

APPLICATION OF NANO ESSENTIAL OILS ON FUSARIUM ROOT ROT DISEASE ON CUCUMBER IN GREENHOUSE

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Abstract: Fusarium root rot disease of cucumber plant is the most epidemic fungal diseases causing significantly losses of plants during growing in greenhouses. Crude and nanoemulsion formulations of some essential oils of clove, black seed, lemon and orange at (5000 ppm) were tested against mycelial linear growth of root rot fungi, *Fusarium oxysporum* (MG018778) and *Fusarium solani* (MG018781), count of conidia and pathological activity on cucumber germinated seeds and seedlings. Essential oils nanoemulsions of clove / black seed (2:1) at (5000 ppm) was the best formulation, significantly reduced mycelia linear growth of both fungal isolates, completely suppress production of conidia spores, reduced seed rotten and seedling mortality of the *Fusarium* fungus was not isolated from cucumber plants with root rot disease. Application of nanoemulsion formulation of clove / black seeds (2:1) by the rate 1% as a soil drench of cucumber plants before and after cultivation in plastic greenhouses were significantly reduced *Fusarium* root rot caused by *Fusarium oxysporum* and *Fusarium solani* on cucumber plants under natural infestation than the systemic fungicide (Topsin M-70). No phytotoxicity of nanoemulsion formulation on cucumber plants as soil amendment by 200 mL/ plant up to (2.5%). So, results indicate that nanoemulsion formulations may be use as eco-friendly alternative fungicides for controlling root rot and wilt diseases causing by soil borne pathogenic fungi of *Fusarium* spp. on cucumber plants.

Key words: cucumber; *Fusarium*; nanoemulsion; oils; management.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important economic vegetable crop cultivation worldwide, significantly losses in total cultivated plants, productivity of fruit yield in quality and quantity, regarding to incidence wilt and root rot diseases caused by several pathogenic soil borne fungi, such as *Fusarium* spp., *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Sclerotinia* spp. [6, 7, 32, 35]. Most fungal diseases are management by synthetic fungicides, but the extensive use of these chemicals could lead to residues in edible plant parts, resistant strains and environmental pollution. Essential oils (EOs) a secondary products of higher plants were extraction from various plant parts by different methods from medical and aromatic plants, it was used in traditional medicine, food and medical on humans, animals and in plants as green pesticides alternatives synthetic pesticides [9, 25, 34]. Thus, the application of safe and eco-friendly antifungal agents, such as plant based on essential oils, is highly encouraged. The antifungal effect of essential oils against pathogenic fungi maybe attributed to the ability of these oils to induce morphological changes in the cell wall and interfere with enzymatic reactions during wall synthesis, thereby affecting fungal growth [5, 21, 23, 26, 27, 36]. Clove essential oil could be used as an antifungal agent against pathogenic fungi responsible against postharvest decay of grape fruit caused by fungi of *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis viticola* and *Rhizopus stolonifer* [29] and banana fruit, such as *Colletotrichum musae* [5, 10, 34, 36], powdery mildew of cucumber

[24]. In addition, several soil borne plant diseases caused by pathogenic bacteria, fungi and nematodes were controlling by crude of essential oils [33]. GC-MS analysis shows that the major components of clove essential oil include eugenol (~83%) [24, 31], while the major components of black cumin seed essential oil were thymoquinone (42.4%), *p*-cymene (14.1%), carvacrol (10.3%), longifolene (6.1%), and 4-terpineol (5.1%) [20], essential oil of lemongrass (*Cymbopogon citratus* (DC) Stapf.) contains geraniol (39.0%), nerol (29.4%), myrcene (18.0%), and limonene (10%) citral is the major component of lemongrass oil possessing antifungal activity against *Colletotrichum musae*, *Lasiodiplodia theobromae* and *Fusarium proliferatum* in banana fruit [19] and *Botrytis cinerea* and *Penicillium* sp. in apple fruit [1].

