RESEARCH Open Access



Evaluation of essential oils nanoemulsions formulations on *Botrytis cinerea* growth, pathology and grey mould incidence on cucumber fruits

El-Sayed H. E. Ziedan^{1*}, Moataza M. Saad², Ahmed A. El-Kafrawy³, Ahmed F. Sahab¹ and Abdel-Tawab H. Mossa⁴

Abstract

Background: Grey mould caused by *Botrytis cinerea* of cucumber immature fruits is the most epidemic fungal diseases causing significantly losses of fruits during development on cucumber plant in winter season.

Material and methods: Crude and nanoemulsions of clove, black seed, lemon and orange essential oils at (5000 ppm) and also different formulations of clove, black seed and lemon oils nanoemulsions as single or in combination were tested against mycelial linear growth of *B. cinerea* (MF996363) as well sclerotial formation and pathological activity on the development of germinated seeds of cucumber.

Results: Essential oils nanoemulsion formulation of clove + black seed (2:1) at 5000 ppm was the best formulation significantly reduced mycelia linear growth of *B. cinerea* by 61.0% followed by 28% formulation (1:1) and (1:2). In addition, nanoemulsions formulations of clove, black seed essential oils (2:1) were highly reduced the count of sclerotial formation of *B. cinerea* and completely (100%) suppress seed rot and seedling mortality of cucumber followed by nanoemulsion of clove only.

Conclusions: Foliar application of nanoemulsion formulation of clove + black seeds is promising than fungicides (Topsin M-70) in controlling grey mould on cucumber fruits caused by *B. cinerea* in plastic greenhouse with no phytotoxicity on cucumber plants.

Keywords: Cucumber, *Botrytis cinerea*, Grey mould, Nanoemulsion, Oils

Background

Cucumber fruit is an important vegetable source high antioxidants, vitamins and mineral for human fresh food, industrial food and medical products (Kumar et al. 2010). Immature fruit of cucumber during growth is attacking by several air-borne fungi genera which causes significantly losses of fruit yield quantity and quality such as

grey mould cucumber of fruit through growing under greenhouses caused by *B. cinerea* (Soliman et al. 2015), and mature cucumber fruit also is attacking by fungi causing spoilage of *Galactomyces candidium*, *Geotrichumsp., Alternaria tenusima, Plerospora alli, Fusarium fujikuroi, F. verticiolides, F. solani, F. geraminearium* and *F. incarnatum* (Ziedan et al. 2018a, b). Due to hazard effects caused by synthetic chemicals fungicides in edible plant parts, resistant strains and environmental pollution, essential oils (EOs) are the safe eco-friendly secondary metabolites of higher plants containing 60, bioactive components of terpenes, alcohol, phenols, aldehydes and

¹ Plant Pathology Department, National Research Centre (NRC), P.O. 12622, 33 El Bohouth Street (Former El Tahrir St.), Dokki, Cairo, Egypt Full list of author information is available at the end of the article



^{*}Correspondence: ziedanehe@yahoo.com

esters (Bakkali et al. 2008) which are compatible with biotic and abiotic agents in controlling different foliar, wilt and root rot diseases, as well as postharvest fungal decay (Abbasi et al. 2003; Kadoglidou et al. 2020; Ziedan et al. 2020).

Clove, orange and lemongrass oils as soil amendment were controlling Fusarium wilt of tomato caused by F. oxysporum f.sp. lycopersici (Ben-Jaber et al. 2015; Selim et al. 2020; Kadoglidu et al. 2020) and Fusarium wilt of chickpea caused by F. oxysporum f.sp. cicer (Moutassem et al. 2019), clove as soaking treatment was to control postharvest decay fungi of grape (Sukatta et al. 2008), fumigation to control spoilage of peach fruits after harvest (Ziedan and Farrag 2008), postharvest rot diseases of banana fruit caused by Colletotrichum musae, Lasiodiplodia theobromae and Fusarium proliferatum (Hossain and Igbal 2016; Zoeir et al. 2017; El-Zahaby et al. 2018). Black seed oil is highly effective against mycelial growth and conidia sporulation of Fusarium spp. and Botryiodiplodia theobromae and significantly reduced root rot incidence of grapevine plants by the rate 4% as soil drench before cultivation transplantings (Ziedan et al. 2020).

