Environmental Friendly Way to Control Root-Knot Nematodes *Meloidogyne Incognita* Infecting Grapevines

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ABSTRACT

This study investigated environmentally friendly alternatives to control the root-knot nematode (*Meloidogyne incognita*) in Thompson seedless grapevines. Three treatments were evaluated over two consecutive growing seasons (2021 and 2022) under field conditions: the bioagent *Trichoderma harzianum*, jojoba plant oil extracts, and the specific nematicide oxamyl (oxafors(R); 24% SL). The study assessed the impact of these treatments on nematode populations (total population, rate of build-up, numbers of juveniles in soil (J_2) (250g), J_2 egg-masses and galls in roots), leaf chlorophyll content and N, P and K contents percentages in leaves. Results showed that all treatments significantly reduced the overall population and build-up of root-knot nematodes, *M. incognita*, in roots and soil, particularly three months - after application. Oxamyl proved most effective in reducing nematode populations and buildup in both soil and roots throughout both seasons, followed by jojoba oil extract where the bioagent (*T. harzianm*) was the least effective treatment overall. Also, oxamyl was the most effective treatment which significantly affected number of second stage juveniles / 250g in soil, followed by (Jojoba oil) and bioagent (*T. harzianm*). Conversely, the *T. harzianm* was the most effective one in reducing the number of egg masses followed by oxamyl and then Jojoba oil. Jojoba oil and oxamyl were superior to *T. harzianum* in increasing nitrogen and potassium levels. Total chlorophyll was at maximum values when vines treated with jojoba oil but had minimum values with *T. harzianum* treatment.

Keywords: ***Meloidogyne incognita*; *Trichoderma harzianum*, Oxamyl.**

INTRODUCTION

Grapes (*Vitis vinifera* L.) hold a significant place in Egypt's agricultural landscape, being one of the most valuable and economically beneficial fruits. Among the various cultivars, Thompson Seedless stands out as one of the top choices both in Egypt and globally.

Soil and plant nematodes are incredibly abundant, with numbers that can astound you. In just a 250 cm^3 soil sample, you might find over 20,000 nematodes, regardless of type of soil (Hartman *et al*., 2016).

Meloidogyne spp. considered one of the most devastating plant parasitic nematodes worldwide, (commonly known as the root-knot nematodes (RKNs)) are obligate sedentary end parasites that establish in the roots, causing hyperplasia and hypertrophy of surrounding cells, triggering the formation of galls (Rusinque *et al*., 2023). The root-knot nematode, specifically the Meloidogyne genus, was the most commonly found in the assays conducted on grapes and guavas (Abdel-Baset *et al.,* 2022). US$173 billion is the economic estimated lose in key crops by plant parasitic nematodes which causes an annual crop yield loss 8.5% (Kumar *et al*., 2020).

The impact of this issue extends beyond significant damage and yield losses affecting numerous crops globally, particularly in developing nations. In Egypt, it has specifically posed challenges for grape production, especially in sandy soil conditions (Netscher and Sikora, 1990). In Egypt, grape growers faced significant challenges due to intense nematode infestations. These infestations led them to uproot their vines and leave the land fallow for several years before they could consider replanting. Utilizing microorganisms that antagonize *Meloidogyne* spp. or the substances they produce may offer a valuable method for mitigating the harm inflicted by root-knot nematodes on grape crops.

Numerous recent studies have focused on biological control agents to manage parasitic nematodes and mitigate the damage they cause, particularly through the application of mycorrhizal fungi (Babu & Suguna, 2000; Duponnois & Plenchette, 2003; Serfoji *et al.*, 2010; Soliman *et al.*, 2011 and Massoud *et al.,* 2021).

Trichoderma harzianum affect the nematodes reduction by many ways such as production antibiotic substances or mycoparasitism and so production of destructive chitinase enzymes. *Trichoderma* species are capable of producing a variety of toxin metabolites and enzymes that enhance the antagonist's photolytic action and help manage nematode populations (Benhamoud & Chet, 1993; Bolar *et al.*, 2000; Sharon *et al*., 2001; Faruk *et al.*, 2002; Siddiqui & Shaukat, 2004; Shawky & Abd El-Moneim, 2005; Sahebani & Hadavi, 2008 and Abd El-Moneim *et al.*, 2010). *Trichoderma* spp. As a living soil fungus is considered as a bio efficient in controlling nematode protecting several vegetable fruits and cash crops (Affokpon *et al*., 2011 and Yao *et al.*, 2023).

