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Nemacur Residue Analysis in Soil Water and Cucumber Samples Collected from the Field in Gaza Strip, Palestine

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Abstract

Application of Nemacur in Gaza strip increased rapidly as a potential alternative to the widely used soil sterilizing agent methyl bromide. Nemacur application may contaminate soil, water and plant systems due to its high solubility in water. The objective of this study was to determine Nemacur residues soil, water, and cucumber samples collected from a field plots applied Nemacur at different field rates (0.0, 0.5 F, 1 F, 2 F) where F is the recommended field rate of Nemacur (4 kg/Hectare). Nemacur residues were determined by chemo-assay and bioassay techniques. Results revealed that considerable Nemacur concentrations were found in cucumber fruits and plant leaves. Nemacur residues were higher in water samples collected from sandy soil (7.2 µg/L) than from clay soil (3.4 µg/L). Furthermore, Nemacur residues in sandy soil (0.23 µg/kg) were lower than those in clay soil (1.3 µg/kg). In addition, Nemacur concentration in top soil layer in clay soil was lower than other layers. Nemacur residues in cucumber fruits grown in sandy soil were lower than those in cucumber fruits grown in clay soil. Nemacur residues in cucumber fruits collected from the market were below detection limit of HPLC technique. Chemo-assay techniques determined lower concentration of Nemacur than bioassay techniques. It can be concludes that considerable concentrations of Nemacur were found in all tested samples. Comparing with maximum residues limits (MRL_S). Nemacur concentrations in various environmental samples were less than the maximum residues limits.

Keywords

Nemacur, Fenamiphos, Chemo-Assay, Bioassay, Soil

1. Introduction

Fenamiphos (ethyl 4-methylthio-m-tolyl isopropylphosphoramidate) is an organo-phosphorus insecticides and nematicide used to control soil born insects and nematode (roundworm). It provides effective control of free living root-knot and cyst-forming nematodes. It is commercialized by Bayer Company under the name of Nemacur and is formulated as a granular product or an emulsifiable concentrate at 400 g active ingredient per liter [1]. There has been a public growing concern on Nemacur use in Gaza Strip due risks associated with its use. So far, risk potential associated with pesticide uses emerged from the fact that pesticides are groups of chemical compounds have heterogeneous chemical structures with divers shapes. Pesticides are widely used to control pests that affect agricultural crops and pests in homes, yards and gardens. Potential risks associated with pesticides includes groundwater contamination [2]-[11], risk to atmospheric contamination [12] [13], food contamination [14]-[20], soil contamination [21] [22] [23] [24] [25] health risks [26]-[31] risk to fish [32] [33] [34] [35] risk to cyanobacteria [36] [37] [38]. The above mentioned reports have a major focal point on herbicides since they are directly applied to the soil and being irrigated with water to be distributed in the top 15 cm of soil layer, the active layer for weed control. So far, few reports investigated risk potential of insecticides in Gaza soils, to the best of our knowledge only one report appeared recently [39] that evaluated the risk potential of Chlorpyrifos. For the case of Nemacur, no reports are available probably due to the fact that Nemacur use may be restricted in many countries. So far, large quantity of Nemacur was used in Gaza strip, Palestine [40] [41] and elsewhere [42] [43] [44]. Nemacur has high solubility in water 400 mg/L [45]. The high solubility of Nemacur in water enables fast distribution in soil, water and plant systems and creates hazards to these systems. Accordingly the authors of this study investigated the distribution of Nemacur in soil, water and plant systems field conditions and provide basic information useful for researchers around the world.

2. Materials and Methods

Study site. Gaza Strip is an important part of State of Palestine. It consists of five Governorates, the northern area, Gaza, the middle (Deir Al-Blah), Khan Yunis and Rafah Governorates. The Gaza Strip, as one of the most densely populated areas in the world (2638 people/km^2), has limited and declining resources and has already started to experience deterioration of environmental quality. Details on study site are shown recently El-Kourdi *et al.* [46]. Commercial formulation of Nemacur was purchased from a local certified pesticide seller. Its biological activity was tested in the laboratory to insure validity of the formulation.