The antifungal activity of essential oils was attributed to morphological changes in the cell wall and interference in enzymatic reactions of cell wall synthesis, which affect fungal growth (Sharma and Tripathi 2008). In this manner, eugenol is a phenolic compound which mainly constitutes (83%) highly antifungal activity in clove essential oil (Tabassum and Vidyasagar 2013; Xing et al. 2012; Moghaddam and Mehdizadeh 2016); meanwhile, the most antifungal components of the black cumin seeds essential oil were thymoquinone (42.4%), p-cymene (14.1%), carvacrol (10.3%), longifolene (6.1%), 4-terpineol (5.1%) (Mahmoudvand et al. 2014). In this respect, the application of nanoparticles becomes important for controlling plant diseases by application in soil, on seeds or plant shoot to protect plants against pathogens of bacteria, fungi, and viruses (Khan et al. 2012). Nanoparticles of silver are one of the best nanoparticles which were used against fungal plant diseases of cereals, viz. spot blotch of wheat caused by Bipolaris sorokiniana, rice blast disease caused by Magnaporthe grisea (Jo et al. 2009), and white rot of green onion caused by Sclerotium cepivorum (Jung et al. 2010). Moreover, nanoparticles of (ZnO NPs) were more effective for controlling postharvest diseases caused by pathogenic fungi of B. cinerea and Penicillium expansum and A. flavus (Krishnaraj et al. 2012; Jayaseelan et al. 2012), silica–silver nanoparticles were completely (100%) suppressive powdery mildew diseases of cucurbits, under field conditions (Park et al. 2006), and nanoformulations of nanosize silver/chitosan are more effective than silver or chitosan nanoparticles against various seed-borne fungal plant pathogens, Rhizoctonia solani, Aspergillus flavus and Alternaria alternata of chickpea (Kaur et al. 2012). Recently, essential oils emulsion of clove and lemongrass were suppressed the growth the causal wilt of tomato caused by *E. oxysporum* f.sp. lycopersici (Sharma et al. 2018) and nanoemulsions formulations of clove and black seed essential oils were application as soaking treatment of cucumber fruit for management of postharvest spoilage caused by Galactomyces candidium, A. tenusima and E. solani (Mossa et al. 2021). This investigation aimed to evaluate the nanoemulsions formulations of some essential oils against fungi attacking fruit during growing season of cucumber in producing greenhouse.

Methods

Essential oils

Essential oils of clove, black seed, lemon and orange were kindly obtained from El- Captain Company, Al-Aobour City, Cairo, Egypt, in comparison with extractions of another two types of black seed essential oil from seeds exported from Turkey and Ethiopia (Mossa et al. 2021).

Essential oil nanoemulsions formulations

Clove, black seed, lemon and orange oils nanoemulsions which provided from El- Captain Company, Al-Aobour City, Cairo, Egypt, were determined, and their droplet size was 82.6, 95.9, 131.9 and 117.4 nm, respectively, which were formulated at 10% concentration using Tween 80 under ultrasonic emulsification. Nanoemulsions stability, characterization and physicochemical studies were described in my previous work (Mossa et al. 2021).

Pathogenic fungal isolate of B. cinerea

Highly pathogenic fungal isolate of *B. cinerea* (MF996363) causing grey mould (blight) was isolated in pure culture from diseased young and immature fruits, showing grey mould (blight) symptoms of yellowish to brownish and dark brown discoloration of cucumber during growing season in greenhouses and identified according to morphological, cultural and molecular biology in previous work (Ziedan et al. 2018a, b).

Effect of oil nanoemulsion on growth of *B. cinerea* and pathological activity

Different nanoemulsion formulations and their crude of essential oils, *i.e.* clove, black seed, lemon and orange, were tested on mycelia linear growth (cm) of *B. cinerea* (MF996363) on plates of potato dextrose agar medium (PDA). Plates were incubated for 4 days at $27\pm2~^{\circ}\text{C}$. Three plates were used as replicates for each treatment, and ten plates free treatment was served as a control. Linear growth (cm) of each plate was measured and the

average diameters were calculated and percentage of fungal reduction was calculated according to formula as follows:

Reduction of mycelial $\% = (A - B/A) \times 100$.

A = diameter of the control hyphal growth; B = diameter of the treated hyphal growth.

Sclerotia count

Sclerotia of *B. cinerea* were counted of petri dish 9 cm in diameter, and their size was calculated 10 days after incubated at 27 ± 2 °C.

Pathogenic potential of B. cinerea

At the end of experiment as mentioned before, colonies of fungal growth produced under nanoemulsion formulations were used at 5000 ppm. Cucumber germinated seeds (cv. Golden) on wetted filter paper were put in Petri dishes for two days and set on fungal mycelium growth for 10 days at 27 ± 2 °C for 7 days. Three plates were used as replicates for each concentration, and ten plates free treatment was served as a control. Rotten syndromes of cucumber germinated seed and mortality of developmental seedlings were visually examined 7 days after incubation according to Mossa et al. 2021.

Phytotoxicity of nanoemulsion formulations on cucumber plants

Different rates of the promising nanoemulsion formulations of clove + black (2:1) oils at different concentrations at 0, 1, 2 and 4% were tested on cucumber plants 30 days old (cv. Golden) by foliar spray 2 times with 1-week intervals. The observation of cucumber plants was recorded such as chlorosis, yellowish, malformation and spotted colour less on leaves of cucumber plants (Abd-Elsalam and Khohlov 2015).