Many researchers used essential plant oils and therapeutic plants, like jojoba leaf extracts, to reduce plant parasitic nematodes (Abd-Aziz *et al.*, 1996; Amin, 1999; El-Nagdi, 2005 and El-Nagdi *et al.*, 2009). Using GC and GC/MS analysis techniques for Egyptian jojoba

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oil showed that it contains Docosenyl docosenoate (C-44, 2%), Eicosenyl docosenoate (C-42, 9%), Docosenyl eicosenoate (C-42, 52%), Eicosenyl eicosenoate (C-40, 30%), and Eicosenyl oleate (C-38, 7%) (Shawky *et al.*, 2010; El-Nagdi *et al.*, 2017 and Mostafa *et al.*, 2017).

This study investigates the efficacy of non-chemical control agents, namely bioagents and plant oil extracts, as alternatives to the synthetic nematicide oxamyl for managing *M. incognita* (root knot nematode) in Thompson vineyards. The research also evaluates the impact of these treatments on leaf photosynthetic pigments and the essential elements required for pigment synthesis.

MATERIAL AND METHODS

This study was carried out on almost similar vigor 10-year-old Thompson seedless vines in a sandy soil supported by the Y system 1.75 x 2.75 m apart were pruned at the first week of January and received similar and regular agricultural practices in a private farm located on the 78th kilometer of the Alexandria-Cairo desert road in an area infested with *M. incognita* for two consecutive seasons (2021 and 2022). 60 vines (4 treatments x 3 replicates x 5 vines/replicate) were chosen in randomized complete blocks design.

The four treatments were as follows:

- Bioagents micro-organisms (*T. harzianum*) (soil application)
- Plant oil extracts jojoba oil (*Simmondsia chinensis* L.) (soil application)
- Oxamyl (oxafors; 24% SL) as nematicide (foliar application)
- Control treatment infested with nematode only

Bioagents and plant oil extract preparation:

- Isolate of bioagents (*T. harzianum*) was sourced from the Central Laboratory of Organic Agriculture, Agricultural Research Center, located in Giza, Egypt, with concentration ($5x10^6$ spore/ml).
- A plant oil extracts (jojoba oil) was used as a commercial product at the concentration 2ml/100ml water, which was applied as liquid formulations (2 ml/vine) (equivalent to 2 L/feddan (Ali *et al.,* 2012).
- Nematicide (oxamyl (oxafors®; 24% SL)) used at the recommended concentration (5 L/600 L water/fed).

After the bud burst, treatments were added where, bioagents were treated weekly for three times at its recommended concentration, whereas oxamyl (24% SL) application repeated two times according to the Ministry of Agriculture and jojoba oil was treated once at the appropriate concentration.

Nematodes determination

After the treatments, the tested nematode *M. incognita* parameters were recorded monthly, this included counting the number of juveniles (J_2) found in 250g of soil, with J_2 in roots, as well as documenting egg masses and galls in the roots of treated grapevines cv. Thompson Seedless, in comparison to the untreated treatment. Nematode juveniles were isolated from grapevine plant soil samples using decanting and sieve techniques (Barker, 1985), using a binocular microscope, and so number of egg masses and galls in roots (5g per plant) was assessed. The following formulas were used to compute the rate of build-up (Oostenbrink, 1966).

Rate of nematode build up (PF/PI) = Total population (PF) (after treatment)/initial population (Pi) (before treatment).

Barker (1985) techniques used for nematode population in soil were extracted by sieving and decanting for 250g soil aliquot. While acid fuchsin in acetic acid used to calculate the nematode population in roots according to Byrd *et al.* (1983), and examined for counting the number of developmental stages and females/5g root.

Hussey and Barker (1973) described *M. incognita* egg masses, eggs/egg-mass extraction by sodium hypochloride (NaOCl). Nematode population in soil and roots calculated as a nematode reduction (%) according to Henderson & Tillton (1955) formula and Puntener (1981).

Efficacy % = [1 - (Total nematode population of treated vines after application x Total nematode population of check vines before application)/ (Total nematode population of treated vines before application x Total nematode population of check vines after application)] x 100.

