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Some Biological Parameters of *Eisenia fetida* (Savigny, 1826) in Pesticide-Applied Vermicompost

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ABSTRACT

The objective of this study was to determine some biological parameters of red California worm (*Eisenia fetida*) in pesticides applied vermicompost. The study conducted under *in-vitro* conditions, as Randomized Block Design with five raplications. Commonly used Granland®, Demond® and Safacol® pesticides in Muş province (Turkey) were used as treatments. The temperature and humidity ratio for the experiment were 20-29°C and 70 to 90%, respectively. The experiments were resumed until the young worms hatched from a cocoon reproduced cocoons again. For investigating the effect of the treatments on specific features One-Way Analysis of Variance (One-way ANOVA) and for determining of different groups TUKEY Multiple Comparison Test were used. Variance analysis indicated that there was no statistically differences among fungicide doses in terms of worm weight (P=0.113); however, there were differences in terms of insecticide and herbicide doses (P=0.000). It was detected that there were significant differences among pesticide doses in respect to the cocoon and worm numbers (P=0.000).

Research Article

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Pestisit Uygulanmış Vermikompostta *Eisenia fetida* (Savigny, 1826)'nın Bazı Biyolojik Parametreleri

ÖZET

Bu çalışmanın amacı, pestisit uygulanmış vermikompostlarda kırmızı Kaliforniya solucanının (*Eisenia fetida*) bazı biyolojik parametrelerini belirlemektir. Çalışma *in-vitro* şartlarda, Tesadüf Parselleri Deneme Düzeninde 5 tekerrürlü olarak yapılmıştır. Pestisit muamelesinde, Muş ilinde çokça kullanılan bazı pestisitler (Granland®, Demond® ve Safacol®) kullanılmıştır. Deney ortamının sıcaklığı 20-29°C ve nem oranı %70-90 arasında tutulmuş ve deney, kokonlardan çıkan yavru bireylerin tekrar kokon vermesine kadar devam etmiştir. Muamelelerinin belirlenen özelliklere etkisinin araştırılmasında Tek Yönlü Varyans Analizi Tekniğinden (One-way ANOVA), farklı grupların belirlenmesinde ise TUKEY Çoklu Karşılaştırma Testi'nden yararlanılmıştır. Yapılan varyans analizi sonucunda; solucan ağırlığı bakımından fungisit dozları arasında istatistiksel olarak önemli farkların bulunmadığı (P=0.113), buna karşın insektisit ve herbisit dozları bakımından söz konusu farkların önemli olduğu görülmüştür (P=0.000). Kokon ve solucan sayıları bakımından ise pestisit dozları arasında önemli farkların bulunduğu tespit edilmiştir (P=0.000).

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INTRODUCTION

It is significant that pesticides are effective in agricultural pest control; but, if they are used randomly and excessively, beneficial organisms and the other constituents of environment would be affected severely (Dığrak et al., 1999). Worms are considered as the significant bioindicators of chemical toxicity in soil ecosystem (Yasmin and D'Souza, 2010). The advantage of using these organisms as bioindicator is that they are easy and affordable to be obtained (Bustos-Obregón and Goicochea, 2002).

Helling et al. (2000) determined that *E. foetida*'s growth and reproducing cocoon decreased considerably in fungicide Copper oxychloride® treatments of ≥ 8.92 mg kg^{-1}. Bustos-Obregón and Goicochea (2002) revealed that Parathion® decreased the body weight and survival rate of *E. foetida*. Espinoza-Navarro and Bustos-Obregón (2005) detected a considerable decrease in the body weight of *E. foetida* subjected to Malathion®. Xiao et al. (2006) determined that Acetochlor® did not have a long-termed effect on the growth and reproduction of *E. foetida* at field doses, however sublethal toxicity effect to *E. foetida* was seen at higher doses. Yasmin and D'Souza (2007) observed that pesticides affected the growth and reproduction of *E. foetida* adversely, and Carbendazim® and Dimethoate® sustained greater harm then Glyphosate®. Correia and Moreira (2010) found that Glyphosate® and 2,4-D® treatments had serious effects on *E. foetida*'s growth and reproduction. Farrukh and S-Ali (2011) stated that Dichlorovos® led to the decrease in the weight of *E. foetida* and reproduction and avoidance behaviors were affected significantly. In pesticide treatments to *E. fetida*, Gupta et al. (2011) detected that Endosulfan®, Aldicarb® and Aarbaryl® were the most eco-hazardous pesticides; Chlorpyrifos® and Monocrotophos® were less toxic and ecologically safe. In the study on the effect of 45 pesticides to *E. fetida,* Wang et al. (2012) pointed out that Clothianidin®, Fenpyroximate® and Pyridaben® were super toxic for *E. fetida* based on LC50 values, and those were followed by Carbaryl®, Pyridaphenthion®, Azoxystrobin®, Cyproconazole® and Picoxystrobin®. Rico et al. (2016) determined the evolution of avoidance behavior in worms after a two-day-exposure; and death, loss in weight, enzymatic activities and histopathologic effects after a fourteen-day-exposure, in their study on the toxicity of five pesticides to *E. fetida*. Wang et al. (2016) determine that the toxic effects of some pesticides to *E. fetida,* stated that Imidacloprid®, Lambda-cyhalothrin®, Atrazine® and Chlorpyrifos®, respectively, had toxic effects. Jovana et al. (2014) stated no death in their insecticide and limacide treatment to the worm *E. fetida* (Savigny, 1826), but Terbis® created the most toxic effect. Vermeulen et al. (2001) detected that Mancozeb® did not have a significantly harmful effect on thereproduction or reproduction of *E. fetida*, at recommended dose or estimated environmental concentration.