Application of nanoemulsion formulations for management grey mould of cucumber fruits

Nanoemulsion formulation of clove + black (2:1) oils by the rate 1 0.0% as foliar application in comparison with systemic fungicide of Topsin M- 70% W.P. (thiophanate) diethyl 4–4 (O-phenylene) bis 3-thiollophanate was manufactured by Nippa Soda, Japan. Treatments were distributed in randomized block design on cucumber plants 3–4 months old, under natural infestation by causal pathogen during growing season 2018–2019 in protective plastic greenhouses in Kafr El Abida village, El Mehalla El Koberea, Gharbeia Governorate, 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 60 (cm). Each treatment with five rows as

replicates (60 cm \times 5 M) and five rows were served as a control as follows:

- 1- Control
- 2- Nanoemulsion formulation of clove + black (2:1) oils by the rate 1 0.0%
- 3- Fungicide treatment of TopsinM-75% by the rate 0.5% as recommended dose.

Assessment of grey mould disease of cucumber fruits

Incidence of grey mould of cucumber young fruits during growing season was determined 7 and 15 days after foliar application as percentage of diseased plants, percentage of diseased fruit and diseases severity on cucumber fruits on the linear scale (0–5) adopted before in previous work (Ziedan et al. 2018a, b) as follows.

0 = no symptoms, 1 = yellowish 50% of fruit, 2 = yellowish 100% of fruit, 3 = grey mould 50% of fruit, 4 = grey mould 100% of fruit and 5 = soften 100% of fruit.

Statistical analysis

Data obtained were statistical analysis using Duncan's multiple range test according to Snedecor and Cocharn 1980.

Results

Grey mould symptoms on immature fruits of cucumber fruits are shown in Fig. 1 as yellowish of fruits developed to brownish, then rotten grey mould and then dried caused by *B. cinerea* fungal.

Effect of some essential oils and nanoemulsions on mycelial growth of *B. cinerea*

Highly aggressive isolate of *B. cinerea* (MF996363) causing fruit blight on cucumber fruits was tested their growth on agar medium amended with nanoemulsions of some essential oil and their crude at (5000 ppm) of clove, black seed (three types), lemon and orange. Data in Table 1 indicated that all perpetrated nanoemulsion and crude of essential oils tested reduced mycelial linear growth of fungal isolate tested than the control with no significances differences between all nanoemulsions and their crude of essential oil tested against mycelial linear growth *B. cinerea*.

Effect of nanoemulsion formulations of some oil on mycelial growth and sclerotia of *B. cinerea*

Nanoemulsions formulations of clove, black seed and lemon essential oils provided from El- Captain Company, Al-Aobour City, Cairo, Egypt, were tested in combination at 5000 ppm on mycelial linear growth of *B.cinerea*. Data in Table 2 indicated that nanoemulsions of clove, black





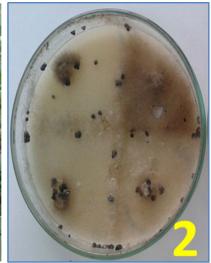


Fig. 1 Cucumber healthy fruits (0), *Botrytis* grey mould disease symptoms cucumber fruits showing yellowish, brownish and death on fruit still attached on cucumber plants fruits (1) and (2) culture of causal fungal pathogen (*B. cinerea*)

Table 1 Effect of some essential oils and their nanoemulsion on growth of *B. cinerea*

Essential oil nanoemulsions (5000 ppm)		Mycelial growth of <i>B. cinerea</i> (4 days at 27 ± 2 °C)		
		Growth (cm)	reduction%	
Control	Non	8.9 a	00.0	
Clove (Egypt)	Crud	b 7.3	17.9	
	Nano	7.3 b	17.9	
Black seed (Turky)	Crud	7.3 b	17.9	
	Nano	b 7.3	17.9	
Black seed (Egypt)	Crud	7.3 b	17.9	
	Nano	7.3 b	17.9	
Black seed (Ethiopia)	Crud	7.3 b	17.9	
	Nano	8.9 a	0.00	
Lemon (Egypt)	Crud	7.3 b	17.9	
	Nano	7.3 b	17.9	
Orange (Egypt)	Crud	8.9 a	0.00	
	Nano	7.3 b	17.9	

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

seed and lemon were significantly reduced mycelial linear growth of $B.\ cinerea$, and formulations by the equal rates of clove+black seed or clove+lemon followed by black seed+lemon were significantly reduced mycelial linear growth of $B.\ cinerea$ than individual nanoemulsions formulations. Clove nanoemulsion in combined with nanoemulsions of black seed+lemon was moderate effective against mycelial linear growth of $B.\ cinerea$.