Chemicals analysis

chlorophyll content in vine mature leaves was measured According to Wood *et al.* (1992), by an acronym for soil plant analysis development (nondestructive Minolta chlorophyll meter model SPAD 502), and so nitrogen (N), phosphorous (P) and potassium (K) percentages were determined according to Wilde *et al*. (1985).

Statistical analysis

ANOVA (analysis of variance) (Gomez and Gomez, 1984) was used for Data analysis and LSD at 5% level of significance were used to compare means.

RESULTS AND DISCUSSION

Evaluating the effectiveness of a bioagent and plant oil extract against the nematicide oxamyl in managing *M. incognita*.

The effect on nematode population

The effectiveness of plant oil extracts as a bioagent was evaluated against oxamyl in a field experiment conducted over two seasons (2021 and 2022), lasting four months. Normally during the two tested seasons the specific nematicide oxamyl was the most effective one between the tested materials. Data in Table (1) demonstrate that every treatment tested during the 2021 season was successful in reducing the ultimate nematode population and the rate at which the root knot nematode accumulated in the soil and roots. Both soil and root samples of the treated trees nematode populations, were decreased gradually indicating that all materials had a suppressive effect on their counts.

Tested treatments resulted in total nematode population reduction, which ranging between 706 and 1303 after one month of application in contrast, the check group was 2314. After three months, there was a significant reduction in nematode populations in both soil and root samples. The differences between the various materials tested were notable, with jojoba oil extract, oxamyl, and *T. harzianum* showing efficacy percentages of 89.5%, 88.0%, and 79.1%, respectively. Additionally, the rates of buildup were recorded at 0.31 for jojoba oil extract, 0.35 for oxamyl, and 0.61 for *T. harzianm*. These results agree with Faruk *et al*. (2002); El-Hamawi *et al.* (2004); Siddiqui & Shaukat (2004); Shawky & Abd El-Moneim (2005); Verma (2006); Khalil & Shawky (2008) and Abd El-Moneim *et al*. (2010).

Data in Table (2) which illustrate results of the season 2022 experiment which gave the same trend of tested materials effects as season 2021 experiment where there were significant differences between all treatments.

After first month of treatments applications oxamyl (nematicide) caused 73.74% decrease of nematodes total population in soil and roots of grapevines, but Plant oil extract (jojoba) caused 59.17% efficiency against tested nematodes which was differ significantly with the effect of bioagent (*T. harzianm*) where the last one affected the tested nematodes total population in soil and roots 43.88%. Data in Table (2) also showed that total population of root knot nematode (*M. incognita*) in root and soil were affected after three months of application where they recorded 86.95, 78.55 and 87.65 by Plant oil extract (jojoba), bioagent and oxamyl respectively with significant differences in between. And so, the tested treatments caused rate of build up after the first month 0.54, 0.74 and 0.35 for Plant oil extract (jojoba), bioagent and oxamyl respectively with significant differences in between, while they caused rate of build up after three monthes 0.34, 0.56 and 0.32 for Plant oil extract (jojoba), bioagent and oxamyl respectively without significant differences between jojoba and oxamyl. These findings concur with Abd-Elgwad & Omer (1995); El-Nagdi (2005); El-Nagdi *et al.* (2009) and Shawky *et al.* (2010).

Table 1. Effectiveness of jojoba oil extract and the *T. harzianum* was evaluated against oxamyl for managing root knot nematodes (*M. incognita*) in soil and roots of Thompson Seedless grapes during the 2021 growing season

parameter / Treatments	Treatment rate	Total population present in soil and root	After first month			After second months			After third months			After fourth months		
			Total population in soil and roots	Efficacy %	rate of build up	Total population in soil and roots	Efficacy %	rate of build up	Total population in soil and roots	Efficacy %	rate of build up	Total population in soil and roots	Efficacy %	rate of build up
Plant oil extract Jojoba oil	2ml/ 100ml	2202	1243	59.1	0.56	837.9	85.6	0.38	672	89.5	0.31	1051	86.3	0.48
Bioagent *T. harzianm*	$5x10^6$ cfu	1488	1303	36.5	0.88	1309	66.8	0.88	904	79.1	0.61	1601	69.0	1.08
Oxamyl oxafors 24% SL	5L/ 600L	1541	706	66.8	0.46	559	86.3	0.36	538	88.0	0.35	822	84.6	0.53
Check (nematode only)		1678	2314		1.38	4443		2.65	4880		2.91	5822		3.47
LSD at 0.05%		41.6	33.8		0.07	41.1		0.13	53.3		0.09	64.2		0.11

