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***Orijinal araştırma* (*Original article*)**

Effects of some inorganic fertilizers on the entomopathogenic nematodes *Steinernema feltiae* (Tur-S3) and *Heterorhabditis bacteriophora* (HBH)

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Bazı inorganik gübrelerin entomopatojen nematodlar (*Steinernema feltiae* Tur-S3 ve *Heterorhabditis bacteriophora* HBH) üzerine etkileri

Öz: Entomopatojen nematodlar (EPN'ler) özellikle toprakta yaşayan zararlı böceklere karşı biyolojik mücadelede kullanılmakta, çevreye ve insan sağlığına olumsuz bir etki göstermemektedirler. Toprak içerisinde uzun süre etkin olup konukçusunu arayabilme özelliğine sahiptirler. Yaşam döngülerinin büyük bir bölümünü toprak içerisinde geçiren EPN'ler, gübreleme, toprak işleme ve sulama gibi tarımsal faaliyetlerden etkilenebilir. Bu çalışmada bazı kimyasal gübrelerin, EPN'lerin canlılığı üzerine gösterdiği doğrudan etkiyi belirlemek için yürütülmüştür. Bu amaçla, tarımda yoğun olarak kullanılan altı inorganik gübre ve iki EPN (*Steinernema feltiae* Tur-S3 ve *Heterorhabditis bacteriophora* HBH) ırkı seçilmiştir. Laboratuvar koşullarında 1gr/l, 5gr/l ve 10gr/l gübre dozları uygulanıp, 10 gün boyunca incelenmiştir. İnorganik gübrelere karşı *S. feltiae* (Tur-S3) ırkının *H. bacteriophora* (HBH)' dan daha dirençli olduğu, ancak DAP, NPK ve NP'nin gübrelerinin her iki EPN ırkını da olumsuz etkilediği tespit edilmiştir. DAP, NPK ve NP' nin öldürücü etkisi diğer gübrelere göre daha yüksek bulunmuştur.

Anahtar sözcükler: Entomopatojen nematodlar, *Steinernema feltiae, Heterorhabditis bacteriophora*, inorganik gübre

Abstract: Entomopathogenic nematodes (EPNs) are used effectively in biological control because of their virulence against soil dwelling pests, long-term efficacy and harmlessness to the environment. EPNs spend most of their life in the soil and have host- finding behavior. They can be affected by agricultural activities such as fertilization, tillage and irrigation. The objective of this study was to determine the direct effects of inorganic fertilizers on EPNs. For this purpose, 6 inorganic fertilizers used extensively in agriculture and two species of EPN (*Steinernema feltiae* Tur-S3 and *Heterorhabditis bacteriophora* HBH) were selected for the study. They were exposed to 1 gr/l, 5 gr/l and 10 gr/l fertilizer doses under laboratory conditions and checked for mortality for 10 days. *Steinernema feltiae* (Tur-S3) was more resistant to inorganic fertilizers than *H. bacteriophora* HBH, and DAP, NPK and NP had more adverse effects than the other fertilizers on both strains.

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Key words: Entomopathogenic nematodes, *Steinernema feltiae*, *Heterorhabditis bacteriophora*, inorganic fertilizers

Introduction

Chemical products used in agriculture are one of the main causes of environmental pollution and they threaten all lifeforms. Alternative methods such as integrated pest management (IPM) and biological control are becoming increasingly important. Entomopathogenic nematodes (EPNs), which spend most of their life in the soil, are effective in biological control (Hazır et al. 2004). EPNs are particularly effective against soil dwelling insects and they are used in many different environments, including grass fields, and in the production of ornamental plants and many fruits and vegetables (Peters 1996; Susurluk et al. 2011; Ulu et al. 2014). Generally, EPNs kill their insect hosts within 24 to 48 hours following infection (Gaugler 1988). There are no negative impacts of EPNs on the environment, human health and non-target organisms when compared to pesticides (Boemare et al. 1996; Ehlers 1996). EPNs can be used within the scope of IPM as they are compatible with some pesticides (Ulu et al. 2016). There are many environmental factors in the soil that effect the viability, reproduction and efficacy of EPNs (Kaya 1990). Agricultural practices such as irrigation, tillage and fertilization influence the abundance of EPNs. Tillage and herbicide applications suppress EPN numbers (Susurluk 2008). In this study, the direct effects of some inorganic fertilizers on EPNs were investigated under laboratory conditions. For that purpose, six different inorganic fertilizers, namely urea, ammonium sulfate, ammonium nitrate, NPK, NP and diammonium phosphate (DAP), which are used extensively in agriculture, were investigated.

