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METALAXYL NANOEMULSION: SYNTHESIS, CHARACTERIZATION AND ITS EFFICACY AGAINST PLANT PATHOGENIC FUNGI



Abd-Elghany, Islam K.F.*, Moustafa A. Abbassy, Entsar I. Rabea, Gehan I. Kh. Marei

Department of Plant Protection, Faculty of Agriculture, Damanhour University, Damanhour 22516, Egypt.

Corresponding Author: islamkhalied259@gmail.com

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ABSTRACT

At the global level, researchers are working on nanoemulsion and nanofungicides to control plant pathogenic fungi; however, farmers are unaware about these nano-based fungicides. Nanoemulsion can act as potential fungicide when compared to the conventional chemical fungicides. Hence, the current study aimed to the synthesis, characterization and study the efficacy of metalaxyl nanoemulsion to control plant pathogenic fungi. The metalaxyl nanoemulsion was characterized by SEM and Zetasizer Nano (ZS) analysis. The results of the study exhibited the metalaxyl nanoemulsion was of spherical shape with an average size of 76.25–104.61 nm. Further, the plant pathogenic fungi including Alternaria alternate, Fusarium oxysporum and Rhizoctonia stolonifer were reacted to metalaxyl nanoemulsion with different efficacies. It showed broad-spectrum antifungal activity against these phytopathogens by the inhibiting the radial growth and spore germination methods. Nanoemulsion of metalaxyl documented high antifungal activity ($EC_{50} = 464.42$ and 851.34 mg/L, respectively) for A. alternata and F. oxysporum. Wattable powder of metalaxyl gave moderate activity against A.

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alternata and *F. oxysporum* (EC₅₀=754.20 and 847.72 mg/L, respectively). While the nanoemulsion of metalaxyl showed high inhibition of spore germination (I (%) = 59.09 and 32.61, respectively) to *A. alternata* and *F. oxysporum*. Wattable powder of metalaxyl gave moderate activity with F. *oxysporum*. On the other hand, Wattable powder of metalaxyl recorded high inhibition of spore germination (I (%) = 38.78) for *R. stolonifer*.

Keywords: Characterizations, Nanoemulsion, Metalaxyl, Antifungal activity, Alternaria alternate, Fusarium oxysporum, Rhizoctonia stolonifer

INTRODUCTION

Fungal diseases cause severe damage to wide variety of crops. *Fusarium oxysporum* is one of the most important soilborne pathogen that causes wilt disease in cumin and geranium plants (Abdel-wahed, 2011). The pathogen has the ability to persist for very long periods in soil. Also, the pathogenic Alternaria fungi cause diseases of various crop plants and are present on all continents (Chase *et al.*, 2005; Perell and Sisterna, 2006). They also infect ornamental plants and fruit trees and shrubs (Andersson *et al.*, 2015). Rhizopus rot caused by *Rhizopus stolonifer* is one of the most important postharvest diseases of stone fruits (Ogawa, 1995). The disease occurs mainly on ripe fruit, which are more brone to wounds and have higher sugar content. After 2–3 days, infected fruits become soft and watery, and they release juices with a fermented or acidic odour.

In recent years, nanotechnological interventions to combat these plant pathogenic diseases have gained attention due to their small size and targeted delivery for sustainable production, crop protection and disease management (**Kalpana** *et al.*, **2010**).Diverse materials have been used alone or along with chemical fungicides, such as metal NPs, polymersmetal conjugates and biopolymers to improve effectiveness against disease

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and protect the chemical pesticides from heat, moisture and premature degradation (Dong *et al.*, 2019; Abd El-Wahab *et al.*, 2025).

Metalaxyl [methyl N-(2, 6-dimethylphenyl) -N- (methoxyacethyl)-DL-alaninate], a broad-spectrum fungicide is widely used to protect a wide range of crops (horticultural crops, vegetables and fruits) from damage by fungal diseases (damping-off, late blight, stem, downy mildew and fruit rots) (**Celis** *et al.*, **2015**). Due to the favourable physicochemical properties such as its nonvolatility and the excellent stability under different conditions of pH, temperature and light, metalaxyl has been broadly used all over the world including the US, Europe, Australia, Asia, Egypt and India (**Malhat**, **2017**). However, the good water solubility of metalaxyl might cause the permeation of metalaxyl into soil to result in potential toxicity by the rain-wash and irrigation (**Wilson** *et al.*, **2001**).

Wanyika (2013), loaded the fungicide metalaxyl onto MSNs and observed leaching in soil between free metalaxyl (76% release) and encapsulated metalaxyl (11.5%) within a period of 30 days. When tested in water, the encapsulated metalaxyl had an increased release rate of 47% compared to the 11.5% seen in the soil, highlighting the importance of testing within the farming environment. Campos *et al.*, (2015) used two different types of nanoparticles, solid lipid or polymeric, and tested the cytotoxicity of carbendazim and/or tebuconazole loaded onto the nanoparticles. A decrease in toxicity with the nanoparticle-loaded pesticides was observed in preosteoblast and fibroblast mouse cell lines. In the soil leaching experiments, the addition of nanoparticles decreased the release rate in soil layer release experiments when compared to the commercial formulation. Also, introduce the nanoformulations technology in pest management.

The objectives of the present study were to a) prepare and characterize the nanoformulation and b) evaluate the antifungal activity of metalaxyl nanoemulsion against some phytopathogenic fungi *A. alternate*, *F. oxysporum* and *R. stolonifer* and to compare efficacy of metalaxyl

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nanoemulsion with that of technical and wettable powder metalaxyl fungicide in prevention of phytopathogenic fungi *in vitro*.

MATERIAL AND METHODS

Fungicide and chemicals

The technical metalaxyl (95%) (Methyl 2-[*N*-(2,6-dimethylphenyl) (methoxy) acetamido] propanoate) was supplied by El-Hoda Company (Wadi El Natrun area, El-Beheira Governorate, Egypt). Tween 80 and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potato dextrose agar (PDA) was purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK).

Tested fungi

Phytopathogenic fungi, Alternaria alternate (Fries), Fusarium oxysporum (Schiech), and Rhizopus stolonifera (Ehren) were used in the present study. They obtained from Department of Plant Pathology, Faculty of Agriculture, Damanhour University, Damanhur, Egypt, and kept during the experiments on PDA medium at $27 \pm 2^{\circ}$ C.

Preparation of nanoemulsion containing fungicide

Nanoemulsion was prepared by the procedure reported by (**Sugumar** *et al.*, **2014**), with some modifications. The nanoemulsion was prepared in two phases. The coarse emulsion was prepared by stirring and then further emulsification using a high-energy ultrasonic process. First, 0.5% of the active ingredient (a.i w/v) of metalaxyl was dissolved in DMSO completely. Flowed by addition of the surfactant (tween 80 %) and mixed with water by stirring at 4000 rpm. The oil phase was added slowly into the aqueous phase with stirring at 4000 rpm for 30 min. The emulsion formed was then sonicated by ultrasonic probe, the tip of the horn was

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symmetrically placed in the coarse emulsion, and the process was carried out (15 min), power (75 % of Sonicator power (20 kHz)) and pulses or cycles (9 cycle/sec) to produce the nanoemulsion at 25°C (Figure 1) (Li and Chiang, 2012).



Fig. 1. Schematic illustration of the preparation of nanoemulsion containing metalaxyl.

Characterization of metalaxyl nanoemulsion

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) analysis was done by using a JEOL JSM-5410 (Japan) electron microscope with a W-source and operating at 80 kV. Sample was prepared on a glass slide $(1 \times 1 \text{ cm})$ after washing it with ethanol. A tiny drop of nanoemulsion was spread evenly over glass slide and allowed to air dry. In order to make it conductive, gold

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coating using the Jeol Quick Auto Coater was performed (JFC-1500). It was then subjected to SEM analysis under ambient conditions.

Particle size and polydispersity index (PDI)

The mean droplet size and polydispersity index (PDI) of nanoemulsion was performed by a dynamic light scattering method using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature. The nanoemulsion was diluted before measurement to 10% with deionized water to avoid multiple scattering effects. Emulsion droplet size was estimated by the average of three measurements and presented as mean diameter in nm. The higher the PDI value refers to the lower uniformity of globules size of nanoemulsion (**Tyagi** *et al.*, **2012**)

Nanoformulation stability tests

Centrifugation assay

The samples were centrifuged for 30 min at 5000 rpm and noticed phase separation, creaming and cracking. The nanoemulsion should have maximum stability, which is not a phase separation (creaming and cracking). Successful formulation exposed to other thermodynamic stability tests (**Kadhim and Abbas, 2015**). The measurements were performed in triplicate.

Freeze thaw cycle test

This test was carried out for the determination of the accelerated stability of the nanoemulsion. The formulation was subjected to two different temperatures (-21°C and 21°C.) for each temperature test of at least 24 h. The measurements were performed in triplicate (Kadhim and Abbas, 2015).

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Heating cooling cycle test

The radiation effect of heating and cooling on the stability of the prepared nanoemulsion was conducted. Where the prepared nanoemulsion was maintained at a temperature of 4°C and 40°C with storage for 48 h for each temperature test. (Kadhim and Abbas, 2015). The measurements were performed in triplicate.

Stability at temperature of 25°C

Twenty-five ml of the freshly prepared nanoemulsion was transferred into a glass tube. The transition from steady state to creaming and coalescence was examined during the storage period of 14 weeks (Badawy *et al.*, 2014).

Viscosity measurement

The dynamic (absolute) viscosity of the nanoemulsion was determined using a Rotary Myr VR 3000 digital viscometer with L1 and L4 spindles at 200 rpm at 25°C. The viscosity of the prepared nanoformulation was measured without further dilution. Each reading was taken after the equilibrium of the sample for two minutes as shown in **Figure 2**. The samples were repeated three times and the data expressed in mPa.s. (**Badawy** *et al.*, **2014**).

pH measurement

The digital pH meter (Mi 151 Martini Instruments, Model Mi 150, UK) was used to determine the pH values of the prepared nanoemulsion. The samples were repeated three times.



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Figure 2. The dynamic (absolute) viscosity of the nanoemulsion was determined using a Rotary Myr VR 3000 digital viscometer.

The antifungal activity of metalaxyl nanoemulsion against phytopathogenic fungi

The antifungal activity was tested by using the mycelia radial growth technique (**Badawy** *et al.*, **2014**). The nanoformulation of metalaxyl was dissolved in water and serial concentrations were tested. The aliquots of the stock solutions were added to the PDA medium and then transferred to Petri dishes. After solidification, the mixtures were inoculated with a 5 mm in diameter mycelium fungi (*A. alternate*, *F. oxysporium* and *R. stolonifer*) at the centre of Petri dishes and these were incubated in the dark at $27 \pm 2^{\circ}$ C. Fungal growth was measured when the control had grown to the edge of the plate. The inhibition of fungal growth was calculated as the percentage of inhibition of radial growth compared to the control. The effective concentration that inhibits 50% of mycelial growth (EC₅₀) for each compound was estimated by probit analysis (**Finney**, **1971**) using SPSS 26.0 software. The percentage of inhibition was calculated according to (**Topps and Wain**, **1957**) as follows:

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Where I (%): is the inhibition percent, A: is the diameter of untreated hyphal growth of fungus and B: is the diameter of treated hyphal growth of fungus.

The activity of metalaxyl nanoemulsion against Spore germination of phytopathogenic fungi

A. alternate, F. oxysporium and R. stolonifer spores were harvested from 2-weeks-old PDA culture grown under fluorescent lights in 9-cm diameter petri dishes at 26° C. An amount of 5 mL of sterile water was added to the petri plate culture. The spores were gently dislodged from the surface with a sterile glass rod and the suspension was filtered through three layers of cheesecloth to remove mycelia fragments. The suspension was diluted with sterile water to an absorbance of 0.25 at 425 nm as determined by a Unico 1200-Spectrophotometer. This suspension contained about 1.0×10^6 conidia/mL. Aliquots of 50 µL of a spore suspension were placed in Eppendorf tubes containing 500 µL of Potato Dextrose Broth (PDB) medium with a compound concentration. Preliminary screening tests were performed at concentrations of 500 and 750 mg/L. The tubes were incubated at $26^{\circ} \pm ??C$ during 24 h. The samples were placed on both chambers of a hemocytometer by carefully touching the edges of cover slip with the pipette tip and allowed capillary action to fill the counting chambers and observed under the microscope for spore germination. Spore counting was done using a Neubauer haemocytometer and light microscopy at 40x. All experiments were conducted in three replicates. A spore was considered germinated when the length of the germ tube equalled or exceeded the length of the spore. The numbers of germinated and non-germinated conidia were recorded and inhibition of spore germination (%) was calculated (Griffin, 1994).

Statistical analysis

Statistical analysis was done using the statistical package SPSS software version 26.0 (SPSS, Chicago, IL, USA). The log dose–response

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curves allowed determination of EC_{50} for the bioassays according to probit analysis (**Finney 1971**), the effective concentration causing a 50% of mortality (EC_{50}). The 95% CL and standard error for the range of EC_{50} values for the compound for assays on growth inhibition were determined by least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration. Statistical significance data was determined with one-way analysis of variance (ANOVA) by comparing means using SNK method at the probability of 0.05 **Steel and Torrie (1980)**.

RESULTS AND DISCUSSIONS

Characterization of metalaxyl nanoemulsion

Stability tests of nanoemulsion

The nanoemulsion was stable at centrifugation of 5000 rpm, heating cycle, and freeze-thaw cycle for 4 weeks. This formulation was not observed in any phase separation. Nanoemulsion was thermodynamically stable systems and formed at a particular composition of oil, surfactant, and water with no separation, creaming, cracking, or coalescence can be seen. Centrifugation can accelerate the rate of sedimentation or incineration, demonstrating that the degradation of an emulsion may be related to the action of the gravitational force (**Tadros** *et al.*, **2004**). These tests were performed to confirm from the stability, low surfactant formulation with a nanoemulsion size droplet and stable physicochemical properties.

Viscosity and pH

It has been measured the viscosity to ensure the better delivery of the formulation, so it has been recorded the viscosity value of metalaxyl nanoemulsion with 39 mPa.s. Viscosity highly influenced by several factors such as disperse phase, volume fraction, colloidal interactions,

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droplet size, archeology of component phases, and droplet charge (McClements, 2015).

SEM estimation

SEM studied the morphology and shape of the nano-metalaxyl and the data are presented in **Figure 3**. The morphology of the nanoemulsion is highly variable, with spherical and occasionally triangular nanoemulsion observed in the micrograph, with a size range of 76 to 104.61 nm. These results agree with (Leng *et al.*, 2012) recorded the particles were circular and well distributed, which was in line with the typical microscopic structure of microemulsion. The particles remained stable but their diameter was becoming smaller after being diluted for 50 times. (Elsharkawy *et al.*, 2022) revealed that the Lambda-cyhalothrin nanoemulsions (LCNs) morphology is nearly spherical and has an average size of 70.3 nm in diameter.



Figure 3. Scanning electron micrograph of prepared metalaxyl nanoemulsion. The SEM was performed on a JEOL JSM-1200EX II scanning electron microscope operating at an acceleration voltage of 25.0 kV.



Figure 4: A typical particle size distribution by a dynamic light scattering of the formulated metalaxyl nanoemulsion.

Antifungal activity of metalaxyl nanoemulsion against A. alternata, F. oxysporum and R. stolonifer

The antifungal activity of metalaxyl fungicide with different formulations against A. alternata, F. oxysporum and R. stolonifer are presented in Table (1) as EC_{50} and 95% Confidence limits. The results indicated that nanoemulsion of metalaxyl was the highest potent (EC₅₀ = 464.42 and 808.34 mg/L, respectively) for A. alternata and F. oxysporum. Wetable powder of metalaxyl gave uppermost effective against R. stolonifer (EC₅₀ = 343.31 mg/L). On the other hand, the technical of metalaxyl was the lowest active one against all tested fungi (Figures 4-6). Hassanin et al. (2017) observed that all essential oil nanoemulsions exhibited higher activities against sporulation than the respective essential oil emulsions. Also, Yao et al. (2018) found that the azoxystrobin nanosuspension reduced the defensive antioxidant capability of F. oxysporum and resulted in the generation of excessive reactive oxygen species. (Ziedan et al., 2022) summarized that foliar application of nanoemulsion of clove and black seeds is more promising than fungicides in controlling gray mold on cucumber fruits caused by Botrytis cinerea in plastic greenhouses with no phytotoxicity on cucumber plants.

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Table 1. Antifungal activity of technical, WP, and nanoemulsion formulations of metalaxyl against *Alternaria alternata*, *Fusarium oxysporum* and *Rhizoctonia stolonifera*

Treatment	EC ₅₀ ^a (mg/L)	95% Confidence limits		Slope ^b	Intercept ^c	(X) ^{2d}	
		Lower	Upper	±SE	± SE	()	
Rhizopus stolonifera							
WP	343.31	223.35	444.04	2.31 ±0.35	-5.85 ±1.2	1.39	
NE	918.34	250.87	1663.89	2.24±0.23	-6.65 ±0.76	5.89	
Т	1633.12	643.15	2887.01	3.1 ± 0.34	-9.23 ± 1.01	4.73	
Fusarium oxysporum							
WP	847.72	338.98	1364.75	3.64 ±0.41	-10.65±1.28	3.85	
NE	808.34	712.12	989.03	2.19±0.23	-6.43 ±0.72	2.82	
Т	3854.52	1926.68	6099.30	1.41 ±0.22	-5.05 ±0.70	3.91	
Alternaria alternata							
WP	754.20	327.26	1141.59	2.73±0.28	-7.86 ± 0.83	4.37	
NE	464.42	347.49	567.02	2.31±0.29	-6.17 ±0.87	1.39	
Т	6712.11	4392.57	15156	1.25 ±0.23	-4.78 ±0.73	1.71	

^aThe concentration causing 50% mycelial growth inhibition. ^b Slope of the concentration-inhibition regression line \pm standard error. ^c Intercept of the regression line \pm standard error. ^d Chi square value. WP: Wattable powder, NE: Nanoemulsion and T: Technical.

Figure 5: Fungicidal activity of the technical, WP, and nanoemulsion of metalaxyl against *A.alternata*

Figure 6: Fungicidal activity of the technical, WP, and nanoemulsion of metalaxyl against *F. oxysporum*

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Figure 7: Fungicidal activity of the technical, WP, and nanoemulsion of metalaxyl against *R. stolonifer* (from right to left 0, 500, 1000, 2000, and 4000 mg/L). A: wattable powder; B: technical and C: nano

Two formulations of azoxystrobin + metalaxyl-M, Premium© 39.1% SC and Uniform© 44.5% SE were evaluated *in vitro* by food poisoning technique against *F. solani*, *F. oxysporum*, *R. solani* and *S. rolfsii* (Azab, 2022). Data indicated that IC₅₀ (mg/L) of Premium was 32.1, 2.5, 1215.5 and 15.2, as well as for Uniform was 40.6, 5.4, 84.4 and 80.7 on *F. solani*, *F. oxysporum*, *R. solani*, *S. rolfsii*, respectively. The obtained results showed that the efficiency of the Premium against *F. solani*, *F. oxysporum* and *S. rolfsii* was significantly more toxic than Uniform, while *R. solani* was significantly more sensitive to Uniform although both formulations have the same active ingredient. It may be concluded that the difference in the efficacy may be due to the difference in the type of the formulation between SC and SE.

The efficacy of different fungicides i.e. metalaxyl+mancozeb, copper oxychloride, benalaxyl+mancozeb, carbendazim and mancozeb at different concentrations against the fusarium wilt of tomato caused by *F. oxysporum*

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f. sp. lycopersici and also to observe the impacts of fungicides on plant height and yield under tunnel condition was conducted (**Baloch** *et al.*, **2021**). The result revealed that copper oxychloride was significantly effective in all its doses to control the Fusarium wilt. The most effective dose was 3 g/l where the disease severity was recorded 6.2 % only, followed by metalaxyl+mancozeb (4 g/l) in which the disease severity was recorded 9.6 %. Other fungicides also showed good result but mancozeb alone was not effective, however it had synergistic effect and could be used as basis with the other product to control the fusarium wilt. Two of the fungicides proved to be the most appropriate chemicals having no severe impacts on plant height and yield. The highest plant height was recorded 3.97 and 3.67 kg in case of copper oxychloride and metalaxyl+mancozeb respectively.

In vitro efficacy of five fungicides; success, copper oxychloride, metalaxyl+mancozeb, topsin M and kumulus against *A. alternata* were tested by using food poisoning technique (**Asim** *et al.*, **2019**). All the fungicides significantly inhibited the mycelial growth of *A. alternata*. Among those, metalaxyl+mancozeb was the most effective as compared to others. Maximum inhibition was observed after 3rd (89%), 5th (91.6%) and 7th (93.3) day by metalaxyl+mancozeb followed by success 43.3, 41.0 and 29.6% respectively. After 3rd and 5th day copper oxychloride was least effective with 19.6 and 9.6% mycelial inhibition respectively while after 7th day the minimum 4.3% mycelial inhibition was observed by topsin M instead of copper oxychloride. Therefore, *A. alternata* is responsible for alternaria leaf spot disease of rose and metalaxyl+mancozeb was found to be the most effective fungicide against *A. alternata* in vitro.

Effect of technical, WP, and nanoemulsion of metalaxyl on spore germination of A. alternata, F. oxysporum and R. stolonifer

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Table 2. Effect of the Technical, WP, and nanoemulsion of metalaxyl on spore germination of *A. alternate, F. oxysporum* and *R. stolonifer*.

Treatments	Concentration (mg/L)	Inhibition of spore germination (%) ± SE				
Alternaria alternata						
Control	0.00	$2.27^{a} \pm 1.31$				
WP	500	$48.86^{cde} \pm 1.14$				
	750	$59.09^{\rm ef}\pm0.00$				
NE	500	$40.91^{\circ} \pm 3.71$				
	750	$51.14^{cde} \pm 2.86$				
Т	500	$23.86^{\text{b}} \pm 2.86$				
	750	$28.41^{b} \pm 2.86$				
Fusarium oxysporum						
Control	0.00	$2.17^{\mathrm{a}} \pm 1.26$				
WP	500	$34.78^{de} \pm 3.55$				
	750	$47.83^{\rm f} \pm 3.55$				
NE	500	$32.61^{cde} \pm 4.44$				
	750	$32.61^{cde} \pm 4.44$				
Т	500	$13.04^{b} \pm 1.77$				
	750	$32.61^{cde}\pm2.66$				
Rhizopus stolonifera						
Control	0.00	$0.00^{a} \pm 4.30$				
WP	500	$36.73^{def} \pm 2.15$				
	750	$38.78^{ef}\pm5.37$				
NE	500	$28.57^{cd} \pm 2.15$				
	750	$34.69^{de} \pm 3.22$				
Т	500	$16.84b \pm 0.54$				
	750	$26.53^{\circ} \pm 3.22$				

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Data are average of four replicates \pm SE. Values within a column bearing the same letter are not significantly different ($P \le 0.05$) according to Student-Newman-Keuls (SNK) test

Inhibition of spore germination of the technical, WP, and nanoemulsion of metalaxyl against *A. alternata, F. oxysporum* and *R. stolonifer* are presented in **Table (2)**. The results showed that the technical metalaxyl was the highest potent in inhabiting spore germination of all tested fungi. Followed in descending order *by* nanoemulsion of metalaxyl. On the other hand, the WP of metalaxyl was the lowest active one against all tested fungi.

CONCLUSION

The nanoemulsion of metalaxyl was successfully prepared and characterized. The biological activity data reported that all formulations showed a significant inhibitory effect on the tested fungi compared to the control. The current study suggests that nanoemulsion of metalaxyl can be used for controlling some of plant pathogens that cause destruction of crops and vegetables instead of current harmful fungicides. However, this kind of compound is worthy further studies.

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الملخص العربى

مستحلب النانو لمبيد الميتالاكسيل: التركيب والوصف والفاعليه ضد الفطريات المسببة لأمراض النبات

اسلام خالد فرج عبدالغني ، مصطفى عبداللطيف عباسي ، انتصار ابر اهيم محمود ربيع ، جيهان ابر اهيم خليل مر عي

قسم وقابة النبات، كلبة الزراعة، جامعة دمنهور، دمنهور 22516، مصر

على المستوى العالمي، يعمل الباحثون على المستحلب النانوي للمبيدات الفطرية لمكافحة الفطريات المسببة للأمراض النباتية. حيث يعمل المستحلب النانوي كمبيد فطريات محتمل عند مقارنته بالمبيدات الفطرية الكيميائية التقليدية. وبالتالي، تهدف الدر اسَّة الحالية إلى تخليق وتوصيف ودراسة فاعلية مستحلب النانو لمبيد الميتالاكسيل لمكافحة الفطريات المسببة للأمراض النباتية. تم توصيف مستحلب النانو لمبيد الميتالاكسيل من خلال تحليل المجهر الإلكتروني الماسح وZetasizer Nano (ZS). أظهرت نتائج الدراسة أن مستحلب النانو لمبيد الميتالاكسيل كان كرويًا بمتوسط حجم 104.61-76.25 نانومتر. علاوة على ذلك، تم تثبيط الفطريات المسببة للأمراض النباتية وهي A. alternata و F. oxysporum و R. stolonifer عند المعاملة بمستحلب النانو لمبيد الميتالاكسيل بدرجات متفاوتة. وقد أظهر مستحلب النانو لمبيد الميتالاكسيل نشاطًا مضادًا للفطريات واسع النطاق من خلال تثبيط النمو الهيفي و إنبات الجراثيم. وقد سجل مستحلب النانو لمبيد الميتالاكسيل نشاطًا مضادًا عاليَّ (EC50 = 464.42 و 851.34 مجم / لتر، على التوالي) لـ A. alternata و F. oxysporum. أعطى مسحوق واتبل من ميتالاكسيل نشاطًا معتدلاً ضد A. alternata و E. oxysporum (EC50 = 754.20 و 847.72 مجم / لتر، على التوالي). بينما أظهر مستحلب النانو من لمبيد الميتالاكسيل تشيطًا عاليًا لإنبات الجراثيم ((%) I 59.09 = و 32.61، على التوالي) لـ A. alternata و F. oxysporum. أعطى مسحوق WP من ميتالاكسيل نشاطًا معتدلاً مع F. oxysporum. ومن ناحية أخرى، سجل مسحوق WP من الميتالاكسيل تثبيطًا عاليًا لإنبات الجر اثيم (I (%) = 38.78) لـ R. stolonifer.

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