▪ Total population tested in 250g soil and 5g roots.
▪ Efficacy % = [1 - (Total nematode population of treated vines after application x Total nematode population of check vines before application)/(Total nematode population of treated vines before application x Total nematode population of check vines after application)] x 100.
▪ Nematode build up rate (PF/PI) = Total population (PF) (after treatment) / initial population (Pi) (before treatment)

Table 2. Effectiveness of jojoba oil extract and the *T. harzianum* was evaluated against oxamyl for managing root knot nematodes (*M. incognita*) in soil and roots of Thompson Seedless grapes during the 2022 growing season

parameter / Treatments	Treatment rate	Total population present in soil and root	After first month			After second months			After third months			After fourth months		
			Total population in soil and roots	Efficacy %	rate of build up	Total population in soil and roots	Efficacy %	rate of build up	Total population in soil and roots	Efficacy %	rate of build up	Total population in soil and roots	Efficacy %	rate of build up
Plant oil extract Jojoba oil	2ml/ 100ml	2002	1081	59.17	0.54	798	82.68	0.40	686	86.95	0.34	1020	82.79	0.51
Bioagent *T. harzianm*	$5x10^6$ cfu	1672	1241	43.88	0.74	1047	72.79	0.63	942	78.55	0.56	1539	68.91	0.92
Oxamyl oxafors 24% SL	5L/ 600L	1693	588	73.74	0.35	621	84.06	0.37	549	87.65	0.32	790	84.24	0.47
Check (nematode only)		1804	2386		1.32	4152		2.30	4738		2.63	5341		2.96
LSD at 0.05%		48.6	66.8		0.04	71.1		0.53	83.3		0.12	94.2		0.01

- Total population tested in 250g soil and 5g roots.
- Efficacy % = 100 x [1 - (Total nematode population of treated vines after application x Total nematode population of check vines before application)/(Total nematode population of treated vines before application x Total nematode population of check vines after application)] x 100.
- Nematode build up rate (PF/PI) = Total population (PF) (after treatment) / initial population (Pi) (before treatment)

The high concentration of specific nematicidal compounds in the tested oils may be the cause of their nematicidal effect. These compounds include oxygenated compounds, which are distinguished by their lipophitic qualities, which allow them to dissolve the cytoplasmic membrane of nematode cells, as well as their functional groups, which interfere with the structure of the nematode enzyme protein (Knoblock *et al.,* 1989). Furthermore, recent theories on the mechanisms of action of plant oils include the degrading and denaturing of enzyme activity, as well as interfering with adenosine diphosphate phosphorylation or electron transport in the respiratory chain (Konstantopoulou *et al.,* 1994).

Both seasons, of tested nematodes parameters measurements.

Data in Figure (1) illustrate the effect of tested materials (plant oil extract and bioagent in comparison with oxamyl) on root knot nematode infecting Thompson seedless grapes parameters [number of second stage juveniles in soil (250g), in roots (5g) and number of egg-masses], under field conditions after three months of treatment during 2021 and 2022 season. Where it seems that the specific nematicides was the most effective one significantly on second stage number juveniles /250 g in soil reduction (%), comparing with plant oil extract (Jojoba oil) and bioagent (*T. harzianm*) respectively. These findings concur with those acquired by Gad-El-Rab (2000); Khalil & Shawky (2008) and Shawky *et al.* (2014).

Also, it described the effect of the tested material on second stage juveniles (J_2) in roots where Jojoba oil was the most effective one causing reduction effect on number of second stage juveniles in roots among the tested materials followed by *T. harzianm* and oxamyl significantly. Diedhiou *et al.* (2003) and Abd El-Wahab *et al*. (2008) reported results agree with above findings.

On the other hand the *T. harzianm* was the most effective one on number of egg masses followed by oxamyl then Jojoba oil respectively, which showed no significant differences between oxamyl and the two other materials and significant differences between *T. harzianm* and Jojoba oil. These results agree with those obtained by Sahebani & Hadavi (2008); Abd El-Moneim *et al.* (2010) and Shawky & Al-Ghonaimy (2015).

