Journal of Plant Protection and Pathology

Journal homepage & Available online at: www.jppp.journals.ekb.eg

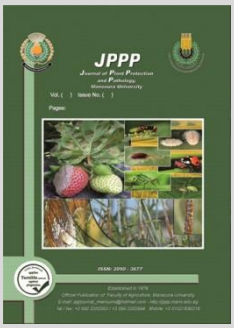
Comparison Between the Effect of Mayonnaise and Some Nanoparticles (NPs) Formulations of Flaxseed oil Against *Alternaria solani* that Infects Tomato Plant

Azza R. Emara[1]; Nosa S. Abd Elattif[2] and Kh. S. Ahmed[* 3]

[1]Bactericide & Fungicide and Nematicide Research Department, Central Agricultural Pesticide Lab. (CAPL), Agricultural Research Center (ARC), Dokki, Giza, Egypt

[2] Formulation Research, Department, Central Agricultural Pesticide Lab. (CAPL), Agricultural Research Center (ARC), Dokki, Giza, Egypt;

[3]Pesticide Residues and Environmental Pollution Research Department, Central Agricultural Pesticide Lab. (CAPL), Agricultural Research Center (ARC), Dokki, Giza, Egypt.



ABSTRACT

The evaluation of the effectiveness of flax oil; mayonnaise oil formulation; AgNPs@oil and CuNPs@oil nanoemulsions against *Alternaria solani* that infects tomato plant. Flax oil was formulated as a mayonnaise formulation (43%). The local formulation overrun all of the laboratory investigations of mayonnaise oil formulation. Preparation of flax oil was AgNPs@oil and CuNPs@oil nanoemulsions. Using transmission electron microscope (TEM) to detect the size, shape and structure in both AgNPs@oil and CuNPs@oil nano-emulsions was conducted. *In vitro* tests, CuNPs@oil nanoemulsion gave the complete inhibition in mycelium growth occurred for *A. solani* at concentration of 200 ppm. Under the greenhouse, azoxystrobin 25% SC was the best, which reduced disease severity at 7, 14 and 21 days after transplanting followed by CuNPs@oil; AgNPs@oil, mayonnaise oil formulation and flax oil. The CuNPs@oil and AgNPs@oil showed high level of peroxidase (PO) and polyphenoloxidase (PPO) compared with the control. Therefore, it can be concluded that CuNPs@oil, AgNPs@oil nanoemulsions, mayonnaise oil formulation and flax oil might have antifungal activity against *A. solani*.

Keywords: Tomato; *A. solani*; flax oil; formulation; nanoparticles.

INTRODUCTION

Tomato (*Solanum lycopersicum*) family Solanaceae is the second most important vegetable crops in the world (Panno *et al.*, 2021). Fungi, *Alternaria solani* causes severe tomato yield and quality losses in Egypt's governorates (Shoaib *et al*., 2019). The application of the synthetic fungicides has led to the deterioration of environmental quality and human health (Pathak *et al.*, 2022).

Medicinal plants once served as the source of all drugs as well as antibacterial; antifungal; insecticidal and antioxidant (Barbosa, 2014 and Behiry *et al.*, 2020).

Flaxseed contains about 36–40% of fixed oil as a rich source of fatty acids, phenolic compounds, peptides, glycosides, alkaloids, polysaccharides, proteins, and its secondary metabolites, which possess several biological activities (Chauhan *et al.* 2015 and Fadzir *et al.*, 2018). Flax oil was applied for antifungal and insecticidal substance (Sharil *et al*., 2022).

Nanotechnology is an innovative methodology that has been widely used in applications to various biological, pharmacy and medical fields (Torabian *et al.*, 2018).

Nanotechnology is being used to develop a more efficient agrochemical industry (Donsì *et al.*, 2011; Gupta and Xie, 2018). The use of nanoparticles (NPs) as soil-borne disease control gives an effective results (Pavoni *et al.*, 2020 and Kutawa *et al.*, 2021).

The present study aimed to prepare mayonnaise flax oil formulation; flax oil nanoemulsion, and evaluate their efficacy against *A. solani* that infects tomato plant.

MATERIALS AND METHODS

1. Source of plant.

Tomato (*Solanum lycopersicum*, cultivar Sara Star) seedling were obtained from Unit of Vegetable Crops, Horticulture Research Institute, Agricultural Research Centre (ARC), Giza, Egypt.

2. Source of oil.

Commercially available flax oil (also known as linseed oil) (*Linum usitatissimum*) was purchased from a local distributor in Taif, Kingdom of Saudi Arabia. The oil was obtained as cold-pressed flax oil.

3. Inoculum preparation.

Old cultures of *A. solani* growing for 7 days using petri dishes with a diameter of 9 cm containing a potato decetrose agar (PDA) medium at 25 ± 2˚C were used to make the inoculum. The mycelial growth was repeatedly rinsed using 50 ml sterile distilled water and ground using ceramic mortar then filtered, the spore's suspension was collected and the concentration was set at 5×10^5 spore/ml. (Derbalah *et al.*, 2018).

4. Fungicide used.

Azo Star 25% SC (Azoxystrobin) was obtained from Central Agricultural Pesticide Lab. (CAPL), Agricultural

* **Corresponding author.**
E-mail address: khal20024sh8@gmail.com
DOI: 10.21608/jppp.2024.336840.1283

Research Centre (ARC), Dokki, Giza, Egypt and used at rate of 50 cm/ 100L.

5. Chemicals.

Surface active agents; silver nitrate ($AgNO_3$); copper sulfate ($CuSO_4$); glucose ($C_6 H_{12} O_6$) and starch ($C_6 H_{10} O_5$) were obtained from EL-Gomhoria Company.

6. Physico-chemical properties of formulation constituents.

Active ingredient.

- Free acidity or alkalinity conducted to: the procedure outlined by WHO standards (1979) were determined.
- Solubility: To ascertain the total solubility or miscibility of one gram of active ingredient (a.i) at 20˚C, the volumes of distilled water, acetone, ethanol, dimethyl formamide (DMF), and xylene were measured (Nelson and Fiero, 1954). The following formula was used to get the percentage of solubility:

% of solubility = W/V x 100

Where, **W means weight of active ingredient, and V means the volume of solvent required for complete solubility.**

Surface active agents.