Table 2 Effect of nanoemulsion formulations of oil on mycelial growth of *B. cinerea*

Nanoemulsion (5000 ppm) of essential oil	Mycelial growth of <i>B. cinerea</i> (4 days at 27 \pm 2 °C)			
	Growth (cm)	% Reduction		
Control	8.9 a	00.0		
Clove	7.3 b	17.9		
Black	7.3 b	17.9		
Lemon	7.3b	17.9		
Clove + Black seed (1:1)	6.4 c	28.0		
Clove + Lemon (1:1)	6.4 c	28.0		
Black + Lemon (1:1)	7.3 b	17.9		
Clove + Black seed + Lemon	7.2 b	19.1		

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

In addition, data in Table 3 indicated that nanoemulsions formulations of clove+black seed oils were significantly reduced mycelial linear growth and the count of sclerotia formation of *B. cinerea* than in the control of fungal free treatment. Formulation of clove+black seed nanoemulsion (2:1) was highly reduced mycelial linear growth and the count of sclerotia of fungal isolate tested as well reduction in the size of sclerotia as shown in Fig. 2) than same formulation of clove+black seed oils with different per cents of 1:1 and 1: 2.

Table 3 Effect of nanoemulsions formulations of clove and black seed oils on mycelial growth and scleroial count of *B. cinerea*

Nanoemulsion (5000 ppm)	Effect of nanoemulsions of clove and black seed essential oils on <i>B. cinerea</i> $(27\pm2^{\circ}\text{C})$				
	% reduction of mycelial growth	Sclerotia (10 days)/plate			
	(4 days)	Count	Size		
Control	00. d	63.0 a	+++		
Clove	17.9 с	39.0 d	++		
Black seed	17.9 d	54.0 b	++		
Clove + Black seed (1:1)	28.0 b	50.0 c	++		
Clove + Black seed (1:2)	28.0 b	66.0 d	++		
Clove + Black seed (2:1)	61.1 a	35.0 e	+		

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

- +++ Large size of sclerotia
- ++ moderate size of sclerotia
- + Little size of sclerotia

Effect of nanoemulsions formulations of clove and black seed oils on seed rot and seedling mortality of cucumber caused by *B. cinerea*

Data in Table 4 and Fig. 3 indicated that all individual and in combinations between nanoemulsions of clove and black seed essential oils were significantly reduced seed rotten and seedling mortality of cucumber of fungi tested after treatment by different nanoemulsions formulation

than its free treatments (control). In general, combined treatment of clove+black seed nanoemulsions significantly than individual against pathological action of *B. cinerea*, followed by clove nanoemulsion alone. Treatment of clove+black seed nanoemulsions at 2:1, respectively, was the best and significantly reduced seed rotten and seedling mortality under stress by fungal isolate tested.

Phytotoxicity of nanoemulsion formulations of oils on cucumber plants

Foliar application of nanoemulsion formulation of clove+black seed essential oils as shown in Fig. 4 was recorded slight syndromes toxicity of cucumber leaves and was observed at 4% as chlorosis, yellowish, malformation wilt and death which decreased by decreasing concentration at 2%, with no toxicity at lower concentration (1%.).

Application of nanoemulsion formulations on grey mould of cucumber fruits

Different nanoemulsion formulations of some essential oil clove, black seed compare the fungicides (Topsin M-70) as shown in Table 5 application of nanoemulsion formulations, and fungicides were reduced fruit blight on young cucumber fruits caused by *B. cinerea* under natural artificial infestation in plastic greenhouses 7 and 15 days after treatments by nanoemulsion formulation of clove + black seed oil (2:1) Meanwhile, another different

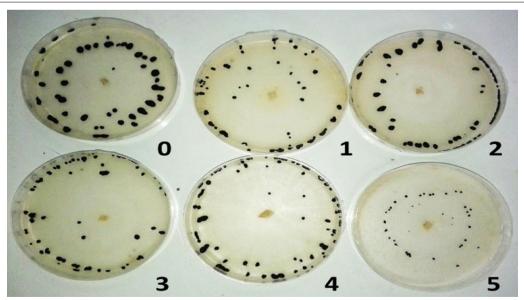


Fig. 2 Effect of nanoemulsions formulations (5000 ppm) on sclerotia formation of *B. cinerea* 10 days at 27 ± 2 °C. The control fungal free treatment (0), treatment with nanoemulsions of clove (1), black seed (2), nanoemulsions formulations of clove + black seed (1:1), (1:2) and (2:1) were (3), (4) and (5), respectively

Table 4 Effect of nanoemulsions formulations of clove and black seed oils on seed rot and seedling mortality of cucumber caused by *B. cinerea*

Nanoemulsion (5000 ppm)	% Seed rot and seedling mortality of cucumber by <i>Botrytis cinerea</i>		
Control	80.0 a		
Clove	30.0 d		
Black seed	50.0 c		
Clove + Black seed (1:1)	40.0 b		
Clove + Black seed (1:2)	40.0 b		
Clove + Black seed (2:1)	20.0 e		

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

treatments tested moderately reduced fruit blight of cucumber at 15 days.