Nanoparticles (NPs) have been applied to control several plant diseases of integrated pest management, these eco-friendly agents are synthesized via different methods to protect plants against pathogens, such as, bacteria, fungi, viruses and nematodes [16, 17]. Nano-silver liquid, has been used to control wilt disease of oak caused by *Raffaelea quercivora* [18]. Nanoemulsions of eugenol was used against wilt disease of cotton caused by *F. oxysporum* f. sp. *vasinfectum* [2] and seed rot, damping off and root rot diseases of cucumber plants caused by seed borne fungi of *F. oxysporum*, *Aspergillus niger* and *A. alternata* [35]. ZnO NPs (22 and 19 nm) are highly effective against pathogenic fungi and bacteria of *Pseudomonas aeruginosa* and *Aspergillus flavus* respectively [13], silver nanoparticles (15 nm) showed highly antifungal against pathogenic fungi of *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *B.*

cinerea and *Curvularia lunata* [13]. Nanoformulations of silver/chitosan was highly effective used against seed borne fungi of chickpea *R. solani*, *Aspergillus flavus* and *A. alternata* [15]. In our previous study, nanoemulsion formulations of some essential oils i.e., clove, black seed, lemon and orange were prepared using ultrasonic emulsification, these nanoemulsions presented as small droplets, particularly, excellent fungicidal activity was noted with (2:1) nanoemulsion formulation of clove + black seed oils against growth of *Galactomyces candidum*, *Alternaria tenuissima* and *F. solani*, were inducing postharvest rots in cucumber fruits [21] and against *Botrytis cinerea* the causal of gray mould of cucumber fruits [32].

Thus, the present study aimed to evaluate the fungicidal activity of clove, black seed, lemon, and orange nanoemulsion formulations and their application for controlling root rot disease incidence, and yield of cucumber plants in greenhouse conditions.

MATERIALS AND METHODS

Fungal isolates

Two pathogenic fungal isolates with accession numbers of *Fusarium oxysporum* (MG018778) and *Fusarium solani* (MG018781) root rot causal of cucumber were kindly provided of Plant Pathology Department, National Research Centre, Egypt which isolated and identified in previous work [3].

Fungicide used

Topsin M-70% W.P fungicide (Thiophanate) Diethyl4-4(O-phenylene) bis 3-thiolllophanate, manufactured by Nippa Soda, Japan.

Essential oils used

Essential oils of clove, black seed, lemon and orange were kindly provided by El-Kaptain Company, Al-Obour City, Cairo, Egypt. In addition, two types of black seeds oil were extracted which exported from Turkey and Ethiopia, GC/MS analysis in previous research, indicated that of clove and black seed oils, the major chemical components of clove oil were eugenol (73.55%), β -caryophyllene (15.02%), (-) caryophyllene oxide (4.16%), caryophyllene oxide (2.08%), α -humulene (1.17%), eugenyl acetate (1.03%) and caryophyllene (0.33%). Meanwhile the major constituents of black seed oil were p-cymene (25.89 %), thymoquinone (19.71 %), citronellal (10.21%), 9,12-octadecadienoic acid (8.94%), α -thujene (7.52%), trans-4- methoxy thujane (4.81.66%), isocaryophyllene (2.42%), β -pinene (2.02 %) and α -pinene (1.52%) [22].

Nanoemulsions

Nanoemulsions of clove, black seed, lemon and orange oils were preparation, characterization, formulation with droplet sizes 82.6, 95.9, 131.9, and 117.4 nm, respectively was described in previous work [22].

Effect of nanoemulsions on fungal growth and pathological activity

Effect on mycelial growth

Different crude and nanoemulsions of oils were prepared at 5000 ppm (0.5%), applied alone or in combination against mycelial linear growth against two isolates of pathogenic fungi, *F. oxysporum* (MG018778) and *F. solani* (MG018781) on (PDA) medium. The plates were incubated at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 5 days. Three plates were used as replicates and three plates without treatment were served as a control. The diameter of mycelium on each plate was measured and the average was calculated [22]. Percentage of fungal reduction was calculated as follows:

$$\text{Reduction \%} = [(A-B)/A] \times 100$$

where as: A=diameter of the hyphal growth of the control, B=diameter of hyphal growth of the treated isolate.

Effect on conidial count

Conidia count of each fungal isolates of *F. oxysporum* (MG018778) and *F. solani* (MG018781), in different treatments were determined using a hemacytometer slide. The average number of spores was calculated per square centimeter area of fungal mycelial growth.

Effect on pathogenic activity

Colonies of fungal isolates *F. oxysporum* (MG018778) and *F. solani* (MG018781) grown before under treatments of nanoemulsion formulations were used. Cucumber seeds (Cv. Baracoda) were germinated on wet filter paper and placed on fungal mycelial growth 10 days old in Petri dishes. Ten seeds were sown in each plate and incubated at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 7 days. Three plates were used as replicates for each concentration, and three plates without the emulsion formulations served as the control. Symptoms of root rot disease on development cucumber seedlings were visually examined [35].