- Hydrophilic-lipophilic balance (HLB): was conducted according to Lynch and Griffin method (1974). The hydrophilic-lipophilic balance (HLB) of the surfactant was approximated by its solubility.
- Critical micelle concentration (CMC) is the concentration at which an increase in surfactant concentration does not cause the surface tension of the solution to decrease. The method outlined by Osipow (1964) was used to determine the CMC of the evaluated surfactants.
- Free acidity or alkalinity was determined as earlier.
- Surface tension was calculated following ASTM D-1331 2001 using a Du-Nouy tensiometer for solutions containing 0.5% (W/V) surfactant.

7. Flax oil prepared mayonnaise concentrate formulation.

The following procedures physicochemical characteristics for mayonnaise formulations:

- Cold stability test: was developed in compliance with FAO/WHO (2010) MT 39.3.
- Emulsion stability test: was followed according the method of FAO/WHO (2010) MT 36.3.
- Accelerated storage was decided: according to CIPAC MT46 (2007).

Spray solution at field dilution rate.

The spray solution flax oil (mayonnaise) formulation's physicochemical characteristics were ascertained as follows:

- Electrical Conductivity (EC): was calculated using the Cole-Parmer PH/Conductivity meter 1484-44, (Dobrat and Martijn, 1995), the unit of electrical conductivity readings was µmhos.
- Surface tension: was decided as previously stated.
- pH was measured: according to Dobrat and Martijn (1995). It was ascertained using the Cole-Parmer pH conductivity meter serial No. 1484-44.
- Viscosity was assayed: according to ASTM D-2196 (2005), the Brookfield viscometer Model DVII+Pro was used to determine it. The unit of measurement was centipoises.

8. Preparation of flax oil nanoparticles.

Synthesis of nanoencapsulation.

The starch-$AgNO_3$ (AgNPs@oil) and starch–$CuSO_4$ (CuNPs@oil) encapsulation of oil was synthesized according to (Nnemeka *et al.,* 2016). The reduction of $AgNO_3$ or $CuSO_4$ was induced by glucose is as follows: add 1% (0.06M) $AgNO_3$ or $CuSO_4$ to glucose solution (0.2 M) in a flask containing starch dispersion (1%), then 30 ml of oil was added. The mixture was stirred and heated at 40 ºC for 3 hrs. The mixture was cooled and centrifuged at 10000 rpm/20 min. The precipitate was mixed with 30ml of acetone, re-centrifuged at 6000 rpm/5 min then the centrifuged pellets were dried at 40 ^{O}C for 24hrs. The nanocomposite was kept in a dark bottle until characterized.

9. Characterization of nanoencapsulation.

Transmission electron microscope (TEM) was used to detect the size and shape of the particles. The flax oil, AgNPs@oil, CuNPs@oil encapsulations and mayonnaise oil formulation were investigated using IR-spectroscopy.

The analysis by UV-visible spectrophotomerter.

The nanoparticles (NPs) were detected using spectrophotometer at range, 200-400 nm (Instrument Cary 5000, Instrument Version 1.12, Software Version: 3.00, 182) at Mammalian & Toxicology Department, Central Agricultural Pesticide Laboratory (CAPL), Dokki, Giza, Egypt, Hassanin *et al.* (2017).

Image by Transmission Electron Microscopy (TEM).

The micrographs were obtained using a high-resolution (HR-TEM) (FEI TECNAI 02), software TECNAI G2 in the National Research Centre, Dokki, Giza, Egypt.

The analysis by Fourier transform-infrared (FTIR).

The spectra were registered within the 400 - 4000 cm^{-1} range in the Central Agricultural Pesticide Lab (CAPL), Dokki, Giza, Egypt.

10. Bioassay *in vitro* test.

Efficacy of flax oil (emulsion was obtained 0.05% Tween-80 v : v) and mayonnaise oil formulation at concentrations (125, 250, 500, 1000 and 2000 ppm), AgNPs@oil and CuNPs@oil at concentrations (12.5, 25, 50, 100, and 200 ppm), against was conducted *A. solani* according to (Mohanty *et al.,* 2012). Added separate concentrations were adusted at 50 ml of potato decetrose agar (PDA) medium, with respect to control PDA medium (without treatment). Then, the samples were inoculated at the center with a mycelial disc 5-mm taken from the margins of 7 days old *A. solni*. Petri- dishes were incubated at 25±2 °C. The colony diameter was measured until the mycelia were fully covered in the check. The percentage of inhibition mycelial was calculated as following equation: (Satya *et al.,* 2014).

$$I\,\% = \frac{R - r}{R} \times 100$$

Where, **R is diameter of the mycelia growth in the control, and r is diameter of the mycelia growth in the treatment.Lpd line program was used to calculate median effective EC_{50} and nightly effective concentrations EC_{90} values according to Finney (1971)**.

11. Greenhouse experiments.

In this experiment, an evaluation of the flax oil (emulsion was obtained 0.05% Tween-80 v : v), mayonnaise oil formulation at three concentrations of 500, 1000, and 2000 ppm was conducted. While, AgNPs@oil and CuNPs@oil nanoparticles at the three concentrations of 50, 100, and 200 ppm were conducted. A zoxystrobin fungicide 25% SC was used as a reference control (positive) examined against *A. solani*. This study was conducted under the greenhouse of the Agricultural Research Center (A.R.C.).

Tomato seedlings cultivar Sara Star (approximately 10 cm tall) were transplanted into plastic pots (30 cm diameter) containing sterilized sandy-clay soil (1:1w/w) using three seedlings/pot. All treatments were applied to tomato plants at four weeks old (3 pots/ treatment). After 24hrs of treatment, the plants were inoculated with mycelial suspension which was sprayed using an atomizer (50 ml/pot) except un-inoculated control. Three replicates were used for each isolate. Clear plastic bags were positioned above the inoculated plants for 24hrs to maintain high relative humidity and promote fungal infection (Derbalah *et al.*, 2018). All pots were arranged in a randomized block design. Disease severity (DS) was estimated at 7, 14 and 21 days after transplanting, the numerical rates of disease symptoms were recorded by Crowe and Hall (1980) using the following equation:

$$DS\% = \frac{DS\ of\ control - DS\ of\ treat.}{DS\ of\ control} \times 100$$

The DS was recorded using the following scale: 0=without clear symptoms; 1=lesions cover the plant with 1-25%/plant; 2=lesions cover the plant with 26-50%/plant; 3=lesions cover the plant with 51-75%/plant; and 4=lesions cover the plant with 76-100%/plant (Cohen *et al.*, 1991).