Discussion

The epidemic distribution of grey mould of cucumber fruit during growing in winter season in plastic greenhouse is caused by the fungal *B.cinerea* causing high losses of developmental cucumber fruits (Yunis et al. 1990; An and Ma 2005–2006; Al-Sadi et al. 2011; Soliman et al. 2015; Elad et al. 2016; Ziedan et al. 2018a, b). In vitro, crude oils of clove, black seed, lemon, orange and single nanoemulsions formulations were tested at (5000 ppm) against mycelia linear growth of fungal isolate of *B. cinerea* (MF996363), with no significant of crude and nanoemulsions of essential oils, while there was slight significant difference in nanoemulsions formulations of clove, black seed and lemon by the equal rate. Meanwhile, nanoemulsions formulations of essential oils of clove+black seed (2:1), respectively, at 5000 ppm

were the best formulations significantly reduced mycelia linear growth of *B. cinerea* followed by the formulation (1:1) and (1:2). In addition, nanoemulsions formulations of (2:1) were highly reduced the count of sclerotial formation and suppress seed rot and seedling mortality of cucumber followed by nanaoemulsion of clove only, and these results are agreement with the evaluation of the same formulation against several fungi causing postharvest spoilage of cucumber fruits (Mossa et al. 2021).

The biological activity of EOs against fungi, bacteria and weeds make by different chemical, physical and biological properties due to the multiple chemical components. The most mode actions of EOs are due to bioactive volatile components towards cells of microorganisms and weeds by suppressing cell wall synthesis enzymes of β-glucans and chitin, increasing permeability in plasma membrane (Viuda-Martos et al. 2007), EOs highly penetrating cell wall, causing dissolvation of chemical components, damaging of cytoplasm and mitochondrial membranes, and death cell bacteria and fungi (Hua et al. 2017; Lagrouh et al. 2017; Bouyaha et al. 2019); in this manner, oregano and fennel EOs cause malformation of fungal mycelia morphology, coagulations of cytoplasm, lysis, and change the shape of sclerotia formation by Sclerotinia sclerotiorum (Soylu et al. 2007). Eugenol strongly reduced mycelial linear growth of the fungus Botrytis cinerea by negative effect on plasma membrane structure, malformation of hyphae morphology, accumulation of cytoplasm and increasing the cell vacuoles (Wang et al. 2010), at the same direction, thymoguinone is a common bioactive constitute in black cumin essential oil had harmful effect on spore germination, germ tube elongation, mycelial growth, cell membrane of Candida spp., (TEM) examination recorded ultrastructure irregular

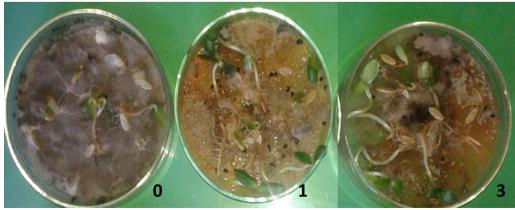


Fig. 3 Effect of nanoemulsion formulations (5000 ppm) of essential on pathogenic activity of *B. cinerea* on seed rot and seedling of cucumber 10 days at 27 ± 2 °C. Fungal free treatment (0), fungal treatment with nanoemulsion of clove (1) and fungal treatment with nanoemulsions (2:1) of clove + black seed oils (3)



Fig. 4 Phytotoxicity test of nanoemulsion formulation of clove + black seed essential oil (2:1), respectively, 0, 1, 2 and 4% foliar application of cucumber plants

Table 5 Effect of nanoemulsion formulation on grey mould of cucumber fruit under greenhouse conditions

Treatment	Grey mould incidence of cucumber (days) after treatment					
	7			15		
	% Diseased plant Diseased fruit		% Diseased plant	Diseased fruit		
		%	D.S		%	D.S
Clove + black seed (2:1) at 1%	20.0 b	2.7 b	0.5 c	28.6 b	13.3 b	2.0 c
Topsin (fungicide) at 0.5%	8.3 c	1.2 c	1.0 b	16.7 c	5.8 c	2.8 b
Control	31.5 a	5.0 a	2.6 a	73.3 a	18.5 a	3.5 a

D. S = disease severity: 0 = No, 1 = yellowish 1/2 fruit, 2 = yellowish hole fruit, 3 = grey mould 50% of fruit, 4 = grey mould of fruit 100% and 5 = fruit completely rotten Values in each column followed by the same letter are not significantly different at $P \le 0.05$ according to Duncan's multiple range test

of cytoplasmic membrane, separation of cell membrane than cell wall, disruption of cytoplasm and irregular nucleus shape were observed (Iscan et al. 2016).

