Suppressive effect of some essential oils on controlling basal rot disease and Biochemistry of garlic cloves

Naglaa G. Ahmed *

Plant pathology Res. Institute, Agric. Res. Center, Giza, Egypt

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chinensis), Mint oil (Mentha
viridis) and Clove oil
(Dianthus caryophllus),
carbohydrate sugar, phenols,
flavonoids and sulphur

Abstract

Garlic plant were attacked by basal rot disease caused by Fusarium oxysporum (Schlecht) . Five isolates were used for pathogenicity test all tested isolates caused high percentage disease severity and disease incidence compared with the control. The highest infected isolates, isolate No 3 followed by isolate No 2 caused by Fusarium oxysporum caused basal rot of under greenhouse conditions. Evaluated chemical analysis of healthy dry cloves and dry disease cloves revealed that low carbohydrate, sugar, phenols, flavonoids and sculphure in healthy dry cloves than in disease dry cloves. Three essential oils namely jojoba (Simmondsia chinensis) mint (Mentha viridis) and clove (Dianthus caryophllus) at different three concentrations (5%, 10% and 15%) in vitro and in vivo this results indicted in vitro all essential oils reduced linear growth of the tested Fungi compared with the control essential oil jojoba (Simmondsia chinensis) gave 23.33 the highest effect, followed by mint (Mentha viridis) 31.00 and clove (Dianthus caryophllus) 58.33 concentration of 15% the highest inhibited of the linear growth of fungi in vitro. Under greenhouse conditions and field conditions all essential oils Jojoba (Simmondsia chinensis), mint (Mentha viridis) and clove (Dianthus caryophllus) concentration 15% reduce percentage disease incidence and percentage disease severity compared with the control while the concentration 15% of jojoba (Simmondsia chinensis) the highest reduce percentage disease severity and percentage disease incidence followed by mint (Mentha viridis) followed by clove (Dianthus caryophllus) under greenhouse conditions and filed conditions during growing seasons 2016 and 2017. This study suggests that to use these essential oils such as Jojoba (Simmondsia chinensis), mint (Mentha viridis) and clove (Dianthus caryophllus) as alternatives to chemical resistance and fungicides because they are cheap, safe to use and do not leave any toxic effects on plants, animals, humans, and the environment, in addition to the easy of obtaining it is available in abundance in nature.

1. Introduction

Garlic (*Allium sativumL*.) is one of the most important vegetable crops in Egypt and all the world. Garlic has many benfinit its used for medical properties and food (Galvez and Palmero, 2021). Fungi which causes many diseases attack garlic cloves in the field during growth season and storage. Fungi

^{*}email: tornado.tornado725@gmail.com

associated with garlic cloves have been reported by several investigators Fusariun solani (Kararah and EL-Tobshy, 1979) and F. oxysporum (Radwan, 1980). Five fungi were isolated from garlic. These fungi included Asprgillus niger, Botrytis allii, Fusarium oxysporum, Penicillium citrimum and Penicillium funiculosum (El-Shabrawy et al., 1981). Clove rot caused by a variety of fungus species, including Fusarium culmorum, Fusarium proliferating, F. oxysporum, Aspergillus niger, A. orchaceus, Botrytis alli (Moharam et al., 2013, Elshawy, et al., 2017). Essential oils, gums, etc. have been shown to exert biological activity against plant fungal pathogens in vitro and in vivo and can be used as bio-fungicidal products (Lozano etal., 2000, Jalili et al., 2010, Bhardwaj, 2012 Romanazzi et al., 2012 and Dewidar et al., 2019). It is recommend to use these plant oils as alternative to chemical resistance and fungicides ,because they are cheap and safe to use, and do not leave any toxic effects on plants, animals ,humans, or structures, environmental, addition to the ease of obtaining them due to their abundance in nature. Oil extract of jojoba seeds at different concentrations from 1 to 10 % gave good control against powdery mildew incidence on different plants mentioned that using garlic and jojoba extracts under field conditions as seed treatment at the concentration 15% significantly reduced disease incidence (Hicks, 2001), (Reyad and Attia 2016), and (Soliman et al.,2013). The aim this work to isolation the causal pathogen of basal rot of garlic and control the disease by using some essential oils and study the relation between garlic in fiction and biochemical changes.