2.1. Experimental Design

2.1.1. Soil Collection

Soil samples were collected from sandy and clayey soil of ten years of history free of Nemacur application. Soil samples were dried for 48 h, and then sieved throughout a 2-mm mish size sieve [24]. Sandy soil was placed in 16 plastic pots, and similarly clay soil. Pot size was 8 L.

2.1.2. Soil Treatment and Cucumber Seedling

The surface of soil in each pot received an appropriate amount of Nemacur through manual spraying process. The concentration of Nemacur in pots corresponds to one of the following application rates 0.0; 0.5 F; 1 F and 2 F where F is the recommended field rate (1.5 L/1000 m^2) [40]. The field concentration was calculated for each pot according to surface area for the pot. The concentrations are as follows, (0.0, 6.6, 13.2, 26.3) mg/L respectively. Cucumber seedlings were then planted in the sandy and clay soil in 32 pots and transferred to a greenhouse to insure normal growth to protect the seedlings from the weather conditions. Four replicates were used to each tested concentration.

2.1.3. Water Irrigation

8.5 L of a regular water were irrigated for each pot along 3 months. Cucumber plant had a normal growth under a normal condition at specified greenhouse.

2.2. Sample Collection

2.2.1. Cucumber Fruits and Plant Collection

Cucumber fruit and leaves were collected from each treatment. One kg of fruits and leaves were collected from each treatment. The samples were washed with distilled water, and mixed separately in homogenizer. Then 100 grams of sample, and 100 ml distilled water were added and mixed together in a homogenizer. After 10 min mixing, the samples were centrifuged at 4000 rpm for 15 min using high speed centrifuge, model TGL-16G. The supernatants were collected and analyzed for Nemacur using HPLC (Agilent 1620 model) under specific condition [46] and bioassay techniques [47].

2.2.2. Soil Sampling

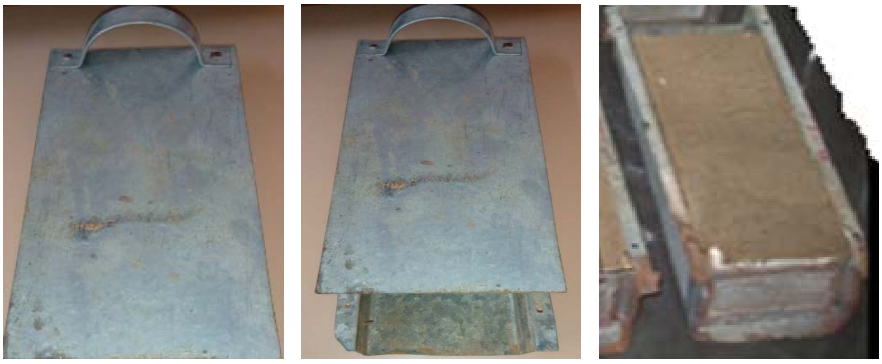
Soil samples were collected from each pot using column techniques **Figure 1**, [48]. In this technique the columns were smoothly inserted in each pot, using large spatula the soil sample was taken out. Then the sand in each column was subdivided to three depths 0 - 5, 5.1 - 10, and 10.1 - 15 cm. Then each soil depth was collected separately, weighted and extracted with distilled water as previously reported [49] [50]. Then filtrate of the sample by filter papers, and then the sample were injected in the HPLC.

2.2.3. Water Collection

Through the irrigation process, special plastic pot was inserted in each plant seeding pot to collect the filtrate (**Figure 2**). Through the growing season, the collected water the samples were analyzed according to previous method [51].

2.2.4. Cucumber Fruits Samples from the Central Market in Deir Al-Balah

Six samples of cucumber fruits were collected randomly from the market. The



(a) (b) (c)

Figure 1. Column techniques used to collect sandy and clay soil samples from the pots. (a), (b) and (c) are large spatula, half column covered with spatula and soil column respectively.