12. Enzyme activities.

Antagonists affect enzyme activities after 7 days of fungal inoculation in the leaves according to Maxwell and Bateman (1967).

Polyphenol oxidase (PPO) assay.

Polyphenol oxidase was determined according to El-Batal *et al.* (2016).

Peroxidase (PO) assay.

Peroxidase activity was determined according to Srivastava (1987).

13. Statistical analysis.

Data were analyzed by (ANOVA) with SAS. The least significant difference (LSD) was 0.05 confidence level.

RESULTS AND DISCUSSION

1. Preparation of Flax oil as Mayonnaise 43% concentrate formulation.

When selecting a pesticide formulation, several elements need to be taken into account. These include whether the formed product will provide effective control, the feasibility of applying a particular formulation in a specific place to control the target pest, and the risks and advantages associated with the options available (Fishel, 2010).

2. Physicochemical properties of Flax oil as active ingredient.

The physicochemical characteristics of flax oil as an active ingredient are displayed in Table (1). It demonstrated total solubility in 100% xylene but no solubility in water or acetone was observed. However, when expressed as a proportion of sulfuric acid, flax oil exhibited a small free acidity (0.098), indicating that it is acidic in nature. These findings demonstrated that it might be expressed as a concentration of mayonnaise.

The physical and chemical properties of the active ingredient largely determine the kind of additives that should be used in this formulation as well as the kind of formulation on which it may be formulated (Abd-Alla and Hamouda, 2021).

Table 1. Physico-chemical properties of flax oil as an active ingredient.

Solubility % (w/v)			Free acidity as % H_2SO_4
Water	Acetone	Xylene	
N.S	N.S	33.3	0.098

N.S*: means insoluble.

As listed in Table (2), surfactant B showed medium solubility in water and complete solubility in DMF, acetone, and xylene, respectively, while surfactant A showed total insolubility in both aqueous and organic solvents. Surfactant C showed full solubility in DMF, acetone, and xylene, as well as the observed degree of solubility in water. All studied surfactants had HLB values of more than 13, and surfactants A, B, and C had relative critical micelle concentrations of 0.3, 0.2, and 0.5, respectively. Furthermore, their respective surface tension values of 50, 36, and 39.2 dyne/cm were lower than those of water, which had a surface tension of 72 dyne/cm. Surfactants B and C showed an acidic nature based on their respective free acidity values of 0.49 and 0.196, which were determined as sulfuric acid percentages. Surfactant A only showed an alkaline property based on its free alkalinity through the assay of sodium hydroxide percentage (2.94). The physicochemical characteristics of the surface-active agents under investigation were carefully assessed to determine surfactant would work best with the active ingredient during the formulation processes Hamouda *et al.*, (2019).

Table 2. Phsico-chemical properties of the tested surface active agents.

Surface active agents	Solubility % (w/v)				HLB	CMC	Surface tension (Dyne/cm)	Free alkalinity as NaOH %	Free acidity as H_2SO_4 %
	Water	DMF	Acetone	Xylene					
Surfactant A	N.S*	N.S	N.S	N.S	> 13	0.3	50	2.94	-
Surfactant B	50	100	100	100	> 13	0.2	36	-	0.49
Surfactant C	20	100	100	100	> 13	0.5	39.2	-	0.196

N.S*: means insoluble

3. Physico-chemical properties of local flax oil 43% mayonnaise formulation before and after hot storage.

The impact of both hot and cold storage conditions on the developed local formulation is displayed in Table (3). The formulation's characteristics before and after storage did not vary in any way. Both processes of the storage passed the mayonnaise test. Additionally, they maintained its free acidity. This suggests that both hot and cold storage conditions can preserve the formulation's qualities (Farag *et al.*, 2021).

Table3. Physico-chemical properties of flax oil 43% mayonnaise formulation before and after hot storage.

Before storage			After storage		
Emulsion stability		Free acidity as H_2SO_4	Emulsion stability		Free acidity as H_2SO_4
Hard pass	Soft pass	0.098	Hard pass	Soft pass	0.098

4. Physico-chemical properties of the spray solution of the formulation of flax oil at field dilution rate.

The physical characteristics of the spray solution at a field dilution rate of 0.5% are displayed in Table (4). In

addition to having a high electrical conductivity (680 µmhos) and viscosity (16.4 cm/poise), the spray solution had an alkaline pH of 8.95 and a low surface tension (52.3 dyne/cm). According to El-Sisi *et al.* (2011), the spray solution's low pH and strong electrical conductivity would cause the insecticide to deionize, increase its deposits, and penetrate the treated surface. All of which would boost the pesticidal efficacy. The low value of surface tension of the spray solution can increase in the spreading on the treated surface with a consequence increase in pesticidal efficacy as stated by Perira *et al.* (2016). Contrary to surface tension, the increase in viscosity can result in an increase in pesticide efficacy through reduction of drift and retention sticking Spanoghe *et al.* (2007).

Table 4. Physico-chemical properties of spray solution at field dilution rate.

Surface tension dyne/cm	Viscosity cm/poise	Conductivity µ-mhos	pH
52.3	16.4	680	8.95

5. UV-visible Absorption Spectroscopy.

Fig. (1) indicates the confirmayion of silver nanoparticles loaded oil (AgNPs@oil) and copper nanoparticles loaded oil (CuNPs@oil) as indicated in UV-visible spectra of the prepared (AgNPs@oil) and (CuNPs@oil) in wavelengths 430 and 424 nm respectively, which due to the excitation of surface plasmon resonance of Ag and Cu. That has been reported to describe the collective excitation of conduction electrons in a metal (Ramakrishna *et al.*, 2012 and Hamedi *et al.*, 2012).