2. Methods and tools Material and methods

2.1. Isolation of the causal pathogen:

Five isolates were isolated on samples of diseased garlic cloves were collected from different localities of Assiut Governorate isolation procedures was carried out using infected garlic cloves. Soaking the infected cloves in 1% sodium hypochlorite solution for 10 minutes followed by complete washing in sterile distilled water was used for surface disinfestation of infected sample before plating on potato dextrose agar (PDA) medium at 27 °C for 5-7 days. Pure culture of the developing fungi was obtained by single spore isolate (Moharam, *et al.*, 2013).

2.2 .Pathogenicity test:

Preparation of the fungal inoculum of 5 isolates of *F. oxysporum* were prepared by inoculating sterilized milk bottels 0.5 L containing barley medium by disca of F.oxysporum incubated at 27°C for 2 weeks described by (Abd-El-Moniem, 1996). Pots 25 cm diameter inoculated by containing sterilization sandy loam soil artificially infested with tested fungi. Soil inoculation was performed by mixing of 3 % of the inoculate with soil in each pot (150g/5Kg soil) and then directly pot was seeded with 5 cloves. Three replicates were used for each tested. Three replicates were used as control without inoculation disease severity and disease incidence were recorded after 60 days.

2.3. Biochemistry of Garlic cloves:-

- Determination of total phenolic contents in the garlic dry cloves.

The concentration of phenolic in plant extracts was determined using spectrophotometric method (singleton et al., 1999)

- Determination of flavonoid concentration in the garlic dry cloves.
- The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (quartier *et al.*, 2000.
- Total soluble sugars found according to the method used by (Yemme Willis 1954). Sulphur according to (Anon, 1985).

Study (Petropoulos et al., 2018) reported that the main carbohydrate.

2.4. Effect of three essential oils on the linear growth of F. oxysporum in vitro:

The concentrations 1.5 ml / later was used of essential oils jojoba (*Simmondsia chinensis*) mint (*Mentha viridis*) and clove (*Dianthus caryophllus*) were prepared by adding suitable amount 0.015 of each tested concentrated to 5 ml, 10 ml and 15 ml (PDA) medium in petri dishes. Inoculation was done with fungal discs, (5mm) in diameter obtained from *F. oxysporum* 7 days old culture Three replicates were used for each tested concentrate. Another group of (PDA) plates free from essential oils inoculated with the fungus as control. All plates were incubated at 27°C for 7 days. Linear growth was recorded. The percentages of reduction in the mycelia growth were calculated. The obtained data were statistically analyzed, according to (Snedecor and Lazzaretti *et al.*, 1995). Statistical analysis: The collected data were statistically analyzed using two factor and 0.01 level probability L.S.D. (Constantinescy. *et al.*, 2002)

2.5 Greenhouse experiment:

This experiment was carried out in Arab EL- Awamer growing 2016 and 2017.

Tow isolates No2 and No3 were used garlic of cloves (baladyc.v.) were sown in pots 25Cm in diameter, were sterilized by dipped in 5% formalin solution for 15 minutes, left to dry for two days to get rid of formalin residues, then filled with soil previously autoclaved for two hours at 121°C. Fungas inoculation of F. oxysporum were added to the sterilized potted soil at rate 3% of the inoculum. Seven days after the inoculation with the pathogen, pots were sown with garlic cloves (balady c.v.) after soaked into oils jojoba (Simmondsia chinensis) mint (Mentha viridis) and clove (Diamnthus caryophllus) for 15 minutes before planting. Disease severity was determined after 60 days from planting. Data were statistically analyzed as Randomized Complete according data (Galal et al., 2002) using the formula as following: percentage of rot severity = $(w1 - w2) / w1 \times 100 w1$ where is: the total weight of the clove, and w2 is the clove's weight after decayed tissue has been removed.

2.6.Field experiment:

Field experiment was carried out in the experimental farm of Arab EL- Awamer research station, Assiut Government, Egypt during growing season (2016 - 2017) and (2017 - 2018). The soil as method before determination of disease severity and disease incidence percentage as described before statically analysis. Randomized complete block design with three replicate plots of 10.5 m² Evaluation of preside recoded along with the yield per plot at harvest time 90 days of planting recorded as percentage of infection and disease severity at harvest. Data presented in Table (2) indicated that the all isolates of *Fusarium oxysporm* causal highest percentage disease incidence and disease percentage every significantly compared with control. Data also indicated that isolate No (3) causes highly percentage disease incidence and disease severity while the isolate No (5) caused lowest percentage disease severity or percentage disease incidence.

