

Nematicidal Potential of Clove Extract Compared to Oxamyl Against *Meloidogyne Incognita* *in Vitro* and *in Vivo*

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Abstract

Chemical nematicides are commonly used to control plant-parasitic nematodes, specifically *Meloidogyne* spp., but they have negative impacts on human health and the ecosystem. In response, nematologists are turning to botanicals for nematode control. In this study, fresh and uniform-sized egg-masses or 100 infective juveniles (J2s) of *Meloidogyne incognita* were exposed to ethanolic bud clove (*Syzygium aromaticum*) extract and the nematicide oxamyl *in vitro*. It was found that, the type of agents tested (clove and oxamyl), concentrations (250, 500, and 1000 µg/ml for clove extract and 0.6, 1.2, and 2.4 µg/ml for oxamyl), and exposure times (one, four, and seven days) were key factors affecting the number of emerged and dead juveniles. The concentrations of oxamyl were based on half the recommended rate (HRR), the recommended rate (RR), and double the recommended rate (DDR). Both clove and oxamyl significantly inhibited egg hatching and increased juvenile mortality of *M. incognita* compared to distilled water treatment. Oxamyl showed a relatively higher effect. At the highest concentrations of clove and oxamyl, the percentage of hatching inhibition after 1, 4, and 7 days was 87.89% (85.31 %), 72.47 % (82.70 %), and 61.73% (67.63 %), respectively. The corresponding values for juvenile mortality were 36.5 % (53.3 %), 52.4 % (70.9 %), and 62.2 % (99.0 %), indicating the effectiveness of both treatments in controlling *M. incognita*. In a greenhouse study, the effects of clove extract and oxamyl on *M. incognita*-infected cucumber plants were investigated. Both treatments were applied at the same concentrations as in the *in vitro* assay. Results showed that both clove extract and oxamyl significantly reduced nematode galling and reproduction compared to the control group, with oxamyl having the most pronounced effect. The control group exhibited the highest levels of galling and reproduction. Higher concentrations of both treatments demonstrated increased efficacy. The percentage reduction in galling and reproduction for clove extract at the three concentrations was 35.4% (87.52%), 41.47% (89.82%), and 47.54% (91.97%), respectively. For oxamyl treatments, the corresponding values were 77.31% (97.46%), 81.21% (97.93%), and 96.96% (99.74%), respectively. Furthermore, both clove extract and oxamyl treatments significantly improved the growth of cucumber plants, as evidenced by increased shoot length and fresh weight.

Keywords: Cucumber, *Meloidogyne* spp., control, clove extract, oxamyl, egg hatching and juvenile mortality.

1. Introduction

Cucumber (*Cucumis sativus* L.) is a widely cultivated vegetable crop due to its high nutritional value, including especially vitamins and minerals. In Egypt, it is grown extensively in newly reclaimed sandy areas as well as old clay soils with a total cultivated area of 19,672 and 20,403 hectares in 2021 and 2022, produced 491,650.0 and

484,424.7 tons, respectively (Faostat, 2023). Cucumber is attacked by many pests including insects, fungi, bacteria, viruses and nematodes. Plant-parasitic nematodes (PPNs) are responsible for about 10% losses in annual global crop yields, equivalent to an estimated US \$173 billion per year (Sasser, and Freckman (1987). On the other hand, root-knot nematodes (RKNs) *Meloidogyne* spp. are considered among the top five major plant pathogens and the first among the ten most important genera of plant-parasitic nematodes in the world (Kayani and Mukhtar, 2018). Root-knot nematodes are particularly damaging to vegetables, causing estimated losses of 50–80% (Sasser, 1979) and contributing to an estimated \$100 billion in losses per year globally (Oka *et al.*, 2000a). It considered to be the most important parasites of cucumber and caused yellow foliage, small slow growing fruits, poor yield, heavy root galling, root decay and reduced root system (Krishnaveni and Subramanian, 2005). In Egypt, *Meloidogyne* species became real threats for most vegetable crops and classified as limiting factors in crop production (Ibrahim *et al.*, 2010). To date about 100 species of *Meloidogyne* have been reported from different parts of the world (Karssen and Moens, 2006). Among these, the most destructive species is *M. incognita* (Kofoid and White) Chitwood, which causes serious damage in most agricultural crops worldwide (Moens *et al.*, 2009). *M. incognita* has a detrimental effect on the sustainable production of cucumbers, reducing plant growth and causing yield losses of 66.23% (Anjali *et al.*, 2022).