Materials and methods

Species and production of EPNs

Two different species of EPNs, *Steinernema feltiae* (Tur-S3) and *Heterorhabditis bacteriophora* (HBH), were used for this experiment. Third stage juveniles (infective juveniles), which have the ability to infect hosts, were allowed to infect wax moth (*Galleria mellonella* Lepidoptera.: Pyralidae) larvae. The last stage larval instars used in the study were obtained with the White trap method (White 1927). Infective juveniles (IJs) of nematode species were produced on wax moth instars at room temperature (25°C). Approximately 2 to 3 day old, newly released IJs were used in the experiments.

Six most commonly used inorganic fertilizers were chosen. These fertilizers were urea (NH_2-N), total N 46%), ammonium sulfate (AS: $(NH_4)_2SO4$, total N 21%,total S 24%), ammonium nitrate (AN: NH4NO3, total N 33%), NPK (15-15-15+20SO_3+Zn, total N 15%, total P_2O_5 15%, total K_2O 15%, total SO_3 20%, total Zn 1%), NP (20-20-0+5.5SO_3+Zn, total N 20%, total P_2O_5 20%, total SO_3 5.5%, total Zn 1%) and (DAP: $(NH_4)_2HPO_4$, total N 18%, total (aqueous solution) P_2O_5 46%). In Turkey, the recommended rate of nitrogen (N) application for field crops generally varies between 30 and 200 kg/ha, depending on the crop and soil requirement. P_2O_5 is recommended in the range of 40 to 180 kg/ha (Kacar & Katkat 2014. However, the amount of N recommended in greenhouse tomato cultivation is 537 kg/ha, phosphorus 234 kg/ha and potassium 383 kg/ha (Demirtas & Yılmaz 2003). The 'standard' amount of NPK applied to the soil corresponds to 1.7 g/l (Bednarek & Gaugler 1997). In the current experiment, three different doses for each of the 6 different fertilizers, namely 1 g, 5 g and 10 g per liter, were used

Methodology

Firstly, the fertilizer granules were broken down into smaller fractions so that they could be easily dissolved in water. The determined doses of the fertilizers were dissolved in one liter of distilled water using a magnetic stirrer. One 24-well culture plate was used for each fertilizer dose. One ml of the particular homogenized fertilizer solution was added to each well (diameter 1.4 cm, volume 3 cm^3) of the plate. However, distilled water only was added to the control wells. An average of thirty IJs (a total of about 720 nematodes per dosage) were added to each well. The initial mortality rate of IJs was determined and subsequent observations were made on the mortality of live juveniles. The solution in each well was covered with canola oil so that the concentration of water was not changed by evaporation. The culture plates were incubated at 25°C.

Experimental Period

Observations and counting were performed at three different times on the nematodes placed in fertilizer solution in each well of 24-well culture plate. The first observation was made after 24 hours and the mortality rate was determined accordingly. Subsequent counts were performed at the end of the fifth and tenth days. The experiment was repeated three times.

Statistical analysis

Statistical differences in the mortality of EPNs were determined by using one-way ANOVA (analysis of variance) in JMP®7.0 software. The LSD (Least Significant Differences) test ($P<0.05$) was used to determine the difference between means.

Results

Mortality of *Heterorhabditis bacteriophora* (HBH)

Compared to the controls, all fertilizer solutions, except for urea doses, appeared to have a significant lethal effect on *H. bacteriophora* (HBH) at the end of the first day (Fig. 1). At 5 days post-exposure, all the fertilizers had caused significant lethal effects and DAP and NP had the most lethal effects for all doses (Fig. 2). Likewise, after 10 days exposure, DAP and NP had the most lethal effects for all doses (Fig. 3).