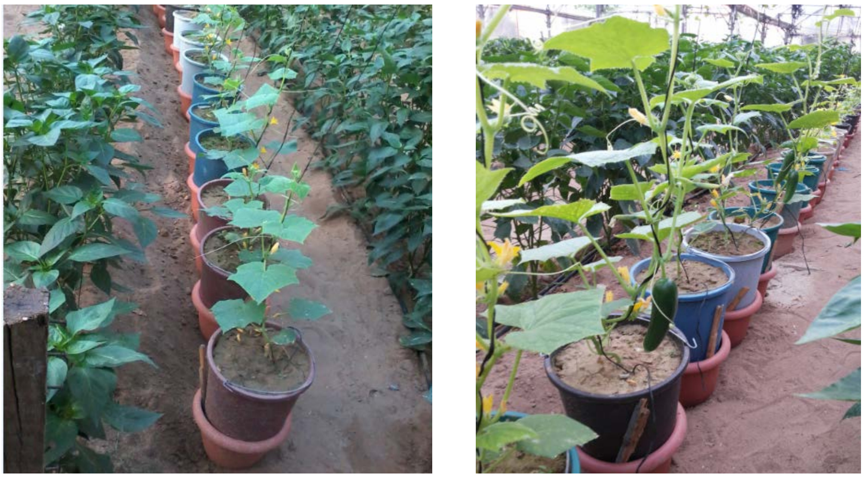


Figure 2. Normal growth of cucumber plants under greenhouse condition.

samples were transferred to the laboratory and prepared as described above for analysis on HPLC. The picture below shows the samples collected from the market in the middle governorate (Deir Al-Balah).

2.3. Extraction and Purification Procedures

Soil water extract was performed by making soil suspension as 1.2.5 (v/v) shaking 24 h under continuous horizontal shaking [52]. Then water filtrates were collected using ash less filter paper. Then the filtrates were centrifuged at 10,000 g for 30 min at 5˚C. The supernatants were then collected and kept in the fridge at 5˚C until determination.

Fruit or plant extracts were collected by mixing 100 g fresh sample with 100 ml distilled water by bender and homogenizer for 10 min. Then the samples were centrifuged at 10,000 g for 30 min at 5˚C. The supernatants were collected and kept in the fridge for HPLC or bioassay determination.

Water filtrates were collected twice during the experimental period. The samples were centrifuged as mentioned above. The supernatants were collected and kept in the fridge for HPLC or bioassay determination.

2.4. Determination or Nemacur Residues in Different Samples

Nemacur was extracted from fruits as previously described [17] [46] and converted into a standard substance, 10 mL of commercial Nemacur were suspended in 20 mL of water, and extracted with 3 * 20 mL of dichloromethane, organic layer was separated and dried with anhydrous sodium sulfate. Dichloromethane was evaporated under stream of nitrogen gas, and the residue was recrystallized using methanol\water system. The white solid was collected by filtration. The purity of Nemacur was tested using HPLC and GC-MS. And Methanol of HPLC grade, purity 99.9%, was purchased from Sigma Aldrich Co., Germany, was purchased from Gaza.

2.4.1. Standard Curve of Nemacur and HPLC-Measurement

HPLC (Agilent 1620) analyses were performed on isocratic system, It was developed way. Nemacur concentrations in the supernatant were determined by Diode Array Detector (DAD) equipped with manual-injection system. The column was Reverse-phase. Packing ODS-BP5 μm (C18), and a 150 mm × 4.6 mm (i.d.). Injection volume is 50 μl and wave length of detection was 250 nm, Mobile phase is water: methanol 20:80. The flow rate was maintained at 2 ml/min. Other conditions were as used for the silica gel column. External calibration was used for quantification of Nemacur.

Standard Curve of Nemacur, as recently described [46] a dose response curve was prepared by a volume of the stock solution 100 ml, containing 1 mL Nemacur, was transferred to a 100 ml volumetric flask and diluted in MeOH (methanol) up to the mark as working standard. A series of Nemacur standards in the range of 0.01 mg/L were prepared. The absorption was measured by HPLC at wavelength 250 nm and retention time 2.004 min.