Control of such nematodes is difficult partly because they have high reproductive potential, sedentary endoparasites and therefore protected in plant tissues and have extensive host range. The nematicides have been applied widely to control plant parasitic nematodes with fast-acting and considerable results. However, they are both expensive and environmentally unfriendly method. Introducing a novel chemical active ingredient to the US and European markets estimated to exceed US\$ 250 million, marking a ten-fold increase compared to the costs observed during the 1960s (Sparks, 2013 and Desaegeer *et al.*, 2020). Reports have shown contamination of groundwater and various food types with nematicides, such as aldicarb residues exceeding reference doses in orange fruits at Sharkia governorate, Egypt (Tchounwou *et al.*, 2002). As a result, research on alternatives to chemical nematicides has received a strong impulse and considered a wide range of options including botanical nematicides. Since, a wide variety of plant species, representing 57 families have been shown to contain nematicidal compounds such as alkaloids, isothiocyanates, phenols, glycosides, thiophenols, tannins, saponins and fatty acids (Gommer, 1981; Chitwood, 2002; and Ntalli & Caboni, 2012). These plant extracts have no negative effect on non-target organisms, humans or animals and are environmentally friendly. Additionally, essential oils have been reported to have a nematicidal effect on root-knot nematodes (Gommers, 1981; Oka *et al.*, 2000b; Chitwood, 2002 and Djiwanti *et al.*, 2019). These natural alternatives provide promising options for controlling nematodes while minimizing environmental impact.

Several studies showed anthelmintic properties of clove, *Syzygium aromaticum* (L.) Merrill & Perry against some species of animal parasitic nematodes i.e., *Haemonchus contortus* (Ahmed and AL-jubori, 2020), *Cotylophoron cotylophorum* (Dhanraj and Veerakumari, 2015) and *Trichinella spiralis* (Fahmy *et al.*, 2020). On the other hand, it showed nematicidal effects in little studies conducted directly against second stage juveniles (J2s) and eggs of *M. incognita*. For instances, Ajith *et al.*, (2020) evaluated the nematicidal activity of essential oils derived from three plants, including *S. aromaticum*, against J2s of *M. graminicola*. The results showed high mortality of J2s in clove and lemongrass oils at 500 µg/ ml after 24 and 96 hours. Furthermore, the dried and unexpanded flower buds of the *S. aromaticum* plant, commercially known as cloves, have been found to be effective against *Radopholus similis* and *Meloidogyne* spp. in greenhouse bioassays on pepper plants. The nematode mortality rate from clove bud mulch was comparable to that of carbofuran, a synthetic pesticide. This suggests that clove bud application could be a promising botanical pesticide in the future (Wiratno *et al.*, 2009 and 2020). Therefore, this study aims to investigate the possible nematicidal efficiency of clove, *Syzygium aromaticum* (L.) Merrill & Perry, extract against eggs and J2s of the root knot nematode, *M. incognita* *in vitro*. Moreover, clove extract was tested *in vivo* on cucumber plants, *Cucumis sativus* L. infected with *M. incognita* under greenhouse conditions compared to the synthetic nematicide, oxamyl.

2. Materials and Methods

1-Culturing of the root knot nematode, *M.incognita* and preparation of nematode inoculum

A pure culture of *M. incognita*, was maintained in the greenhouse using the susceptible tomato cultivar Super Strain B as the inoculum source. A single egg-mass was used to establish the nematode population. Species identification was based on juvenile measurements and examination of perineal pattern system of adult females according to Jepson, (1987) and Eisenback & Triantaphyllou, (1991). Infected tomato roots were cut into 2-cm pieces and placed in a 600-ml flask containing 200 ml of 0.5% sodium hypochlorite solution (180 ml water + 20 ml Clorox). The flask was tightly capped and shaken for 3 minutes to partially dissolve the gelatinous matrix and free the eggs from egg-masses (Hussey and Barker, 1973). The liquid suspension of eggs was poured through a 200-mesh sieve nested upon a 500-mesh sieve. Eggs collected on the 500-mesh sieve were immediately washed free of residual sodium hypochlorite solution under a slow stream of tap water and incubated in Petri dishes at $25\pm3^{\circ}\text{C}$ until hatching. Newly hatched juveniles were collected using a micropipette.

