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Comparative Fungicidal Activity of some Pure and Nanoemulsion Monoterpenes Effects on Soil Borne Plant Pathogenic Fungi

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ABSTRACT

Comparative study between pure and nanoemulsions (NEs) monoterpenes, cinnamaldehyde, citral, geraniol pulegone, and (*R*)-carvone for their fungicidal activity against three of soil borne pathogenic fungi namely, *Pythium digitatum*, *Rhizoctonia solani* and *Fusarium solani* by using poisoned food technique were evaluated. Results indicated that all examined monoterpenes have displayed antifungal activity under different tested concentrations. Further more. NEs- monoterpenes reduced significantly the linear growth of all tested pathogens than pure monoterpenes. NEs citral and geraniol ranked the first for their effective antifungal compounds on the tested fungi with EC_{50} values of 31.31 and 49.41 µg/ml on *R. solani,* 48.21 and 54.16 µg/ml on *P. digitatum* and 83.61 and 100.75 µg/ml on *F. solani,* respectively. Carbendazim fungicide was the reference in this study. NEs citral and geraniol were induced effective defense responses *in vivo* in tomato plants against *R. solani* at a rate of 1000 mg/L. Determination in the leaves after 0, 1, 3, 7 and 15 days of inoculation for polyphenoloxidase (PPO), peroxidase (POD) as well as total phenolic content activities as defense-related enzymes were measured. A significant increase in PPO, POD activities and total phenol content were detected. Results, also, showed that to reflect the resistance and susceptibility nature of tomato cultivars against the pathogenic fungi, *R. solani*., POD and PPO activities can be considered as biochemical markers. The obtained results indicated that the elicitor's NEs-citral and geraniol have brought about defense reaction in tomato plants towards the pathogenic fungi. These effective NEs monoterpenes could be effective potentially and environmentally safe to control tomato damping- off disease.

Keywords: Nanoemulsion, monoterepenes, phytopathogenic fungi, Induced resistance.

INTRODUCTION

Soil borne pathogenic fungal diseases are causing significant economic losses in agricultural production. Plant diseases caused by *Rhizoctonia* spp., *Fusarium* spp., *Verticillium* spp., *Sclerotinia* spp., *Pythium* spp., and *Phytophthora* spp. affect asmany economic crops, notably, wheat, cotton, vegetables and temperate fruits. Soil borne pathogenic agents are regarded as the most important diseases that affect the tomato crop causing damages to such crop (Song *et al.,* 2004).The frequent use of fungicides resulted in serious environmental pollution and development of resistance. Therefore, it is prompted to find out safe natural products and/or artificial chemicals to control phytopathogenic fungi. However, more interest was oriented to the use of essential oils and their major constituents as plant secondary metabolites for integrated pest control (Costa *et al.,* 2000). In this direction, monoterpenes are the most promising plant-derived products for diseases control. Monoterpenes are a class of natural products which contain ten carbons atoms that commonly used, detected in many different plants. Monoterpenes have been shown to possess remarkable fungicidal (Cärdenas-Ortega *et al*., 2005) and bactericidal (Cristani *et al.,* 2007) activities. Studies in relation to the antifungal activities of essential oils against soil borne pathogens are very few (McMaster *et al*., 2013; Turkolmez and Soylu, 2014). Citral and geraniol are antiseptic and suppress the growth of bacteria and fungi (Ganjewala, 2009).

Nanoemulsions(NEs) are emulsified materials that can be incorporated from essential oils (such as monoterpenes), water, and emulsifier with particle sizes ranged in 10-100 nm (McClements, 2011). NEs have several properties, such as high physical stability, high bioavailability, and low turbidity, so that they are helpful in lowering degradation and volatilization of active ingredient and improve their bioavailability for long time period (Mason *et al*., 2006; Anton *et al*., 2008). Nanoemulsions are used as agrochemicals in pesticide drug-delivery formulas (Jianguo *et al.*, 2016).