Nnemeka *et al.* (2016) found that, the first confirmation of silver nanodichlorvos and nanochlorpyrifos on spectroscopy. The report describes the collective excitation of conduction electrons in a metal.

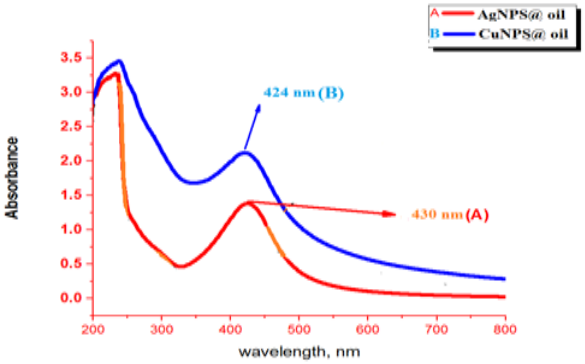
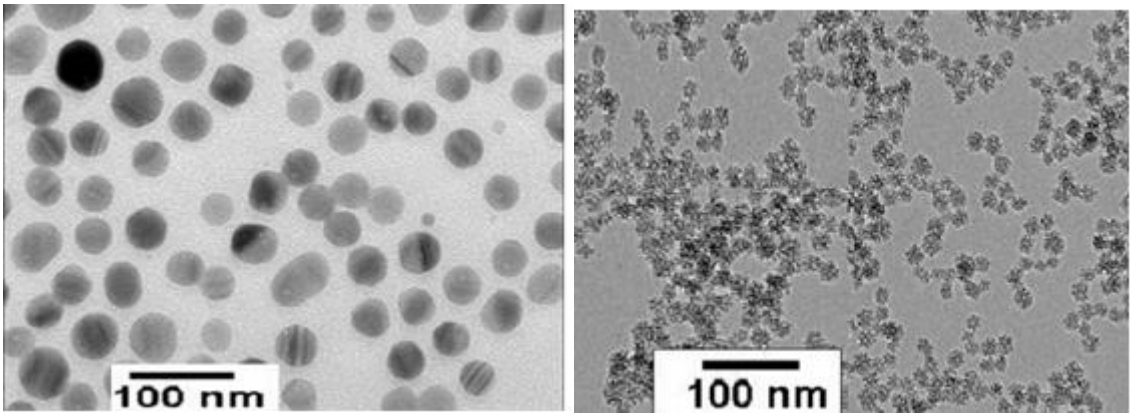


Fig. 1. The spectrum of (AgNPS@oil) & (CuNPS@oil).

6. Transmission Electron Microscopy (TEM).

Fig. (2) shows the shape and size of the Ag and Cu colloid particles by TEM imaging. The size (diameter) of a great number of the silver nanoparticles loaded oil (AgNPs@oil) measured on the TEM images ranged from 9.42 to 33.62 nm (Fig.2A), while the size of copper nanoparticles loaded oil (CuNPs@oil) ranged from 4.19 to 29.27 nm (Fig.2B), with mostly spherical shape. The change of color is retained to by the effect of agglomeration, as reported by (Hamedi *et al.*, 2012 and Mohamed *et al.*, 2012). The TEM determines the morphology of nanoparticles as spherical. The same results were obtained by (Nnemeka *et al.*, 2016; Hammad and Hassanin 2022). The HR-TEM image of the nano-dichlorvos revealed spherical particles with an average size range of 23–30 nm, with an average particle size of 23.2 nm.



A **B**

Fig. 2. The transmission electron micrographic image of AgNPs@oil (A) and CuNPs@oil (B).

7. Fourier Transform Infrared Spectroscopy (FT-IR).

Fig. (3) illustrates the FTIR spectroscopy of both AgNPs@oil nanoemulsion (A), CuNPs@oil nanoparticles (B), mayonnaise oil formulation (C), flax oil (D):

AgNPs@oil (A) appeared peaks at 3911.0, 3896.8, 3853.4, 3795.5, 3769.1, 3703.5, 3619.3, 3357.5 and 2035.6 cm^{-1}; while peaks disappeared at 3808.5, 3660.3, 3638.2 and 1945.9 cm^{-1}. The peaks increased in bond length at 1643.5 cm^{-1}, all compared to oil.

CuNPs@oil (B) appeared peak at 3885.3, 3834.6, 3790.8, 3759.7, 3732.9, 3686.5, 3649.4, 2029.3 and 1644.9 cm^{-1}; while the peaks disappeared at 1945.9 and 1652.9 cm^{-1}. The peak at 1644.9 cm^{-1} increased in bond length, this all compared to oil.

Formulation (C) appeared peaks at 3949.5, 3878.7, 3745.9, 3729.6, 3717.4 and 3334.2 cm^{-1} and peaks disappeared at 3660.3 and 3638.2 cm^{-1}. The peaks decreased in bond length at 3009.5, 2923.4 and 2853.5 cm^{-1}, while the peak at 1652.9 increased in bond length and moved to 1641.9 cm^{-1}, this all compared to flax oil.

The O–H stretching of broad and strong bands at 3416.4 and 3416.0 cm-^{1} vibration for profenofos and

AgNPs@P (Khaled *et al.,* 2019). Also, it's appeared peaks at 2960.26 and 2927.63 cm^{-1} corresponding to the methylene groups for profenofos, while disappeared these peaks and appeared peak at 2360.98 cm^{-1} in case of profenofos nanoparticle . This may be indication for nitrile group in linkage with AgNPs (Abouelkassem *et al.,* 2016).

In the region 1600-1400 $cm^{-1.}$ the profenofos and nano- profenofos showed these bands at 1618.20 and 1637.58 cm^{-1} and 1618.37 and 1637.58 cm^{-1}, respectively (Abouelkassem *et al.,* 2016). While, AgNPs@P was attached to the functional groups that presented in starch. (Hamedi *et al.,* 2012).