2-Preparation concentrations of the utilized clove botanical extract and oxamyl

Buds of clove, *Syzygium aromaticum* (L.) Merrill & Perry, were purchased from a supermarket in Zagazig city, Egypt. The collected buds were cleaned and dried in an oven at 40°C , ground to a fine powder in a mill and stored in a cool place. The clove botanical extracts were prepared at the Biochemistry Lab. of Agricultural Biochemistry Department, in the Faculty of Agriculture, Zagazig University, Egypt. Ten grams of ground cloves were extracted using 100 ml of 80% ethanol with an ultrasonic device at 140 watts (40 KHz) for 30 minutes at 40°C . The extract was then filtered using Whatman No. 1 filter paper. The combined filtrate was evaporated in a rotary evaporator at 40°C and the residue was freeze-dried. The dried extract was weighed to calculate the yield and stored at -20°C until further use. Three concentrations of 250, 500, 1000 $\mu\text{g}/\text{ml}$ of clove extract were utilized in comparison to the chemical pesticide oxamyl (Vydate 24%EC), methyl 2- (dimethylamino)-N-[(methylcarbamoyl) oxy]-2-oxoethanimidothioate. Also three concentrations of oxamyl were used i.e., half of the recommended rate (HRR)= 1.5 L/600 L water / feddan= 0.6 $\mu\text{g}/\text{ml}$, recommended rate (RR) =3L/600 L water/feddan =1.2 $\mu\text{g}/\text{ml}$ and double recommended rate (DRR) = 6L/600L water /feddan=2.4 $\mu\text{g}/\text{ml}$. A commercial nematicide was obtained from the Central Laboratory of Pesticides, Dokki, Giza, Egypt.

3-Effect of clove extract and oxamyl on egg hatching and juvenile mortality of *M. incognita* in vitro

A-Effect on egg hatching

Five fresh and uniform size egg-masses of *M.incognita* were transferred to 9-cm diameter Petri dishes contained 10 ml of serial concentrations of 250, 500, and 1000 $\mu\text{g}/\text{ml}$ of the clove extract, compared to 0.6, 1.2 and 2.4 $\mu\text{g}/\text{ml}$ of oxamyl. Control treatment was prepared using distilled water. Each treatment was replicated five times. All treatments were left under room temperature $25\pm3^{\circ}\text{C}$. Numbers of hatched juveniles were counted after 1, 4 and 7 days of treatment using a research microscope (100X magnification). The following formula was used to determine the percentages of hatching suppression:

$$\text{E. I. (\%)} = \frac{\text{N. E. C.} - \text{N. E. T.}}{\text{N. E. C.}} \times 100$$

where:

E.I. % = Egg hatching inhibition % , N.E.C.= Number of eggs hatched in control, N.E.T.= Number of eggs hatched in tested treatment.

B-Effect on juvenile mortality

The suspension of second- stage juveniles (J2s) of *M.incognita* was adjusted to 1000 infective juveniles per ml. About 100 juveniles in 0.1 ml of the suspension was added to final volume of 10 ml of prepared concentrations (250, 500, and 1000 $\mu\text{g}/\text{ml}$ for clove extract and 0.6, 1.2 and 2.4 $\mu\text{g}/\text{ml}$ for oxamyl) in 9- cm diameter Petri dishes. Control treatment was done using only distilled water. All treatments were left under room temperature ($25\pm3^{\circ}\text{C}$) to assess the effect of these treatments on juvenile mortality. Each treatment was replicated five times. Juvenile mortality was entailed after 1, 4, and 7 days post treatment. According to Ishibashi and Takii (1993),

the juveniles were deemed dead when they had a static straight posture and remained immotile even after being prodded. The mortality percentages were calculated using the formula below.

$$\text{J2s M. \%} = \frac{\text{Dead J2s}}{\text{T. J2s}} \times 100$$

where:

J2s M. % = J2s mortality % , T. J2s = Total number of J2s.

Abbott's formula (1925) was used to adjust the percentage of deaths in treatments based on mortality under control.