EOs are low residues, toxicity and high compatibility with a wide range of beneficial microorganisms and are useful in managing plant pests and diseases caused

by biotic and abiotic agents (Irshad et al. 2018; Ziedan et al. 2020). The slight toxicity was observed on cucumber plant after application of the rate 2 and 4% of nanoemulsion formulation of clove + black seed essential oils (2:1) at (5000 ppm), while no phytotoxicity was observed of foliage cucumber plant at 1%. These results that are agreement with result reported to the phytotoxic effect of neem oil were chlorosis and stunted by the rate more than 0.5% (Abbasi et al. 2003) and up to 3% of lemon essential oil on leaves and roots on corn plant (Hollingsworth 2005). Eugenol nanoemulsion had no phytotoxicity at low concentrations on cotton seedlings (Abd-Elsalam and Khokhlov 2015) and on maize plants by nanoemulsion formulations of carvacrol and linalool up to 2.5 mg/ ml (Campos et al. 2018). In addition, essential oils of thyme and cotton lavender were lower phytotoxicity to major cereal and vegetable crops (Benchaa et al. 2019).

In greenhouses, application of nanoemulsion formulation of clove + black seed oils (2:1) compared to fungicides of Topsin M-70 was reduced fruit blight on young cucumber fruits caused by B.cinerea under natural artificial infestation in plastic greenhouse 7 and 15 days after treatments. These results are agreement with nanosilver which is one of the best nanoparticles used against white rot of green onion caused by Sclerotium cepivorum (Jung et al. 2010) and wilt disease of oak caused by Raffaelea quercivorus (Kim et al. 2011). Moreover, nanoparticles of (ZnO NPs) were more effective for controlling postharvest caused by pathogenic fungi, B.cinerea, P.expansum and A. flavus (Krishnaraj et al. 2012; Jayaseelan et al. 2012). Recently, the same nanoemulsions formulations of clove and black seed essential oils were controlling the incidence of postharvest spoilage fungal diseases of cucumber fruits (Mossa et al. 2021). So, nanotechnology tools have been promising eco-friendly agents, cheap cost, more effective to control plant diseases and enhance quality and quantity.

Conclusions

In plastic greenhouse, foliar application of cucumber plants with 7- and 15-day intervals, during growing season by 1% of nanoemulsion formulation of clove + black seeds oils (2:1), was reduced grey mould of young cucumber fruits caused by *B. cinerea* under natural artificial infestation than compared with the application of fungicide Topsin M-70. So, nanoemulsion formulation considered as eco-friendly agents of environmental resources will be considered in programme as alternative fungicides for controlling plant diseases.

Abbreviations

EOs: Essential oils; D.S: Disease severity; PDA: Potato dextrose agar; F. solani: Fusarium solani; B. cinerea: Botrytis cinerea; A. tenusima: Alternaria tenusima.

Acknowledgements

The authors extend their appreciation to the National Research Centre, Egypt for funding this work through research Project No. P11030135.

Authors' contributions

ZE contributed to conceptualization, investigation, experimental and submission to journal; MS contributed to vivo experiment, editing and submit manuscript to publishing; AA reviewed and supervised the study; SA contributed to statistical analysis; MA achieved experiment in vitro and writing draft. All the authors read and approved the final version of the manuscript.

Funding

Funding this work through research Project No. P11030135.

Availability of data and materials

The data sets generated and/or analysed during the current study are included in this published manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Plant Pathology Department, National Research Centre (NRC), P.O. 12622, 33 El Bohouth Street (Former El Tahrir St.), Dokki, Cairo, Egypt. ²Microbial Chemistry Department, National Research Centre (NRC), P.O. 12622, 33 El Bohouth Street (Former El Tahrir St.), Dokki, Cairo, Egypt. ³Plant Pathology Department, Plant Pathology Institute, Agriculture Research Centre, Giza, Cairo, Egypt. ⁴Pesticide Chemistry Department, National Research Centre (NRC), 33 El Bohouth Street (Former El Tahrir St.), P.O. 12622, Dokki, Cairo, Egypt.

Received: 8 February 2022 Accepted: 14 March 2022 Published online: 01 April 2022