This research was carried out with the objective to determine some biological parameters of red California worm (*E. fetida*) in widely used some certain pesticides applied vermicomposts in Muş province (Demond®, Safacol® and Granland®)

MATERIAL and METHOD

The study was conducted under *in-vitro* conditions, in 2018. The vermicompost needed for the experiment was obtained from 100% cow manure; cocoons from regenerating from stock culture; and pesticides purchased from trading companies. The study was carried out in Randomized Block Design with five replications. The recommended dose and 4 sub-doses of the pesticides (herbicide Granland®, insecticide Demond® and fungicide Safacol®), which are widely used in Muş province (in Turkey), were applied. The steps given below were followed in the experiment;

1) For pesticide treatments; 100 gr vermicompost was placed into 300 cm^{3}sized containers; to each sample, 10 ml pesticide solution [for Granland®: Normal dose (0.0125 g 100 ml), one-sub-dose (0.006 g 100 ml), two-sub-dose (0.003 g 100 ml), three-sub-dose (0.0016 g 100 ml), four-sub-dose (0.0008 g 100 ml), and the control group (with no treatment but only tap water is provided); for Demond®: Normal dose (1.25 g 100 ml), one-sub-dose (0.625 g 100 ml), two-sub-dose (0.313 g 100 ml), three-sub-dose (0.156 g 100 ml), four-sub-dose (0.078 g 100 ml) and the control group to which no treatment but only tap water is provided; for Safacol®: Normal dose (0.05 g 100 ml), one-sub-dose (0.025 g 100 ml), two-sub-dose (0.013 g 100 ml), three-sub-dose (0.006 g 100 ml), four- sub-dose (0.003 g 100 ml) and the control group to which no treatment but only tap water is provided], and to the control group, tap water were provided.
2) After treatments, 10 cocoons per container were placed.
3) The temperature and humidity rate of experiment environment were maintained at 20-29°C and 70 to 90%, respectively (Gunadi et al., 2002). For conserving the ambient air humidity, 10 ml tap water was added periodically to the samples every other day.
4) The weight of worms, the number of produced cocoons and the number of young members hatching from each cocoon were determined on a weekly basis until the young worms hatching from a worm cocoon would then reproduce cocoons.

In the statistical analyses of data obtained from this research, One-Way Analysis of Variance (One-way ANOVA) and in the determination of different groups Tukey Multiple Comparison Test were applied. For the mentioned statistical analyses to be carried out, Minitab (Version 17) statistical package programs were benefitted from (Winer et al., 1971).

RESEARCH FINDINGS and DISCUSSION

Effects of insecticide (Demond®) treatment

Results of variance analysis on the effect of insecticide doses on worm weight were presented in Table 1 and Figure 1. When Table 1 is considered, it is seen that the effect of insecticide doses on the worm weight is statistically significant (P=0.000). It was also determined that 92.96% of the variation observed in worm weight could be explained by the doses

(R^2=92.96%). Results of Tukey Multiple Comparison Test revealed that differences among doses were significant and normal dose treatment had toxic effect (Table 2). The average worm weight was found that none of the worm survived at Recommended-dose whereas It was maximum at Two-sub-dose (0.512 g).

Table 1. Results of variance analysis by the effect of insecticide doses on worm weight
Tablo 1. İnsektisit dozlarının solucan ağırlığına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	0.862	0.173	63.340	0.000
Error (*Hata*)	24	0.065	0.003		
Total (*Genel*)	29	0.928			

R^2 =%92.96

Table 2. Descriptive statistics and Tukey multiple comparison test for weights of worms
Tablo 2. Solucan ağırlıkları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Insecticide Treatments *İnsektisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
Two sub-dose (*İki alt doz*)	5	0.512	A	0.022	0.455	0.590
One sub-dose (*Bir alt doz*)	5	0.468	A B	0.030	0.408	0.546
Three sub-dose (*Üç alt doz*)	5	0.445	A B C	0.024	0.369	0.507
Four sub-dose (*Dört alt doz*)	5	0.374	B C	0.036	0.273	0.464
Control (*Kontrol*)	5	0.348	C	0.005	0.332	0.363
Recommended-dose (*Önerilen doz*)	5	0.000	D	0.000	0.000	0.000

