

**Orijinal araştırma (Original article)**

**Side effects of some crop protection products on non-target soil invertebrates<sup>1</sup>**

Bazı bitki koruma ürünlerinin toprakta yaşayan hedef dışı omurgasızlar üzerine etkileri

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**Summary**

Soil living invertebrates play crucial roles in the terrestrial ecosystem. Plant protection products are known to affect soil organisms and might have negative impacts on soil functions influenced by these organisms. The main goal of this study was to estimate the prompt effects of various pesticides applied against soil pests, weeds and diseases agents on some soil populations. Four plant protection products were applied to soil in a trial at the Plant Protection Department of Ankara University demonstration field and the variation of population in samples was then observed. The samples were collected at different periods and soil depths. The soil samples taken were washed through sieves and the invertebrates were isolated, counted and classified.

The results obtained from this study indicated that the most sensitive invertebrate groups to pesticides were collembolans (Insecta: Collembola). Ants (Hymenoptera: Formicidae), earthworms (Clitellata: Haplotaxida) and soil mites (Arachnida: Acarina) were also monitored. According to the results obtained from this work, the changes of distributed products, sampling period, soil depth and applied plant protection products statistically were detected.

**Key words:** Collembolans, earthworms, mites, ants, plant protection products

**Özet**

Toprakta yaşayan omurgasızlar, karasal ekosistemde çok önemli roller üstlenirler. Bitki koruma ürünlerinin topraktaki organizmalar üzerinde etkili olduğu ve bunun sonucunda bu organizmaların popülasyonlarında görülen değişimin, bu canlıların topraktaki fonksiyonları üzerinde olumsuz etkileri olduğu bilinmektedir. Çalışmanın temel hedefi, toprağa uygulanan pestisitlerin toprakta yaşayan hedef dışı organizmalara olan olumsuz etkilerinin belirlenmesidir. Çalışmalar Ankara Üniversitesi Ziraat Fakültesi, Bitki Koruma Bölümünün deneme parsellerinde dört farklı bitki koruma ürünü ile yürütülmüştür. İlaçlamalardan önce ve sonra deneme parsellerinden farklı derinliklerden alınan toprak örneklerinde hedef dışı omurgasız örnekleri incelenmiştir. Toprak örnekleri özel eleklerde yıkanmış ve elde edilen omurgasızlar sınıflandırılıp, sayılmıştır.

Çalışma sonuçları, denemede kullanılan pestisitlerden en fazla etkilenen hedef dışı omurgasızlar grubunun collembolalar olduğunu göstermiştir. Farklı pestisitler, farklı örnekleme zamanları ve farklı toprak derinliklerinde, hedef dışı omurgasızlar üzerinde önemli ölçüde farklı etkiler göstermişlerdir.

**Anahtar sözcükler:** Collembolalar, topraksolucanları, akarlar, karıncalar, bitki koruma ürünleri

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## Introduction

Soils are essential sources of a wide diversity of ecosystem services defined as the goods and ecosystem functions that provide benefit to human populations (Daily et al., 1997). Soil invertebrates are enormously diverse. According to recent estimations, soil animals may represent as much as 23% of the total diversity of living organisms that has been described to date (Decaëns et al., 2006). Fauna is an important part of soil environments. It is involved in many aspects of organic matter decomposition, partial regulation of microbial activities, nutrient cycles and crumbly structure. Depending on their diet (saprophagous, phytophagous or predation), soil fauna are closely linked both to each other and to microorganisms, plants and soil (Tomlin, 1975). They are a vital key to understanding the soil ecosystem. Springtails (Collembola), earthworms, mites and ants contribute to the decomposition process in soil environments by facilitating the breakdown of organic matter and release of nutrients. Collembolans play a major role in the formation of the soil microstructure in the early succession stages of weakly developed soils. They are also food sources for many predators like carabid beetles and mites. Earthworms have a major role in the breakdown of organic matter and are associated with dung degradation (Svendsen et al., 2003).

The term pesticides are used to cover a range of chemicals that kill organisms that humans consider undesirable. Pesticides include the more specific categories of insecticides, herbicides, rodenticides, and fungicides. Farmers use pesticides for the following reasons: protect crops from insect pests, weeds, and fungal diseases while they are growing; prevent rats, mice, flies, and other insects from contaminating foods while they are being stored; and safeguard human health by stopping fungal contamination of food crops. However, chemical pesticides are usually not target-specific and, therefore, may cause harm to non-target species, and many of them are quite persistent for long periods in the environment.

Indeed, pesticides are needed to meet the world's demand for foodstuffs and no other alternative can compete on such a large scale. Slow degradation of pesticides in the environment and extensive or inappropriate usage by farmers can lead to environmental contamination of water, soil, air, several types of crops and indirectly to humans (Navalón et al., 2002).

The unwanted mortality of natural enemies of pests is a key concern in developing effective pest management. Most chemical pesticides used earlier were broad-spectrum and simply 'killed insects' (or other invertebrates) in the areas to which they were applied. Side effects are a potential, largely inevitable consequence of this practice, with all coexisting species in the crop environment being potentially susceptible. Many studies have investigated pesticide effects on natural enemies, but wider studies of impacts on innocuous taxa are relatively rare (Moreby et al., 1997).

A side effect of usage of some pesticides results in unfortunate consequences to nontarget organisms. There have been numerous attempts to predict the environmental impacts of pollutants on soil systems, particularly those associated with the application of pesticides to agroecosystems. Progressively, national and international pesticide registration authorities are requiring more data on the effects of pesticides, particularly on some soil organisms such as earthworms, echytraeids, nematodes and springtails, and on soil processes such as rates of organic matter or cellulose breakdown, but such data are often required only on an ad-hoc basis, and are often required for only those pesticides that are applied to soils to control soil-inhabiting pests. There is an urgent need for a more comprehensive, holistic, hierarchical and integrated approach to assessing the impacts of chemical pollutants on soil ecosystems (Morgan & Knacker, 1994; Edwards et al., 1996; Edwards et al., 1998).

Many chemical pollutants are directly toxic to particular taxa of soil invertebrates. This can have major effects on populations and communities of soil organisms, as well as on their functional roles in soil ecosystems (Edwards & Thompson, 1973).

To better understand the activity of pesticides in soil and management of the associated risks, we determined the effects of a number of pesticides on some soil invertebrates in a study at the demonstration farm of the Department of Plant Protection, Ankara University.

## Material and Methods

The experiment was carried out during 2010. The trial at the Plant Protection Department demonstration farm (latitude 39° 55' N, longitude 32° 51' W), Faculty of Agriculture, University of Ankara, Turkey. The demonstration farm had not been sprayed with pesticides at least for 5 years. The trial was divided into five randomly placed blocks, each block divided into five replicates. Distances between each replicate about 0.5m to avoid spray drift when applied. The study area received two applications of various pesticides, the first one on 18<sup>th</sup> May 2010 and second on 11<sup>th</sup> August 2010. Chemical products sprayed to control insects, weeds and diseases in the experiment, such as Bordeaux (300g/daa) (Bor-Dox®) as a fungicide, Methiocarb (100g/daa) (METCAR 500 WP), a broad spectrum insecticide from the carbamate group, Glyphosate (300mL/daa) (ARMAGEDDON 48 SL) as a herbicide and Imidacloprid 60% (20mL/daa) (Merit) a broad spectrum neonicotinoid, were applied. Pesticides were applied two times in the evening using a knapsack sprayer. No plants were deliberately cultivated in the experimental area, only weeds as volunteers.

Irrigation was applied two times per week over all the trial area. Soil samples were taken once before treatments as a pre-control. The temperatures and humidity were recorded when soil samples were collected from the trial. The soil samples were taken from two different upper layer depths (0- 7.5 cm and 7.5-15 cm) and 10 cm diameter. From each depth, five replicate samples were randomly collected in plastic bags and transported to the laboratory. From each bag, 1kg of soil was weighed, washed through sieves and soil invertebrates collected.

Furthermore, soil samples were taken randomly after one week of spraying pesticides in each period of application. The second collection was made in July 2010, about 45 days after the first spray application. The third collection was made in 11<sup>th</sup> August. About 1 cm of the soil surface was removed before the collection of soil samples.

The soil samples were washed and soil arthropods were collected, counted and identified under a binocular microscope. Petri dishes, each one divided into four section made out from rubber bands, were used to count soil arthropods (this technique is used for the first time).

A randomized complete block design was used to determine the interactions between fixed factors (pesticides, periods and depths) and soil invertebrates. All data were presented as replicates using Henderson- Tilton's formula so as to calculate the corrected efficacy %. All analyses were performed with the General Linear Models (GLM) of the Statistical Analysis System (SPSS 18 Statistics Program). Later, the MSTAT program and Duncan's multiple range tests were used to determine if there were any significant differences in pesticide effects on non-target soil arthropods and their interaction between different periods and depths.

## Results

Statistical analysis showed that for the three sampling times, the interaction between different pesticides and some soil invertebrates (F: 3.733, P: 0.000 and N: 30), as well as the interaction between periods and soil invertebrates (F: 2.675, P: 0.016, N: 40), was significant. However, the interaction between pesticides and different soil depths (F: 2.386, P: 0.072 and N: 10), as well as the interaction between sampling periods, pesticides, soil invertebrates and depths, was not significant (F: 0.759, P: 0.747). There were also no significant differences between the soil depths and sampling periods (F: 0.67, P: 0.512).

From Table 1, it is apparent that the sensitivity of collembolans to all pesticides (Bordeaux, Glyphosate, Methiocarb and Imidacloprid) gave a significantly higher mean when compared with mites and ants, as well as earthworms to bordeaux and methiocarb, but no significant difference with earthworms in sensitivity to glyphosate and imidacloprid. In contrast, no effects observed of all pesticides applied to mites except imidacloprid which showed higher susceptibility in this study.

Table 1. The effects of four different pesticides (bordeaux mixture, glyphosate, methiocarb and imidacloprid) applied to soil on some soil invertebrates (collembolans, earthworms, mites and ants)

Pesticides	Collembolans Mean + SE (Min – max)	Earthworms Mean + SE (Min – max)	Mites Mean + SE (Min – max)	Ants Mean + SE (Min – max)
Bordeaux	84.11±2.97 (44.6 – 97.88) A a	55.83 ± 5.13 (0.00 – 88.89 ) B b	19.45 ± 5.49 (0.00 – 94.44) B c	72.89 ± 6.02 (0.00 – 99.51) A ab
Glyphosate	79.03 ± 3.08 (37.87 – 97.23) A a	81.60 ± 5.32 (0.00 – 100.00) A a	8.55 ± 3.27 (0.00 – 59.00) B c	52.84 ± 7.27 (0.00 – 100.00) BC b
Methiocarb	82.39 ± 4.46 (0.00 – 97.62) A a	62.09 ± 5.53 (0.00 – 92.00) B b	18.65 ± 4.75 (0.00 – 79.24) B c	42.65 ± 6.92 (0.00 – 100.00) C b
Imidacloprid	87.81 ± 3.35 (23.08 – 98.66) A a	82.58 ± 4.48 (0.00 – 100.00) A a	67.63 ± 4.58 (0.00 – 93.75) Ab	65.35 ± 6.00 (0.00 – 99.60) AB b

Capital letters show the differences in columns and small letters show the differences in rows. Means followed by similar letters show no significant difference in susceptibility ( $P < 0.05$ ).

The results in Table 2 indicate that the mean of collembolan abundances were highly sensitive to all applied pesticides for three periods (May, July and August) when comparisons made between soil invertebrates and periods. However, the earthworms showed a significant difference in susceptibility in August compared to May and a slight difference in July. Furthermore, a higher significant difference was observed in May and July compared to August in same year. Comparisons between collembolans and the remaining organisms after different periods showed highly significant differences in the collembolans sensitivity to all chemical products compared with mites and ants, as well as earthworms in May and July, but no significant difference between collembolans and earthworms in August. In addition, there were highly significant effects on collembolan abundances among different periods for different pesticides.

Table2. Interactions between some soil invertebrates (collembolans, earthworms, mites and ants) and different periods (May, July and August 2010)

Periods	Collembolans Mean + SE (Min – max)	Earthworms Mean + SE (Min – max)	Mites Mean + SE (Min – max)	Ants Mean + SE (Min – max)
May	80.09 ± 3.56 (0.00 – 97.88) A a	64.31±5.67 (0.00 – 100.00 ) B b	23.29 ± 5.34 (0.00 – 93.75 ) B c	56.64 ± 6.10 (0.00 – 99.60) A b
July	85.16 ± 2.34 (34.69 – 97.87) A a	71.68 ± 4.41 (4.00 – 100.00) AB ab	33.52 ± 5.84 (0.00 – 100.00) A c	68.01 ± 5.76 (0.00 – 99.51) A b
August	84.75 ± 3.14 (6.93 – 98.66) A a	75.59 ± 4.02 (0.00 – 100.00) A a	29.79 ± 5.19 (0.00 – 91.5) AB c	49.56 ± 5.53 (0.00 – 100.00) B b

Capital letters show the differences in columns and small letters show the differences in rows. Means followed by similar letters are not significantly different susceptibility ( $P < 0.05$ ).

## Discussion

The available information during this study suggests that the soil invertebrate (collembolans, earthworms, mites and ants) sensitivity may be relatively frequent when different chemical products were used. It is concluded that collembolans were affected detrimentally and showed highly sensitivity by all pesticides applied within different periods, this probably due to collembolans little mobility in soil as well as pesticides risks (Figure 1). Moreover, our data demonstrated that collembolans susceptibility to all pesticides, were significantly difference compared to mites and ants beside earthworms treated with fungicide (bordeaux), neonicotinoid (imidacloprid) and carbamate (methiocarb), but no significant difference observed when applied with herbicide (glyphosate). This result is not difference from Reinecke & Reinecke (2007) who concluded that the collembolans were affected detrimentally by the pesticides due to chronic (chlorpyrifos) and intermittent (azinphos methyl) exposure.

Our results were no far from Geoff et al. (2006) who recorded than the using of data obtained in a systematic review, the present study investigates the relevance of species sensitivity distributions (SSD) and 5% hazardous concentrations (HC5) for predicting pesticide risks to soil invertebrates. Altogether, 1,950 laboratory toxicity data were obtained, representing 250 pesticides and 67 invertebrate taxa. The majority (96%) of pesticides have toxicity data for fewer than five species. Based on a minimum of five species, the best available endpoint data (acute mortality median lethal concentration) enabled SSD and HC5 to be calculated for 11 pesticides (atrazine, carbendazim, chlorpyrifos, copper compounds, diazinon, dimethoate, hexachlorocyclohexane, lambda-cyhalothrin, parathion, pentachlorophenol, and propoxur). He also recorded that, arthropods and oligochaetes exhibit pronounced differences in their sensitivity to most of these pesticides. The standard teste arthworm species, *Eisenia fetida sensu lato*, is the species that is least sensitive to insecticides based on acute mortality, where as the standard collembolan test species, *Folsomia candida*, is among the most sensitive species for a broad range of toxic modes of action (biocide, fungicide, herbicide, and insecticide). These findings suggest that soil arthropods should be tested routinely in regulatory risk assessments.

Earthworms had no higher effects in sensitivity compared to collembolans when treated with fungicide for three times in this study. This result is supported by Cong et al. (2010) who found that Fungicides did not show significant toxicity to earthworms when applied only once, but their toxicities increased as application frequency increased.

The current, deterministic pesticide risk assessment scheme for soil invertebrates assumes that effects on soil invertebrate communities may be predicted using single species tested under a narrow range of exposure conditions (Anonymous, 1984). Santi et al. (2001) recorded that the massive and inadequate use of organophosphorus and carbamate pesticides has produced some water bird mortality events, and may have direct and indirect effects on other non-target organisms.

Some previous studies reported that significant suppression of earthworms can be achieved by one single application of pesticides for a period of over 3 weeks in Lexington, Kentucky (Potter et al., 1990; 1994). However, our current results showed that multiple applications of the pesticides were reduced the abundances of collembolans (high sensitivity) followed by ants, earthworms and mites respectively, even similar application doses were applied .For earthworms the negative effects of pesticides seen in August, but no longer negative observed in May and July. In additional, no adverse effects of toxicants were seen on mites and ants through all trail periods (Figure 2).

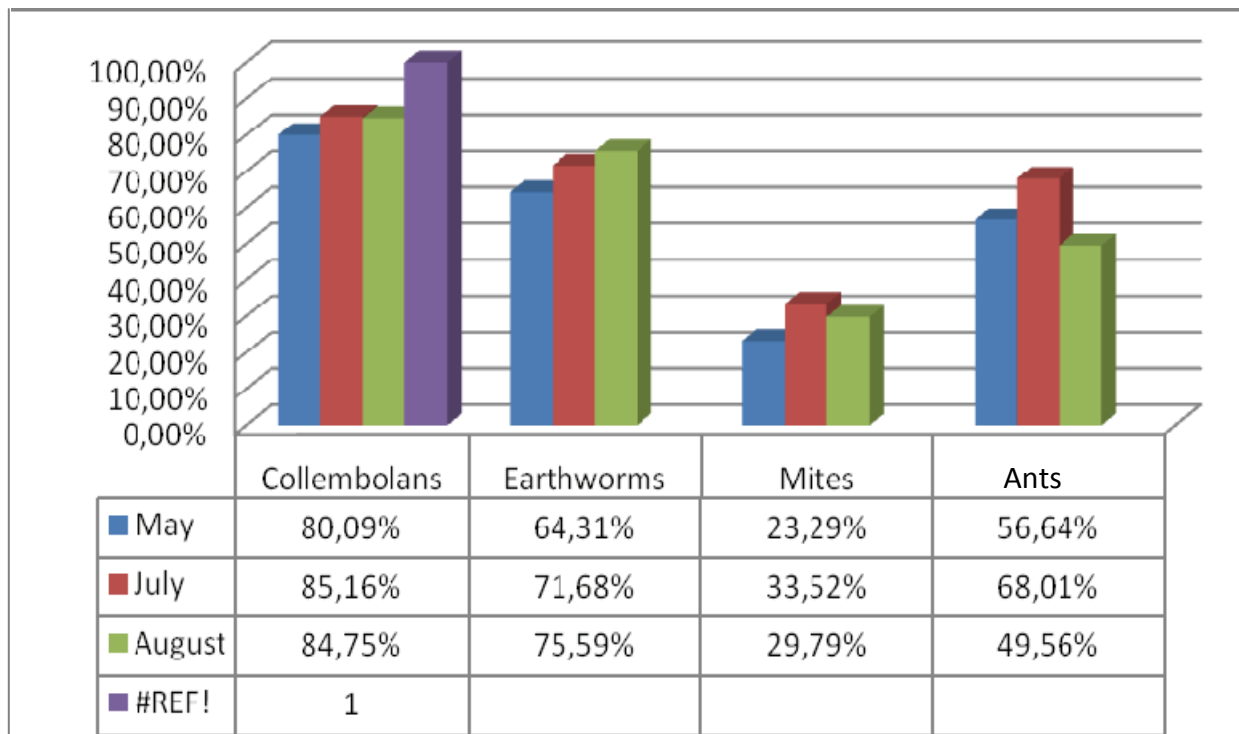


Figure 1. The effects of different pesticides (bordeaux mixture, glyphosate, methiocarb and imidacloprid) applied for three different times (May, July and August 2010) to some soil invertebrates (collembolans, earthworms, mites and ants), Y-axis shows organisms % survival when exposed to different pesticides.

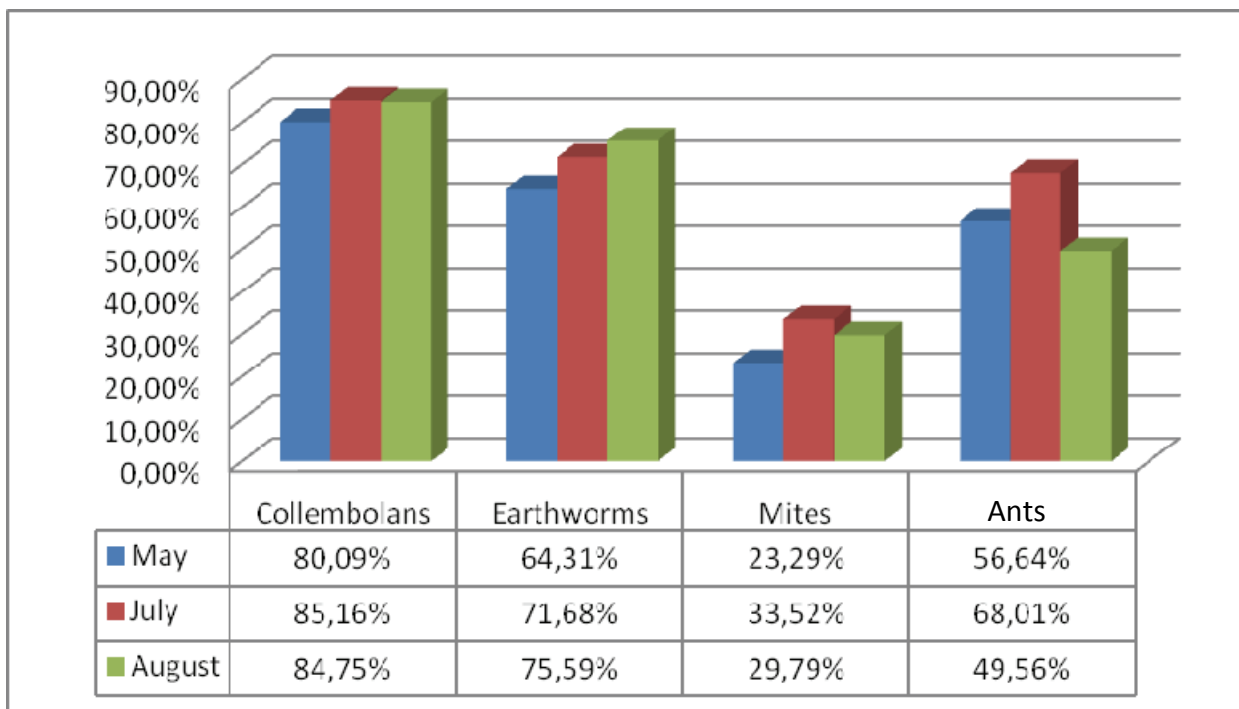


Figure 2. Interaction between some soil invertebrates and different periods. Y-axis shows organism's % survival for three different periods when exposed to different pesticides.

The results similar with Geof (2007) who studied that the effects of chlorpyrifos on arthropod abundance and taxonomic richness were consistently negative to some soil invertebrates, whereas effects of cypermethrin were negative for predatory arthropods but positive for other soil organisms.

## Conclusions

This work revealed that collembolans sensitivity to toxicants is higher compared with remaining arthropods (earthworms, mites and ants) when treated with fungicide (Bordeaux), herbicide (Glyphosate) and insecticides (Imidacloprid and Methiocarb) throughout Plant Protection Demonstration farm, Faculty of Agriculture, University of Ankara, Turkey.

In details, it is apparent that the sensitivity of collembolans to all different applied pesticides gave a significantly higher mean when compared with mites and ants as well as earthworms when applied with bordeaux and methiocarb, but no significant differ with earthworms applied with glyphosate and Imidacloprid. In contrast, no effects observed of all pesticides applied to mites except Imidacloprid which showed higher susceptibility in this study.

The study provided that the highest sensitivity in mean over all soil arthropod abundances to all applied pesticides upon three various periods (May, July and August) was collembolans.

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