4-In vivo nematocidal activity of three concentrations of clove extract compared to oxamyl on galling and reproduction of *Meloidogyne incognita* infecting cucumber under greenhouse conditions

Pot experiment was conducted in the greenhouse, Faculty of Agriculture, Zagazig University, Egypt on cucumber plants *Cucumis sativus* L., as a host plant. Seeds of cucumber (cultivar Mayar) were soaked in distilled water in Petri dishes and kept in an incubator at $28 \pm 1^\circ\text{C}$. After 48 hours, seeds were sown in formalin sterilized 20-cm diameter plastic pots filled with approximately 2000 g of sterilized sandy soil (71.3% sand, 11.2% clay, and 8.2% silt) mixed with 150 g of peat moss and 3 g of urea. Three weeks after sowing, seedlings were thinned to one plant per pot. Approximately 1000 second-stage juveniles (J2s) of *M. incognita* were introduced into pots of all treatments except the negative control treatment (healthy plants) using a micropipette. The J2s were placed near the fine roots of the tested seedling through three holes made at a depth of 5 cm using a pencil. Plants in the positive control group were only exposed to *M. incognita* J2s alone. Simultaneously, three concentrations of 250, 500, 1000 $\mu\text{g/ml}$ of clove extract were utilized in comparison to 0.6, 1.2 and 2.4 $\mu\text{g/ml}$ of the nematicide oxamyl. The top 5 cm of soil surrounding each cucumber plant was mixed with aliquots of 10 ml of each concentration. The temperature and relative humidity in the greenhouse were recorded at $27 \pm 5^\circ\text{C}$ and $65 \pm 5\%$, respectively. Each treatment was replicated five times. All pots were arranged in a randomized complete design in the greenhouse and received similar horticultural treatments.

Forty-five days after inoculation, plants were removed carefully from pots and data on plant growth as indicated by length and fresh weight of shoots were recorded. An aliquot sample of 100 g soil were processed for nematode extraction using a combination of sieving and Baermann trays technique (Hopper *et al.*, 2005). Roots were soaked in tape water for one hour to facilitate removing adhering soil and to keep egg-masses on root surface. During the steps of evaluation, roots were wrapped in tissue paper to prevent drying out. The nematode parameters, such as the number of galls and egg-masses per root system. Moreover, number of J2s in 100 g soil, were assessed. In addition, reproduction factor (RF) was calculated according to Oostenbrink (1968) as follows:

Reproduction factor (RF) = final juvenile population/initial juvenile population.

5-Statistical analysis

A completely randomized design was used in the experiments. All the data were subjected to one-way analysis of variance (ANOVA) using MSTAT VERSION 4 (1987). Means were compared by Duncan's multiple range test at $P \leq 0.05$ probability (Duncan 1955).

3. Results and Discussion

1-The *in vitro* effects of clove extract concentrations compared to oxamyl on egg hatching and juvenile mortality of *M. incognita*

A-Effect on egg hatching

In this study, the nematocidal effect of clove crude extract was compared to the recommended rate of oxamyl in terms of its ability to inhibit egg hatching and cause mortality in second-stage juveniles (J2s) of *M. incognita* after different exposure periods. The nematocidal effect of different concentrations of *S. aromaticum* extract and oxamyl on the number of emerged *M. incognita* J2s is presented in Table (1). It was found that, the type of tested agents (clove and oxamyl), concentrations (250, 500, and 1000 $\mu\text{g/ml}$ for clove extract and 0.6, 1.2 and

2.4 µg/ ml for oxamyl) and exposure times (one, four, and seven days) were the main factors affecting the number of emerged juveniles. The concentrations of clove and oxamyl significantly ($P \leq 0.05$) inhibited egg hatching of *M. incognita* at all exposure periods compared to the distilled water treatment. Oxamyl showed a relatively higher effect compared to clove extract at all the tested concentrations. Unfortunately, the low concentration of 250 µg/ml of clove extract and 0.6 µg/ml of oxamyl exhibited the least impact on egg hatching at various exposure times. For instance, numbers of emerged juveniles in treatments of clove and oxamyl one, four and seven days after treatment were 34.00 (17.20), 110.80% (65.80) and 123.40 (83.00) juveniles with percent inhibition of 66.26% (82.93%), 30.05% (58.45%) and 36.19% (57.08%) % respectively. The highest values were detected at the highest concentration with percent reduction of 87.89% (85.31%), 72.47% (82.70%) and 61.73% (67.63%) for clove and oxamyl, respectively. Generally, As the concentrations of the tested agents and time of exposure were increased, numbers of emerged juvenile were obviously decreased.