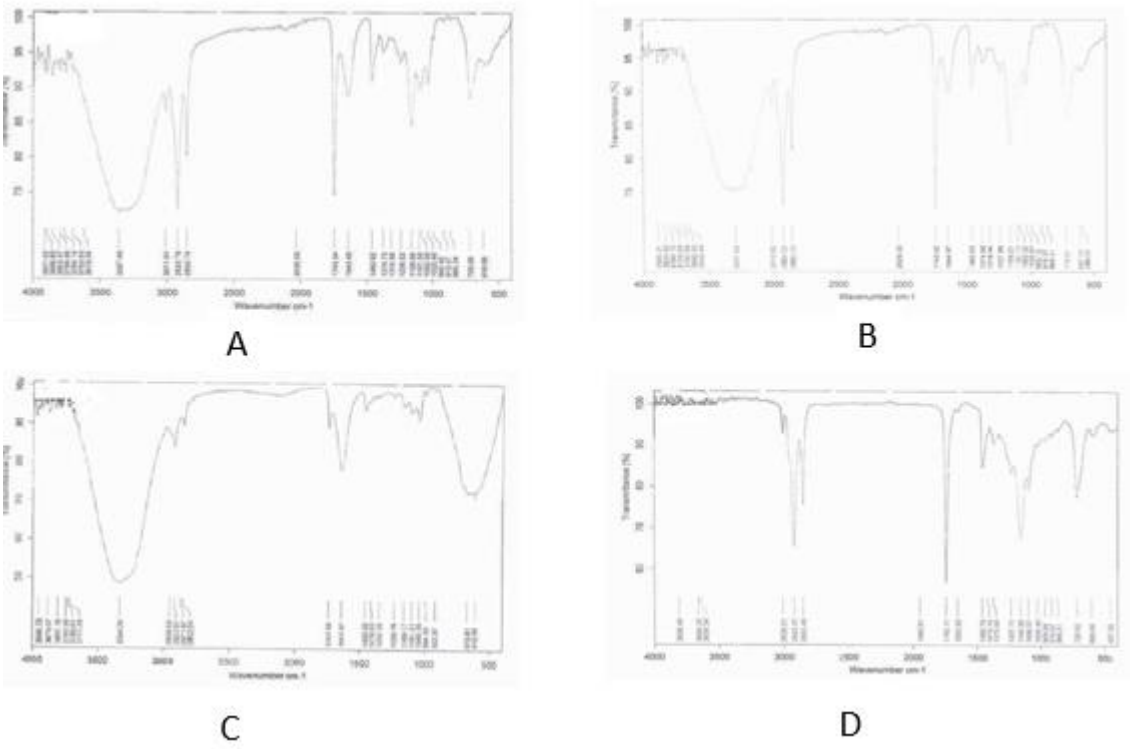


Fig. 3. FT-IR spectra for AgNPs@oil (A), CuNPs@oil (B), Mayonnaise oil formulation (C) and flax oil (D).

8. Evaluation effect of flax oil and its mayonnaise oil formulation AgNPs@oil; CuNPs@oil nanoparticles on *A. solani in-vitro* test.

Table (5) shows the effect of flax oil and mayonnaise oil formulation against the tested *A. solani* under laboratory conditions. Flax oil and its mayonnaise oil formulation had an inhibiting impact on the linear growth fungal mycelial at all tested concentrations. Mayonnaise oil formulation gave the inhibition percent (83.1%) for *A. solani* at concentration of 2000 ppm.

Table 5. Effect of flax oil and mayonnaise oil formulation on *A. solani in-vitro* test.

	FO		MOF	
Conc. (ppm)	**Linear growth (mm)**	**Mycelial growth inhibition (%)**	**Linear growth (mm)**	**Mycelial growth inhibition (%)**
125	77.5	12.5	65.7	24.3
250	69.7	20.7	56.4	33.6
500	54.1	35.9	41.7	48.3
1000	44.4	45.6	26.3	63.7
2000	33.7	56.3	6.9	83.1
Control	90.0	0.0	90.0	0.0
EC50		483.04		20.68
EC90		4338.91		366.59
Slope		1.34 ±0.15		1.30 ±0.15

FO: Flax oil; MOF: Mayonnaise oil formulation; ppm: parts per million Control was: (PDA medium without any component)

While, flax oil gave the inhibition percent (56.3%) at the same concentration. The results indicated that the *A. solani* showed the response to mayonnaise oil formulation (EC_{50} values 20.68 ppm), greater than that of flax oil (483.04 ppm). The increased effectiveness of the formulation may be due to the serves active agents that used in the formulation. These results showed that the flax oil reduced the growth of fungal mycelial. This finding is in accordance with that previously obtained by (Guilloux *et al.,* 2009; Raouf and Mir, 2023) who showed that the bioactive compounds in flax oil were effective against *A. alternata*; *A. solani; Penicillium chrysogenum,* and *F. graminearum* under *in-vitro* conditions. Zuk *et al.* (2011) explained that the flax seed has many antimicrobial agents, including flavonoids.

The results showed that there is relationship between tested concentrations of AgNPs@oil and CuNPs@oil nanoparticles of flax oil and its inhibition percentages on fungi growth are displayed in Table (6). CuNPs@oil nanoemulsion gave the complete inhibition in mycelium of *A. solani* at concentration of 200 ppm. While AgNPs@oil nanoemulsion gave the inhibition percent (74.3%) for the same fungi.

It was obtained that *A. solani* was greatest responded to CuNPs@oil nanoemulsion followed by AgNPs@oil nanoemulsion (EC_{50} 3.85 and 9.921 ppm), respectively. Such finding is in accordance with those of Raouf and Mir (2023) who showed that the effect of bioactive compounds nano-emulsion is due to the particle size and their stability. It was clear that nano-emulsions of flax oil at all concentrations were higher antifungal impact on mycelial growth than mayonnaise flax oil formulation.

Table 6.Effect of AgNPs@oil and CuNPs@oil nanoparticles of flax oil on *A. solani in vitro* test.