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

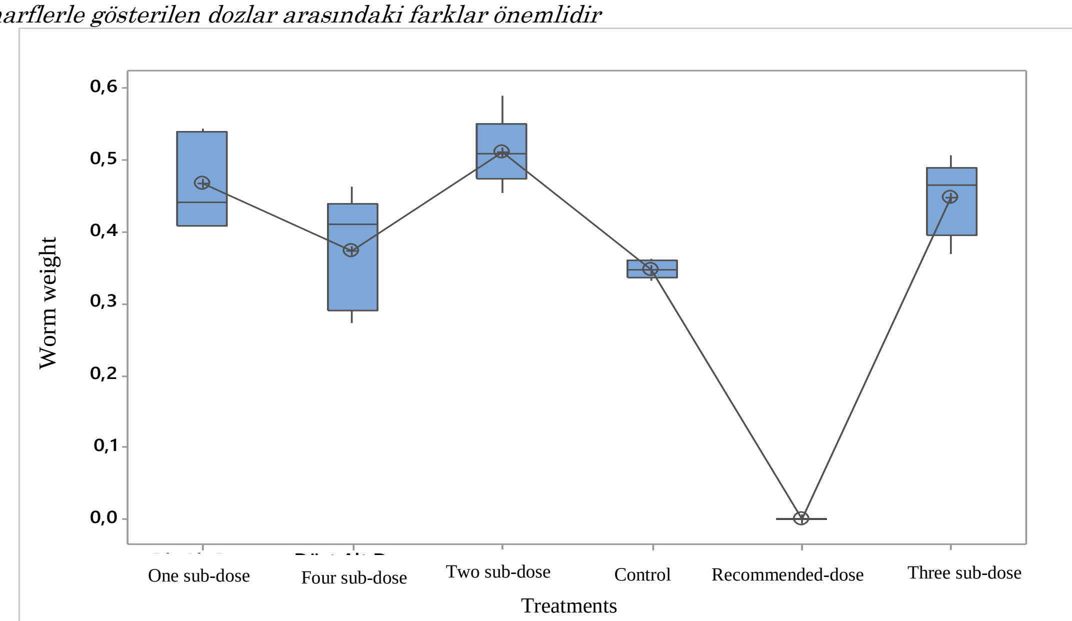

Figure 1. Effect of insecticide doses on worm weight
Şekil 1. İnsektisit dozlarının solucan ağırlığına etkisi

Results of variance analysis on the effect of insecticide doses on cocoon number were presented in Table 3 and Figure 2. The results of Tukey Multiple Comparison Test to determine doses causing the differences were given in Table 4. Variance analysis resulted that the effect of insecticide doses on cocoon number was significant (P=0.000) and 93.06% of the variation observed in cocoon number could be explained by doses (R^2=93.06%). Seeing the results of Tukey test, it was detected that the most toxic effect emerged when normal and one-sub-dose were applied. The averages of cocoon number was found minimum at Recommended-dose (0.000 pcs) and maximum in control treatment (7.600 pcs) (Table 4).

Results of variance analysis for the effect of insecticide doses on the number of worms were presented in Table 5 and Figure 3, and Tukey test results were given in Table 6.

Table 3. Results of variance analysis by the effect of insecticide doses on cocoon number
Tablo 3. İnsektisit dozlarının kokon sayısına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	209.070	41.813	64.330	0.000
Error (*Hata*)	24	15.600	0.650		
Total (*Genel*)	29	224.670			

R^2=%93.06

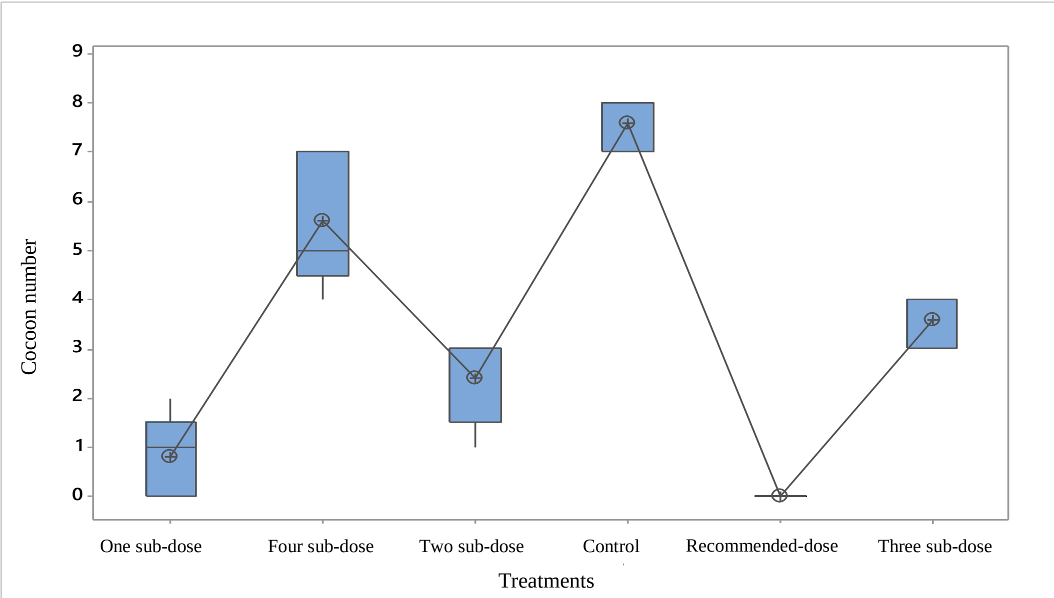

Figure 2. Effect of insecticide doses on cocoon number
Şekil 2. İnsektisit dozlarının kokon sayısına etkisi

Table 4. Descriptive statistics and Tukey multiple comparison test for numbers of cocoons
Tablo 4. Kokon sayıları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Insecticide Treatments *İnsektisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
Control (*Kontrol*)	5	7.600	A	0.245	7.000	8.000
Four sub-dose (*Dört alt doz*)	5	5.600	B	0.600	4.000	7.000
Three sub-dose (*Üç alt doz*)	5	3.600	C	0.245	3.000	4.000
Two sub-dose (*İki alt doz*)	5	2.400	C	0.400	1.000	3.000
One sub-dose (*Bir alt doz*)	5	0.800	D	0.374	0.000	2.000
Recommended-dose (*Önerilen doz*)	5	0.000	D	0.000	0.000	0.000

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

Table 5. Results of variance analysis by the effect of insecticide doses on worm number
Tablo 5. İnsektisit dozlarının solucan sayısına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	824.000	164.800	36.900	0.000
Error (*Hata*)	24	107.200	4.467		
Total (*Genel*)	29	931.200			