One of the effective disease control strategy is enhancing host resistance using elicitors. Inducible or stimulation of plant defenses are triggered by the perception of a pathogen or elicitors which takes place in receptors located in pathogen cells either external and internal. (Dardick and Ronald, 2006). This recognition of elicitors triggers overlapping signaling responses in the plant (Kim *et al.,* 2005). Recognition of the elicitor induces several reactions in plants. The oxidation of polyphenols into quinons (antimicrobial compounds) is performed by Polyphenoloxidase (PPO), enzyme which result in lignifications of plant-cell wall during microbial infection, and also may contribute in the defense reaction and hypersensitivity by inducing plant resistance against fungi (El-Khallal, 2007). Positive correlation between levels of PPO and the resistance to pathogens is frequently detected (Mayer, 2006). Peroxidase (POD) is a defense-related enzyme that plays a key role in plant-pathogen interactions and is suggested to be one of the most important agents of the plant's biochemical defense against pathogenic microorganisms (Saravanan *et al*., 2004). POD is one of the defense agent that are set up in plants as a response to pathogenic infection (Chittoor *et al*., 1999; Morkunas and Gmerek, 2007). Plant phenolics compounds play key roles in plant development, particularly in lignin synthesis (Bhattacharya *et al.,* 2010). Polyphenolic compounds protect plants from biotic and a biotic stress agent, especially under stress factors amongst which, phytoalexins, that exhibited in defense mechanisms and are synthesized up on pathogen or predator attack or injury (Cantos *et al*., 2003). Therefore, the aim of this study was focused on the optimal conditions for preparing geraniol, cinnamaldehyde, pulegone, carvone, and citral in water nanoemulsions with mixed surfactants using ultrasonic emulsification. Evaluation the comparative antifungal activity of NEs-monoterpenes against three plant pathogenic fungi, *R. solani*, *F. solani,* and *P. digitatum in*

vitro and *in vivo* studies was considered. Additionally, the fungal pathogens and the host plant interaction were, also, investigated.

MATERIALS AND METHODS

Isolation and identification of tomato soil-borne fungi.

Pure cultures of highly virulent isolates of tomato soil borne fungi such as, *Rhizoctonia solani*, *Fusarium solani,* and *Pythium digitatum* were obtained from the microbiology laboratory, department of plant pathology, faculty of agriculture, damanhour university, Egypt. Samples of tomato infected roots were prepared, purified and identified according to Barnett and Hunter (1987). The fungi were maintained during the experiment on potato dextrose agar medium (PDA: potato 200, dextrose 20 and agar15 g/l in distilled water) at 25 °C.

Tested nanoemulsion monoterpenes

Five monoterpenes, geraniol (98%), cinnamaldehyde (98%), pulegone (92%) were obtained from Acros Organics Company (USA), while, (*R*)-carvone (98%), and citral (95%) from Sigma–Aldrich Chemical Co., Steinheim, Germany Figure (1).

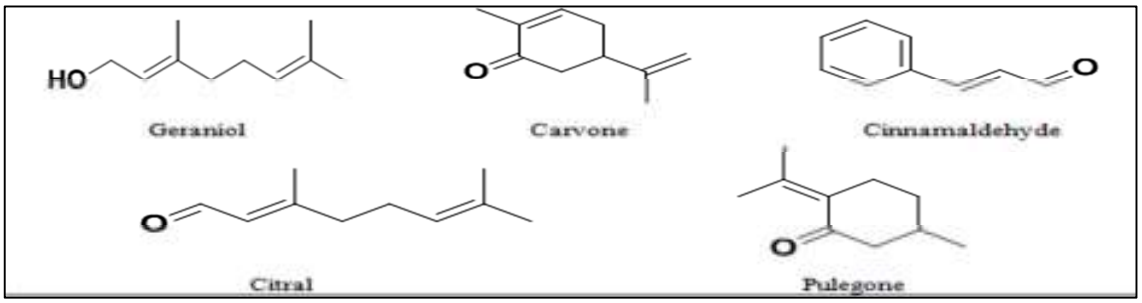

Figure 1. The chemical structure of monoterpenes used for *in vitro* and *in vivo* biological assays.