The treatments studied clearly had a significant impact on the number of clusters in the second season and on the yield per vine across both seasons. Specifically, oxamyl (24% EC), jojoba oil extract, and the bioagent *T. harzianum* greatly enhanced the number of clusters in the 2021 season and increased yield when compared to the control group.

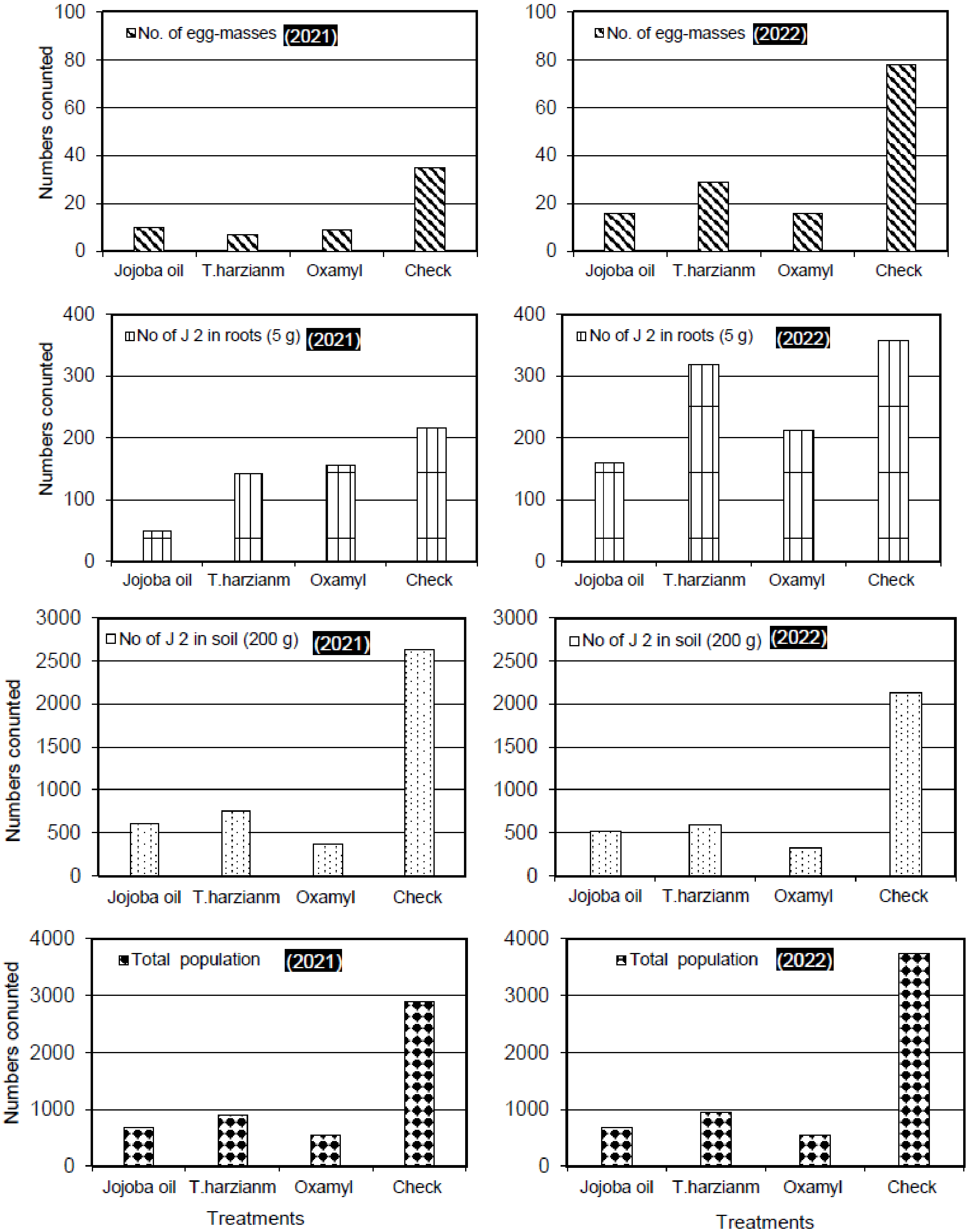

Fig. 1. Efficacy of plant oil extract and bioagent in comparison with oxamyl against root knot nematode (*M. incognita*) parameters infecting Thompson seedless grapes under field conditions during 2021 and 2022 season.