Table (1). Inhibitory effect of clove extract and oxamyl concentrations on *M. incognita* eggs hatching *in vitro*

Treatments	Mean number of juveniles emerged after			General mean
	One day	Four days	Seven days	
Control (Distilled water)	100.80a	158.40a	193.40a	150.86
Clove extract (250 µg/ ml)	34.00b	110.80b	123.40b	89.40
	(66.26)	(30.05)	(36.19)	
(500 µg/ ml)	27.00c	86.60c	108.60c	74.06
	(73.21)	(45.32)	(43.84)	
(1000 µg/ml)	12.20de	43.60e	74.00de	43.26
	(87.89)	(72.47)	(61.73)	
Oxamyl Half recommended rate (HRR), (0.6 µg/ml)	17.20d	65.80d	83.00d	55.33
	(82.93)	(58.45)	(57.08)	
Recommended rate (RR), (1.2 µg/ml)	16.60d	56.00e	69.80de	47.46
	(83.53)	(64.64)	(63.90)	
Double recommended rate (DRR), (2.4µg/ml)	14.80de	27.40f	62.60e	34.93
	(85.31)	(82.70)	(67.63)	
*Figures in parenthesis are percentages of egg hatching inhibition in comparison to control of distilled water				
**Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan's multiple range test.				

B-Effect on juvenile mortality

Mortality of *M. incognita* J2s *in vitro* treatments after exposure to three concentrations of clove extract compared to oxamyl was illustrated in Table (2). Entailed data after one, four and seven days post treatment of clove extract and oxamyl at the tested concentrations offered proper dead mortality effect against J2s of *M. incognita*. All concentrations of the tested agents showed significant ($P \leq 0.05$) effect on numbers of dead

juveniles compared to treatment of distilled water at the three periods post application. In another words, numbers of dead J2s at the tested periods were concentrations dependent. Treatment of oxamyl gave significantly higher effect compared to crude extract of clove at all the tested concentrations. One day after treatment, numbers of *M. incognita* dead juveniles in clove and oxamyl treatments at the lowest tested concentration were 54.40 (94.8), 60(95.6) and 73(06.6) with percent mortality of 27.20% (47.40%), 30.0%(47.80%) and 36.50%(53.30%), respectively. However, the highest effect was detected at seven days after treatment. Since, values of J2s percent mortality of clove and oxamyl treatments at the tested concentrations were 51.40% (88.70%), 52.40% (92.30%) and 62.20% (99.00%), respectively. Generally, oxamyl treatment exhibited significant ($P < 0.05$) potency effect surpassed clove extract at different times of exposure and concentrations. Moreover, results revealed that, the supplication of clove extract, was more effective against J2s at 1000 µg/ml and showed the best combating for the management of *M. incognita*.

Table 2. Effect of three concentrations of clove extract and oxamyl on mortality of *M. incognita* infective juveniles *in vitro*

Treatments	Numbers of dead juveniles after			General Mean
	One day	Four days	Seven days	
Control (Distilled water)	5.80j	7.20 j	9.40j	7.46
Clove extract (250 µg/ ml)	54.40 e	84.80e	102.80e	81.33
	(27.20)	(42.40)	(51.40)	
(500 µg/ ml)	60.00d	89.80e	104.80e	84.86
	(30.00)	(44.90)	(52.40)	
(1000 µg/ml)	73.00c	104.80d	124.40d	100.73
	(36.50)	(52.4)	(62.20)	
Oxamyl, Half recommended rate (HRR), (0.6 µg/ml)	94.80b	120.40c	177.40c	130.86
	(47.40)	(60.20)	(88.70)	
Recommended rate (RR), (1.2 µg/ml)	95.60b	130.80b	184.60b	137.00
	(47.80)	(65.40)	(92.30)	
Double recommended rate, (DDR) (2.4 /ml)	106.60a	141.80a	198.00a	148.80
	(53.30)	(70.90)	(99.00)	
*Figures in parenthesis are percentages of egg hatching inhibition in comparison with control of distilled water				
**Different letters in the same column indicate significant differences ($P \leq 0.05$) according to Duncan's multiple range test.				