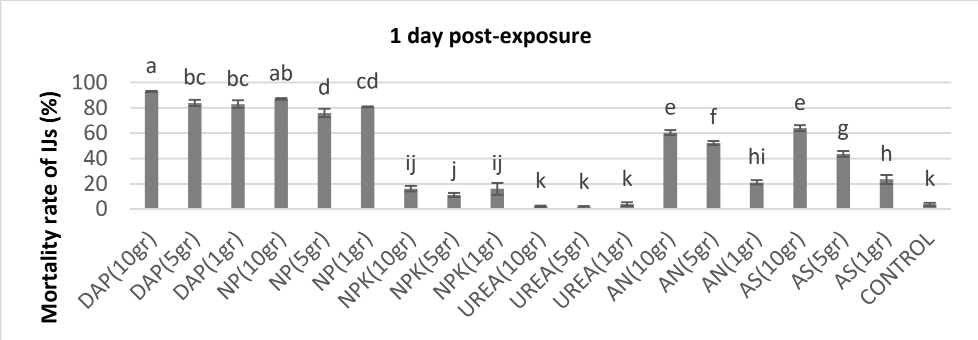

Figure 1. The mortality effects of three different doses (1 g / l, 5 g / l, 10 g/ l) of inorganic fertilizers on *H. bacteriophora* (HBH) 1 day post-exposure (F: 237.69; df: 18 P<0001). Different letters indicate a significant difference between means.

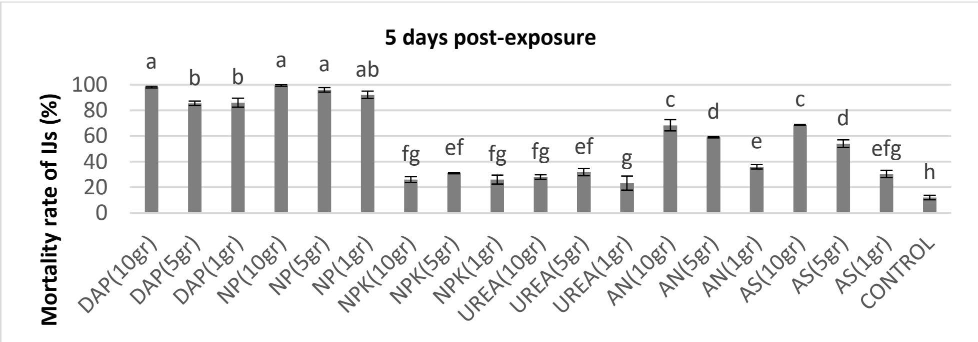

Figure 2. The mortality effects of three different doses (1 g / l, 5 g / l, 10 g/ l) of inorganic fertilizers on *H. bacteriophora* (HBH) 5 days post-exposure (F: 135.41; df: 18 P<0001). Different letters indicate a significant difference between means.

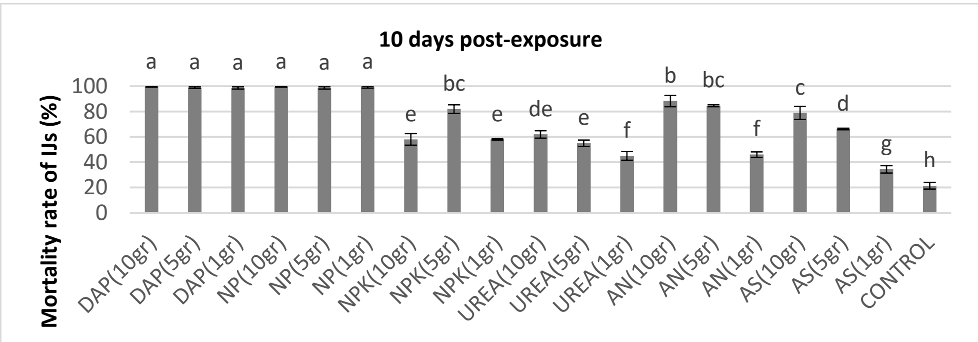

Figure 3. The mortality effects of three different doses (1 g / l, 5 g / l, 10 g/ l) of inorganic fertilizers on *H. bacteriophora* (HBH) 10 days post-exposure (F: 91.62; df: 18 P<0001). Different letters indicate a significant difference between means.

Mortality of *Steinernema feltiae* (Tur-S3)

Compared to the controls, only NPK (1 g/l) and all doses of Urea, AN and AS did not show significant effect on *S. feltiae* (Tur-S3) after 1 day (Fig. 4). After 5 days exposure, DAP and NP had the most lethal effects for all doses and NPK for 5 g/l and 10 g/l (Fig. 5). After10 days exposure, all fertilizers except for AN, AS and Urea for all doses had a significant lethal effect on the EPNs (Fig. 6).

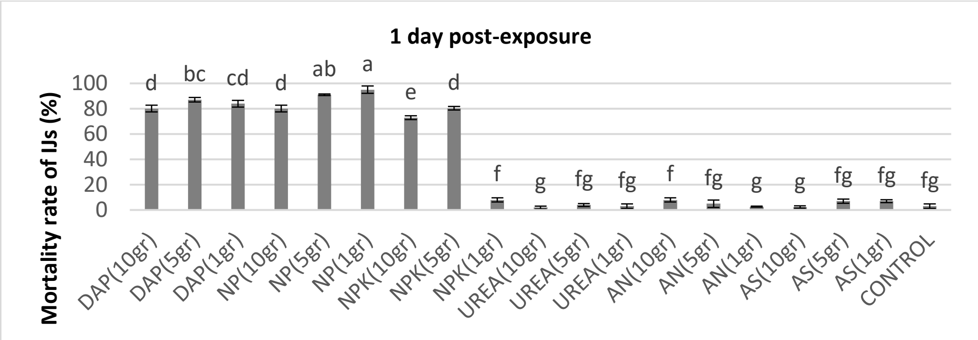

Figure 4. The mortality effects of three different doses (1 g / l, 5 g / l, 10 g/ l) of inorganic fertilizers on *S. feltiae* (Tur-S3) 1day post-exposure (F: 476.68; df: 18 P<0001). Different letters indicate a significant difference between means.

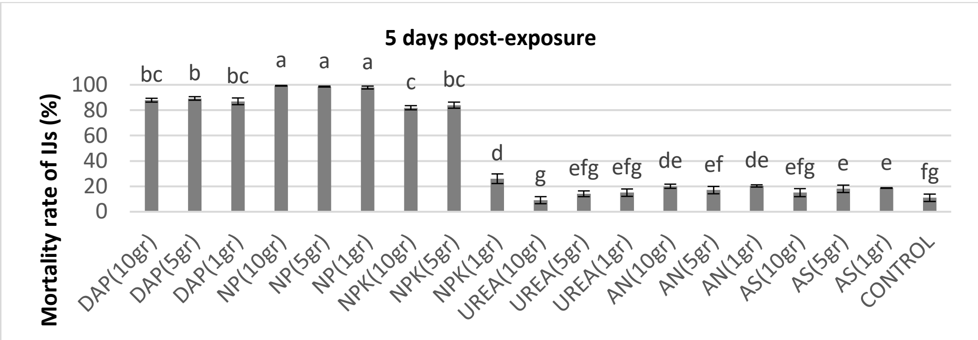

Figure 5. The mortality effects of three different doses (1 g / l, 5 g / l, 10 g/ l) of inorganic fertilizers on *S. feltiae* (Tur-S3) 5 days post-exposure (F: 284.80; df: 18 P<0001). Different letters indicate a significant difference between means.

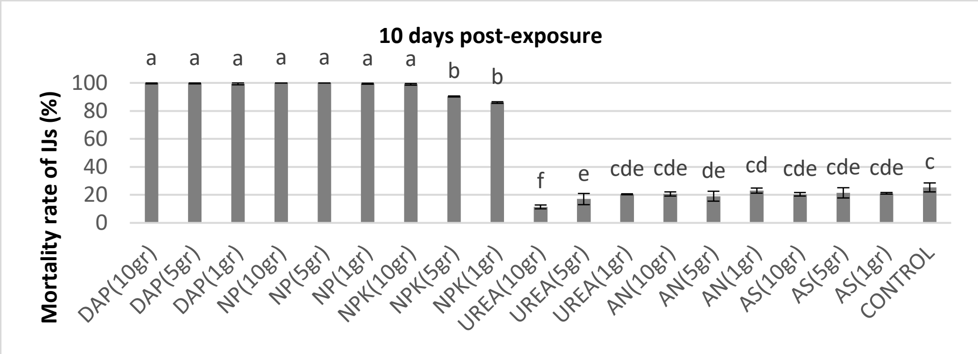

Figure 6. The mortality effects of three different doses (1 g / l, 5 g / l, 10 g/ l) of inorganic fertilizers on *S. feltiae* (Tur-S3) 10 days post-exposure (F: 483.46; df: 18 P<0001). Different letters indicate a significant difference between means.

Discussion

The excessive use of inorganic fertilizers causes a variety of environmental problems such as soil salinity, heavy metal accumulation, greenhouse effect, eutrophication in water bodies and nitrate accumulation (Sönmez et al. 2008). When exposed to soil salinity, the movement of EPNs is restricted and their ability to find and recognize their host is reduced (Nielsen 2011). There is more accumulation of heavy metals in the areas where agricultural activities such as fertilizing are conducted than in natural areas (Campos-Herrera et al. 2010). Some heavy metals have a direct lethal effect on EPNs and, if not lethal, reduce their ability to infect hosts (Jaworska et al. 1996; Jaworska et al. 1997; Sun et al. 2016). In addition, EPNs were found to be more susceptible to heavy metals in the soil than plant parasitic nematodes (Sun et al. 2016). Excessive inorganic fertilizer use reduces the pH of the soil, so some nutrients, such as phosphorus (P) and calcium (Ca), cannot be absorbed by plants. Thus, nutrient deficiency is seen in plants and it becomes necessary to apply additional fertilizer (Bilen & Sezen 1993; Bellitürk 2011). EPNs are highly exposed to the effects of fertilizers due to excessive and prolonged use of inorganic fertilizers. In the current research, the direct effects of fertilizers on *S. feltiae* (Tur-S3) and *H. bacteriophora* (HBH) were investigated under laboratory conditions. EPNs were affected by inorganic fertilizer according to their their ingredients and concentrations and the species of nematode (Bednarek & Gaugler 1997). According to the present research, *S. feltiae* (Tur-S3) was not adversely affected by the following fertilizers, urea, AN and AS, over the 10 day trial period. However, all inorganic fertilizers at different doses were lethal to *H. bacteriophora* (HBH) over the 10 day period, and only urea for all doses had no effect after 1 day. *Steinernema feltiae* was more resistant than *H. bacteriophora* (HBH) to inorganic fertilizers. Similar results were reported by Bednarek & Gaugler (1997). Susurluk (2008) reported that when agricultural activities such as fertilization (organic and NPK), tillage, irrigation and herbicide (Trifluralin EC) were undertaken together during a 2-year field study, *Heterorhabditis* was adversely affected in the second year, but *Steinernema* was not adversely affected. In addition, Shapiro-Ilan (1999) stated that urea (280 kg/ha or less of nitrogen) did not adversely affect *S. carpocapsae* during 10 days exposure. In the present study, NPK fertilization was highly lethal to nematodes. Only *S. feltiae* (Tur-S3) was not adversely affected after 1 day with a 1 gr/l dose of NPK (Fig. 4). The results of the present study support those of Susurluk (2008) who stated that prolonged exposure to high concentrations of inorganic NPK fertilizers inhibit the activities of *Steinernema* and *Heterohabditis*. Bednarek & Gaugler (1997) stated that NPK increases infectivity after 1 day and decreases it between 10 and 20 days. Moreover, it was determined in this study that NPK was more lethal to *S. feltiae* (Tur-S3) than AN and AS at 10 days. Even at 24 hours, the fertilizers containing 20% and 46% P_2O_5 were highly lethal to both strains, Tur-S3 and HBH. This study showed that overall the two EPNs were negatively affected by inorganic fertilizers. However, further studies on the effects of fertilizers on EPNs need to be conducted in the field.

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