Chemicals analysis

Based on the data presented in Figure (2), it's evident that the total chlorophyll content in the leaves improved significantly with the application of oxamyl, jojoba oil, and bioagents when compared to the control group. Notably, the treatments with plant oil extract and oxamyl were more effective than the bioagent in enhancing the percentages of key elements in the leaves, particularly nitrogen and potassium. The highest levels of total chlorophyll were observed in the vines treated with jojoba oil, while the lowest levels were found in the bioagent treatment compared to the control group. The impact of *M. incognita* on the photosynthetic pigments in leaves could stem from the compromised capacity of damaged roots to take up sufficient amounts of essential nutrients like nitrogen, zinc, iron, and magnesium, which are crucial for pigment synthesis. The finding here is in harmony with those obtained by El-Shenawy & Fayed (2005); El-Gendy & Shawky (2006); El-Nagdi *et al.* (2009); Shawky *et al.* (2010) and Soliman *et al.* (2011), who have highlighted the positive impact of plant oils and bioagents on the overall chlorophyll content in leaves.

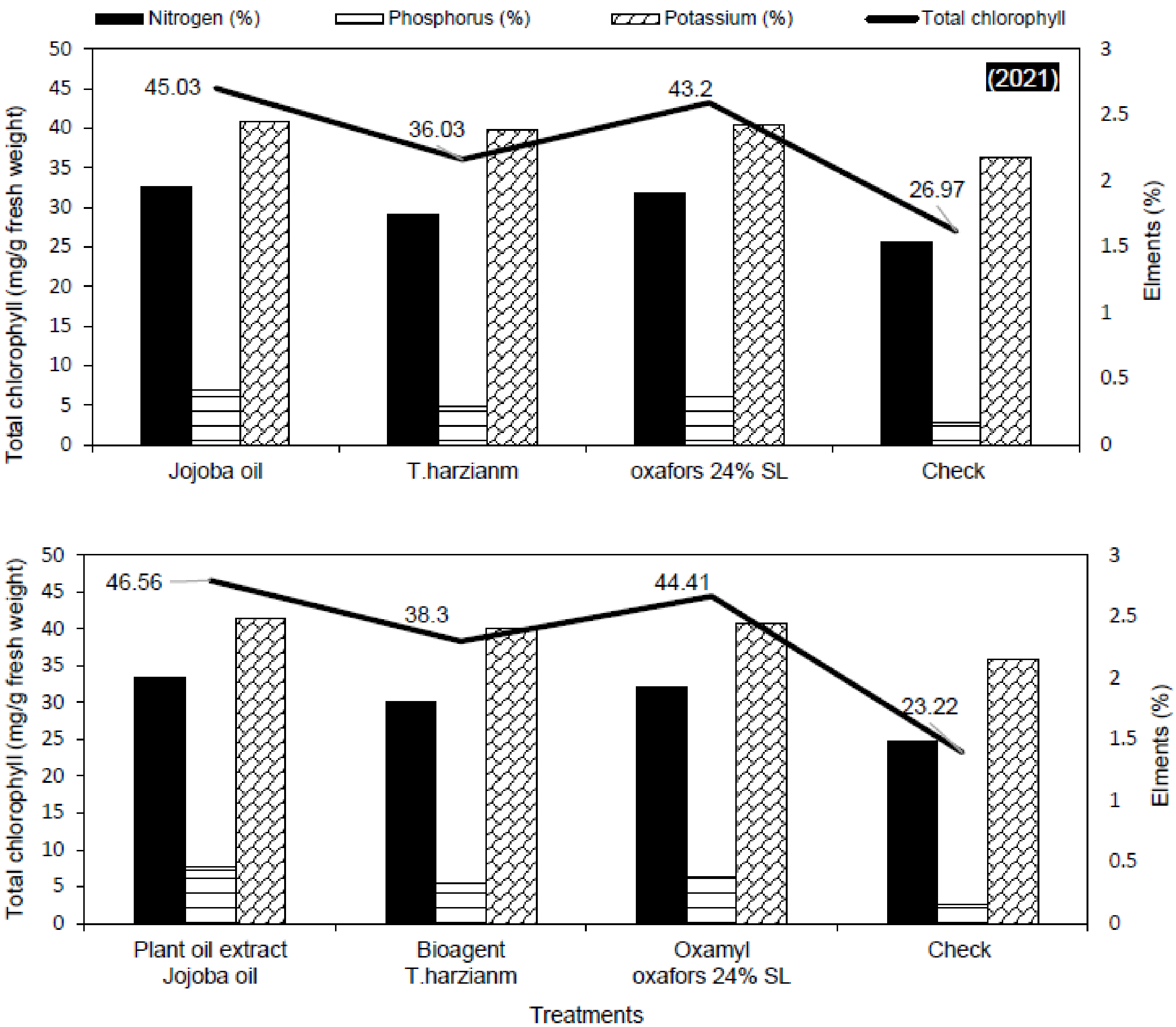

Fig. 2. The impact of tested bioagents and plant oil extracts compared to oxamyl on the chemical properties influencing the vegetative growth of Thompson Seedless grapevines during the 2021 and 2022 growing seasons

CONCLUSION

The results indicate that jojoba oil significantly impacted the control of the root-knot nematode *(M. incognita).* Jojoba oil demonstrated superior efficacy and safety compared to oxamyl (24% EC). Furthermore, jojoba oil treatment resulted in the best yield and fruit quality.

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الملخص العربي

طريقة صديقة للبيئة لمكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* التي تصيب العنب