2.4.2. Bio Assay Technique and Residue Determination

The used Nemacur was biologically active as demonstrated recently [46]. The reason behind this test is to insure possible reaction between Nemacur and Acetylcholinesterase as shown below for bio-determination of Nemacur risk in different samples [47]. The bioassay technique based on the reaction of acetylcholine esterase and Nemacur as previously described by Elman *et al.* [53] using thiochline. The reaction produced yellow color indicating the activity of the enzyme. Linear relationship between yellow color and enzyme activity was used to Nemacur concentration in the tested samples. Moreover, we followed the technique previously developed [47] [54] [55] [56].

In this technique, 650 μl of phosphate buffer pH8 were added to each well containing 50 μl AchE to equilibrate the enzyme, 200 μl of phosphate buffer containing 2 mM ACTh-I and 6% DTNB were added then 100 μl sample was added to the micro plate and incubated for 30 min under constant orbital stirring (300 rpm).

In the blank test 100 µl distilled water was added instead of sample to get the relationship between enzyme activity and substrate as measured by the yellow color produced due to enzyme reaction. In case on enzyme inhibition, the yellow color is reduced or disappeared. This reflects the concentration of Nemacur in sample.

So far, the absorbance was then measured at 405 nm using 100 µl of the solution taken from each samples. Inhibition experiments were performed by incubating magnetic beads (1 µl) with 100 µl of different concentration of different samples during 10 min. according to the procedure described by Ell man *et al*, The thiocholine reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) yielding a yellow complex absorbing at 412 nm. The inhibition rate was calculated according to El-Nahhal *et al.* [35] using the following formula:

$$I(\%) = 100 * \frac{(ODc - ODt)}{ODc} \tag{1}$$

where ODc and ODt are the optical density of yellow color in the control and treatment samples respectively.

The intensity of yellow color indicates the free activity of AchE. Subtracting the free enzyme from the added are the gives bond enzyme. Application with standard curve gives the concentration of Nemacur in the samples. The bioassay data were calculated based on Equation (1).

2.5. Statistical Analysis

The experiment was arranged as randomized complete block design using two types of soil. Each concentration was repeated four times. Average and standard deviation was determined to each concentration. Difference between treatment was done using T-test. Values of Nemacur residues in tested samples were compared by Turkey's test at $\alpha = 0.05$.

3. Results

3.1. Cucumber Fruits and Plant Collection

Growth of cucumber in the experiment is shown in **Figure 2**, which clearly shows normal growth of cucumber plants and field conditions in greenhouse.

It is obvious that fruits are reaching normal size (15 - 20 cm) according to the local standards

3.1.1. Chemo Assay Technique

Water filtrate

Nemacur residues in water filtrate collected two times during the growth season are shown in **Table 1**.

It is obvious that Nemacur residues are higher in water filtrate obtained from sandy soil than from clayey soil. Furthermore, the residues in the 2nd filtrate are higher than the 1st one.

3.1.2. Soil

Nemacur residues in soil are shown in Table 2. Generally, Nemacur residues are higher in the top soil layer than in deeper depths. Furthermore, the residues are higher in sandy soil than in clayey soil.

Concentration of Nemacur in clay soil has different behavior. It can be seem that the concentration is higher in layers 5 - 10 cm in all applied rate. This trend is similar in all layers.

3.1.3. Bioassay Measurement of Nemacur Residues

Nemacur residues in soil samples measured by bioassay technique are shown in Table 3. It is obvious that Nemacur concentrations in both soil types are significantly different from each other but in the same soil type concentrations are not significantly different at p value = 0.05 in terms of soil depths for each applied rate.

Nemacur residues in 1st and 2nd water filtrate measured by bioassay technique are shown in Table 4. It is obvious that Nemacur concentrations in both filtrate are not significantly different from each other but in the same soil type. Furthermore, Nemacur concentrations in 1st and 2nd filtrate are significantly different at p value = 0.05 in few cases marked with a *.

3.1.4. Nemacur Residues in Different Cucumber Fruits and Leaves

Nemacur residues in cucumber fruits and leaves are shown in Table 5. It can be seen that Nemacur concentration in the 1st harvest of fruit was lower than in the 2nd harvest. Moreover, fruits from sandy soil have divers of Nemacur residues but in general the concentrations are not significantly different at p-value 0.05. Furthermore, residues in plant leaves are higher in leaves from clay soil than leave from sandy soil. Significant differences between both leaves were found at p-value 0.05.

Table 1. Nemacur concentration (μg/L) in filtrate water (average ± standard deviation).

Soil type	1st filtrate			2nd filtrate		
	2 F	1 F	0.5 F	2 F	1 F	0.5 F
Clay	3.4 ± 0.93	2.3 ± 0.2	1.4 ± 0.14	5.8 ± 0.16	4.2 ± 0.25	1.4 ± 0.21
Sand	7.2 ± 0.26	5.4 ± 0.19	2.2 ± 0.35	9.0 ± 0.97	6.1 ± 0.23	1.6 ± 0.35

Table 2. Nemacur concentration in sandy and clay soils average and standard deviation (μg/kg soil) measured by chemo-assay.

soil depth	Sandy			Clay		
	0.5 F	1 F	2 F	0.5 F	1 F	2 F
0 - 5 cm	11.0 ± 1.3	2.9 ± 0.22	4.2 ± 0.40	2.9 ± 0.15	1.1 ± 0.25	1.5 ± 0.24
5 - 10 cm	6.4 ± 0.86	1.5 ± 0.37	2.7 ± 0.57	1.9 ± 0.14	2.1 ± 0.27	2.2 ± 0.72
10 - 15 cm	2.3 ± 0.75	1.7 ± 0.12	2.0 ± 0.20	1.3 ± 0.30	1.8 ± 0.25	7.2 ± 0.96

Table 3. Nemacur concentration in sandy and clay soil average and standard deviation (mg/kg) soil measured by bioassay.

Depth cm	Sandy			clay soil		
	0.5 F	1 F	2 F	0.5 F	1 F	2 F
0 - 5	4.01 ± 0.31	3.95 ± 0.070	3.87 ± 0.17	2.01 ± 0.18	1.87 ± 0.11	1.87 ± 0.23
5 - 10	4.36 ± 0.26	4.12 ± 0.09	3.50 ± 0.33	2.16 ± 0.07	2.91 ± 0.75	2.21 ± 0.29
10 - 15	4.34 ± 0.49	4.02 ± 0.52	5.80 ± 0.25	2.14 ± 0.69	2.33 ± 0.45	2.22 ± 0.07

P-values for clay soil columns ranged between 0.06 - 0.5 indicating no significant difference. However, the p-value of 0.06 is very low to the border of significant difference.

Table 4. Nemacur concentration in water average and standard deviation mg/L.

Water filtrate	1st filtrate			2nd filtrate		
	0.5 F	1 F	2 F	0.5 F	1 F	2 F
Clay	1.00 ± 0.53*	4.30 ± 0.17	4.01 ± 0.59	1.21 ± 0.23*	3.53 ± 0.56	3.21 ± 0.28
Sand	4.01 ± 0.50	4.56 ± 0.58	4.45 ± 0.41	3.84 ± 0.70	3.87 ± 0.56	3.63 ± 0.77

Table 5. Nemacur concentration in cucumber fruits average and standard deviation mg/kg.

Cucumber	1st harvest			2nd harvest			Leaves		
	0.5 F	1 F	2 F	0.5 F	1 F	2 F	0.5 F	1 F	2F
Clay	0.24 ± 0.17	0.80 ± 0.05	1.26 ± 0.12	1.93 ± 0.46	2.09 ± 0.22	2.26 ± 0.46	1.16 ± 0.02	1.14 ± 0.12	2.02 ± 0.30
Sand	0.74 ± 0.13	0.04 ± 0.04	0.94 ± 0.15	1.60 ± 0.61	2.97 ± 0.36	1.93 ± 0.21	0.95 ± 0.29	1.15 ± 0.04	1.30 ± 0.05

4. Discussion

Applied rate of Nemacur in the range of 0.5 - 2 F was chosen because mechanical application or manual application can add 2 folds of recommended rate under difficult field conditions during application. This is in agreement with previous reports [2] [46] [57] that used bioassay and chemo-assay to determine pesticide residues in soil and water samples. Moreover, the data in **Figure 2** clearly show that cucumber grows normally under greenhouse house conditions and the highest applied rate (2 F) of Nemacur did not induce Phytotoxicity to cucumber plants or its fruits. This result agrees with previous reports [58] [59] that demonstrated no effects of insecticides to plants or cyanobacteria at the tested concentrations. Furthermore, determination of Nemacur in different homogenates agrees with chemo assay methods [41] and bioassay methods [47] [60]. Moreover, bioassay method indicates strong positive association between Nemacur concentration and reduced yellow color.