Table (1): Scientific English and Arabic names of Essential oils and their natural component:

Essential oils	English name	natural component	Arabic names
Simmondsia chinensis	Jojoba	Gadolic acid	زيت الجوجوبا
Mentha viridis	Mint	Menthend	النعناع
Dianthus caryophyllus	Clove	Eugenol	القرنفل

This is parallel line with results reported by (Ibrahim and Zein El-Abdee 2000) and (Osman 2004). Among natural plant oils tested, was the most efficient. These results were confirmed by (Ata, 2005)

and Ata, *et al.*, 2006) on suger beet rust disease. Garlic extract showed high suppressive effect on linear growth of *R. solani* and *F. solani* (Kuruchve and Padmavathi, 1997) and (Abd El-Moniem, 2001). Jojoba seeds oil extract at different concentration 1% and 10% gave good control for powdery mildew disease - incidence on different plants (Hicks, 2001). Data presented in Table (2) indicated that all isolates of *F. oxysporum* causes root rot disease of garlic. Data also show that isolate (No.3) was the highly pathogenic followed by isolate (No.2) These results were in agreement with the finding of (Hicks, 2001) and (Reyad and Attia, 2016), (Soliman *et al.*, (2013).

Table (2): Pathogenicity test five isolates of F. oxysporum causal pathogen of root rot of garlic under greenhouse coditions:

Fusarium oxysporm	Disease incidence %	Disease severity%
(1)	20.00	13.30
(2)	23.33	14.43
(3)	26.67	19.63
(4)	16.67	11.47
(5)	13.33	9.20
Control	0	0
S.D 5% 6	.22 2.2	0

L.S.D 5% 6.22 2.20 L.S.D 1% 8.83 3.13

Data presented in Table (3) indicated that SO4 9.27% more and phenols indisease dry clove compared with healthy dry cloves. While total carbohydrates 47% and total sugar 0.98% more in healthy dry cloves compared with disease dry cloves. Data presented in Table (3) indicated that total phenols more in infected dry clove compared with healthy dry cloves this result agree with (Manal El- Shazly and Abd El Wahab 2017) which said the increased quantity of phenolics in infected plant may be contributing to the resistance against the infection. Data presented in Table (3) also indicated that flavionids 113.74% more in infeedted dry clove compored with healthy dry cloves this results agree with (Sicilant *et al.*, 2015) . The biosynthesis of sakuranetin (a flavanone) in rice was shown to increase the plant resistance to infection against bakanaa caused by fusarium (Hasegawa fujikuroirce *et al.*, 2014) cenblast cause by *Magnaporthe oryzae*.

Flavonoid, such as kaempferol inhibits auxin transport, which enhances auxin concentration in the corticap cells and accelerates cell division and growth. Together these physiological a chemical processes lead to the for mation grycosyration and acyration of flavonoids have been identified, which collectively contribute to the diversity of the flavonoids synthedsized by plants (Alseekh, *et al.*, 2020). Data presented in Table (4) indicated that all three essential oils inhibited linear growth of *F. oxysporum in vitro* compared with the control untreated with essential oils the most effective in habiting the linear growth of *F. oxysporum* Jojoba oil (*simmondsia chinensis*) followed by mint oil (*Mentha viridis*) and clove oil (*Dianthus caryophllus*) in all concentration while the concentration of 15% the heights inhibition linear growth of the pathogen compared with all the concentration.

Data also indicted that concentration 15% in all essential oils more effective in reducing linear growth of *F.oxysporum in vitro*. Also indicated that isolate No (3) more effective than isolate No (2). Data also indicated that Jojoba more effective inreducing linear growth of *Fusarium oxysporum in vitro* this results agree with (Elshaer *et al.*, 2019) who said that Jojoba (*simmondsia chinensis*) were tested for their ability to inhibit mycelial growth of the pathogenic fungi *Fusarium solani*, *Macrophomina phaselonia* and *Rhizoctonia solania* under the lab condition.

THE CHEMISTRY OF GARLIC



Normale clove



showing rot lesions penetrating the cloves include polygonal light brown centrally depressed spots that are not easily detected.



showing brown discoloion of the stem plat tissue later, the stem plate.

figur (1) showing Normal clove and dry rot.