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تهدف الدراسة الحالية إلى اختبار مواد صديقة للبيئة لمكافحة نيماتودا تعقد الجذور *Meloidogyne incognita*، التي تصيب أشجار العنب البناتى (الخالية من البذور) طومسون. حيث تم اختبار ثلاثة معاملات هي: العامل الحيوي (*Trichoderma harzianum*) ، ومستخلص الزيت النباتى (الجوجوبا)، مقارنة بمبيد نيماتودا متخصص أوكساميل (اوكسافورس ٢٤% S.L). وقد تضمن البحث تقييم تأثير المعاملات المختبرة على إجمالي التعداد الكلى للنيماتودا، ومعدل التراكم، وأعداد العمر اليرقى الثانى في التربة، وفي الجذور، وكذلك كتل البيض والعقد الجذرية، بالإضافة إلى محتوى الأوراق من صبغة الكلوروفيل، والنسب المئوية لمحتويات أوراق العنب من النيتروجين والفوسفور والبوتاسيوم على مدار موسمين متتاليين للنمو (٢٠٢١ و ٢٠٢٢) في ظل الظروف الحقلية.

أشارت النتائج إلى أن جميع المعاملات المستخدمة خلال موسمين التجارب قللت بشكل كبير من إجمالي تعداد نيماتودا تعقد الجذور ومعدل تعدادها التراكمى، في كل من التربة والجذور، خاصة بعد ثلاثة أشهر من التطبيق، حيث كان مبيد النيماتودا المتخصص أوكساميل هو الأكثر فعالية بين المواد المختبرة في تقليل إجمالي تعداد نيماتودا *M. incognita* ومعدل تعدادها التراكمى في كل من جذور العنب والتربة المحيطة بها حتى وقت الحصاد متبوعا بمستخلص الزيت النباتى (الجوجوبا)، ثم العامل الحيوي (*T. harzianm*) كان الأقل فعالية، كما كان الأوكساميل هو العلاج الأكثر فعالية الذي أثر بشكل كبير على النسبة المئوية لتعداد العمر اليرقى الثانى في التربة، يليه مستخلص الزيت النباتي (الجوجوبا) ثم العامل الحيوي (*T. harzianm*).

من ناحية أخرى، كان العامل الحيوي *T. harzianm* هو الأكثر فاعلية في عدد كتل البيض يليه الأوكساميل ثم زيت الجوجوبا النباتى. وكان استخدام مستخلص الزيت النباتي والأوكساميل متفوقا على العامل الحيوي في التأثير على النسب المئوية للعناصر في الأوراق وخاصة النيتروجين والبوتاسيوم. وقد تم الحصول على أعلى قيم للكلوروفيل الكلي في العنب المعامل بزيت الجوجوبا بينما تم تسجيل أقل القيم عند استخدام العوامل الحيوية مقارنة بالغير معامل (الكنترول).