Phytotoxicity of nanoemulsions formulations on cucumber plants

Different nanoemulsion formulation concentrations of, clove + black seed oils at the ratio (2:1) respectively at (0.0, 2.5%, 5.0% and 10.0%) were tested as a soil drench for cucumber plants (Cv. Baracoda) 30 days old in a greenhouse. The formulation was applied at the rate, 50 mL/plant. Two week after application plants were view the syndromes on cucumber plants for phytotoxicity observation.

Application of nanoemulsion formulations on root rot disease incidence on cucumber plants

Treatments

Nanoemulsion formulation of clove + black seed oils (2:1) was applied to cucumber (Cv. Baracoda) by the rate 200 mL/tray included 96 seedlings of cucumber by each 0.5% and 1% as a soil drench of transplantings (15 days old), 2 days before cultivation in greenhouse and second treatment 30 days after their cultivation in greenhouse field. The treatments were applied by the rate 200 mL/plant of both concentrations (0.5% and 1.0%). Treatments were distributed in a

randomized block design during the 2018–2019 growing season in protective plastic greenhouses at the Agriculture Research Center, Dokki, Egypt, the size of plastic greenhouses is (9 × 25 M), with drip irrigation at 50 cm intervals between each two plants, five rows with distance 100 (cm). Each treatment with five rows as replicates (60 cm × 5 M) and five rows were served as a control as follows:

0 – Control;

1 - Soil drench of transplanting with nano emulsion formulation (0.5%);

2 - Soil drench of transplanting with nano emulsion formulation (1.0%);

3 - Soil drench of transplanting and plants in field with nano emulsion formulation (0.5%);

4 - Soil drench of transplanting and plants in field with nano emulsion formulation (1.0%);

5 - Soil drench of transplanting with (Topin M-70) fungicide (0.5%);

6 - Soil drench of transplanting and plants in field with (Topin M-70) fungicide (0.5%).

Root rot disease assessment

Percentage of wilt and root rot on cucumber plants was demonstrating 5 months after cultivation. Disease severity was determined in shoots by the linear scale (0-4) as a grades of wilt syndromes on leaves, 5 months after cultivation i.e., 0 = healthy, 1 = yellowish and 1/3 plant wilted, 2 = 2/3 plant wilted, 3= whole plant wilted, 4 = dead [32].

$$\text{Disease severity} = [\Sigma (n \times r) / N] \times 100$$

where: n = number of plants in each numerical disease grade; r = number of the disease grade; N = total number of plants multiplied by the maximum numerical disease grade

Morphological characteristics and yield of cucumber plants

Plant height, fresh shoot weight, number of buds, total number of cucumber fruits, and total yield (kg) were estimated 5 months after cultivation.

Statistical analysis

Data were statistically analyzed by analysis of variance, and means were compared using least-significant differences at $P = 0.05$ according to the procedures outlined by [28].

RESULTS

Effect of essential oils and nanoemulsions on fungi growth

The effect of nanoemulsions of clove, black seed (three types), lemon, and orange and their crude oils (5000 ppm) on the growth of *F. solani* and *F. oxysporum* were evaluated. The data in (Table 1) indicate that all prepared nanoemulsions and their crude materials were significantly reduce the mycelial growth of fungal isolates. Nanoemulsions of black seed, clove and lemon from Egypt, followed by their crude materials, showed the best effects and significantly reduced mycelial growth of tested fungal isolates. Nanoemulsions of clove and black seed revealed excellent effects against *F. oxysporum* and *F. solani*, respectively. Nanoemulsions and crude essential oils of black seed from Turkey and Ethiopia revealed the least effect against mycelial growth of tested fungi.

Effect of nanoemulsions formulations on mycelial growth of fungi

The effect of nanoemulsion formulations of clove, black seeds essential oils by the rate (5000 ppm) on mycelial growth of *F. oxysporum* (MG018778) and *F. solani* (MG018781) were tested. Data in (Table 2) indicate that all individual and nanoemulsion formulations significantly reduced mycelial growth of fungal isolates compared the control. Nanoemulsion formulations of clove / black seed, followed by the formulation of clove alone, reduced growth of *Fusarium oxysporum* and *Fusarium solani* as shown in (Figure 1). In addition, data in (Table 2) indicate that nanoemulsion formulation of clove, black seed oils, and their formulations were significantly reduce mycelial growth and conidial production by fungal isolates. Moreover, nanoemulsion formulation of clove / black seed oils (2:1) was highly greatest reduction of mycelial growth and completely suppress (100%) conidia spores of both fungal isolates followed by the formulation (1:2).