R^2=%88.49

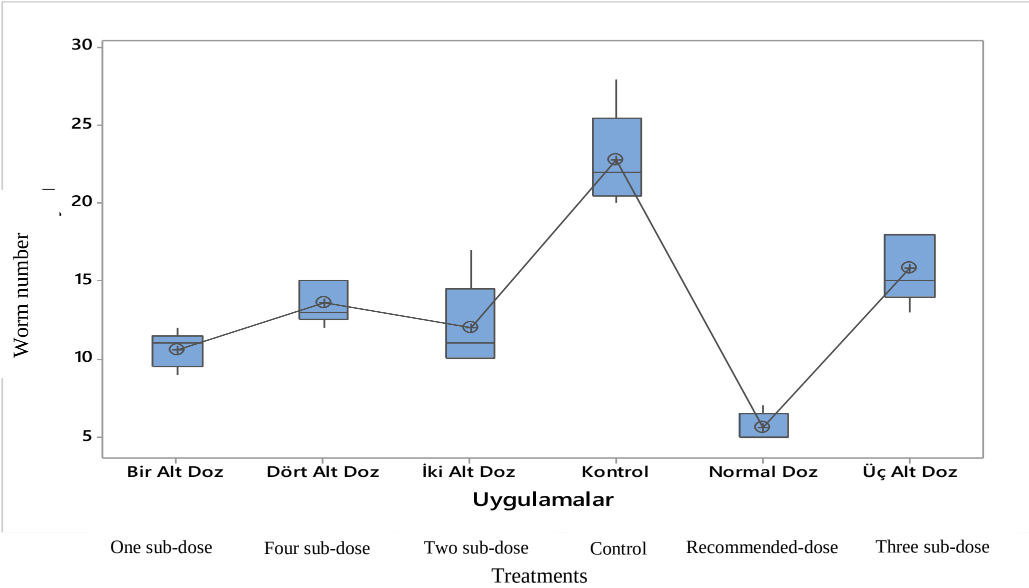

Figure 3. Effect of insecticide doses on worm number
Şekil 3. İnsektisit dozlarının solucan sayısına etkisi

Table 6. Descriptive statistics and Tukey multiple comparison test for numbers of worms
Tablo 6. Solucan sayıları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Insecticide Treatments *İnsektisit Uygulamaları*	N	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. H*	Minimum *En Az*	Maximum *En Çok*
Control (*Kontrol*)	5	22.800	A	1.390	20.000	28.000
Three sub-dose (*Üç alt doz*)	5	15.800	B	0.970	13.000	18.000
Four sub-dose (*Dört alt doz*)	5	13.600	B C	0.600	12.000	15.000
Two sub-dose (*İki alt doz*)	5	12.000	B C	1.300	10.000	17.000
One sub-dose (*Bir alt doz*)	5	10.600	C	0.510	9.000	12.000
Recommended-dose (*Önerilen doz*)	5	5.600	D	0.400	5.000	7.000

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

Based on variance analysis, it was determined that the effect of insecticide doses on worm number was statistically significant (P=0.000) and 88.49% of the variation observed in the number of worms could be explained by doses (R^2=88.49%). Concerning Tukey test results, it was detected that the most toxic effect emerged when Recommended-dose was applied. The average of worm number was found minimum at Recommended-dose (5.600 pcs) and maximum in control treatment (22.800 pcs) (Table 6).

Effects of herbicide (Granland®) treatment

Results of variance analysis on the effect of herbicide doses on worm weight were presented in Table 7 and Figure 4. The effects of herbicide doses on the worm weight is statistically significant (P=0.000) (Table 7). It was also determined that 64.47% of the variation observed in worm weight could be explained by the doses (R^2=64.47%). Results of Tukey Multiple Comparison Test showed that differences among doses were significant and one-sub-dose treatment had toxic effect (Table 8). The average worm weight found minimum at One-sub-dose (0.136 g) and maximum at Three-sub-dose (0.428 g) (Table 8).

Results of variance analysis on the effect of herbicide doses on cocoon numbers were presented in Table 9 and Figure 5. Also, the results of Tukey Multiple Comparison Test to determine dose differences were given in Table 10. Based on variance analysis results, the effect of herbicide doses on cocoon number was significant (P=0.006) and 47.57% of the variation observed in cocoon number could be explained by doses (R^2=47.57%). Based on the Tukey test, the most toxic effect emerged when Three-sub-dose were applied. The average of cocoon number was found minimum at Three-sub-dose (3.200 pcs) and maximum at Four-sub-dose (7.000 pcs) (Table 10).

Results of variance analysis for the effect of herbicide doses on worm number were presented in Table 11 and Figure 6, and Tukey test results were given in Table 12. As a result of variance analysis showed that the effect of herbicide doses on worm number was statistically significant (P=0.000) and 63.81% of the variation observed in the number of worms which could be explained by doses (R^2=63.81%). The average number of worm was found minimum in control treatment (13.000 pcs) and maximum at Recommended-dose (20.200 pcs) (Table 12).

Table 7. Results of variance analysis by the effect of herbicide doses on worm weight
Tablo 7. Herbisit dozlarının solucan ağırlığına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	0.256	0.051	8.710	0.000
Error (*Hata*)	24	0.141	0.006		
Total (*Genel*)	29	0.397			