2-Influence of clove extract compared to oxamyl on galling and reproduction of *M. incognita* infected cucumber plants under greenhouse conditions

The effect of clove extract applied at three concentrations, i.e., 250, 500 and 1000µl/ml, and the nematicide oxamyl applied at three concentrations, i.e., half of the recommended rate (HRR), the recommended rate (RR)

and double the recommended rate (DRR), on galling (as indicated by the number of galls per root system) and reproduction (as indicated by the R factor) of *M. incognita* infected cucumber plants was studied under greenhouse conditions. The data obtained indicated that clove extract and oxamyl significantly ($P < 0.05$) reduced nematode galling and reproduction compared to the control (Table 3). Higher concentrations led to a greater reduction in nematode parameters. Oxamyl treatments exhibited the greatest reduction in *M. incognita* parameters. The lowest effect was found in the oxamyl HRR treatment followed by the RR treatment while the DRR treatment gave a relatively higher effect. The numbers of galls and egg-masses per root system for oxamyl treatments were, 31.40 (34.20), 26.00 (28.60) and 4.20 (10.80), respectively. The corresponding values for the number of J2s in 100 g of soil and the R factor were 11.80 (0.206), 9.6 (0.168) and 1.20 (0.021) respectively. The percent reduction in galling (number of galls per root system) and nematode reproduction (R factor) in the lowest and the highest concentration of oxamyl were 77.31% (97.48%) and 96.96% (99.74%), respectively.

At the endpoint of the current study, the three concentrations of clove extract significantly ($P < 0.05$) reduced galling and reproduction of *incognita* infected cucumber plants. At 250 and 1000 $\mu\text{g/ml}$, the number of galls and egg-masses per root, J2s per 100 g of soil and R factor were 89.40(72.60), 52.20 (48.60), 58.00(37.33) and 1.015 (0.653) with percent reduction reaching 35.40% (47.54%), 51.93% (55.24%), 87.52% (91.97%) and 87.52% (91.97%), respectively compared to the control treatment. In the negative control, *M. incognita* scored maximum galling and reproduction parameters (138.40 galls and 108 egg-masses /root system, 465 J2s/100g of soil, 8137 final population (P_f) and 8.137 RF.

Application of clove extract and oxamyl at the tested concentrations significantly improved cucumber plant growth parameters as indicated by shoot fresh weight and shoot length. Treatments of oxamyl surpassed treatments of clove extract at the same concentrations. In most cases, the improvement in shoot length was more pronounced than that of fresh weight. Inoculating cucumber seedling with 1000 J2 of *M. incognita* reduced shoot fresh weight and shoot length by 39.10% and 44.96 % respectively. Percent increase in shoot fresh weight of cucumber plants treated with clove extract and oxamyl at the three concentrations were, 23.45%(37.80%), 34.52%(50.84%) and 37.34%(47.09%). While the corresponding values for shoot length were, 15.94%(47.09%), 31.27% (59.75%) and 39.45%(59.62%), respectively

Table 3. Effects of root cucumber application with 250 ,500, 1000 $\mu\text{g/ml}$ of clove extracts and 0.6, 1.2, 2.4 $\mu\text{g/ml}$ of oxamyl on galling and reproduction of *M. incognita* infecting cucumber after 45 days of inoculation under greenhouse conditions

Treatments	Shoot fresh weight (g) (% Increase)	Shoot length (cm)	Number of galls / root system (% Reduction)	Number of egg -masses / root system (% Reduction)	Number of J2S/100 g soil (% Reduction)	Reproduction factor (RF) (% Reduction)
Healthy cucumber plants (HCP)	38.42 a	68.72 a	0.00 f	0.00e	0.00f	0.00
Infected cucumber plants with J2s of <i>M.incognita</i> (ICP Mi)	23.78 f (38.10)	37.82 f (44.96)	138.40a	108.60a	465.00a	8.137
ICP Mi + 250 $\mu\text{g/ml}$ of clove extract	29.38e (23.54)	43.85e (15.94)	89.40b (35.40)	52.20b (51.93)	58.00b (87.52)	1.015 (87.52)
ICP Mi + 500 $\mu\text{g/ml}$ of clove extract	31.99 cd (34.52)	49.65 cd (31.27)	81.00b (41.47)	51.20b (52.85)	47.33bc (89.82)	0.828 (89.82)
ICP Mi + 1000 $\mu\text{g/ml}$ of clove extract	32.66cd (37.34)	52.74 cd (39.45)	72.60bc (47.54)	48.60bc (55.24)	37.33cd (91.97)	0.653 (91.97)