Table (3): Biochemistry composition constituents of healthy garlic dry cloves and diseased garlic dry cloves:

Biochemistry composition					
46.11.1	Healthy garlic dry cloves	diseased garlic dry cloves			
1-Carbohydrates	7.40	6.04			
2- sulfer SO4 %	9.27	15.82			
3- Phenols %	1473.22	8872.06			
4- Flavonoids %	113.74	444.31			
5- Sugars %	0.98	0.65			

Table (4): Effect of three essential oils on the linear growth of F. oxysporum in vitro:

Essential oils	Concentration		Linear growth (mm)		
Essential ons	Concentration	on	Isolate 2	Isolate 3	
Jojoba			30.00	31.00	
Mint	5%		43.00	43.00	
Clove			62.67	66.67	
Mean			45.22	46.89	
Jojoba			30.67	29.00	
Mint	10%		41.33	38.33	
Clove			60.67	64.00	
Mean			44.22	43.78	
Jojoba			23.33	26.67	
Mint	15%		31.00	33.33	
Clove			58.33	60.67	
Mean			37.56	40.22	
Control			89	89	
SD 5%	Conc.	14.251	15	.422	
	E. oils	4.3892	3.	7577	
	Conc. x E. oils	4.2018	4.′	7036	
SD 1%	Conc.	19.313	20	.899	

E. oils 5.9481 5.0923 Conc. x E. oils 5.7569 6.4443

Conc.: Concentration - **E. oils:** Essential oils

Data presented in Table (5) indicated that Jojoba oil more effective in reducing % disease severity and % disease incidence this results agree with (Manal El-Shazly and Abd El-Wahab 2017) who said that all treatment with Jojoba seeds extract caused virus inhibition when plant were sprayed before the virus transmission in onion plant Egypt. (Soliman et al., 2013) mentioned that using garlic and jojoba extracts under field conditions seed treatment at the concentration 3% significantly reduced disease incidence and also increased the percentage of yield components, e., the number and weight of pods/plat and dry weight of 100 seeds followed by jojoba extracts.

Data presented in Table (5) indicated that concentration 15% of Jojoba more effective in reducing disease incidence percentage and disease severity percentage followed by mint and clove under green house conditions during growing seasons (2016 and 2017).

Data presented in Table (5) also indicated that isolate No (3) more effective in reducing disease severity percentage and disease incidence percentage than isolate No (2) during growing seasons (2016 and 2017) under green house conditions. Role in the ability of Jojoba, in controlling Fusarium oxysporum_may be attributed to the presented of Jojoba oil wax esters which are derived from esterification of monoethylenic acids and monoethyfenic alcohols, these compounds block the movement of the pathogenic fungi (Hicks, 2001). Data presented in Table (5) indicated that the tested natural essential oils tested significantly reduced the development of Basal rot disease of garlic during two tested seasons when compared with control this result agree with (Hicks, 2001), (Reyad and Attia, 2016) and Soliman *et al.*, (2013). Data presented in Table (6) indicated that concentration 15% of Jojoba more effective in reducing disease incidence percentage and disease severity percentage followed by mint and clove under filed conditions during growing seasons (2016 and 2017).

Table (5): Effect of Essential oils on the disease incidence and disease severity at concentration 15% of Basal of Basal rot disease in green house during 2016 and 2017 growing season:-

Isolate No	Essential oils	2016 season		2017 season	
		Disease incidence %	Disease severity %	Disease incidence %	Disease severity %
2	Ioiobo	33.50	12.75	34.50	11.75
3	Jojoba	35.00	14.18	36.00	13.50
Mean		34.25	13.46	35.25	12.62
2	Mint	40.75	14.87	41.75	15.00
3	Mint	41.50	15.18	42.00	16.18
Mean		41.12	22.46	41.87	15.59
	C1	46.50	16.90	46.00	14.90
	Clove	44.50	15.90	43.50	20.90
Mean		45.5	16.40	44.75	17.90
	control	21.50	20.50	20.50	25.40
. S. D	5% 4.75	5 1.33		•	

L.S.D 5% 4.75 1.33 1% 2.54 1.36

Table (6): Effect of Essential oils on the disease incidence and disease severity at concentration 15% of Basal of Basal rot disease in under filed conditions during 2016 and 2017 growing season:-

	Essential oils	2016 season		2017 season	
Isolate		Disease incidence %	Disease severity %	Disease incidence %	Disease severity %
2	Ioiobo	30.00	14.50	33.00	18.75
3	Jojoba	34.00	20.00	35.00	17.50
Mean		32	17.25	34.00	18.12
2	Mint	36.00	13.75	44.25	19.00
3	Mint	40.00	15.00	46.20	18.00
Mean		38	14.37	45.22	18.50
2	Classa	45.00	15.70	45.70	20.70
3	Clove	44.00	17.90	47.50	22.90
Mean		44.50	16.80	46.60	21.80
	control	21.50	20.50	20.50	25.40

L.S.D

4.75 7.33 6.57 10.16

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