R^2=%64.47

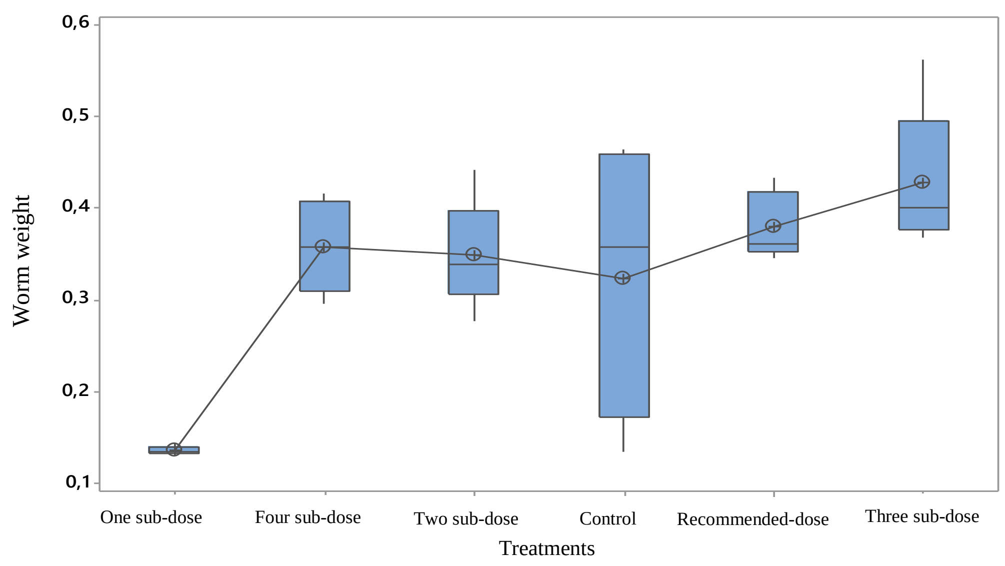

Figure 4. Effect of herbicide doses on worm weight
Şekil 4. Herbisit dozlarının solucan ağırlığına etkisi

Table 8. Descriptive statistics and Tukey multiple comparison test for weights of worms
Tablo 8. Solucan ağırlıkları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Herbicide Treatments *Herbisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
Three sub-dose (*Üç alt doz*)	5	0.428	A	0.035	0.368	0.563
Recommended-dose (*Önerilen doz*)	5	0.380	A	0.016	0.346	0.433
Four sub-dose (*Dört alt doz*)	5	0.358	A	0.022	0.296	0.416
Two sub-dose (*İki alt doz*)	5	0.349	A	0.027	0.277	0.442
Control (*Kontrol*)	5	0.320	A	0.066	0.134	0.464
One sub-dose (*Bir alt doz*)	5	0.136	B	0.002	0.133	0.143

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

Table 9. Results of variance analysis by the effect of herbicide doses on cocoon number
Tablo 9. Herbisit dozlarının kokon sayısına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	41.370	8.273	4.350	0.006
Error (*Hata*)	24	45.600	1.900		
Total (*Genel*)	29	86.970			

R^2=%47.57

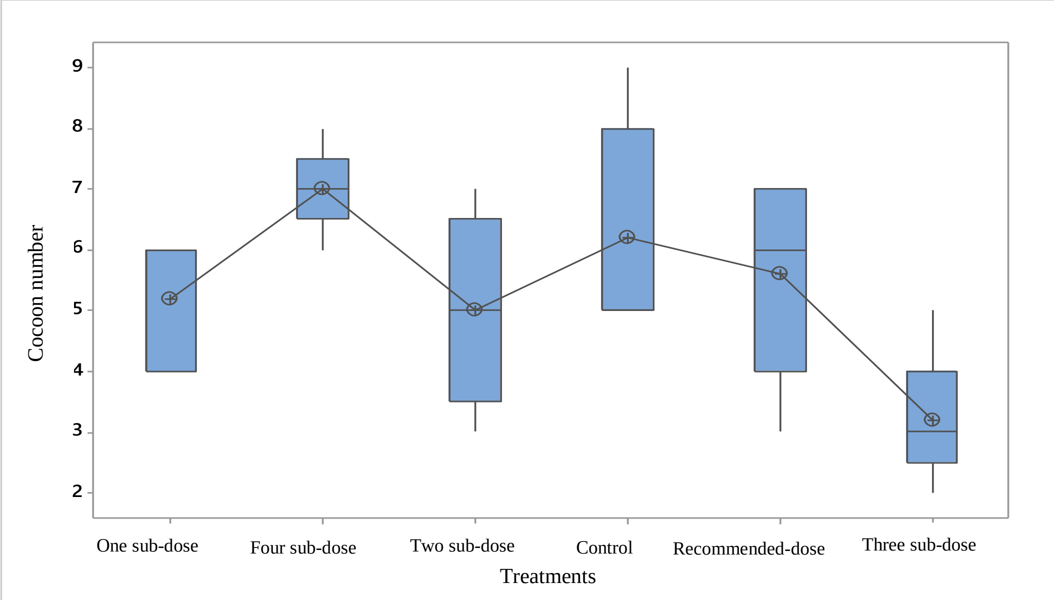

Figure 5. Effect of herbicide doses on cocoon number
Şekil 5. Herbisit dozlarının kokon sayısına etkisi

Table 10. Descriptive statistics and Tukey multiple comparison test for numbers of cocoons
Tablo 10. Kokon sayıları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Herbicide Treatments *Herbisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
Four sub-dose (*Dört alt doz*)	5	7.000	A	0.316	6.000	8.000
Control (*Kontrol*)	5	6.200	A	0.800	5.000	9.000
Recommended-dose (*Önerilen doz*)	5	5.600	A B	0.748	3.000	7.000
One sub-dose (*Bir alt doz*)	5	5.200	A B	0.490	4.000	6.000
Two sub-dose (*İki alt doz*)	5	5.000	A B	0.707	3.000	7.000
Three sub-dose (*Üç alt doz*)	5	3.200	B	0.490	2.000	5.000

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

Table 11. Results of variance analysis by the effect of herbicide doses on worm number
Tablo 11. Herbisit dozlarının solucan sayısına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	197.500	39.493	8.460	0.000
Error (*Hata*)	24	112.000	4.667		
Total (*Genel*)	29	309.500			