ICP <i>Mi</i> + HRR of oxamyl, (0.6 µg/ml)	32.77c (37.80)	55.63 c (47.09)	31.40 d (77.31)	34.20d (68.50)	11.80 d (97.46)	0.206 (97.48)
ICP <i>Mi</i> + RR of oxamyl,(1.2 µg/ml)	35.87 b (50.84)	60.42 b (59.75)	26.00e (81.21)	28.60 d (73.66)	9.60e (97.93)	0.168 (97.93)
ICP <i>Mi</i> + DRR of oxamyl, (2.4 µg/ml)	34.98 b (47.09)	60.37 b (59.62)	4.20 f (96.96)	10.80 de (90.05)	1.20ef (99.74)	0.021 (99.74)
*Means followed by the same letter(s) within a column are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test **Figures in parenthesis are percentages of increase or reduction in comparison with ICP <i>Mi</i> treatment. ***Means in the same column followed by same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.						
Reduction (%) = $\frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$				Increase (%) = $\frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$		

4. Discussion

Historically, conventional nematicides have been used to control plant parasitic nematodes but with the growing popularity of organic farming and environmentally friendly practices, researchers are exploring non-chemical control methods that include using plant-derived materials. Chemical fumigation, especially with methyl bromide (MeBr), has been widely used in agriculture for many years. However, the global ban on the production and use of MeBr by the Montreal Protocol in 2015 has necessitated the development of safe and effective alternatives to maintain agricultural production (Ajwa *et al.*, 2003). The potential of applying botanicals in management of root-knot nematodes has been reported by many authors (Goswami & Vijayalakshmi, 1986; Akhtar & Mahmood, 1994 and Asif *et al.*, 2013). The potential use essential oils in controlling root-knot nematodes *Meloidogyne* spp. has been recommended by many authors (Ajith *et al.*, 2020; Catani *et al.*, 2023 and Sarri *et al.*, 2024). Among plants that possess essential oils, *Syzygium aromaticum* (Family Myrtaceae) commonly known as clove is the second most valuable spice in world trade. Eugenol is the major compound present along with saponins, alkaloids, flavonoids, glycosides, tannins and steroids. Clove exhibits various biological activities including antibacterial, antifungal, herbicidal, nematicidal, antitumor and anti-inflammatory properties (Kaur & Kaushal, 2019 and Batiha *et al.*, 2020). Moreover, both clove oil and clove extract exhibit strong antimicrobial activity, with clove extract being more potent at lower concentrations than clove oil (Chinelo and Omebere, 2024). On the other hand, rarely studies have been conducted on clove extract as a novel nematicide tool in the management of plant parasitic nematode, particularly *Meloidogyne* spp. Therefore, we tested its effects compared to oxamyl on *M. incognita* *in vitro* and *in vivo*.

The three concentrations of clove extract and oxamyl treatments reduced *M. incognita* egg hatching and increased J2s mortality by about 50% relative to the untreated control at extended time intervals of 7 days after application with 500 and 1000 µg/ml. This result support findings of authors who tested the effect of clove oil *in vitro*. For instance, clove oil showed nematotoxicity to J2 of *Anguina tritici*, *Tylenchulus semipenetrans*, *Meloidogyne javanica* and *Heterodera cajani* (Sangwan, et al. 1990). Als, the concentration of clove oil that resulted in 50% mortality (LC50) of J2 of *M. javanica* was 413 µgmL⁻¹, while the highest LD50 value was 1040 µgmL⁻¹ for *H. cajani*. Extracts from clove also caused mortality in *Xiphinema americanum*, *Longidorus* sp., *Hoplolaimus indicus*, *Pratylenchus* sp. and *Helicotylenchus indicus* when nematodes were exposed to 50, 75 and 100% concentrations (Pandey and Dwivedi 2000). Additionally, clove oil showed strong dose-response effects against *M. incognita* eggs and J2s. The mean effective clove oil concentration that reduced egg hatch by 50% (EC50) was 0.097% (v/v) clove oil; the EC50 for J2 viability was 0.145% clove oil (compared with carrier control treatments). Volatiles from 5.0% clove oil reduced nematode egg hatch in water by 30%, and decreased