References

- Abbasi PA, Cuppels DA, Lazarovits G (2003) Effect of foliar applications of neem oil and fish emulsion on bacterial spot and yield of tomatoes and peppers. Can J Plant Pathol 25:41–48
- Abd-Elsalam KA, Khokhlov AR (2015) Eugenol oil nanoemulsion: antifungal activity against *Fusarium oxysporum* f. sp. *vasinfectum* and phytotoxicity on cotton seeds. Appl Naosci 5:255–265
- Al-Sadi AM, Al-Said FA, Al-Kaabi SM, Mohammed S, Al-quraini SM, Al-Mazroui SS, Al-Mahmooli IH, Deadman LD (2011) Occurrence, characterization and management of fruit rot of immature cucumbers under greenhouse conditions in Oman. Phytopathol Mediterr 50:421–429
- An RP, Ma Q (2005–2006) Control of cucumber grey mold by endophytic bacteria. Cucurbit Genetics Cooperative Report 28–29, pp 1–6
- Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46:446–475
- Benchaa S, Hazzit M, Zermane N, Abdelkrim H (2019) Chemical composition and herbicidal activity of essential oils from two *Labiatae* species from Algeria. J Essent Oil Res 31:335–346
- Ben-Jabeur M, Ghabri E, Myriam M, Hamada W (2015) Thyme essential oil as a defense inducer of tomato against gray mold and *Fusarium wilt*. Plant Physiol Biochem 94:35–40
- Bouyaha A, Abrini J, Dakka N, Bakri Y (2019) Essential oils of *Origanum compactum* increase membrane permeability, disturb cell membrane integrity, and suppress quorum-sensing phenotype in bacteria. J Pharm Anal 9:301–311
- Campos EVR, Patrícia LF, Oliveira JL, Pereira AES, Ribeiro LN, Fernandes FO, Gonçalves KC, Polanczyk RA, Pasquoto-Stigliani T, Lima R (2018) Carvacrol

- and linalool co-loaded in β -cyclodextrin-grafted chitosan nanoparticles as sustainable biopesticide aiming pest control. Sci Rep 8:1
- El-Zahaby HM, Maswada HF, Ziedan EH, Zoeir HA (2018) Safe integrated control of postharvest rot diseases on banana fruit. Plant Arch 18:1345–1351
- Elad Y, Vivier M, Fillinger S (2016) Botrytis, the good, the bad and the ugly. In: Fillinger S, Elad Y (eds) Botrytis—the fungus, the pathogen and its management in agricultural systems. Springer, Bern, p 1
- Hollingsworth RG (2005) Limonene. A citrus extract, for control of mealybugs and scale insects. J Econ Entomol 98:772–779
- Hossain MS, Iqbal A (2016) Effect of shrimp chitosan coating on postharvest quality of banana (*Musa sapientum* L.) fruits. Int Food Res J 23:277–283
- Hua Y, Zhang J, Kong W, Zhao G, Yang M (2017) Mechanisms of antifungal and anti- aflatoxigenic properties of essential oil derived from turmeric (*Curcum alonga* L.) on *Aspergillus flavus*. Food Chem 220:1–8
- Irshad M, Aziz S, Ahmed MN, Asghar G, Akram M, Shahid M (2018) Comparisons of chemical and biological studies of essential oils of stem, leaves and seeds of *Zanthoxylum alatum* Roxb growing wild in the state of Azad Jammu and Kashmir. Pak Rec Nat Prod 6:638–642
- Iscan G, Iscan A, Demirci F (2016) Anticandidal effects of thymoquinone: mode of action determined by transmission electron microscopy (TEM). Nat Prod Commun 11:977–978
- Jayaseelan C, Rahuman AA, Kirthi AV, Marimuthu S, Santhoshkumar T (2012) Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi. Spectrochim Acta A Mol Biomol Spectrosc 90:78–84
- Jung J-H, Kim S-W, Min J-S, Kim Y-J, Lamsa K, Kim KS, Lee YS (2010) The effect of nano-silver liquid against the white rot of the green onion caused by Sclerotium cepivorum. Mycobiology 38:39–45
- Jo YK, Kim BH, Jung G (2009) Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Dis 93:1037–1043
- Kadoglidou K, Chatzopoulou P, Maloupa E, Kalaitzidis A, Ghoghoberidze S, Katsantonis D (2020) Mentha and oregano soil amendment induces enhancement of tomato tolerance against soilborne diseases, yield and quality. Agronomy 10:406
- Kaur P, Thakur R, Choudhary A (2012) An *in vitro* study of the antifungal activity of silver/chitosan nanoformulations against important seed borne pathogens. Int J Sci Technol Res 1:83–86
- Khan MM, Khan MR, Mohiddin FA (2012) The relative performance of different inoculation methods with *Alternaria brassicae* and *A. brassicicola* on Indian mustard. Plant Pathol J 11:93–98
- Kim S-W, Jung J-H, Lamsal K, Kim Y-S, Sim S-J, Kim H-S, Chang S-J, Kim J-K, Kim K-S, Lee O-S (2011) Control efficacy of nano-silver liquid on oak wilt caused by *Raffaelea* sp. in the field. Res Plant Dis 17:136–141
- Krishnaraj C, Ramachandran R, Mohan K, Kalaichelvan PT (2012) Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochim Acta A Mol Biomol Spec 93:95–99
- Kumar D, Kumar S, Singh J, Narender R, Vashistha BD, Singh N (2010) Free radical scavenging and analgesic activities of *Cucumis sativus* L. fruit extract. J Young Pharm 4:365–368
- Lagrouh F, Dakka N, Bakri Y (2017) The antifungal activity of Moroccan plants and the mechanism of action of secondary metabolites from plants. J Mycol Méd 27:303–311
- Mahmoudvand H, Sepahvand A, Jahanbakhsh S, Ezatpour B, Mousavi SA (2014) Evaluation of antifungal activities of the essential oil and various extracts of *Nigella sativa* and its main component, thymoquinone against pathogenic dermatophyte strains. J Mycol Med 24:155–161
- Moghaddam M, Mehdizadeh L (2016) Essential oil and antifungal therapy. In: Basak A et al (eds) Recent trends in antifungal agents and antifungal therapy. Springer India, New Delhi, pp 29–74
- Mossa AA, Mohafrash SMM, Ziedan EHE, Abdelsalam IS, Sahab AF (2021)
 Development of eco-friendly nanoemulsions of some natural oils and evaluating of its efficiency against postharvest fruit rot fungi of cucumber. Ind Crops Prod 159:113049–113051
- Moutassem D, Belabid L, Bellik Y, Ziouche S, Baali F (2019) Efficacy of essential oils of various aromatic plants in the biocontrol of *Fusarium wilt* and inducing systemic resistance in chickpea seedlings. Plant Protect Sci 55:202–217
- Park HP, Kim SH, Kim HJ, Choi HS (2006) A new composition of nanosized silica-silver for control of various plant diseases. Plant Pathol J 22:295–302