R^2=%63.81

Table 12. Descriptive statistics and Tukey multiple comparison test for numbers of worms
Tablo 12. Solucan sayıları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Herbicide Treatments *Herbisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
Recommended-dose (*Önerilen doz*)	5	20.200	A	0.663	18.000	22.000
Four sub-dose (*Dört alt doz*)	5	18.000	A	0.894	16.000	20.000
One sub-dose (*Bir alt doz*)	5	17.800	A	0.800	15.000	20.000
Three sub-dose (*Üç alt doz*)	5	16.400	A B	0.748	15.000	19.000
Two sub-dose (*İki alt doz*)	5	13.400	B	0.510	12.000	15.000
Control (*Kontrol*)	5	13.000	B	1.700	7.000	17.000

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

Effects of fungicide (*Safacol®*) treatment

Results of variance analysis of the effect of fungicide doses on worm weight were presented in Table 13 and Figure 7. The effect of fungicide doses on the worm weight was not significant (P=0.113) (Table 13). It was determined that 29.55% of the variation observed in worm weight could be explained by the doses (R^2=29.55%). The average worm weight was found minimum at Four-sub-dose (0.172 g) and maximum at Two-sub-dose (0.934 g) (Table 14).

Results of variance analysis of effect of fungicide doses on cocoon number were presented in Table 15 and Figure 8. The results of Tukey Multiple Comparison Test to determine doses causing the differences were given in Table 16. The effects of fungicide doses on cocoon numbers was statistically significant (P=0.004) and 49.66% of the variation observed in cocoon number could be explained by doses (R^2=49.66%). The average cocoon number was found minimum at Recommended-dose (5.800 pcs) and maximum at Three-sub-dose (11.200 pcs) (Table 16).

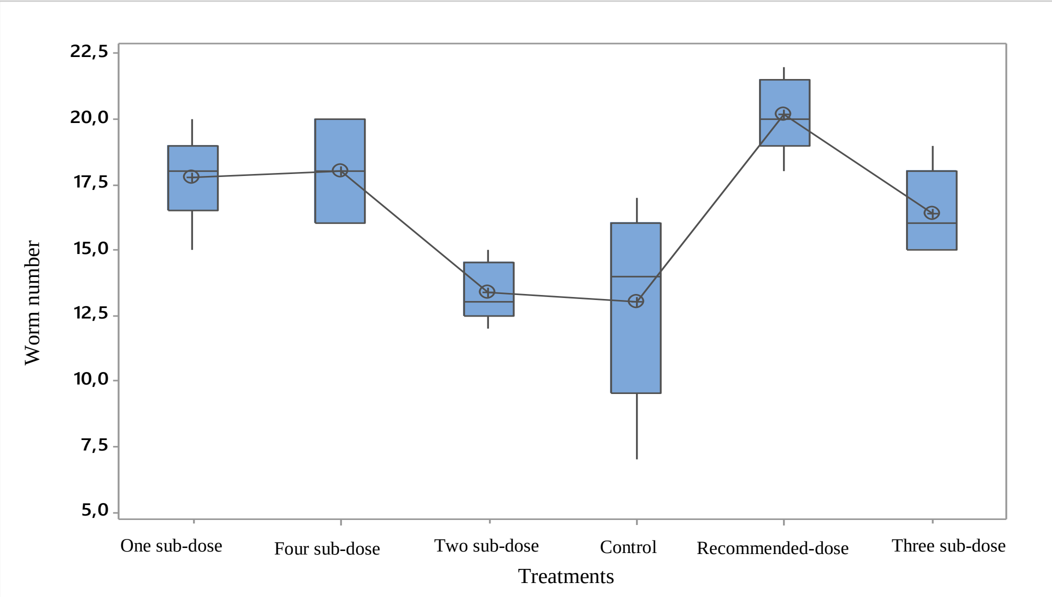

Figure 6. Effect of herbicide doses on worm number
Şekil 6. Herbisit dozlarının solucan sayısına etkisi

Table 13. Results of variance analysis by the effect of fungicide doses on worm weight
Tablo 13. Fungisit dozlarının solucan ağırlığına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	0.177	0.035	2.010	0.113
Error (*Hata*)	24	0.423	0.018		
Total (*Genel*)	29	0.600			

R^2=%29.55

Table 14. Introductory statistics for the worm weight according to fungicide doses
Tablo 14. Fungisit dozlarına göre solucan ağırlığı bakımından tanıtıcı istatistikler

Fungicide Treatments *Fungisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
One sub-dose (*Bir alt doz*)	5	0.347	0.033	0.252	0.426
Four sub-dose (*Dört alt doz*)	5	0.172	0.002	0.167	0.181
Two sub-dose (*İki alt doz*)	5	0.398	0.135	0.218	0.934
Control (*Kontrol*)	5	0.263	0.014	0.218	0.304
Recommended-dose (*Önerilen doz*)	5	0.349	0.025	0.289	0.439
Three sub-dose (*Üç alt doz*)	5	0.239	0.030	0.175	0.323

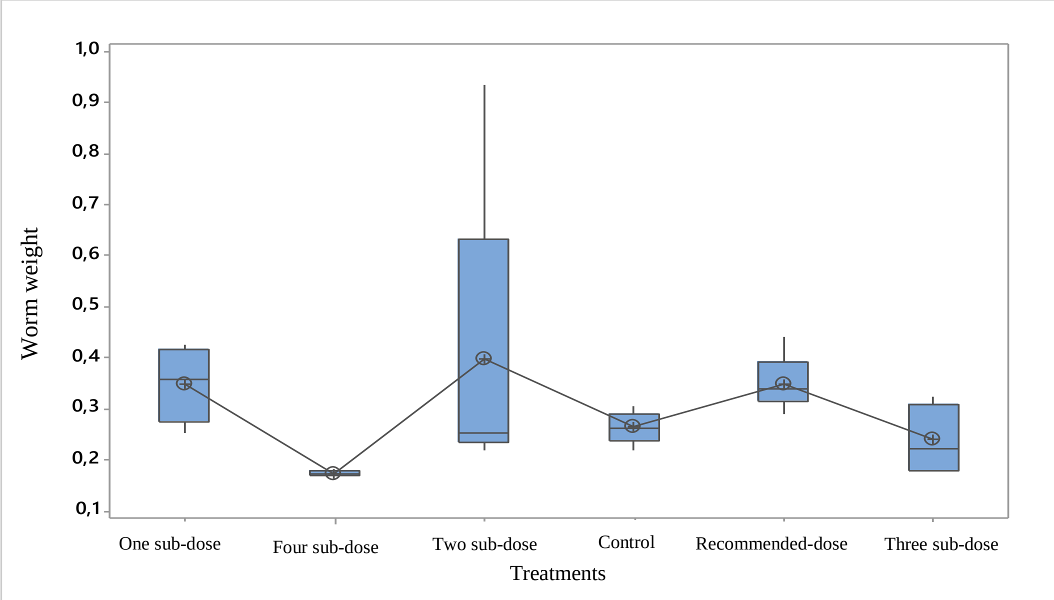

Figure 7. Effect of fungicide doses on worm weight
Şekil 7. Fungisit dozlarının solucan ağırlığına etkisi

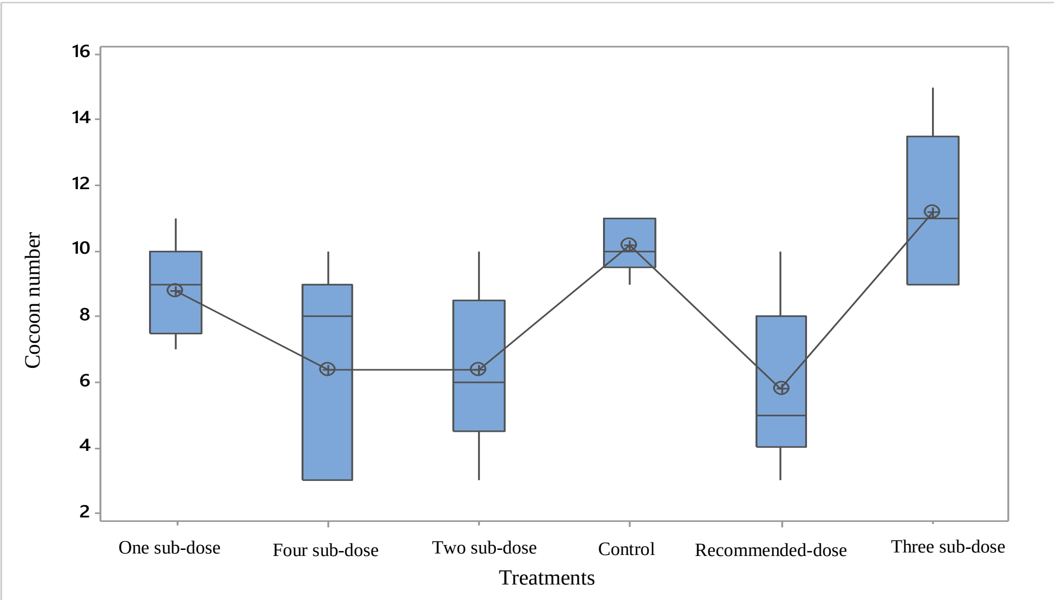

Figure 8. Effect of fungicide doses on cocoon number
Şekil 8. Fungisit dozlarının kokon sayısına etkisi

Table 15. Results of variance analysis by the effect of fungicide doses on cocoon number
Tablo 15. Fungisit dozlarının kokon sayısına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	127.900	25.573	4.740	0.004
Error (*Hata*)	24	129.600	5.400		
Total (*Genel*)	29	257.500			

R^2=%49.66

Results of variance analysis for the effect of fungicide doses on worm number were presented in Table 17 and Figure 9, and Tukey test results were given in Table 18. The effects of fungicide doses on worm number were statistically significant (P=0.003) and 51.14% of the variation observed in the number of worms could be explained by doses (R^2=51.14%). Tukey test showed that the most toxic effect emerged when Recommended-dose were applied. The average of worm number were found minimum at Recommended-dose (16.000 pcs) and maximum at Three-sub-dose (21.600 pcs) (Table18).

Table 16. Descriptive statistics and Tukey multiple comparison test for numbers of cocoons
Tablo 16. Kokon sayıları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Fungicide Treatments *Fungisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
Three sub-dose (*Üç alt doz*)	5	11.200	A	1.110	9.000	15.000
Control (*Kontrol*)	5	10.200	A B	0.374	9.000	11.000
One sub-dose (*Bir alt doz*)	5	8.800	A B	0.663	7.000	11.000
Two sub-dose (*İki alt doz*)	5	6.400	B	1.120	3.000	10.000
Four sub-dose (*Dört alt doz*)	5	6.400	B	1.440	3.000	10.000
Recommended-dose (*Önerilen doz*)	5	5.800	B	1.160	3.000	10.000

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

Table 17. Results of variance analysis by the effect of fungicide doses on worm number
Tablo 17. Fungisit dozlarının solucan sayısına etkisi bakımından varyans analizi sonuçları