Conc. (ppm)	AgNPs@oil		CuNPs@oil	
	Linear growth (mm)	Mycelial growth inhibition (%)	Linear growth (mm)	Mycelial growth inhibition (%)
12.5	90.0	NE	36.1	53.1
25	69.5	20.8	28.1	61.9
50	57.5	32.5	18.3	71.3
100	36.2	58.8	9.9	80.1
200	25.7	74.3	0.0	100.0
Control	90.0	0.0	90.0	0.0
EC_{50}		9.921		3.85
EC_{90}		87.031		40.42
Slope		1.30± 0.2		1.28± 0.11

NE: no effect

9. Evaluation of effects of flax oil and its mayonnaise oil formulation AgNPs@oil; CuNPs@oil nanoparticles on disease severity in leaves of tomato under greenhouse conditions.

Table (7) shows that all treatments had significant reduction in disease severity (DS %) compared to untreated control. Azoxystrobin was the best one, which reduced disease severity to 16.24, 21.20 and 21.56 % after 7, 14 and 21 days, respectively of fungal inoculation. Regarding CuNPs@oil; AgNPs@oil nanoparticles concentration 200 ppm reduced disease severity to 25.72, 30.15 and 41.20 % and 31.57, 37.31 and 47.53%, after 7, 14 and 21 days, respectively, of fungal inoculation. Mayonnaise oil formulation at concentration of 2000 ppm recorded in the next order, followed by flax oil reduced disease severity to 45.76, 50.82 and 56.24 % at 7, 14 and 21 days, respectively, of fungal inoculation.

Table 7. Effect of different treatments on disease severity in leaves of tomato under greenhouse conditions.

Treatments	Conc. (ppm)	Disease severity (%)		
		7^{th} day	14^{th} day	21^{th} day
Flax oil	500	53.20	60.33	68.75
	1000	49.51	54.10	59.13
	2000	45.76	50.82	56.24
Mayonnaise oil formulation	500	46.35	51.32	63.12
	1000	44.13	48.61	59.51
	2000	40.61	45.73	54.30
AgNPs@oil nanoemulsion	50	41.32	46.22	52.34
	100	38.21	42.54	50.11
	200	31.57	37.31	47.53
CuNPs@oil nanoemulsion	50	30.18	36.46	49.23
	100	28.60	33.42	46.13
	200	25.72	30.15	41.20
Azoxystrobin		16.24	21.20	21.56
Infected control		68.13	81.32	92.41
Untreated control		0.0	0.0	0.0
L.S.D (0.05)		4.732	4.960	4.231

10. Enzymes activities.

Results in Table (8) show that the treated tomato leaves had higher levels of peroxidase (PO) and polyphenol oxidase (PPO) activities than untreated ones. However, plants treated with CuNPs@oil nanoemulsion showed the highest activity of peroxidase and polyphenol oxidase, recording 4.495, 1.280 (U/ml), respectively, followed by AgNPs@oil nanoemulsion, (4.38 and 0.874 U/ml), respectively. While mayonnaise oil formulation recorded the activities: 4.159 and 0.532 (U/ml), respectively. Flax oil recorded 4.103 and 0.500 (U/ml) compared to control group.

Table 8. Effect of different treatments on peroxidase, polyphenol oxidase enzymes in leaves of tomato after 7 days fungal inoculation under greenhouse conditions.

Treatments	(Activity enzyme/ml)	
	Peroxidase (PO)	Polyphenol oxidase (PPO)
Flax oil	4.103	0.500
Mayonnaise oil formulation	4.159	0.532
AgNPs@oil nanoemulsion	4.385	0.874
CuNPs@oil nanoemulsion	4.495	1.280
Azoxystrobin	4.076	0.501
Control	4.016	0.498
L.S.D at 0.05	0.459	0.251

CONCLUSION

The results of persent study revealed the high antagonistic effect of flax oil, mayonnaise oil formulation, AgNPs@oil and CuNPs@oil nanoparticles against *A. solani* that infected tomato plant under laboratory and greenhouse. These findings support the possibility to use such substances, where they more potent as antifungal against *A. solani*, with respect to recommended synthetic compounds.

REFERENCES

Abd-alla, H. I. & Hamouda, S. E. S. (2021). Study of potential activity of clove oil 10 % emulsifiable concentrate formulation on Two-spotted spider Mite Tetranychus urticae Koch (Acari: Tetranychidae), *Journal of Applied and Natural Science*, 13(4): 1414 - 1419. https://doi.org/10.31018/jans.v13i4.3124

Abouelkassem S., Ola M. El-Borady and Mona B. M., Remakable (2016). Enhancement of Cyhalothrin upon Loading into Silver Nanoparticles as Larvicidal. International Journal of Contemporary Applied Sciences Vol. 3, No. 1.

ASTM (2001). American Society of Testing Materials. Standard Test Method for Surface and Interfacial Tension of Solution D-1331.

Barbosa, A. D. (2014). An overview on the biological and pharmacological activities of saponins. Int. J. Pharm Pharm Sci, Vol 6, Isse 8, 47- 50.

Behiry, S. I.; Nasser, R. A.; Abd Elkareem, M. S. M.; Ali, H. M. and Salem, M. Z. M. (2020). Mass spectroscopic analysis, MNDO quantum chemical studies and antifungal activity of essential and recovered oil constituents of lemon-scented gun against three common molds. Processes, 8(3)1-24.

Chauhan, R.; Chester, K.; Khan, Y.; Tamboli, E. T. and Ahmad, S. (2015). Characterization of *Linum usitatissimum* L. oil obtained from different extraction technique and in vitro antioxidant potential of supercritical fluid extract. Journal of Pharmacy and Bio Allied Science 7(4), 284 – 288.

CIPAC MT 46 (2007). Collaborative International Pesticides Analytical Council. Physicochemical Methods for Technical and Formulated Pesticides, Printed in Great Britain by the Block Boar Press LTD. Kings Hedg-es Cambridge CB492, England.

Cohen, Y., Gisi, U., & Mosinger, E. (1991). Systemic resistance of potato plants against *Phytophthora infestans* induced by unsaturated fatty acids. Physiological and Molecular Plant Pathology, *38*(4): 255 – 263.

Crowe, F. J. and Hall, H. D. (1980). Soil temperature and moisture effects on Sclerotium germination and infection of onion seedlings by *Sclerotium cepivorum*. Phytopathol., 70: 74 -78.

