCUSSIONS

The results of species identification based on 16S ribosomal RNA of selected 3 strains by 50 strains isolated from soil and leaf samples are given in Table-1.

Table-1. Species isolated from the stations.

Strain Code	Name of the StationandIsolation Place	EMBL/Gen Bank Number	Name of the Species
DY6	Station1 Leaf	KT719870.1	*Bacillus cereus*
DT1	Station 1 Soil	KT719656.1	*Micrococcus yunnanensis*
DT2	Station1 Soil	KT720350.1	*Bacillus tequilensis*

This 3 isolate can be found in soil and leaf flora often.

Primarily minimum inhibition concentration was determined to show the effects of pesticides on identified 3 bacteria. Accordingly, the data obtained are shown in Table-2.

Table-2. MIC concentrations of pesticides on test microorganisms

Test Microorganisms	Pesticides				
	Biological Pesticides	Natural Pesticides	Synthetic Pesticides		
	Companion	Bordeaux mixture	Basudin 60 EM	Cupravit OB 21	Dursban 4
Escherichia coli (KKÜ)	12 ml/L	10 mg/ml	3ml/L	128 mg/ml	-
Pseudomonas aeeruginosa ATCC 9027	12 ml/L	13 mg/ml	0.70 ml/L	5 mg/ ml	3 ml/L
Salmonella sp. (KKÜ)	8 ml/L	15 mg/ml	0.65 ml/L	256 mg/ml	24 ml/L
Staphylococcus aureus ATCC 6538	2 ml/L	15 mg/ml	0.55 ml/L	6.5 mg/ml	3 ml/L
Bacillus spizenii ATCC 6633	12 ml/L	15 mg/ml	3 ml/L	128 mg/ml	24 ml/L

According to the data obtained the inhibitory concentrations of all pesticides on the test bacteria were found close to each other. While MIC dose of bordeaux mixture which is a natural pesticide was determined to be applied dose in the field and MIC value of Dursban 4 which is a synthetic pesticide was found to be high than land dose. Application doses on agricultural land and properties of pesticides on the bacteria isolated and identified from the stations are given in Table-3.

Table-3. The properties of synthetic, biological and natural pesticides used in the study.

The name of pesticide	Product Group	Manufacturer	Land dose	High dose
Basudin 60 EM	Insecticide	Bayer	0,75ml/ L	12 ml/L
Cupravit OB 21	Fungicide	Bayer	8 mg/ ml	256 mg/L
Dursban 4	Insecticide (organophosporous)	Dow Agro Sciences	1,5 ml/ L	24 ml/L
Bordeaux mixture	Fungicide	Lenafruit 20 WP	15mg/ml	240 mg/ml
Componion (*Bacillus Subtilis* GB03)	BiologicalFungicide	Growth Products	0.5 ml/L	16 ml/L

Despite of MIC concentrations of tested pesticides determined, antibacterial activity of application doses in the agricultural areas (land dose and high dose) on the test bacteria was investigated (Tables 4a and 4b).

Tablo-4a. The antibacterial activities of synthetic, biological and natural pesticides used in the study (high dose).

The name of pesticide	Test Microorganisms				
	P. aeruginosa	***S. aureus***	***B. spizenii***	***E. coli***	***Salmonella sp***
Componion (high dose)	24 ± 0.02	10 ± 0.01	-	-	10 ± 0.01
Bordeaux mixture (high dose)	18 ± 0.01	21 ± 0.00	27 ± 0.02	22 ± 0.01	12 ± 0.02
Cupravit OB 21 (high dose)	9 ± 0.01	9 ± 0.02	-	4 ± 0.03	-
Basudin 60 EM (high dose)	12 ± 0.01	21 ± 0.01	30 ± 0.02	-	10 ± 0.02
Dursban 4 (high dose)	-	14 ± 0.02	29 ± 0.02	-	14 ± 0.02

Tablo-4b. The antibacterial activities of synthetic, biological and natural pesticides used in the study (land dose).

	Test Microorganisms				
The name of pesticide	***P. aeruginosa***	***S. aureus***	***B. spizenii***	***E. coli***	***Salmonella sp***
Componion (land dose)	8 ± 0.03	-	10 ± 0.00	-	8 ± 0.03
Bordeaux mixture	-	-	7 ± 0.01	9 ± 0.02	-
Cupravit OB 21 (land dose)	10 ± 0.04	10 ± 0.02	-	-	-
Basudin 60 EM (land dose)	-	-	10 ± 0.02	-	-
Dursban 4 (land dose)	-	14 ± 0.01	-	-	-

Whereas synthetic pesticides effect to *S. aureus,* natural and or biological pesticides don't. It has been found that land dose of biological and natural pesticides are more effective for *B. spizenii*. It has been found that the most effective pesticide in the dose of agricultural practice for *Salmonella sp. and B. spizenii* isbiopesticide. It was found that the natural pesticide (bordeaux mixture) was the most effective pesticide on *E. coli* one of pathogens tested. It was determined also applications of bordeaux mixture in high doses had inhibition effect in all tested bacteria.

Antibiotic susceptibility changing before and after application of pesticide on 3 strain isolated from stations are shown in Table-5 and Table-6.

Table-5. As a control group, the antibiotic susceptibility of *Bacillus cereus DY6, Micrococcus yunnanensis DT1, Bacillus tequilensis DT2* (zone diameter - mm).

Antibiotics	**Control Group (*Bacillus cereus* DY6)**	**Control Group (*Micrococcus yunnanensis* DT1)**	**Control Group (*Bacillus tequilensis* DT2)**
Erythromycin	27 ± 0.02	-	12 ± 0.01
Gentamycin	26 ± 0.03	23 ± 0.02	22 ± 0.03
Penicillin	-	10 ± 0.01	9 ± 0.02
Tetracycline	30 ± 0.02	40 ± 0.00	13 ± 0.02
Vancomycin	21 ± 0.03	24 ± 0.01	20 ± 0.03
Ampicillin	10 ± 0.01	-	12 ± 0.01

(-): No inhibition

Table-6. The antibiotic susceptibility of Bacillus cereus DY6, Micrococcus yunnanensis DT1, Bacillus tequilensis DT2 (zone diameter - mm).

		Bacillus cereus DY6		*Micrococcus yunnanensis DT1*		*Bacillus tequilensis DT2*	
Pesticides	**Antibiotics**	**Land dose**	**High dose**	**Land dose**	**High dose**	**Land dose**	**High dose**
Componion (Bacillus subtilis GB03)	Erythromycin	27 ± 0.01	25 ± 0.02	25 ± 0.02	23 ± 0.01	12 ± 0.02	12 ± 0.01
	Gentamycin	24 ± 0.02	25 ± 0.00	22 ± 0.01	26 ± 0.02	22 ± 0.01	23 ± 0.02
	Penicillin	-	-	30 ± 0.03	20 ± 0.02	11 ± 0.03	8 ± 0.03
	Tetracycline	27 ± 0.02	30 ± 0.02	45 ± 0.01	40 ± 0.01	14 ± 0.00	13 ± 0.01
	Vancomycin	18 ± 0.03	23 ± 0.00	22 ± 0.01	26 ± 0.02	20 ± 0.01	20 ± 0.01
	Ampicillin	-	8 ± 0.02	-	25 ± 0.03	9 ± 0.03	-
Bordo Mixture	Erythromycin	25 ± 0.02	28 ± 0.00	25 ± 0.01	25 ± 0.01	10 ± 0.01	25 ± 0.03
	Gentamycin	20 ± 0.03	27 ± 0.01	21 ± 0.02	30 ± 0.03	25 ± 0.03	25 ± 0.01
	Penicillin	-	16 ± 0.02	27 ± 0.02	17 ± 0.01	-	31 ± 0.00
	Tetracycline	29 ± 0.00	37 ± 0.02	43 ± 0.00	42 ± 0.00	15 ± 0.02	40 ± 0.01
	Vancomycin	17 ± 0.01	23 ± 0.01	27 ± 0.01	27 ± 0.03	19 ± 0.01	27 ± 0.03
	Ampicillin	-	15 ± 0.03	18 ± 0.03	22 ± 0.01	12 ± 0.03	23 ± 0.01
Dursban 4	Erythromycin	14 ± 0.01	25 ± 0.01	30 ± 0.02	30 ± 0.02	17 ± 0.03	20 ± 0.02
	Gentamycin	30 ± 0.00	29 ± 0.02	27 ± 0.01	30 ± 0.01	24 ± 0.01	21 ± 0.03
	Penicillin	10 ± 0.01	16 ± 0.03	41 ± 0.00	33 ± 0.02	-	31 ± 0.01
	Tetracycline	22 ± 0.02	35 ± 0.02	42 ± 0.03	48 ± 0.00	18 ± 0.01	
	Vancomycin	24 ± 0.03	24 ± 0.00	28 ± 0.02	25 ± 0.01	21 ± 0.03	22 ± 0.01
	Ampicillin	-	14 ± 0.01	43 ± 0.00	22 ± 0.02	-	
Basudin 60 EM	Erythromycin	20 ± 0.01	25 ± 0.02	29 ± 0.00	28 ± 0.02	-	-
	Gentamycin	26 ± 0.02	25 ± 0.00	25 ± 0.01	22 ± 0.01	19 ± 0.01	21 ± 0.01
	Penicillin	-	15 ± 0.02	48 ± 0.00	29 ± 0.01	10 ± 0.03	9 ± 0.03
	Tetracycline	34 ± 0.01	35 ± 0.00	43 ± 0.02	38 ± 0.00	12 ± 0.01	30 ± 0.00
	Vancomycin	27 ± 0.02	24 ± 0.01	25 ± 0.02	25 ± 0.03	20 ± 0.02	20 ± 0.03
	Ampicillin	9 ± 0.01	15 ± 0.03	36 ± 0.01	18 ± 0.02	8 ± 0.01	-
Cupravit OB 21	Erythromycin	24 ± 0.01	21 ± 0.01	30 ± 0.00	25 ± 0.02	28 ± 0.02	18 ± 0.01
	Gentamycin	25 ± 0.02	25 ± 0.03	30 ± 0.01	30 ± 0.02	33 ± 0.01	25 ± 0.03
	Penicillin	-	16 ± 0.02	47 ± 0.00	21 ± 0.03	-	23 ± 0.01
	Tetracycline	38 ± 0.03	42 ± 0.01	43 ± 0.01	30 ± 0.02	44 ± 0.01	33 ± 0.00
	Vancomycin	29 ± 0.02	22 ± 0.00	28 ± 0.03	26 ± 0.01	27 ± 0.02	22 ± 0.01
	Ampicillin	10 ± 0.03	15 ± 0.01	45 ± 0.03	26 ± 0.03	21 ± 0.01	20 ± 0.02

(-): No inhibition

It has been determined that susceptibilities *of Bacillus cereus* DY6 to erythromycin in all pesticide groups has resistance after culturation. It is noteworthy that also *Bacillus cereus* DY6 has sensitivity to penicillin after culturation with bordeaux mixture widely used in especially organic agriculture of *Bacillus cereus* DY6 resistant to penicillin. It has been determined that ampicillin susceptibility changedin all pesticide groups and reduced according to the control.

It was found that *Micrococcus yunnanensis* DT1 resistant to ampicillin and erythromycingained sensitivity after culturation with pesticides. While *Bacillus tequilensis* DT2 was resistant to erythromycin, ampicillin and penicillin according to NCCLS (2011) data, it was found that this strain gained sensitivity after culturation with pesticides especially bordeaux mixture.

Bacillus tequilensis DT2 shows the maximum change in antibiotic susceptibility cultured with cupravit (high dose) from synthetic pesticides, bordeaux mixture (land dose and high dose) from natural pesticides and companion (MIC dose) from biopesticides in order to determine the effect of pesticides on bacterial growth. The change in generation number and generation time compared with the control, are shown in Table-7.

Table-7. Determined generation time and numbers after culturation with pesticides of *Bacillus tequilensis* DT2.

	Generation numbers (cfu/ml)	Generation time (min.)
Control Group	$20x10^4$	48
Cupravit-high dose (synthetic)	$10x10^4$	96
Componion- land dose (biopesticide)	$6x10^4$	144
Bordeaux mixture-land dose (natural)	$13x10^4$	72

According to the data obtained as much as synthetic pesticides, natural and biopesticides used in organic farming have been changed in bacteria generation number and generation time. It has been identified areduction in generation number and an increase in generation time similar to synthetic pesticides.

Bordeaux mixture detected the maximum change in susceptibility to antibiotics for detection of genetic modifications and bacterial growth was chosen as an indicator pesticide. Cultures obtained for the detection of genetic modifications that occur in *Bacillus cereus* DY6 treated with bordeaux mixture were sent to Gazi University Life Sciences Application and Research Center. While it is determined that homology in the base sequence fall from 100% to 99%, it has been observed the third nucleotide of GTC sequence become GTG in 126. codon in the comparison *of Bacillus cereus* DY6 cultured under standard conditions in Research Center and *Bacillus cereus* DY6 cultured with bordeaux mixture (EMBL/Gen Bank No: KT719870.1).

--CGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTG
--CGGGTGGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTG

In this study based on the effects of pesticidesto the microbial floraas considering the changes in metabolic activity; *Bacillus cereus* DY6, *Micrococcus yunnanensis* DT1 ve *Bacillus tequilensis* DT2 were chosen as an indicator from isolates obtained from leaf and soil samples. Antibiotic susceptibility of these microorganisms as metabolic activity, changes in generation time and genetic modifications occured were investigated. As a result, as much as conventional applications pesticides used in organic farming (natural and biopesticide) cause change in the metabolic activity of bacteria and genetic modifications.

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