Table 1. Screening of some crude and nanoemulsions of oils on fungi causing root rot of cucumber

Treatment	5000 ppm	% Reduction of mycelial growth (5 days)	
		<i>F. oxysporum</i>	<i>F. solani</i>
Control	Check	0.0 a	0.0 a
Clove (Egypt)	Crude	26.3 h	28.9 f
	Nano	40.2 j	42.2 i
Black seed (Turkey)	Crude	7.8 d	12.8 d
	Nano	10.6 g	20.0 e
Black seed (Egypt)	Crude	26.3 hi	29.4 f
	Nano	32.9 k	53.3 g
Black seed (Ethiopia)	Crude	2.2 b	3.2 b
	Nano	2.2 b	7.2 b
Lemon (Egypt)	Crude	35.8 j	30.6 h
	Nano	43.6 j	41.1 j
Orange (Egypt)	Crude	7.0 f	16.7 c
	Nano	5.8 e	15.0 d

The same letters in each column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Table 2. Effect of essential oils nanoemulsions on mycelial linear growth and conidia count of fungi causing root rot of cucumber

Nanoemulsion (5000 ppm)	% Reduction of mycelial growth (5 days at 27±2°C)		Conidia count 1x10 ⁵ /cm ²	
	<i>F. oxysporum</i>	<i>F. solani</i>	<i>F. oxysporum</i>	<i>F. solani</i>
Control	00.0a	00.0a	5.5d	6.8 e
Clove	41.7 c	36.3d	2.0b	2.0 b
Black seed	31.9 b	39.7c	2.5c	4.0 d
Clove + Black seed (1:1)	47.2d	32.4f	2.0b	2.0 b
Clove + Black seed (1:2)	42.0 c	34.6c	2.2 cb	2.3 c
Clove + Black seed (2:1)	47.9 d	53.1f	0.0 a	0.0 a

The same letters in each column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test

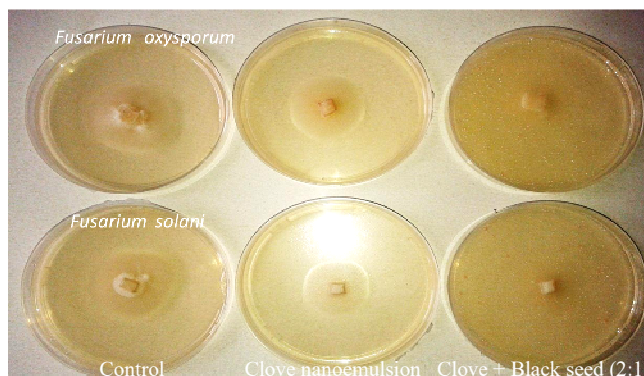


Figure 1. Effect of clove nanoemulsion and clove / black seed essential oils formulation, on mycelial growth of *Fusarium* spp.

Effect of oils nanoemulsion formulations on pathogenic potential on fungi

The pathological potential of *F. oxysporum* (MG018778) and *F. solani* (MG018781) was tested after treatment with different nanoemulsions formulations of clove and black seed essential oils (5000 ppm). Data in Table 3 indicate that the pathological potential of fungal isolates on the cucumber germinated seeds and their developed to seedlings remarkably decreased. In general, all

nanoemulsions formulation significantly reduced percentage of seed rotten and seedling mortality of cucumber compared with the control. Treatment with nanoemulsions formulations of clove alone and clove / black seed revealed the greatest inhibition of pathological potential (100%) of *F. oxysporum*. As shown in Figure 2, nanoemulsion formulations of clove / black seed (2:1) was the best formulation, significantly reduced the rot of germinated seeds and seedling mortality in cucumber.

Table 3. Effect of nanoemulsions on seed rot and seedling mortality of cucumber

Nanoemulsion (5000ppm)	% Seed rot and seedling mortality of cucumber	
	<i>F. oxysporum</i>	<i>F. solani</i>
Control	80.0 a	60.0 a
Clove	50.0 c	30.0 b
Black seed	70.0 b	30.0 b
Clove + Black seed (1:1)	40.0 d	10.0 d
Clove + Black seed (1:2)	40.0 d	30.0 b
Clove + Black seed (2:1)	20.0 e	20.0 c

Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.