Nanoemulsions were firstly prepared by stirring monoterpene, solvent, surfactant and deionized water and then further emulsification using a high-energy ultrasonic process (Figure 2), under optimal conditions as it described by (Badawy *et al.* 2017).The mixed monoterpenes, solvent (DMSO), surfactant (tween80) and deionized water concentration were incorporated by 5%, 5%, 10% and 80%, respectively, thus the final oil concentration was 5000 ppm. NEs of *R*-carvone, cinnamaldehyde, citral, geraniol and pulegone have droplet size of 67.23, 128.07, 56.64, 176.00 and 58.83 nm orderly, while, PDI values were 0.177, 0.322, 0.130, 0.630 and 0.311 respectively**,** (Abdelrasoul *et al.* 2018).These nanoemulsions were immediately used for *in vivo* and *in vitro* biological assay.

Antifungal assay

The fungal toxicity of the tested normal and NEs-monoterpenes were tested by the poisoned food technique reported by Bajpai *et al.* (2007). The monoterpenes were incorporated into the PDA medium with 0.5% Tween 20 as an emulsifying agent to final concentrations that ranged from 10 to 1000 µg/ml. Each concentration was tested in triplicates. The controls were maintained with 0.5% Tween 20 in PDA medium. The discs of mycelia felt (0.5 cm diameter) of the plant pathogenic fungi, taken by cork borer from 7-day-old cultures on PDA plates, were transferred aseptically to the centre of petri dishes. Carbendazim was used as a reference fungicide. The fungal cultures were incubated at 25±2C°in the dark. As the fungal colony extended As the fungal colony extended towards margin of petri dishes, radial growth was measured and percentages of mycelia growth inhibition (MGI) were calculated according to (Pandey *et al.* 1982). The concentration of the monoterpene that inhibiting the fungi mycelia growth by 50% (EC50) was determined.

Fig. 2. Formulated monoterpenes nanoemulsion used for *in vitro* and *in vivo* biological assays.

Evaluation of NEs citral and geraniol on enhancing resistance of tomato against *R. solani in vivo*.

Nanoemulsion of citral and geraniol were applied to evaluate their efficacies on soil borne fungi, *R. solani* at the concentration of 1000 mg/L and carbendazim (1.5 g/L) as a common comparable fungicide. Uniform 30-day-old tomato (*Lycopersicone sculentum Mill cv.* Elisa) transplants, were singly transplanted in a plastic pot (25- cm diameter) filled with sandy soil (clay and sand, 1:1, 3 Kg soil / pot). The pots were previously sterilized with 5 % formalin and left for one week to eliminate the traces of formalin. Transplants were allowed to recover from transplanting shock for 10 days. The fungal pathogens were grown according to Fatouh (2012). Infected soil was achieved by mixing the inoculums of fungi with soil (Marwa *et al*., 2014). The inoculated pots were irrigated and left for 7 days before transplanting to enhance fungal growth in the soil. The pots were irrigated and fertilized as usual but no fungicides were applied. Three pots for each treatment were used and control divided to untreated inoculated (control positive, UI) and untreated un-inoculated pots (control negative, UU) were planted with transplants used as an respectively. Roots of the transplants were treated by the tested NEs-citral, geraniol singly (5 ml/seedling), for 5 minutes dipping, then immediately planted in pots artificially inoculated planted with the fungi.

Plant growth parameters expressed by fresh and dry shoot and root weights (in grams), were recorded.