Source *Varyasyon Kaynakları*	DF *Serbestlik Derecesi*	Adj SS *Kareler Toplamı*	Adj MS *Kareler Ortalaması*	F-Value *F-Değeri*	P-Value *Önemlilik Düzeyi (P)*
Treatment (*İlaç Uygulaması*)	5	104.270	20.853	5.020	0.003
Error (*Hata*)	24	99.600	4.150		
Total (*Genel*)	29	203.870			

R^2=%51.14

Table 18. Descriptive statistics and Tukey multiple comparison test for numbers of worms
Tablo 18. Solucan sayıları bakımından tanımlayıcı istatistikler ve Tukey çoklu karşılaştırma testi

Fungicide Treatments *Fungisit Uygulamaları*	N *Tekrar Sayısı*	Mean *Ortalama*	Grouping *Gruplandırma*	SE Mean *Ort. St. Hatası*	Minimum *En Az*	Maximum *En Çok*
Three sub-dose (*Üç alt doz*)	5	21.600	A	1.210	18.000	25.000
One sub-dose (*Bir alt doz*)	5	21.200	A	1.020	18.000	24.000
Two sub-dose (*İki alt doz*)	5	20.600	A	0.872	18.000	23.000
Four sub-dose (*Dört alt doz*)	5	20.600	A	0.872	18.000	23.000
Control (*Kontrol*)	5	20.400	A	0.812	18.000	23.000
Recommended-dose (*Önerilen doz*)	5	16.000	B	0.548	15.000	18.000

Note: Differences among doses which are represented by different letters are significant
Not: Farklı harflerle gösterilen dozlar arasındaki farklar önemlidir

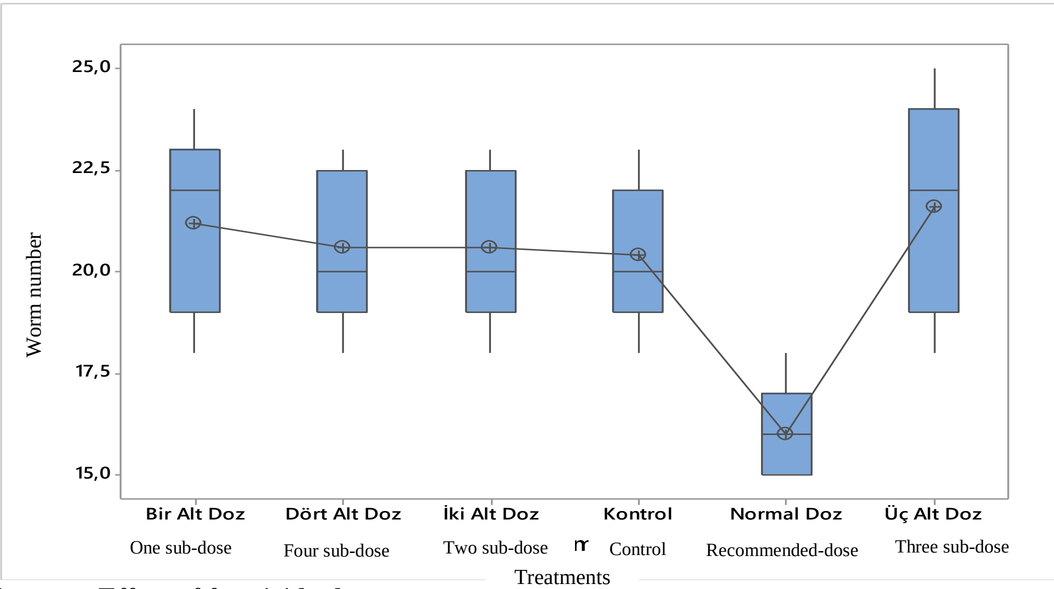

Figure 9. Effect of fungicide doses on worm number
Şekil 9. Fungisit dozlarının solucan sayısına etkisi

Compared to the control, insecticide treatments, particularly at recommended field doses and other doses, resulted negative effects on the worms weight, cocoon numbers and worm numbers. These results was supported by the findings by Haque and Ebing (1983), Bustos-Obregón and Goicochea (2002), Espinoza-Navarro and Bustos-Obregón (2005), Farrukh and S-Ali (2011), Rico et al. (2016), Wang et al. (2016) and Gupta et al. (2011). However, as slightly different from the results obtained from others, Jovana et al. (2014) detected that insecticide Galition G-5®did not cause any death in *E. foetida* members, albeit showing some sensitivity to the insecticide. Findings obtained from fungicide treatments were supported by the findings by Helling et al. (2000); yet, they differ from the results of Vermeulen et al. (2001). The findings obtained from herbicide treatments in this research were not very similar to those of Haque and Ebing (1983), Xiao et al. (2006), Correia et al. (2010), Jovana et al. (2014) and Wang et al. (2016), yet, It was considered that the results were partially compatible. In the light of findings obtained from this research, it has been determined that insecticide (Demond®) and fungicide (Safacol®), especially at the recommended dose for the field use have negative effects on *E. fetida* weight, the number of produced cocoon and the number of hatching from the cocoons; but, herbicide (Granland®) showed less negative effect. It has been realized that these results share similarity with the findings obtained by Heimbach (1992), Yasmin and D'Souza (2007), and Wang et al. (2012).

CONCLUSIONS

Consequently, it has been detected that among the pesticides tested, especially insecticide and fungicide have negative effects on *E. fetida*. With the awareness that pesticides regarded as a must in agricultural activities in present-day conditions effect every individual and every single thing, it is recommended that;

1) Pesticides should be definitely subjected to a wide range of toxicity tests before they are launched to the market,

2) Should be specific to the target pest,

3) Should not be used above the recommended dosing rate, and

4) Should be considered as measure of the last resort in pest control.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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