Figure 2. Effect of nanoformulations of clove and black seed essential oils on pathological activity of fungi causing *Fusarium* root rot of cucumber plants

Phytotoxicity of oils nanoemulsion formulation on cucumber plants

The phytotoxicity effect of different concentrations of the (2:1) nanoemulsions formulation of clove / black seed respectively on grown cucumber plants following soil drench by 200 mL/plant was tested for 15 days after treatment Figure 3, indicate that no phytotoxicity of cucumber plant drenched their soil by 200 mL/plant at the rate 2.5%, of formulation, while the slight chlorosis was reported with application by the rate (5%), meanwhile, the clear toxicity was observed by the rate used at 10% as chlorosis, yellowish, necrotic spots in leaf edges and stunting growth and on apical meristem.

Application of oils nanoemulsion formulation on *Fusarium* root rot disease incidence and growth of cucumber plants

Soil drenched by nanoemulsion formulation of clove / black seed essential oils (2:1) at the rates (0.5 and 1%) compare systemic fungicide (Topsin M-70) (0.5%) as recommended dose. Soil drench of transplantings 2

days before their cultivation and 30 days after cultivation in greenhouse field. Data in Table 4 indicated that all treatments were reduced root rot disease incidence on cucumber plants five months after cultivation under natural greenhouse infestation as shown in Figure 4. In addition, data in Table 5 revealed that treatment with the nanoemulsions formulation, clove / black seed essential oils (2:1) as soil drench of transplantings in combination with soil drench in field by the rate (1.0%) of both treatments, were enhanced percentage of survival plants, morphological characteristics, increasing shoot length, fresh shoot weight, number of buds, numbers of cucumber fruits/plants, and total yield of cucumber fruits.

DISCUSSION

Root rot disease of cucumber plants, caused by several soil borne pathogenic fungi, of *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Sclerotinia* spp. [6, 7, 32, 35]. In this study,



Figure 3. Phytotoxicity of nanoemulsion formulation of clove / black seed essential oils, soil drench of cucumber plants

Table 4. Effect of application oils nanoemulsion formula on *Fusarium* root rot incidence on cucumber plants under natural infestation in plastic greenhouses

Root rot disease incidence			Soil drench		
Disease severity*	% infection	Survival plant %	Treatments	Rate	Materials
3.7 a	70.0 a	65.0 d	T		
2.6 b	45.0 b	80.0 b	T + S	0.5 %	Nanoemulsion
1.0 f	30.0 e	80.0 b	T		Clove / black seed oils (2:1)
2.4 c	42.5 c	92.0 a	T + S	1.0%	
1.5 e	25.0 f	90.0 a	T		
2.0 d	46.0 b	75.0 c	T + S	0.5 %	Topsin M -70
1.6 e	37.5 d	80.0 b	----	0.0	Control

T = transplanting soil drench in 2 days before cultivation, S = soil drench of plants 30 days after cultivation in greenhouse field

Disease severity was determined in shoots by using the following scale: 0 = healthy, 1 = yellowish + 1/3 plant wilted, 2 = 2/3 plant wilted, 3 = whole plant wilted, 4 = dead plant. The same letters in each column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Table 5. Effect of oils nanoemulsion formulation treatments on growth characters of Fusarium root rot incidence on cucumber plants under natural infestation in plastic greenhouses

Morphological characters of cucumber plant				Soil drench		
Kg	fruit/plant	Plant		Treatments	Rate	Materials
No	weight fresh (g)	length (cm)				
110.0d	14 f	40.0 f	110.0d	T		
135.0b	17 d	64.3 d	135.0b	T + S	0.5 %	Nanoemulsion
150.0a	28 a	99.9 b	150.0a	T		Clove / black seed oils (2:1)
106.0e	25 b	109.3 a	106.0e	T + S	1.0%	
150.0a	24 b	108.5 a	150.0a	T		Topsin M -70
120.0c	15 e	51.6 e	120.0c	T + S	0.5 %	
120.0c	20 c	74.8 c	120.0c	----	0.0	Control

T = transplanting soil drench in y2 days before cultivation, S = soil drench of plants 30 days after cultivation in greenhouse field.