Assessments of Disease

Symptoms of pre- and post-emergence damping off were monitored after 15 and 45 days of planting, respectively, according to El-Shafey *et al*., (1988). Pre-emergence damping off (%) = (No. of non-emerged seed/Total No. of seeds sown) x100. Post-emergence damping off (%) = (No. of wilted plant /Total No. of emerged plant) x100. Ninety days after planting, disease severity was recorded based on Rowe (1980) and Liu *et al*. (1995) with the following ranking: no internal or external browning. 0: No internal or external browning, 1: No internal browning, with superficial lesions (≤25%) on the tap root, 2: Slight internal browning with (<25 to ≤50%) surface covered with cankers, 3: Moderate internal browning with <50 to ≥75% cankers, 4: Severe internal and external (<75%) browning.

$$\text{Disease severity (\%)} = \frac{\text{Sum. (n x r0) + (n x r1) + ….. + (n x r4)}}{\text{4 N}} \text{ x100}$$

Where: n= No. of plants in each numerical rate (r0….r4).

N= Total No. of plants multiplied by the maximum numerical rate (4).

The activity of defense-related enzymes

Polyphenoloxidase (PPO) and peroxidase (POD) activities were determined in the leaves after 0, 1, 3, 7 and 15 days day after inoculation, each treatment was represented by three replicates. The activity of polyphenol oxidase (EC 1.14.18.1) was determined according to Mayer *et al*., (1965) and was expressed as change in OD min^{-1} mg^{-1} protein. Peroxidase activity (EC 1.11.1.7) was assayed as it described by Chen *et al*. (2000) and was expressed as change in the OD min^{-1} g^{-1} of fresh tissue. Total phenolic content of leaf was estimated by FolinCiocateau method (Zieslin and Ben Zaken, 1993) and expressed as catechol equivalent/ g of fresh tissue.

Statistical analysis

One -way analysis of variance (ANOVA) was used to analyze data by using SPSS 20.0 software (Statistical Package for Social Sciences, USA). The EC_{50} values and their 95% confidence limits for the fungal bioassay were calculated using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Antifungal activity of monoterpenes against plant pathogenic fungi

Marked inhibitions of mycelial growth of the three plant pathogenic fungi, *R. solani, P. digitatum,* and *F. solani* as a result of the tested pure monoterpenes were detected . Citral exhibited the highest inhibitory effect with EC_{50} value, followed by geraniol, while cinnamaldehyde, pulegone and carvone were less effective compounds (Tables 1). It was ,also, noticed that, *R. solani* was the most sensitive fungus to all tested pure monoterpenes compounds as it recorded the lowest EC_{50} values compared with *P.digitatum,* and *F. solani*. However, *F. solani* was less responded. Results revealed that the EC_{50} values of both citral and geraniol were more closed to carbendazim as a reference fungicide as compared to cinnamaldehyde, pulegone and carvone. These previously findings are in harmony with those reported by Shi *et al.,* (2016), who found that the citral was the most effective monoterpenes on plant pathogenic fungi.

Table 1. Effect of pure monoterpenes against the tested soil borne pathogenic fungi.

Compound	Fungi	EC_{50} [a] (µg/ml)	95% Confidence Limits(µg/ml)		Slope ± S.E [b]	Chi-Square [c]
			Lower	Upper		
Citral	*Rs*	69.45	41.59	107.72	1.63±0.13	6.45
	Pd	109.47	88.79	135.19	1.35±0.12	2.60
	Fo	154.53	120.65	203.56	1.11±0.11	2.26
Geraniol	*Rs*	71.712	59.278	85.810	1.65±0.13	5.05
	Pd	103.629	83.002	129.276	1.275±0.12	1.286
	Fo	168.622	133.569	219.072	1.19±0.12	5.285
Cinnamaldehyde	*Rs*	185.13	156.23	222.31	1.77±0.16	3.85
	Pd	121.56	97.90	152.11	1.29±0.12	3.31
	Fo	310.415	244.107	420.710	1.34±0.14	0.93
Pulegone	*Rs*	241.512	199.712	301.250	1.619±0.16	1.999
	Pd	245.93	202.80	308.19	1.59±0.16	2.12
	Fo	256.189	214.098	315.823	1.763±0.17	0.97
Carvone	*Rs*	311.32	253.11	402.81	1.59±0.16	1.49
	Pd	316.29	261.09	400.66	1.75±0.18	3.55
	Fo	403.41	311.44	572.34	1.386±0.16	2.623
Carbendazim	*Rs*	48.614	42.053	56.139	2.04±0.16	0.49
	Pd	65.75	55.11	79.46	1.57±0.15	1.69
	Fo	77.32	60.31	104.21	1.08±0.13	1.55