- Selim EMI, Ammar MM, Amer GA, Awad HM (2020) Effect of some plant extracts, plant oils an Trichoderma spp. on tomato Fusarium wilt disease. Menoufia J Plant Prot. 5:155–167
- Sharma A, Sharma NK, Srivastava A, Kataria A, Dubey S, Sharma S, Kundu B (2018) Clove and lemongrass oil based non-ionic nanoemulsion for suppressing the growth of plant pathogenic *Fusarium oxysporum* f. sp. *lycopersici*. Ind Crops Prod 123:353–362
- Sharma N, Tripathi A (2008) Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. Microbiol Res 163:337–344
- Snedecor GW, Cochran WG (1980) Statistical methods, 7th edn. Iowa State University Press, Ames
- Soliman HM, El Metwall MA, Elkahky MT, Badawi W (2015) Alternative to chemical control of grey mold disease on cucumber caused by *Botrytis cinerea* Pers. Asian J Plant Pathol 9:1–15
- Soylu S, Yigitbas H, Soylu EM, Kurt S (2007) Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. J Appl Microbiol 103:1021–1030
- Sukatta U, Haruthaithanasan V, Chantarapanont W, Dilokkunanant U, Suppakul P (2008) Antifungal activity of clove and cinnamon oil and their synergistic against postharvest decay fungi of grape *in vitro*. Kasetsart J Nat Sci 42:169–174
- Tabassum N, Vidyasagar GM (2013) Antifungal investigations on plant essential oils. A review. Int J Pharm Pharm Sci 5:19–28
- Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-álvarez JA (2007) Antifungal activities of thyme, clove and oregano essential oils. J Food Saf 27-91–101
- Wang C, Zhang J, Chen H, Fan Y, Shi Z (2010) Antifungal activity of eugenol against *Botrytis cinerea*. Trop Plant Pathol 35:137–143
- Xing Y, Xu Q, Li X, Che Z, Yun J (2012) Antifungal activity of clove oil against Rhizopus nigricans, Aspergillus flavus and Penicillium citrinum in vitro and in wounded fruit test. J Food Saf 32:84–93
- Yunis H, Elad Y, Mahrer Y (1990) Effects of air temperature, relative humidity and canopy wetness on gray mold of cucumbers in unheated greenhouses. Phytoparasitica 18:203–215
- Ziedan EHE, Farrag ESH (2008) Fumigation of peach fruits with essential oils to control postharvest decay. Res J Agric Biol Sci 4:512–519
- Ziedan EH, Khattab AA, Sahab AF (2018a) New fungi causing postharvest spoilage of cucumber fruits and their molecular characterization in Egypt. J Plant Prot Res 58:362–371
- Ziedan EH, Attallah AG, Abd-El-Aal SK, Sahab AF (2018b) Molecular identification and pathogenic potential of *Botrytis cinerea* isolates causing fruit blight of cucumber under protective greenhouse in Egypt. Plant Arch
- Ziedan EH, Saad MM, El-Naggar MA, Hemida KA, El Samman MGA, Mostafa HM (2020) Efficacy of compatibility between endophytic biocontrol agents and abiotic agents as fungicides alternatives for controlling root rot of grapevine. Acta Sci Agric 4:10–17
- Zoeir HA, El Zahaby HM, Ziedan EH, Maswada HF (2017) Efficacy of antifungal activity of essential oils, salts and antioxidants acids on pathogenic fungi and their application methods for controlling postharvest diseases on banana fruits. Afr J Microbiol Res 11:1162–1170

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.