Derbalah, A., Shenashen, M., Hamza, A., Mohamed, A., & El Safty, S. (2018). Antifungal activity of fabricated mesoporous silica nanoparticles against early blight of tomato. Egyptian journal of basic and applied sciences, 5(2):145-150.

Dobrat W. and Martijn A. (1995). CIPAC Hand Book, Vol. F, Collaborative International Pesticides Analytical Council Limited.

Donsì, F., Annunziata, M., Sessa, M., and Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT-Food Sci. Technol.* 44, 1908–1914. doi: 10.1016/ j.lwt. 2011.03.003.

El-Batal, A. I.; N. M. Sidkey; A. A. Ismail; R. A. Arafa and R.M. Fathy (2016). Impact of silver and selenium nanoparticles synthesized by gamma irradiation and their physiological response on early blight disease of potato. J. Chem. Pharm. Res; 8(4), 934-951.

El-Sisi A., El-Mageed A., El-Asawi T. F. & El-Sharkawy R. (2011). Improvement the physico-chemical properties and efficiency of some insecticides formulation by using adju-vants against cotton laefworm *Spodoptera littoralis (BOISD.),* Journal of Plant Protection and Pathology, 2 (8), 757-764.

Fadzir, U. A.; Darnis, D. S.; Mustafa, B. E. and Mokhtar, K. I. (2018). *Linum usitatissimum* as an antimicrobial agent and a potential natural healer: A review, Archives of Orofacial Science 13(2), 55 – 62.

FAO/WHO (2010). Manual on Development and Use of FAO and WHO Specifications for Pesticides, 1st Ed. 3rd Rev. FAO Plant Production and Protection, FAO, Rome.

Farag E. M., Sahar A. Attia, Abd-Elattif, N. S. & Hamouda, S. E. S. (2021). Palm oil formulation as 34 % mayonnaise and evaluation of its biological efficacy against itrus mealybug, Planococcus citri under laboratory and field conditions, Journal of Applied and Natural Science, 15(2): 783 – 79.

Finney, D. J. (1971). Probit analysis 3rd ed. Cambridige Univ. Press, London.

Fishel F. M. (2010). Pesticide Formulations UF/IFAS EDIS Publication PI231. http://edis.ifas.ufl.edu/pi081

Guilloux K, Gaillard I, Courtois J, Courtois B and Petit E. (2009). Production of arabinoxylan oligosaccharides from flaxseed (*Linum usitatissimum*). J Agric Food Chem 57 (23): 11308-11313. DOI: 10.1021/jf902212z.

Gupta R & Xie H. (2018). Nanoparticles in daily life: applications, toxicity and regulations. *J Environ Pathol Toxicol Oncol.* 37: 209–30.

Hamedi S, Masumeh S, Shojaosadati S and Soheila S (2012). Comparative study on silver nanoparticles properties produced by green methods. Iran J Biotechnol 10(3):191–197

Hammad, E. A., & Hasanin, M. M. H. (2022). Antagonistic effect of nanoemulsions of some essential oils against *Fusarium oxysporum* and root-knot nematode *Meloidogyne javanica* on coleus plants. Pakistan Journal of Nematology, 40(1): 35-48.

Hamouda, S. E. S., Abd-Elattif, N. S. & Abd-Alla, H. I. (2019) Nematicidal Efficiency of 10 % Emulsifiable Concentrate Formulation of Propolis on Root-Knot Nematode *Meloidogyne Spp*., International Journal for Research in Agricultural and Food Science, 5(6): 24 - 34.

Hassanin, M. M. H., Abd-El-Sayed, M. A., & Abdallah, M. A. (2017). Antifungal activity of some essential oil emulsions and nanoemulsions against *Fusarium oxysporum* pathogen affecting cumin and geranium plants. Scientific Journal of Flowers and Ornamental Plants, 4(3): 245-258.

Khaled S. Ahmed; Wafai Z. A. Mikhail; Hassan M. Sobhy; Eman Mohamed Mostafa Radwan; Taher Salah El Din and Ahmed M. Youssef (2019). Effect of *Lambda-Cyhalothrin* as Nanopesticide on Cotton Leafworm, *Spodoptera littoralis* (Boisd.). Egyptian Journal of Chemistry. Egypt.J.Chem. Vol. 62, No. 7. pp.1- 14.

Kutawa, A. B., Ahmad, K., Ali, A., Hussein, M. Z., Abdul Wahab, M. A. and Adamu, A., *et al*. (2021). Trends in nanotechnology and its potentialities to control plant pathogenic fungi: a review. Biology 10:881. doi: 10.3390/biology10090881.

Lynch M. I. and Griffin W. C. (1974). Food Emulsions in: Emulsion Technology, by Lissant K. J., Marcell Decker, Inc., New York.

Maxwell, D. P. and Bateman, F. D. (1967). Changes in the activities of some oxidase in extracts of Rhizoctonia - infected have been hypocotyls in retation to Pesion motuation. Phytopathol,75 : 132 -136.

Mohamed M. B., Adbel-Ghani N. T., El-Borady O. M. and El-Sayed M. A.(2012). 5-Fluorouracil Induces Plasmonic Coupling in Gold Nanospheres: New Generation of Chemotherapeutic Agents. *J. of Nanomedicine & Nanotechnology*, 3, 7, 146-153.

Mohanty, R. C.; Ray, P. and Rath, S. (2012). In vitro antifungal efficacy study of plant leaf extracts against three dermatophytes CIB Tech Journal of Microbiology 1(2-3), 27-32.

Nelson F. G. & Fiero G. W. (1954). A selected Aromatic Fraction Naturally Occurring in Petroleum as Insecticides Solvents. *J. Agric. Food Chem.*,4:735-737.

Nnemeka E. I., Rufus S., Terrumun A. T., Lami A. N., Philip B., Bertrand S. and Malik M. (2016). Facile formulation of starch–silver-nanoparticle encapsulated dichlorvos and chlorpyrifos for enhanced insecticide delivery. *New J. Chem.*, 40, 1777-1784.

Osipow L. I. (1964). Surface Chemistry Theory and Application. Reinhold Publishing Crop, New York, Pp. 4736 – 4739.