[a] The concentration causing 50% inhibition of the fungi mycelial growth, [b] Slope ± standard error of the concentration–inhibition regression line, [c]Chi square, *R. solani(Rs), P.digitatum (Pd), F.solani(Fs*)

Results in Table (2), generally, indicated that all evaluated NEs-monoterpene exhibited marked antifungal activity against all tested fungi as the EC_{50} values ranged from 31.31 to 175 mg/L. It was noticed that the nanoemulsion was more effective than pure tested monoterpenes. These findings could be referred to the thermodynamically linked between NEs-particles and lipid containing organisms which enhanced by the chemical reaction between the emulsion (cationic charge) and the pathogen (anionic charge) as outlined by Kadhim and Abbas (2015).When enough nanoparticles fuse with the pathogen, they release a part of energy entrapped in the emulsion. Both the active ingredient and the energy released destabilize the pathogen lipid membrane, resulting in cell lysis and death. The inhibitory effect of monoterpenes on microorganism cells could be ascribed to cytoplasm granulation, cytoplasmic membrane rupturing and inactivation and/or synthesis inhibition of intracellular and extracellular enzymes (Cowan, 1999)

Table 2. Effect of nanoemulsion monoterpenes against the testes soil borne fungi.

Compound	Fungi	EC_{50}[a] (µg/ml)	95% Confidence Limits(µg/ml)		Slope ± S.E[b]	Chi-Square[c]
			Lower	Upper		
Citral	*Rs*	31.31	27.61	35.29	2.72±0.21	3.14
	Pd	48.21	41.68	55.72	2.03±0.16	2.53
	Fo	83.61	71.38	99.72	1.86±0.16	2.74
Geraniol	*Rs*	49.41	42.81	57.00	2.06±0.16	3.12
	Pd	54.16	46.66	63.027	1.94±0.15	2.82
	Fo	100.75	82.45	128.56	1.48±0.15	4.44
Cinnamaldehyde	*Rs*	98.11	86.14	113.30	2.47±0.21	1.07
	Pd	100.21	87.08	117.41	2.25±0.19	1.49
	Fo	150.79	120.71	202.51	1.56±0.17	0.29
Pulegone	*Rs*	121.52	103.24	147.88	2.02±0.19	1.82
	Pd	128.31	110.52	153.45	2.29±0.21	1.72
	Fo	174.33	138.99	237.36	1.66±0.19	1.71
Carvone	*Rs*	97.51	82.14	119.16	1.75±0.16	3.151
	Pd	110.34	78.36	183.42	1.90±0.18	5.322
	Fo	175.11	138.88	240.64	1.62±0.18	3.737
Carbendazim	*Rs*	48.614	42.053	56.139	2.04±0.16	0.49
	Pd	65.75	55.11	79.46	1.57±0.15	1.69
	Fo	77.32	60.31	104.21	1.08±0.13	1.55

[a] **The concentration causing 50% inhibition of the fungi mycelial growth,** [b] **Slope ± standard error of the concentration–inhibition regression line,** [c] **Chi square,*R. solani(Rs), P.digitatum(Pd), F.solani(Fs)***

Effect of NEs-citral and geraniol on enhancing resistance of tomato plants against *R. solani in vivo*

It was noticed that NEs-citral and geraniol were highly effective compounds for the three tested fungi. Defense reactions were evaluated after the 0, 3, 7, 15 day post inoculations by *R. solani* after the treatment with tested nanoemulsion.