The same letters in each column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test



Figure 4. Effect of nanoemulsion of clove/black seed oils on Fusarium root rot on cucumber plants under natural field infestation by pathogens

nanoemulsions of clove, black seed, and lemon essential oils were significantly than crude materials reduced mycelial growth of tested fungal isolates. Nanoemulsions of each clove and black seed essential oils were high significant effects against mycelial growth of *F. oxysporum* and *F. solani* respectively. In addition, nanoemulsion formulation with equal ratio (1:1) of clove + black seed essential oils then nanoemulsion formulation of clove oil only were highly significantly reduced growth of *F. oxysporum*, meanwhile, nanoemulsion formulation of black seed oil was the best effect against mycelial growth of *F. solani*. Moreover, nanoemulsion formulation of clove / black seed oils (2:1) was highly greatest reduction mycelial growth and completely suppress (100%) conidia spores production of both fungal isolates and greatest inhibition of pathological potential of both fungal isolates on the germinated seeds and seedlings of cucumber, in this manner, in previous study, this formulation of clove / black seed at 5000 ppm by the ratio (2:1) respectively was highly reduced mycelial growth and conidial spore production of several fungi caused spoilage of cucumber fruit [22]. The antifungal activity of black cumin seed essential oil attributed to thymoquinone (42.4%), as the major and high bioactive antimicrobial components of clove essential oil eugenol (83%) is the common antifungal component agent against pathogenic fungi causing postharvest decay of grapefruit [24, 29, 31]. In addition, two main constituents of lemongrass are geraniol (39.0%), nerol (29.4%), showed the antifungal activity against several fungi causing postharvest

spoilage on banana fruit [19], *Botrytis cinerea* and *Penicillium* sp. on apple fruit [1]. The biological mechanisms of eugenol and thymoquinone are the main and effective constituents in clove and black seed essential oils against mycelia growth and conidial sporulation of *Fusarium* spp. [2, 22, 37], in addition, other essential oils are causing, several morphological, physiological and ultrastructures on the cells of several fungi were reported such as *B. cinerea*, *Candida* spp. and *Aspergillus niger* were including malformation of hyphae growth, lysis of cell wall components, increasing permeability of plasma membrane, deterioration membrane of mitochondrial, coagulation of cytoplasm and increase vacuolation size in cells, death cells, moreover, formation of irregular ultrastructure of cytoplasmic membrane and nucleus [4, 11, 12, 27, 30]. No phytotoxicity was observed on cucumber plants grown in oil drenched with 2.5% of nanoemulsion formulation of clove / black seed essential oils (2:1), meanwhile, slight chlorosis was observed at the rate 5% and clear toxicity, including yellowing of the cucumber leaves, was noted at 10%. These results agree with previous findings indicating that low concentrations of eugenol nanoemulsion was no phytotoxicity to cotton seedlings [2]. So, application nanoemulsion formulation at 1% of clove / black seed essential oils (2:1) as a soil drench of transplantings before cultivation and plants grown in a greenhouse 30 days after cultivation remarkably reduced root rot disease incidence and enhanced survival plants, morphological characteristics, such as plant height, fresh weight and fruit yield. These results are

agreements with application of nano-silver is one of the best nanoparticles was used against wilt disease of oak caused by *Raffaelea quercivora* [18], root rot of cucumber seedlings [33], white rot of green onion caused by *Sclerotium cepivorum* [35]. In this respect, nanoemulsion formulation of eugenol is the main active component of clove essential oil and has antifungal effect against a wide range of fungal genera [8, 14], in pot experiments nanoemulsion of eugenol oil was protect different genotypes of cotton against *Fusarium* wilt incidence caused by *Fusarium oxysporum* f. sp. *vasinfectum* [29]. Application of nanoemulsion formulation of clove and lemongrass oils as seed soaking and soil amendment on seedlings of tomato significantly reduced *Fusarium* wilt disease of tomato plants caused by *F. oxysporum* f. sp. *lycopersici* [14, 26]. Recently, the same nanoemulsions formulations of clove / black seed essential oils were controlling the incidence of postharvest spoilage fungal diseases of cucumber fruits [22], and gray mould of cucumber fruit caused by fungal of *Botrytis cinerea* under greenhouse conditions [33]. EOs, nanoemulsions of are the promising eco-friendly biological agents, in integrated pest management its low residues, low phytotoxicity than traditional pesticides. So, the nanoemulsion formulation of clove / black seed oil (2:1) as oil drench may be used as eco-friendly and promising alternative fungicides to control *Fusarium* root rot disease and enhance fruit yield of cucumber plants grown in greenhouses.

Conflict of interest: There is no actual or potential conflict of interest in relation to this article.

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