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# Effects of commonly used pesticides (demond, granland and safacol) on non-targeted organisms (wheat plant, soil nematodes, microfungi and aerobic mesophilic bacteria) in Muş Province, Turkey

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## ABSTRACT

Chemical drugs (pesticides) that are xenobiotic to the nature of agricultural agro-ecosystems affect the target organism and non-target factors. The efficiency and reliability of agricultural areas are important not only for living creatures in the environment, but also for community. This study was carried out to determine the effects of Demond EC 2.5 (insecticide), Granland DF (herbicide) and Safacol 70 WP (fungicide) pesticides, which are widely used in the agricultural areas of Muş, on the development of non-target wheat plant, free-living nematodes, microfungi and aerobic mesophilic bacteria communities. The study was performed in three replicates in the variance analysis method for repeated measurements in laboratory conditions. Pesticides were applied by spraying to the pots including one kg of soil as recommended and upper doses, respectively, with the help of an injector. Repeated Measurement ANOVA and Profile Analysis Technique were used in the comparison of treatments in terms of nematode, bacteria and microfungi numbers (before-after), and the Analysis of Means (ANOM) Technique in comparing treatments in terms of plant parameters. As a result of the experiments; While the effect of only periods is important for the bacteria combination ( $P=0.001$ ); for the microfungi community, both period ( $P=0.004$ ) and Period  $\times$  Treatment interaction were found significant ( $P=0.050$ ). Periodic  $\times$  Treatment interaction was statistically significant for plant-parasitic, omnivore-predator, bacterivore and total nematodes respectively ( $P=0.002$ ;  $P=0.004$ ;  $P=0.001$ ;  $P=0.000$ ). As a result; the pesticides used had more or less positive effects on the microfungi community, while they had a negative effect on the bacteria community; According to trophic levels, soil nematodes and plant parameters were found to have a positive / negative effect. It is thought that pesticides should be used in the last resort and recommended dosage, without forgetting that the soil is complex and all living things share this environment.

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## 1. Introduction

Agro-ecosystems are complex environments and the activities carried out here affect productivity, as well as sustainability, environmental and human health. Manufacturers prefer using

chemical pesticides to get more crops from agricultural areas. In this context, Özbek and Fidan (2014), although the differences from time to time, between 1979 to 2007 pesticide use increased by 270% in Turkey. Quinn et al. (2011) reported that although the benefit provided by pesticides cannot be denied, the negativities it gives to the environment and human

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health cannot be ignored. The indiscriminate use of pesticides affects soil microflora, fauna and flora (Arora et al., 2019). AL-Ani et al. (2019) reported that pesticides, in which they applied different doses, negatively affect the microbial activities of bacteria, microfungi and actinomycetes in the soil, and significantly reduced their number. Ekundayo (2003) found that among the pesticides treated to the garden soil at the recommended doses, Agrosan is the most negative pesticide affecting bacteria density. Ubuoh et al. (2012) observed that glyphosphate treatments caused a significant reduction in microfungi and bacteria population compared to control. Ören et al. (2009) stated that the pesticides they use show different effects on bacteria and yeast-molds in the soil. Demirci et al. (2002) have listed the fungicides they use against fungi to be effective (Flusilazole, Tebuconazole, Diniconazole, Penconazole, Cyproconazole, Triticonazole). Arora and Sahni (2016) reported that microorganisms react differently to different pesticides. Heinonen-Tanski et al. (1989) reported that soil microorganisms were affected by pesticides used in their studies in some cases, but not in all cases. Lo (2010) stated that pesticides have different effects against soil microorganisms due to their different structure. Küçük et al. (2009) concluded that some fungicides may negatively affect the development of fungal isolates (*T. harzianum*, a biocontrol factor). Koç and Yardım (2019) reported that the pesticides (herbicides and fungicides) they used did not significantly affect soil microfungi and aerobic mesophilic bacteria density. Güven and Koç (2020a) found that the bacteria population in the pesticide treated samples compared to the control treatment showed a decrease in other treatments, while the microfungi population increased only in the treatment of Pesos 100 EC. Wesley et al. (2017), they observed that different results were obtained according to the parameters considered.

Arora and Sahni (2016) predicted that chemical pesticides disrupt the activities of soil microorganisms and therefore the nutritional quality of the soil may be affected, and this situation may lead to serious ecological consequences. Küçük et al. (2016) reported that it is inevitable to use fungicides in the fight against pest and therefore it should be used considering the effect of the fungicides to be used on soil microorganisms. Johns (2017) stated that soil microbiology should be better understood in order for agricultural production to meet the needs of the growing world population. Yardım and Edwards (1998) stated that nematodes (according to trophic groups) respond positively / negatively to pesticides used in field trials. Daramola et al. (2015) reported that Carbofuran's nematode population was significantly suppressed. Römbke et al. (2009) stated that the pesticides they used reduced the number of nematodes by 48% compared to the control. Koç et al. (2020), according to their compliance analysis, found significant relationships between pesticide treatments and nematode groups. Güven and Koç (2020b) declared that the pesticides they use affect soil nematodes. Demircioğlu (2007) stated that herbicides tested in different environments and shapes affect the number of seed germination, sugar beet wet, dry and root weight. Fındıklı and Türkoğlu (2010) found that all doses of pesticides they use have an inhibitory effect on cell division at the root ends of *Allium cepa*. Siddiqui and Ahmed (2006) observed that the pesticide concentrations they use have combined effects on plant growth and the nutritional

composition of seeds. Niewiadomska and Klama (2005) have demonstrated that the pesticides they apply have a toxic effect on the nodulation and root growth of the tested plants. Boutin et al. (2014) stated that as a result of the use of pesticides, a large number of non-targeted plants and vegetation have changed significantly, in addition, there is a delay in flowering and a decrease in seed production. İlkhan and Koç (2020) reported that Demond EC 2.5 and Safacol 70 WP had a toxic effect on *E. fetida*. This study was conducted for the purpose of determining the effects of Demond EC 2.5 (insecticide), Granland DF (herbicide) and Safacol 70 WP (fungicide), which are widely used in agricultural areas of the province of Muş, Turkey on the free-living nematodes, microfungi and aerobic mesophilic bacteria communities.

## 2. Material and Methods

This study was carried out in in-vitro conditions (mean temperature: 23 °C) between 2019-2020. The soil used in the study is from the pasture environment (Latitude: 38° 28'54.75", Longitude: 42° 9'51.93") and 0-30 cm depth (Yıldız et al., 2019) for the agricultural activities in the Bitlis Eren University Campus area. Taken on August 2, 2019. Samples were passed through a 2 mm sieve and kept in clean nylon bags at +4 °C in the refrigerator until experimenting. Soil texture, according to Bouyoucos (1951) (clay ratio; 32%, silt ratio; 5%, sand ratio; 63%, lime ratio; 1.43%, pH; 7.8, EC; 181 µS/cm<sup>-1</sup>); Moisture content (%) was determined before and after treatment (12.7%, 14.6%) according to Craze (1990). For 2017, pesticides commonly used in the province of Muş were determined by asking the Muş Provincial Directorate of Agriculture and Forestry, pesticides and farmers. A total of three [Demond EC 2.5 (insecticide, Deltamethrin 25 g/l active substance), Granland DF (herbicide, 75% Tribenuron-methyl) and Safacol 70 WP (fungicide, 70% Propineb)] were used to represent the pesticide groups. Trial measurements were carried out in three replications according to the variance analysis method (Figure 1). Before the pesticide treatment, Ceyhan-99 bread wheat seeds (twenty piece) were planted 3 cm deep. Determined pesticides were used with the help of injector (5 cc) as twice the recommended dose and the recommended dose. Demond EC 2.5 (200 ml distilled water + 0.1 ml drug; 200 ml distilled water + 0.2 ml drug), Granland DF (150 ml distilled water + 0.02 mg drug; 150 ml distilled water + 0.04 mg drug), and Safacol 70 WP (200 ml of distilled water + 0.5 g of drug; 200 ml of distilled water + 1 g of drug) was prepared by spraying 50 ml of solution (distilled water + drug) into pots placed in a kg of soil.



Figure 1. Sample image from trial.

The average sprouting time of the seeds was 4 days. After sprouting, 10 plants were left in each pot. The experiment lasted about a month (January 7 – February 3, 2019) and soil samples were taken before and after pesticide treatment, according to nematode counts (trophic groups), microfungi, and aerobic mesophilic bacteria were examined. The determination of the total number of microfungi and aerobic mesophilic bacteria was done according to Benson (2001). Plate Count Agar for bacteria count and Rose Bengal-chloramphenicol Agar media were used for microfungi count. For extraction and counting of nematodes, “Improved-Baermann Funnel Method” according to was used (Baermann, 1917; Whitehead and Hemming, 1965; Southey, 1986), the microscope’s lens (×10) counts according to Yeates (1971) and Yeates et al. (1993). The dismantling process was removed from the soil by carefully washing it thoroughly with water after the soil in the pots was removed together with the plants. For each pot, five randomly selected plants were taken to represent the whole of the cleaned plants, and measurements were made. Wheat plants root length (cm), seedling length (cm), root weight (g), seedling weight (g), dry root weight (g), dry seedling weight (g), dry root/seedling ratio and root volume (cm<sup>3</sup>) measurements (Geçit et al., 1987; Sönmez, 2001; Sipan, 2014). In the investigation of the effects of pesticide treatments (before and after treatment), Repeated Measured Variance Analysis and Profile Analysis Techniques were used to compare the treatments in terms of plant parameters (ANOM) Technique (Mendes et al., 2007; Mendes, 2012). SPSS (ver. 19.0) and Minitab (ver. 17) statistical package programs were used in making statistical analyzes.

### 3. Results and Discussion

#### 3.1. Effect of pesticide treatments on soil-free nematodes

##### 3.1.1. Effect of treatments on plant-parasitic nematode count

According to the repeatedly measured variance analysis on the data obtained, the Period × Treatment interaction effect was found statistically significant (P=0.002). In other words, the effect of pesticide treatments on the number of plant-parasitic nematodes varied significantly in periods (Table 1).

Table 1. Introductory statistics in terms of plant-parasitic nematode number.

Pesticide Treatments	N	Before Treatment	After Treatment
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
Demond recommended d.	3	2.00±0.47	7.33±0.90
Demond a single high d.	3	9.67±0.67	3.33±0.60
Granland recommended d.	3	8.00±0.94	4.33±0.69
Granland a single high d.	3	16.00±1.33	3.67±0.64
Safacol recommended d.	3	8.00±0.94	11.00±1.10
Safacol a single high d.	3	4.67±0.72	5.67±0.79
Control	3	15.00±1.29	4.33±0.69
Total	21	9.05±1.00	5.67±0.79

When the results of the profile analysis are examined, the granland did not affect any toxic effect, except for the relative negative in the recommended dose. Even, it has been determined that demond has a positive effect on the increase in the number of plant-parasitic nematodes in recommended doses and safacol treatments (Table 1, Figure 2).

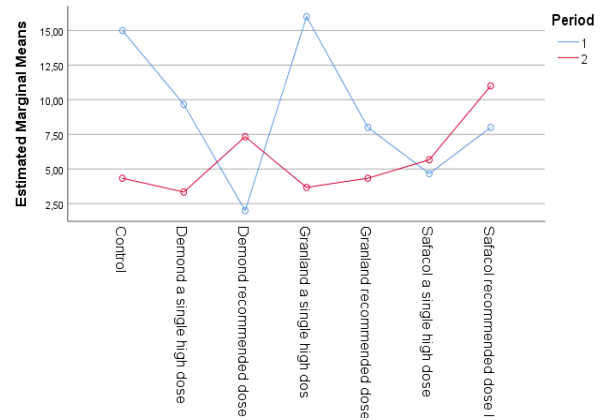


Figure 2. Profile analysis in terms of plant-parasitic nematode number.

\* Period 1: Before treatment, Period 2: After treatment

##### 3.1.2. Effects of treatments on fungivore nematode count

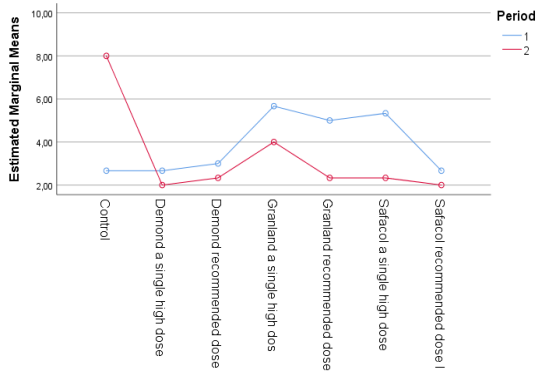
As a result of repeated measured variance analysis on the obtained data, the Period × Treatment interaction effect was not statistically significant (P=0.265). Therefore, the effect of pesticide treatments on fungivore nematode numbers did not change significantly according to periods (Table 2).

Table 2. Effects of treatments on fungivore nematode count.

Pesticide Treatments	N	Before Treatment	After Treatment
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
Demond recommended d.	3	3.00±0.57	2.33±0.51
Demond a single high d.	3	2.67±0.54	2.00±0.47
Granland recommended d.	3	5.00±0.74	2.33±0.51
Granland a single high d.	3	5.67±0.79	4.00±0.67

Safacol recommended d.	3	2.67±0.54	2.00±0.47
Safacol a single high d.	3	5.33±0.77	2.33±0.51
Control	3	2.67±0.54	8.00±0.94
Total	21	3.86±0.65	3.29±0.60

When the results of the profile analysis on the data are examined; according to the control, all pesticide treatments seem to have a negative effect on fungivore nematodes (Table 2, Figure 3).



**Figure 3.** Profile analysis in terms of the number of fungivore nematodes.  
\* Period 1: Before treatment, Period 2: After treatment

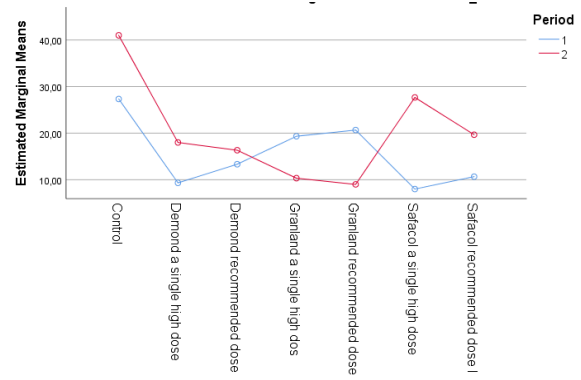
### 3.1.3. The effect of treatments on the number of bacterivore nematodes

As a result of repeated measured variance analysis, the Period × Treatment interaction effect was found statistically significant (P=0.001). Therefore, the effect of pesticide treatments on the number of bacterivore nematodes varied significantly over periods (Table 3).

**Table 3.** Introductory statistics in terms of bacterivore nematode number.

Pesticide Treatments	N	Before Treatment	After Treatment
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
Demond recommended d.	3	13.33±1.22	16.33±1.35
Demond a single high d.	3	9.33±1.02	18.00±1.41
Granland recommended d.	3	20.67±1.51	9.00±1.00
Granland a single high d.	3	19.33±1.46	10.33±1.07
Safacol recommended d.	3	10.67±1.09	19.67±1.48
Safacol a single high d.	3	8.00±0.94	27.67±1.75
Control	3	27.33±1.74	41.00±2.13
Total	21	15.52±1.31	20.29±1.50

Looking at the results of profile analysis; according to the control, it was observed that especially granland had a negative effect on bacterivore nematodes (Table 3, Figure 4).



**Figure 4.** Profile analysis in terms of bacterivore nematode count.  
\* Period 1: Before treatment, Period 2: After treatment

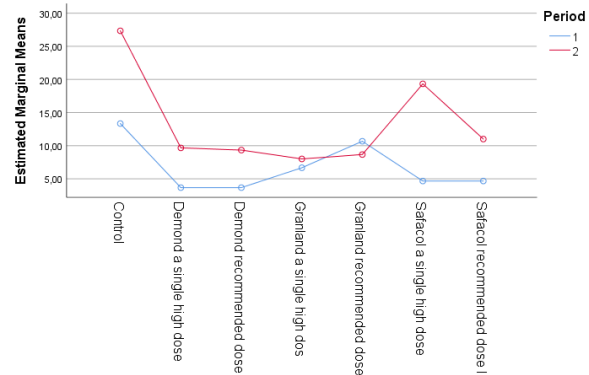
### 3.1.4. The effects of treatments on the number of omnivore-predator nematodes

As a result of repeated measured variance analysis, the Period × Treatment interaction effect was found statistically significant (P=0.004). In other words, the effect of pesticide treatments on the number of omnivore-predator nematodes varied significantly in periods (Table 4).

**Table 4.** Introductory statistics in terms of the number of omnivore-predator nematodes.

Pesticide Treatments	N	Before Treatment	After Treatment
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
Demond recommended d.	3	3.67±0.64	9.33±1.02
Demond a single high d.	3	3.67±0.64	9.67±1.04
Granland recommended d.	3	10.67±1.09	8.67±0.98
Granland a single high d.	3	6.67±0.86	8.00±0.94
Safacol recommended d.	3	4.67±0.72	11.00±1.10
Safacol a single high d.	3	4.67±0.72	19.33±1.46
Control	3	13.33±1.22	27.33±1.74
Total	21	6.76±0.87	13.33±1.22

Looking at the results of profile analysis; according to the control, it was found that demond and granland had negative effects on omnivore-predator nematodes (Table 4, Figure 5).



**Figure 5.** Profile analysis in terms of number of omnivore-predator nematodes.  
\* Period 1: Before treatment, Period 2: After treatment

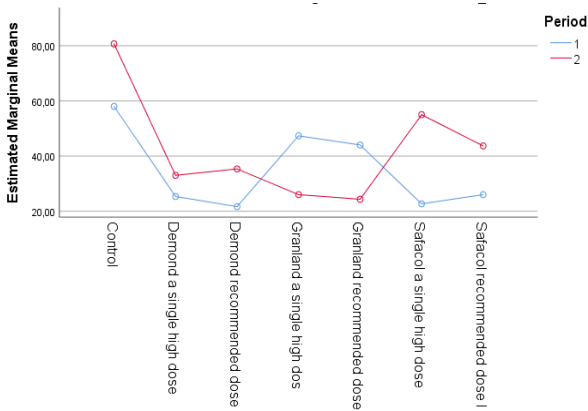
**3.1.5. The effects of treatments on the total number of nematodes**

As a result of repeated measured variance analysis, the Period × Treatment interaction effect was found statistically significant (P=0.000). Therefore, the effect of pesticide treatments on the total number of nematodes varied significantly over periods (Table 5).

**Table 5.** Introductory statistics in terms of total number of nematodes.

Pesticide Treatments	N	Before Treatment	After Treatment
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
Demond recommended d.	3	21.67±1.55	35.33±1.98
Demond a single high d.	3	25.33±1.68	33.00±1.91
Granland recommended d.	3	44.00±2.21	24.33±1.64
Granland a single high d.	3	47.33±2.29	26.00±1.70
Safacol recommended d.	3	26.00±1.70	43.67±2.20
Safacol a single high d.	3	22.67±1.59	55.00±2.47
Control	3	58.00±2.54	80.67±3.00
Total	21	35.00±1.97	42.57±2.17

Looking at the results of profile analysis; according to the control, it was observed that especially granland had a negative effect on total nematodes (Table 5, Figure 6).



**Figure 6.** Profile analysis in terms of total number of nematodes.  
\* Period 1: Before treatment, Period 2: After treatment

**3.2. Effect of pesticide treatments on soil microorganisms**

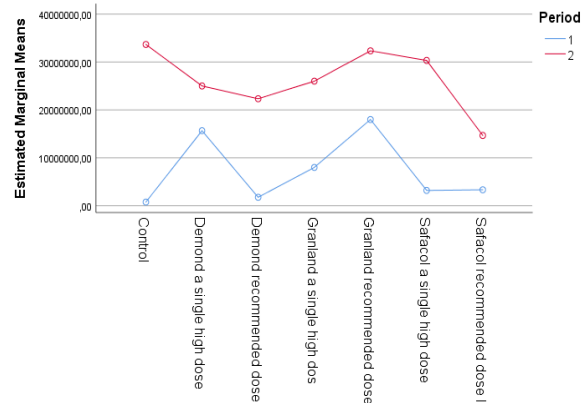
**3.2.1. The effects of treatments on the number of aerobic mesophilic bacteria**

As a result of repeated measured variance analysis, the Period × Treatment interaction effect was not found statistically significant (P=0.835). Therefore, the effect of pesticide treatments on aerobic mesophilic bacteria number did not change significantly according to periods (Table 6).

**Table 6.** Introductory statistics in terms of the number of aerobic mesophilic bacteria.

Pesticide Treatments	N	Before Treatment	After Treatment
		$\bar{X} \pm S_{\bar{x}}$	$\bar{X} \pm S_{\bar{x}}$
Demond recommended d.	3	1766666.67±443.05	2233333.33±1575.27
Demond a single high d.	3	1566666.67±1319.37	2500000.00±1666.67
Granland recommended d.	3	1800000.00±1414.21	3233333.33±1895.41
Granland a single high d.	3	800000.00±942.81	2600000.00±1699.67
Safacol recommended d.	3	3333333.33±608.58	1466666.67±1276.57
Safacol a single high d.	3	320000.00±596.28	3033333.33±1835.86
Control	3	766666.67±291.86	3366666.67±1934.10
Total	21	7247619.05±897.38	2633333.33±1710.53

When the profile analysis results are examined, it has been found that pesticide treatments have more or less negative effects on the number of aerobic mesophilic bacteria compared to the control (Table 6, Figure 7).



**Figure 7.** Profile analysis in terms of the number of aerobic mesophilic bacteria.  
\* Period 1: Before treatment, Period 2: After treatment

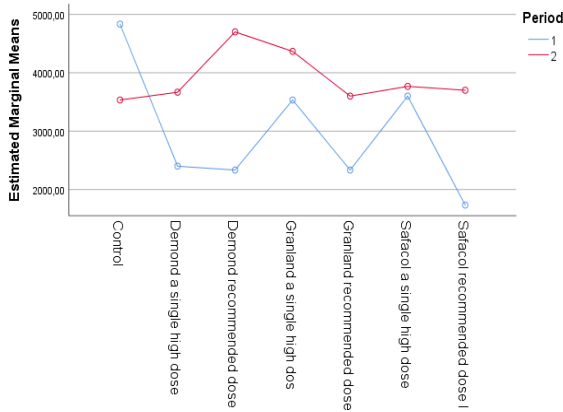
**3.2.2. The effect of treatments on the number of microfungi**

As a result of repeated measured variance analysis, the Period × Treatment interaction effect was found statistically significant (P=0.050). Therefore, the effect of pesticide treatments on the number of microfungi varied significantly in periods (Table 7).

**Table 7.** Introductory statistics in terms of microfungi number.

Pesticide Treatments	N	Before Treatment	After Treatment
		$\bar{X} \pm S_x$	$\bar{X} \pm S_x$
Demond recommended d.	3	2333.33 ± 16.10	4700.00 ± 22.85
Demond a single high d.	3	2400.00 ± 16.33	3666.67 ± 20.18
Granland recommended d.	3	2333.33 ± 16.10	3600.00 ± 20.00
Granland a single high d.	3	3533.33 ± 19.81	4366.67 ± 22.03
Safacol recommended d.	3	1733.33 ± 13.88	3700.00 ± 20.27
Safacol a single high d.	3	3600.00 ± 20.00	3766.67 ± 20.46
Control	3	4833.33 ± 23.17	3533.33 ± 19.81
Total	21	2966.67 ± 18.15	3904.76 ± 20.83

When the profile analysis results were examined, it was found that pesticide treatments had more or less positive effects on the number of microfungi according to the control (Table 7, Figure 8).

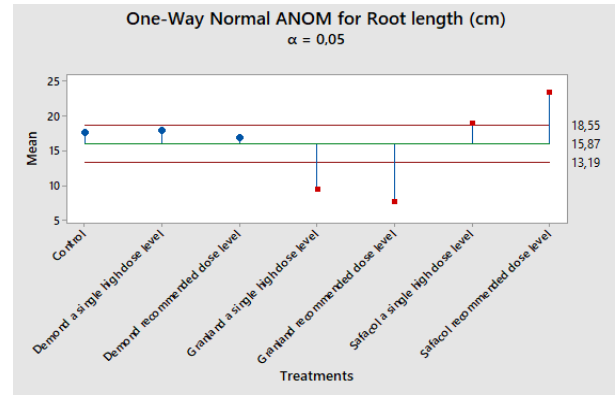


**Figure 8.** Profile analysis in terms of microfungi number.  
\* Period 1: Before treatment, Period 2: After treatment

**3.3. The effect of pesticide treatments on the development of root and above-ground parts in the first development period of wheat**

**3.3.1. Effect of treatments on plant root length (cm)**

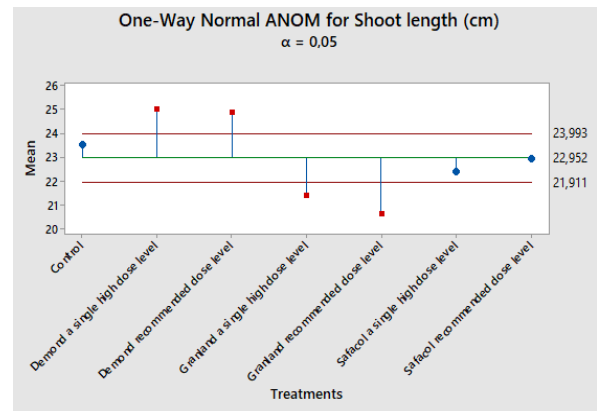
According to the ANOM test performed in terms of the effect of pesticide treatments on plant root length, it was observed that safacol had a positive effect and granland had a negative effect compared to the control (Figure 9).



**Figure 9.** ANOM graphic in terms of plant root length.

**3.3.2. Effect of treatments on plant shoot length (cm)**

According to the ANOM test conducted in terms of the effect of pesticide treatments on plant shoot length, it was found that demond had a positive effect and granland had a negative effect when compared with the control (Figure 10).



**Figure 10.** ANOM graphic in terms of plant shoot length.

**3.3.3. Effect of treatments on plant root weight (g)**

According to the ANOM test results in terms of the effect of pesticide treatments on plant root weight, it was found that other treatments that granland had a positive effect compared to the control had a relatively positive effect (Figure 11).



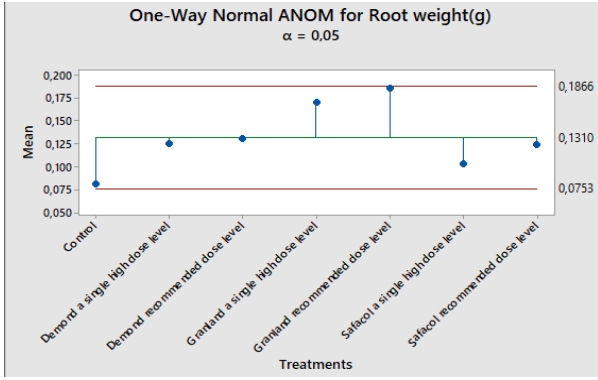


Figure 11. ANOM graphic in terms of plant root weight.

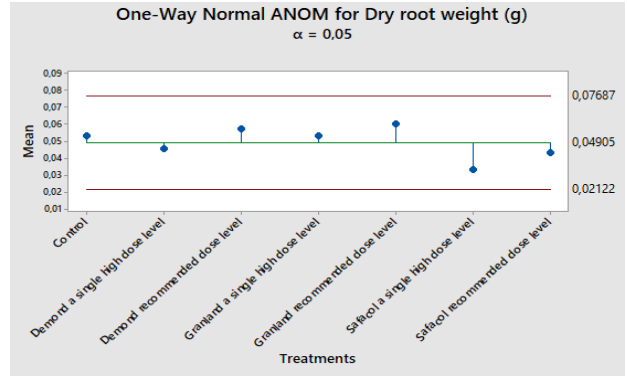


Figure 13. ANOM graphic in terms of plant dry root weight.

3.3.4. Effect of treatments on plant shoot weight (g)

According to the ANOM test performed in terms of the effect of pesticide treatments on plant shoot weight, safacol was found to be positive while other treatments had a positive effect compared to the control (Figure 12).

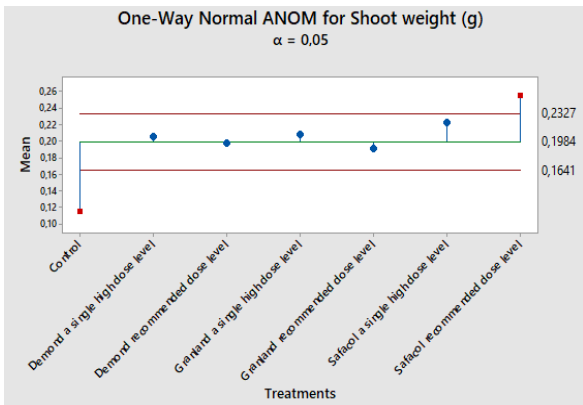


Figure 12. ANOM graphic in terms of plant shoot weight.

3.3.6. Effect of treatments on plant dry shoot weight (g)

According to the ANOM test performed in terms of the effect of pesticide treatments on plant dry shoot weight, compared to the control, safacol suggested overdose treatment positively and other treatments affected relatively (Figure 14).

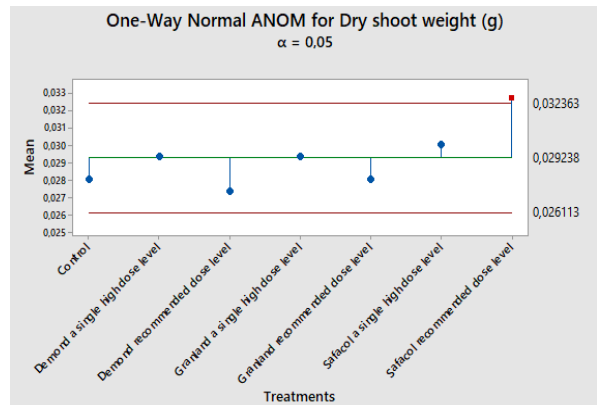


Figure 14. ANOM graphic in terms of plant dry shoot weight.

3.3.5. Effect of treatments on plant dry root weight (g)

According to the ANOM test performed in terms of the effect of pesticide treatments on plant dry root weight, it was found to be relatively affected compared to the control (Figure 13).

3.3.7. Effect of treatments on plant dry root / shoot ratio

According to the ANOM test performed in terms of the effect of pesticide treatments on the plant dry root / shoot ratio, it was observed that all treatments were relatively affected when compared with the control (Figure 15).

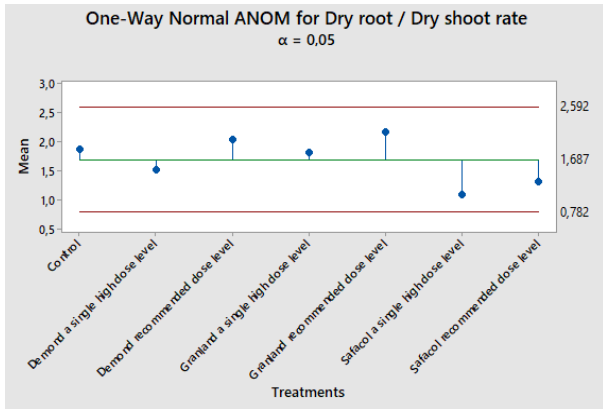


Figure 15. ANOM graphic in terms of plant dry root / shoot ratio.

### 3.3.8. Effect of treatments on plant root size (cm<sup>3</sup>)

According to the ANOM test results in terms of the effect of pesticide treatments on plant root size, all pesticide treatments were found to be partially effective compared to the control (Figure 16).

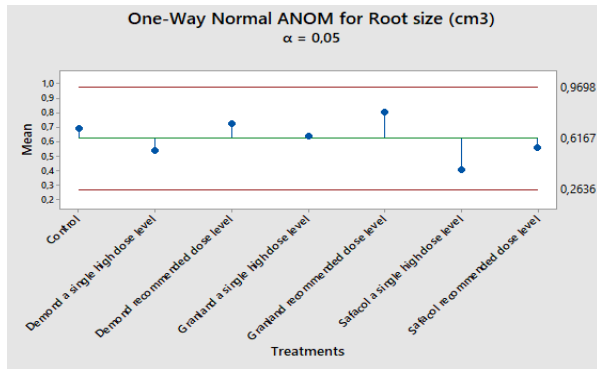


Figure 16. ANOM graphic in terms of plant root size (cm<sup>3</sup>).

Chemical drugs (pesticides) used in disease, pest and weed control not only affect the target parameter, but unfortunately also affect the biotic and abiotic parameters that are not targeted and whose assets are indisputably indispensable. Treatments performed in this study showed positive, negative and neutral effects compared to the control and dosage used in terms of plant growth parameters. The results obtained in this study were partially in agreement with Demircioğlu (2007), Fındıklı and Türkoğlu (2010), Siddiqui and Ahmed (2006), Niewiadomska and Klama (2005) and Boutin et al. (2014). As free-living nematodes in soil, grandland, which is one of the pesticide treatments, generally shows toxic effects; with the recommended dose of demond, safacol has also been found to have a positive effect on plant-parasitic nematodes and other treatments have different effects. Further, similar outcomes with previous studies including Yardim and Edwards (1998), Römbke et al. (2009) and Koç et al. (2020) were obtained in the laboratory experiments. When evaluated in terms of microfungi; the treatments were found to have more or less positive effects on the number of microfungi. Although some of

the findings in this study were contrary to the suggestions proposed by AL-Ani et al. (2019), Ubuoh et al. (2012) and Küçük et al. (2009), they were substantially consistent with Wesley et al. (2017), Arora and Sahni (2016), Arora et al. (2019), Ören et al. (2009), Heinonen-Tanski et al. (1989) and Koç and Yardım (2019). In terms of aerob mesophilic bacteria, pesticides were found to have more or less toxic effects. The findings of this study were relatively parallel with Ubuoh et al. (2012), Güven and Koç (2020a) and AL-Ani et al. (2019, Ören et al. (2009), Ekundayo (2003), Heinonen-Tanski et al. (1989), Yousaf et al. (2013), Lo (2010), Arora and Sahni (2016), Aries and Aid (2019) and Wesley et al. (2017). Based on the findings obtained, different results (such as positive, negative and neutral) were obtained, depending on the type and dose of the drug used and the parameter considered. It is thought that this situation may be caused by the variability of factors such as the type of drug used, dosage, parameter taken into account and the environment studied.

As a result; It can be stated that the negative effects of pesticides to the environment cannot be ignored (Quinn et al., 2011; Arora and Sahni, 2016; Küçük et al., 2016) and soil microbiology should be better understood in order for agricultural production to meet the needs of the growing world population (Johns, 2017).

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