Disease severity: *In vivo* experiment, using NEs-citral and geraniol and carbendazim against damping off and root rot severity in tomato plants has revealed different responses after the inoculation with *R. solani* as shown in Table (3).

Table 3. *In vivo* response of *R.solani* to NEs-citral and geraniol on damping off and root rot severity of tomato plants.

Treatment	Pre-emergence (%)	Post-emergence (%)	Root Rot Severity (%)
UU	0.00±0.00[d]	0.00±0.00[c]	0.00±0.00[d]
UI	39.75±0.85[a]	20.25±0.85[a]	25.79±0.40[a]
Citral	18.25±1.11[c]	6.50±0.645[b]	5.44±0.16[c]
Geraniol	23.00±1.29[b]	7.25±0.47[b]	6.15±0.08[b]
Carbendazim	17.25±0.32[c]	6.38±0.11[b]	6.14±0.19[b]

*** UU control : untreated un-inoculated control,**
UI control :untreated inoculated controls.
***Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.**

A significant difference was noticed among treatments in all parameters and inoculated control, where significant protective effects against *R. solani* were evidenced. NEs-citral ranked the first effective compound, as it decreased pre-emergence damping-off level to 18.25% compared to 39.75% for the inoculated control and with no significant difference with that the carbendazim fungicide. However, application of NEs-citral, geraniol and carbendazim, significantly, decreased post emergence damping-off to 6.38, 6.50 and 7.25% compared to 20.25% for the untreated inoculated control. Meanwhile, no significant difference between the two NEs and tested fungicide were observed. Root rot severity was deceased significantly by all treatments, where NEs-citral was the superior effective, as it reduced disease severity to be 5.36%, compared to 30.54% for the untreated inoculated control. These findings are in accordance with Elamawi and Al-Harbi (2014).

Fresh and dry weight for shoot and root: Fig. 3 showed that both shoot and root weights of tomato plant was markedly increased by most of the used treatments. However, *R. solani* supressed growth parameters in the untreated inoculated treatments as compared with the treated or uninoculated plants. Both NEs-citral and geraniol exhibited highly significant effect as it significantly increased the fresh shoot and root weight. These effects were not significantly differing than carbandazim.

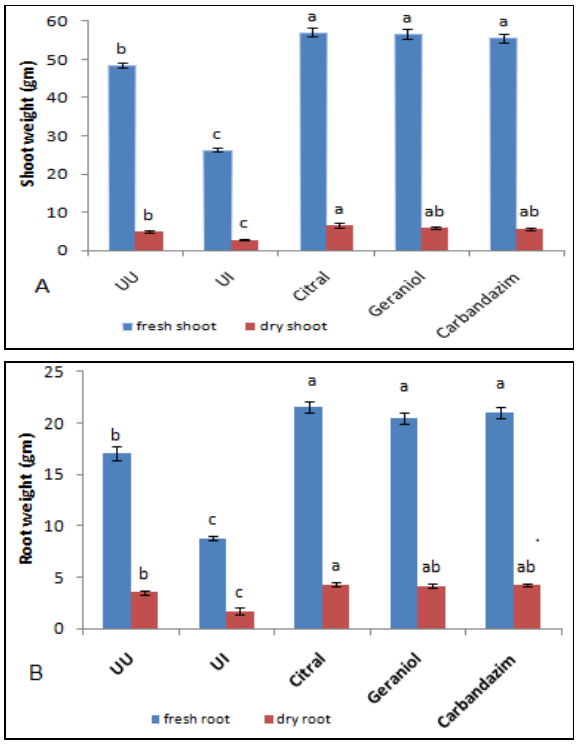

Fig. 3. Effect of NEs-citral and geraniol on fresh and dry shoot and root weights (in grams) of tomato plant growing in soil infested with R. solani in pot experiments. *Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.

Defense-related enzymes and induced resistance

Polyphenol oxidase activity (PPO)

Two fold increase of PPO activity was noticed relative to the control after treatment of tomato plants compared to positive control (Fig. 4A). Carbendazim ranked the first effect as it increased PPO activity by 65.3% compared to the positive control. The effect was significantly higher than NEs-citral (49.4%) and geraniol (28.85).

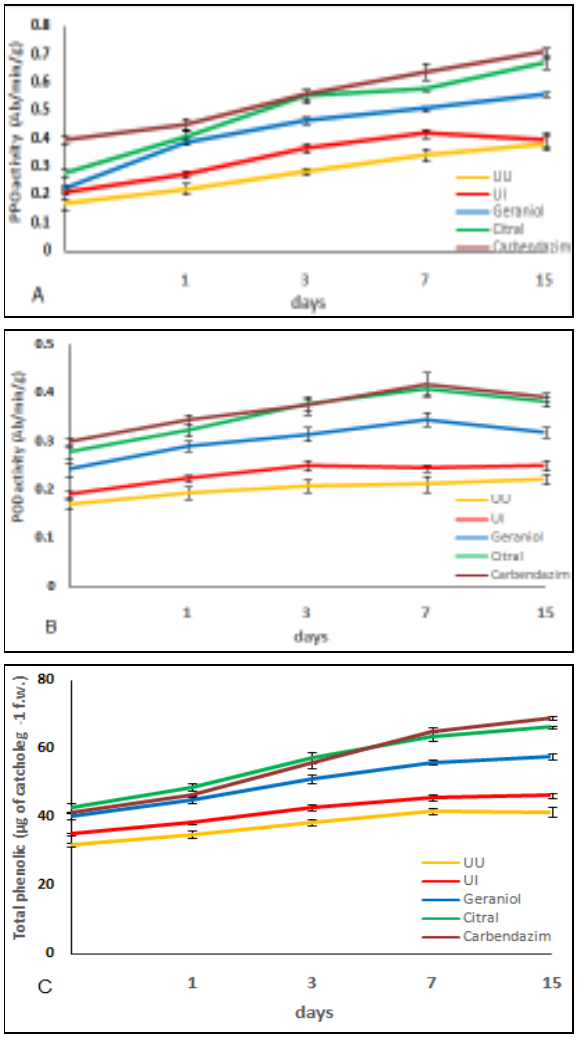

Figure 4. Polyphenol oxidase (PPO, A) , Peroxidase (POD, B) activity, and total phenolic content(C) in tomato plants post the inoculation with R. solani.

Peroxidase activity (POD)

Results in Fig.(4B) displayed that POD activity (OD min^{-1} g^{-1} FW) was increased up to 2-fold compared to the control. POD activity was significantly higher in induced plants compared to both negative control (UU) and positive control (UI). The highest POD enzyme activity was noticed after seven days after infection, and then declined. These findings agreed with those reported by Latha *et al.* (2009), who showed that the defense enzymes: POD, PPO in tomato plants recorded higher values after the treatment with *Alternaria solani* compared to control. The increase in the activities of such defensive oxidanting enzymes, and the accumulation of phenols were correlated with resistance to *Alternaria solani* and *Botrytis cinerea* fungi (Dixon and Paiva, 1995; Gill and Tuteja, 2010; Zhu *et al.*, 2010).

Total phenolic content in tomato plants

Data in Fig. (4C) revealed that the total phenolic content was significantly increased using the elicitors NEs-citral and geraniol compared with that found in negative (UU) and positive (UI) control. NEs-citral was the most effective, followed by geraniol . Results, also, revealed that total phenolic content was increased with time. Plant phenols are well-known as an antifungal, antibacterial and antiviral compounds that play an important role in determination resistance or susceptibility of a plant host to parasitic infection (Galeotti *et al*., 2008).

CONCLUSION

Evaluation the impacts of the fungicidal activity both of five normal and NEs-monoterpenes, geraniol, cinnamaldehyde, pulegone, carvone, and citral against three soil borne plant pathogenic fungi, *R. solani*, *F. oxysporum,* and *P. digitatum in vitro* and *in vivo*. Out of all the tested pure or nanemulsion monoterpenes, both citral and geraniol were the most potent antifungal, indicating that they are very helpful in plant protection, non-pollutant and environmental friendly alternative methods to fungicides. Finally, it could be concluded that to achieve a safe to control tomato damping-off disease, and application of citral and graniol as natural compounds are the best option.

Conflict of interest

None.

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مقارنة التأثيرات الأبادية الفطرية لبعض مركبات التربينات الاحادية النقية والنانوية على الممرضات النباتية الفطرية التى تصيب التربة

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تم مقارنة النشاط الابادى الفطرى بين المركبات النقية ومستحلبات النانو للتربينات الاحادية مثل الكارفون والسينامالدهيد والسيترال والجيرانيول والبوليجون على ثلاثة انواع من الممرضات النباتية الفطرية القاطنة بالتربة وهي *Rhizoctonia solani* و *Fusarium solani* و *Pythium digitatum* وذلك بطريقة تسمم البيئة الغذائية. وأشارت النتائج إلى أن كل مركبات التربينات الاحادية لها تاثير ابادى فطرى مع التركيزات المختلفة المختبرة. واظهرت النتائج بشكل ملحوظ ان مستخلصات النانو للتربينات الاحادية اعلى كفاءة ابادية فطرية عن التربينات الاحادية الاصلية لجميع مسببات الأمراض الفطرية التي تم اختبارها. وكان مستحلب النانو لكلا من مركب السترال والجيرانيول اعلى كفاءة على الثلاثة انواع من الفطريات المختبرة حيث كانت قيم EC_{50} (٣١.٣١ و٤٩.٤١ ميكروجرام/مل لفطر *R. solani* , ٤٨,٢١ و٥٤,١٦ ميكروجرام/مل لفطر *P. digitatum* و ٨٣.٦١ و ١٠٠.٧٥ ميكروجرام/مل لفطر *F. solani*) على التوالى. وتمت مقارنه التأثير الأبادى الفطرى بالمبيد الفطري الكيميائي (كاربيندازيم). وتم استخدام مستحلب النانو للسترال والجيرانيول بمعدل ١٠٠٠مجم/لتر كمستحثات دفاعية فعالة على نبات الطماطم المعدى بفطر *R. solani*. وتم قياس نشاط الانزيمات الدفاعية المتعلقة بالمقاومة مثل: بوليفينولوكسيداز (PPO) ، البيروكسيديز (POD) وكذلك محتوى الفينول الكلي في الأوراق بعد فترات زمنية (٠ ، ١ ، ٣ ، ٧ و ١٥) يومًا من التلقيح. وتشير النتائج عن زيادة معنوية فى نشاط انزيمات PPO و POD ومحتوى الفينول الكلي. وأظهرت النتائج أيضا أن أنزيمات POD و PPO يمكن اعتبارها علامات بيوكيميائية لتعكس طبيعة مقاومة نبات الطماطم ضد الفطر الممرض *R. solani*. ولقد أشارت النتائج إلى أن مستحلب النانو للسيترال والجيرانيول اظهر مقاومة مستحثة في نبات الطماطم المعدي بالفطريات الممرضة تحت الدراسة. وبناءا على النتائج المتحصل عليها يمكن القول ان مستحلب النانو للتربينات الاحادية فعالة وآمنة بيئياً لمكافحة الفطريات التي تصيب نبات الطماطم.