Panno S, Davino S, Caruso AG, Bertacca S, Crnogorac A, Mandi´c A, Noris E and Mati´c S (2021). A review of the most common and economically important diseases that undermine the cultivation of tomato crop in the Mediterranean Basin. Agronomy 11:2188.

Pathak, V.M.; Verma, V.K.; Rawat, B.S.; Kaur, B.; Babu, N.; Sharma and A. *et al.* (2022). Current status of pesticide effects on environment, human health and i' eco-friendly management as bioremediation: A comprehensive review. Frontiers in Microbiology. pp. 2833.

Pavoni, L., Perinelli, D. R., Bonacucina, G., Cespi, M., and Palmieri, G. F. (2020). An overview of micro-and nanoemulsions as vehicles for essential oils: formulation, preparation and stability. Nanomaterials 10:135. doi: 10.3390/nano10010135.

Pereira V. J., da Cunha, J. P. A. R., de Morais, T. P., de Oliveira, J. P. R., & de Morais, J. B. (2016). Physical-chemical properties of pesticides: concepts, applications, and interactions with the environment. *Bioscience Journal*, *32* (3), 627-641.

Ramakrishna V., Rima P. and Apurba K. and Nanomater (2012). Green Synthesis of Silver Nanoparticles and the Study of Optical Properties. Nanomaterials and Nanotechnology 2(1):1-6. DOI: 10.5772/52329 LicenseCC BY 4.0.

Raouf, A. MIR and MIR, KH. A. (2023). Fungicidal effect of flax seed oil against several fungal strains. Asian J. Nat. Prod. Biochem. 21 (1): 1-5.

Satya, P. R.; S. P. Manisha and S. Khushboo (2014). Evaluation of the antifungal activity of the potent fraction of hexane extract obtained from the bark of Acacia nilotica. International Journal of Science and Research) IJSR) vol 3 issue (10) 730- 738.

Sharil ATM, Ezzat MB, Widya L, Nurhakim MHA, Hikmah ARN, Zafira ZN and Haris MS. (2022). Systematic review of flaxseed (*Linum usitatissimum* L.) extract and formulation in wound healing. J Pharm Pharmacogn Res 10 (1): 1-12. DOI: 10.56499/jppres21.1125 (10)1.1.

Shoaib A, Awan ZA and Khan KA. (2019). Intervention of antagonistic bacteria as a potential inducer of disease resistance in tomato to mitigate early blight. Scientia Horticulturae, 252, 20-28.

Spanoghe P. D., Schampheleire, M., Van Dermeeren P and Steurbaut W. (2007). Influence of Agricultural Adjuvants on Droplet Spectra; Pest Management Science. 63(1): 416.

Srivastava, S. K. (1987). Peroxidase and Polyphynol oxidase in Brassica juncea plants infected with *Maerophomina phaseolina* (Tassi. Gold.) and their implication in disease resistance phytopathol, 120: 249-254.

Torabian S, Farhangi-Abriz S and Zahedi M (2018) Efficacy of $FeSO_4$ nano formulations on osmolytes and antioxidative enzymes of sunflower under salt stress. Indian J Plant Physiol 23:305–315.

World Health Organization, WHO (1979). Specification of Pesticides Used in Public Health, 5th Ed. Geneva.

Zuk M, Kulma A and Dymińska L *et al.* (2011). Flavonoid engineering of flax potentiates its biotechnological application. BMC Biotechnol 11: 10. DOI: 10.1186/1472-6750-11-10.

مقارنة تأثير مستحضرالمايونيز وبعض الجسيمات النانوية لزيت بذور الكتان ضد *Alternaria solani* الذي يصيب نبات الطماطم

عزة رسمي عمارة [1] ، نوسه شعبان عبد اللطيف [2] وخالد سيد أحمد[3]

[1] قسم بحوث المبيدات الفطرية والبكتيريه والنيماتودية - المعمل المركزى للمبيدات، مركز البحوث الزراعية، الدقي، الجيزة، مصر.

[2] قسم بحوث مستحضرات المبيدات - المعمل المركزى للمبيدات، مركز البحوث الزراعية، الدقي، الجيزة، مصر.

[3] قسم بحوث متبقيات المبيدات وتلوث البيئة – المعمل المركزي للمبيدات ، مركز البحوث الزراعية، الجيزة، مصر.

الملخص

تهدف هذه الدراسة إلى تقييم فعالية زيت الكتان ومستخلص المايونيز للزيت والجسيمات النانوية AgNPs@oil وCuNPs@oil ضد فطر *A. solani* فى الطماطم. تم تحضير زيت الكتان كمستحضرمايونيز (43٪). اجتاز المستحضر المحلي جميع الخصائص الفيزيائية والكيميائية بنجاح. تم تحضير زيت الكتان باستخدام الجسيمات النانوية AgNPs@oil و CuNPs@oil. أظهرت الصور التي تم الحصول عليها عن طريق المجهر الإلكتروني النافذ الثبات للجسيمات النانوية لزيت الكتان أنه فى شكل كروي في الغالب. تراوح حجم الجسيمات النانويه للزيت بين 36.0 و51.3 نانومتر، وبين 25.1 و44.6 نانومتر في حالة الفضة النانوية وجسمات النحاس النانوية.فى الاختبارات المعملية، أعطت الجسيمات النانوية CuNPs@oil تثبيطًا كاملاً لنمو ميسليوم *A. solani* عند تركيز 200 جزء في المليون.أجريت تجربة تحت ظروف الصوبة كان Azoxystrobin 25% SC هو الأفضل، أحدث إنخفاض لشدة الإصابة عند 7، 14 و 21 يوم من تاريخ التلقيح الفطري يليه الجسيمات النانوية CuNPs@oil ثم مستحضر زيت المايونيز وزيت الكتان. من ناحية أخرى، كان للجسيمات النانوية CuNPs@oil و AgNPs@oil نشاط فائق في زيادة أنزيم البيروكسيديز والبولي فينولواكسيديز مقارنتا بالمعاملات الأخرى. لذلك، يمكن اقتراح أنه يمكن استخدام هذه الجسيمات النانوية ومستحضر المايونيز لزيت الكتان كمركبات فعالة ضد فطر: