Chemical control of Root-Knot Nematode (Meloidogyne javanica) On Olive in the Greenhouse conditions

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Abstract

Root knot nematodes of Meloidogyne spp. are among the most damaging plant pathogens worldwide. Meloidogyne Species Known of this genus which damage olive trees are sporadically found in wild olives, olive nurseries and established orchards; they generate gall on the roots and delay plant growth. In this study, the impact of nematicide toxins of Aldicarb, Enzon, Oxamyl, and Cusadafos with concentrations of 6 and 8 ppm on olive’s root knot nematode in one-year old olive seedlings, collected from Dezful, was studied in a greenhouse. The results of this study showed that Rugby toxin with concentration of 8 ppm (79/24 percent reduction in nematode population) and Enzone toxin with concentration of 8 ppm (50/38 percent reduction in nematode population) had, respectively, the most and least effect in the control of nematode. This experiment was done by inoculation of 5000 eggs and second instar larvae in a completely randomized design with three replications.

Keywords: Meloidogyne javanica; Nematode; Olive; Concentration

Introduction

Olive, whose scientific name is Olea europea L., is a plant from Oleaceae family and Olea genus [1]. Olive is among the trees that have multiple benefits and nearly all of its parts are used. Its main products are oil and conserve [1]. According to the statistics provided by Food and Agriculture Organization of United Nations (FAO) in 2009 A. D., olive cultivation in Iran has been 31,114 hectares and the rate of the product 40,025 ton [2]. Cultivation of aqueous olive in the Khuzestan province, in 1387 has been 4789/79 hectares and its product, in the same year, 606230/30 kilogram per hectare whose product yielded 1442/52 kilogram per hectare [3]. More than 100 species belonging to 47 genera have been reported from plant parasitic nematodes associated with olive [4]. Among the nematodes, the root-knot nematodes of M. arenaria, M. incognita, M. javanica are of great importance [5]. Also, according to Nicol et al. [6], species of M. arenaria, M. incognita, M. javanica, M. histanica possess the highest potential to damage olive cuttings in the orchards of Indonesia and South of Spain. Barouti and Holami [7] reported the nematodes of M. javanica, Tylenchulus semipenetrans and Xiphinema pachtaicum from olive orchards of Gilan for the first time. Hoseininejad et al. [8] separated 26 species and 19 genera of nematodes from olive trees by which dominant genera of olive parasite nematode, M. javanica, and two species of Helicotylenchus were identified. Mahdikhani Moghaddam et al. [9] collected the root-knot nematode called M. cruciatus from Rudbar’s roots of olive.

Nematodes management in ideal agricultural systems can be achieved by combining different tactics like preventing nematode’s entrance, using resistant varieties, and chemical and biological controlling. In Integrated Pest Management (IPM), using nematicides can be combined with other control tactics by the purpose of the use of low doses of toxin [4]. All nematicides were effective in reducing the number of nematodes both in the soil and on the roots. But Fenamiphos made the greatest reduction in the number of nematodes in the soil and the number of galls, females and egg masses while Carbophuran caused the lowest rate of reduction in this sense [10]. Hoseininejad [11] showed that Temik toxin has possessed the best effect in controlling nematodes and Oxamyl, Rugby, and Mucap, respectively, were in the next categories. Rostami et al. [12] reported that treatment of Vapam has encompassed the highest impact on controlling the nematodes.

The traditional methods used to protect crops from diseases, mostly, have been based on the use of chemicals. The use of fungicides and fumigatory nematicides can have devastating effects on the environment and consumer and are often used in agriculture more than herbicides and pesticides. Chemical methods are not economical in the long run because, in addition to polluting the air, soil, and environment, they can result in developing resistant strains in the organism of the objective by frequent uses [13].

This experiment was conducted regarding the Fifth Development Plan, suggesting that olive cultivation in the province, Khuzestan, is developing and according to reports of the researchers of Research Center for Agriculture and Natural Resources of the province announcing that 75 percent of greenhouses and seedling production centers of the province have been infested and polluted by the root-knot olive nematode and that the infected plants, due to production costs, must somehow be disinfected and free of nematodes. Also, due to the difficulties of using granular pesticides by farmers, three liquid toxins which are easier to use by farmers were applied in this study.

Materials and Methods

Nematodes’ Sampling, Extracting, and Reproducing in Greenhouse Experiments

Sampling was done from the surrounding roots and soil of olive and also olive orchards and greenhouses in some towns of Khuzestan like Bagh Malek, Shoush, Ramhormoz, and Dezful. The samples were...
placed in plastic bags and transported to the laboratory with the name, location, and date of collection. For this purpose, first, nematode-infected roots of the olives were gently washed with water and cut into pieces of 2 to 3 centimeters. Then, below binocular using sharp and arrowhead needle, egg masses were carefully removed and placed in distilled water. After that, disinfection was done by placing the removed egg masses in commercial vaytks (10%) for four minutes. For reproducing nematode, first, tomato (Lycopersicon esculentum Mill. (cv. Rutgers) was implanted in pots with diameter of 12 centimeters containing sterilized soil with a 1:1:1 ratio (soil, sand, leaf compost) in the greenhouse of the faculty of Shahid Chamran University. After the seedlings reached to the stage of having 3 to 4 leaves, a 1/5–2 centimeters deep pit was created near the seedlings and then one disinfected egg mass was placed in each pot and the pots were irrigated when needed. After 2-3 months, plants were taken from the soil and symptoms of nematode infection were observed in the roots. Extraction of nematode egg and second instar larvae of the tomato plants were removed from pots and the roots were washed with water and cut into smaller parts. Five grams of roots were placed in the box of an electric mixer. By adding 100 ml of water and 500 ml of 1% sodium hypochlorite solution at high speed of the mixer for about 40 seconds, the roots were completely crushed and the eggs released. After this stage, materials inside the mixer were passed through two sieves of 100 and 400 meshes and the content of 400 meshes sieve were collected within a beaker after being washed several times with distilled water. Then these materials were transferred into centrifuge tubes and after two stages of centrifuge, the eggs were collected [14].

The impact of nematicides on olive seedlings in the greenhouse

For this experiment, three liquid nematicides of Enzone (403 gr/l), Cadusafos (Rugby) (1200 gr/l), and Oxamyl (Vydate) (240 gr/l) and granule nematicide of Aldicarb (Temik, 10 G) were used. First, one-year old olive seedlings, Dezfool figure, obtained from a greenhouse free of nematode infection in Khouzestan, were implanted in pots with diameter of 18 centimeters containing disinfected soil with a 1:1:1 ratio (soil, sand, leaf compost) in the greenhouse. To inoculate the nematode, first, few holes were established around the seedlings’ roots. Then, the number of intended nematodes was added to the foot of the seedlings and then the disinfected egg mass was placed in each pot and the pots were irrigated when needed. Meanwhile, to inoculate Temik toxin used in a granule, Cadusafos toxins with the commercial names of Rugby and Traden, Oxamyl under the name of Vydate, and Enzone toxin in liquid form were used in the study.

To evaluate the effect of toxins, percent reduction of nematode’s population for each treatment was calculated by comparing the initial population of nematode with final population according to the following formula:

\[
\text{Percent reduction of nematode’s population} = \left( \frac{\text{initial population-final population}}{\text{initial population}} \right) \times 100
\]

The pots were kept and irrigated when required in the greenhouse of Agricultural Research Center for 6 months at a constant temperature of 25 ± 2°C. After this period, the seedlings were removed from the soil and after cutting the shoots, the roots of each pot were separately washed and weighed; and the number of egg mass and its index, population of egg and second instar larvae of the soil and vase was extracted and counted using Jenkin method [15]. Gall level was assessed based on gall index.

### Results and Discussion

Aldicarb toxin with the commercial name of Temik in the form of granule, Cadusafos toxins with the commercial names of Rugby and Traden, Oxamyl under the name of Vydate, and Enzone toxin in liquid form were used in the study.