4.1. Chemo Assay Results

The data in **Tables 1-4** clearly show the concentration of Nemacur in filtrate water at different collection times, and in different depth in sand and clay soils. It can be seem that the concentration in sandy soil is lower than the concentration in clay soil, regardless to some discrepancy. The explanation of these variations is that clay soil can absorb Nemacur more than sandy soil. This explanation is in accordance with previous reports [61] [62]. Moreover, clay fraction in soil can interact with Nemacur and retain it in the top soil layer available for plant uptake or for bacterial degradation [37] [63]. Furthermore, the organic fraction in soil may retain high fraction of Nemacur through binding process that may take place either with the organic matter functional groups or with the intimately associated clays [24] [25].

In addition, Nemacur in soil and aquatic systems may form hydrogen bonding through van der Waals interactions or hydrophobic interactions with soil organic matter through phenyl rings [64].

The adsorption interactions of pesticides in soil may involve either the mineral or organic components, or both. In soil that have higher organic matter levels (>5%) pesticide adsorption depend on organic matter contents (Spark, and Swift, 2009). Also micro-organisms play an important role in breaking down the pesticide in the soil surface.

So far, bioassay techniques have previously been used to determine concentration of herbicides in Gaza soils [65]. These techniques have wide application for pesticide residue determination [47].

In addition, Nemacur concentration in filtrate water from sandy soil is higher than those from clay soil, because the sandy soil is high permeability more than clay soil, in accordance with previous reports [29] [43].

It can be seem that the concentration rate of Nemacur in sandy soil is lower than the concentration in clay soil. Furthermore, Nemacur residues in top layer (0 - 5 cm) are lower than the concentration in deeper layers (5 - 10), (10 - 15 cm), in accord with Ref [39]. Moreover, it can be suggested that Nemacur may undergo degradation is soil consequently low concentration may be detected. This suggestion is supported by many reports [55]-[62]. Nemacur residues were detected in cucumber fruit and plant leave grown in clay soils higher than plants grown in sandy soil. These results agree with chemo assay results and previous studies [41] who found low pesticides residues in fruit and vegetables collected in Arab countries.

4.2. Environmental Relevance of Pesticide Residues

It is well known in the literature that Nemacur is a strong acetylcholinesterase inhibitor, accordingly it's residues in cucumber fruits, water samples and or soil sample may cause of morbidity and mortality to local inhabitants .

So far, the environmental relevance of this work emerges from the fact that residues of Nemacur may reach the population and cause toxicity to them as revealed by previous investigations [66] [67] [68].

5. Conclusion

The results revealed that Nemacur residues were higher in water samples collected from sandy soil (7.2 μg/L) than from clay soil (3.4 μg/L). Furthermore, Nemacur residues in sandy soil (0.23 μg/kg) were lower than those in clay soil (1.3 μg/kg). In addition, Nemacur concentration in top soil layer in clay soil was lower than other layers. On the other hands residues in cucumber fruits grown in sandy soil were lower than those in cucumber fruits grown in clay soil. Chemo-assay techniques determined lower concentration of Nemacur than bioassay techniques. It can be concluded that considerable concentrations of Nemacur were found in all tested samples. Comparing with maximum residues limits (MRL_S), Nemacur concentrations in various environmental samples were less than the maximum residues limits. So far, this study is a unique of its kind but it is not enough, further studies are required to evaluate the effects of seasonal variation, crop variations and climate variation in Nemacur residues.

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Author Contribution

YE designed and supervised the experimental work and wrote the manuscript. MAL performed the experimental work. SK participated in developing the analytical methods and MRA revised the statistical analysis and proofread the manuscript.

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