viability of the hatched J2 by as much as 100% (Meyer *et al.*, 2008a). In laboratory assays extracts from tobacco (*Nicotiana tabacum* L), clove (*S. aromaticum* L), betel vine (*Piper betle* L), and sweet flag (*Acorus calamus* L) were most effective in killing the nematode, with an EC50 that was 5-10 times lower than the EC50 of the synthetic pesticides chlorpyrifos, carbosulfan and deltamethrin (Wiratno *et al.*, 2009). the clove oil extract stopped the embryogenesis of eggs and showed a complete nematicidal action against second-stage juveniles both free and of the egg masses. These properties make this product an interesting tool for a novel control strategy in nematode pest management (Carletti *et al.*, 2011).

Under greenhouse conditions, the obtained results indicated that clove extract and oxamyl significantly ($P \leq 0.05$) reduced galling and reproduction of *M. incognita* compared to the control. Higher concentrations led to a greater reduction in nematode parameters. Both treatments improved the growth of cucumber plants, as evidenced by increased shoot length and fresh weight. This result is in agreement with the findings of Meyer *et al.* (2008b) who reported the nematicidal suppression of *M. incognita* infected certain vegetable crops including cucumber when seedlings were treated with clove oil at concentrations ranging from 0.1 to 0.3% without phytotoxicity in greenhouse Wiratno *et al.*, (2019) tested clove bud and betelvine as mulch against *Radopholus similis* and *Meloidogyne* spp. in pepper plants in a greenhouse bioassay. The number of live nematodes treated with clove bud mulch did not differ significantly from that of plants treated with carbofuran (recommended synthetic pesticide).

Eugenol, is a major constituent of clove oil (*S. aromaticum*, Myrtaceae), and other plants belonging to the Lamiaceae family. In an *in vitro* study complete inhibition of egg hatch was observed at a concentration of 1.25 mg mL⁻¹, and at a lower concentration of 0.20 mg mL⁻¹, more than 50% mortality of *M. incognita* J2 juveniles was recorded within 24 h of immersion (Kaur *et al.*, 2018). Ajith *et al.*, (2020) evaluated the nematicidal activity of essential oils and their major isolated compounds against J2 of *M. graminicola* to determine whether the nematicidal activity of the oils is due to their major compounds or not. Among which, clove oil and its major compound eugenol were found to possess potent nematicidal activity against J2 stage of *M. graminicola* and could be used to develop environmentally friendly nematicidal product of plant origin for the management of this nematode in rice. On the other hand, under greenhouse conditions, Bala and Sukul (1987), showed that eugenol as a major constituent of clove effectively reduced *M. incognita* infection of *Hibiscus esculentus* and promoted plant growth at a concentration of 0.2 ml/l. Generally, the effect of essential oils including clove on nematodes is through a range of mechanisms, such as disrupting their nervous systems, inducing harmful effects on plasma membrane permeability, penetrating the gelatinous matrix of nematode eggs, and damaging intracellular redox status (Kalaiselvi *et al.*, 2019, Dutta *et al.*, 2021 and Goyal *et al.*, 2021). Since several essential oils have shown nematicidal and insecticidal activity and acetylcholine is a neurotransmitter in nematodes, it may be suppose that they are involved in interrupting the nematode nervous system (Oka, 2001). Additionally, El-Maati *et al.*, 2016 indicated that clove extract had higher phenolic content with stronger antibacterial capacity and ethanol extract damaged the morphology and membranes of tested bacterial cells.

5. Conclusions

Clove, (*S. aromaticum*) extract exhibited strong nematicidal activity *in vitro* by inhibiting egg hatching and causing high mortality of *M. incognita* J2. Moreover, the study demonstrated that the application of clove extract and oxamyl significantly reduced galling and reproduction of *M. incognita* on cucumber plants in greenhouse conditions. This approach shows promise for controlling root-knot nematodes, specifically *M. incognita*. Future research should focus on identifying the active chemicals responsible for the nematicidal effects and elucidating their mode of action.

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