According to the table of variance analysis of eggs and second instar larvae of nematode in the soil, there is a significant difference between the treatments in the one percent level but the number of eggs and larvae of nematode in the roots of all experimented treatments was statistically identical (Table 1). Comparing the mean of the data with Duncan test put the treatments into two groups A and B (Table 2). The results showed that Rugby toxin with concentration of 8 ppm had the best effect in controlling the root-knot nematode of M. javanica (with

| Treatment | Degrees of freedom | Total F | F-value | C.V | F-value |
|-----------|--------------------|---------|---------|-----|---------|
| Enzone    | 1                  | 8.11    | 3.97**  | 32.20% | 6.17** |
| Rug     | 2                  | 2.84    | -       | -    | -       |
| Total    | 29                 | 10.91   | 3.97**  | 32.20% | 6.17** |

** : Treatments are significant at the 1% level.

n.s: non significant

Table 1: Variance analysis of the nematicide effects testing on control of Meloidogyne javanica on olive trees cv Dezfool.
using granular toxins is difficult for farmers, using liquid toxin of Rugby granular Temik toxin is more used in Khouzestan’s greenhouses and reducing nematode population in the soil; regarding the point that instar larvae of the nematode seems necessary. The research showed and its application concurrently with inoculation of egg and the second repeating this experiment by both the other concentrations of the toxin applied toxins. But, because it was the first time to evaluate this toxin, toxin had a less effect in reducing nematode population than the other its higher concentration. According to the results of the research, this of plant with nematode’s second instar larvae had a better effect than of 6 ppm of the toxin and its application one week after inoculation of olive for the first time, the results showed that the concentration showed a better effect.

In the case of Oxamyl toxin, its lower concentration toxin, its rate in the soil becomes further which results in the better concentration of toxin had a better effect in Temik toxin that seems a direct relationship with the increase of toxin concentration. Higher larvae of roots and population of larvae in soil; this decrease has had egg and larvae caused a significant decrease in the number of egg and reproduction factor

| Treatment                              | Root weight | Number gall/Gall index | Population of root eggs and larvae | Number of eggs and larvae in the soil total | Reproduction factor | reduction Percent in nematode population |
|----------------------------------------|-------------|------------------------|-----------------------------------|---------------------------------------------|--------------------|------------------------------------------|
| Control without nematode and toxin     | 40          | 0                      | 0                                 | B 0                                         | 0                  | -                                        |
| Control nematode and without toxin     | 31          | 1 (1)                  | 730                               | A 8167                                      | 1.68               | -                                        |
| Temik ppm 6                            | 32          | 1 (1)                  | 167                               | A 1424                                      | 0.32               | 68.18                                    |
| Temik ppm 8                            | 30          | 1 (1)                  | 133                               | A 1336                                      | 0.28               | 72.28                                    |
| Oxamyl ppm 6                           | 29          | 1 (1)                  | 117                               | A 1600                                      | 0.38               | 70.66                                    |
| Oxamyl ppm 8                           | 33          | 1 (1)                  | 378                               | A 1064                                      | 0.29               | 71.16                                    |
| Rugby ppm 6                            | 33          | 2 (1)                  | 150                               | A 888                                       | 0.21               | 79.24                                    |
| Enzone ppm6                            | 28          | 1 (1)                  | 78                                | A 2136                                      | 0.44               | 55.79                                    |
| Enzone ppm8                            | 36          | 2 (1)                  | 217                               | A 2264                                      | 0.49               | 50.38                                    |

Table 2: The effect of different nematicides on control of olive root-knot Meloidogyne javanica on old olive seedling varieties Dezfuli.

![Figure 1: The effects of different pesticides on olive root-knot Meloidogyne javanica.](image)

which is applied easily and together with irrigation water can be offered to the farmers. Based on the results of the research, all the studied toxins had a positive impact on the control of this nematode resulted in the reduction of nematode population between 50 to 80 percent.

In Hoseininejad’s experiment, it was shown that the most effective nematicide was Temik and the toxins, Oxamyl, Nemacur, Rugby, and Mucap were placed in the next categories. In addition, according to this researcher, Lamberti and Di Vito stated that disinfection of olive seedling infected by root-knot nematode with Aldicarb and Motomyl has significantly reduced the nematode population without adverse effects on plants while Carbofuran has cause phytotoxicity and Nemacur has reduced plant growth [11]. In the experiment of Acosta and colleagues, on chemical control of *M. incognita*, was also shown that the higher concentrations of the toxins, Fenamiphos and Oxamyl, had the maximum reduction in egg and larvae of nematode population [16].

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