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Study the Efficacy of Formulated Malic Acid Alone and its Mixture with Thiourea against *Fusarium oxysporium* in *Cucumis sativus* Plants

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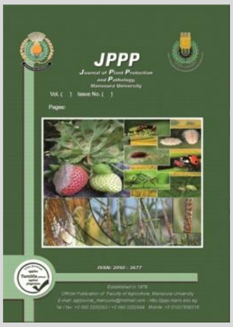

ABSTRACT

Malic acid lowers the cytoplasmic pH of fungi and inhibits their ability to reproduce, it also encourages plant growth. Physico-chemical properties of malic acid, thiourea, and malic acid mixture with thiourea as an active ingredient were measured, then prepared as soluble powder formulation and passed successfully all specified tests. The efficacy of malic acid, thiourea, and the mixture of malic acid with thiourea (active ingredient) was tested against *F. oxysporum* under laboratory conditions, and their inhibition percent of mycelial growth values were: 49.6, 72.4, and 75.7% respectively at 2000 ppm, while their formulations (SP) displayed higher inhibition of mycelial growth and its values were: 86.9, 83.7 and 61.7% for malic acid thiourea mixture (SP), thiourea (SP) and malic acid (SP) at 2000 ppm respectively. A greenhouse experiment was done and the results revealed that malic acid with all tested concentrations decreased significantly the percentage of disease incidence and increased the percentages of plant survival followed by a mixture of malic acid with thiourea (SP) and thiourea (SP), where the efficacy values were: 94, 92 and 84% at 4000 ppm respectively, against fusarium wilt on cucumber plants. Malic acid (SP) at 4000 ppm displayed the highest plant promotion shown clearly in the results of parameters measured. Finally from the previous results malic acid (SP) could be used as safe alternative of chemical fungicides against fusarium wilt on cucumber plants after complete the needed studies.

Keywords: Malic acid, thiourea, *Fusarium oxysporium*.

INTRODUCTION

Wilt diseases in cucumber (*Cucumis sativus*) are caused by a *Fusarium oxysporum* and obstruct the tissues that conduct water, leading to a decrease in the yield and quality (Zhai *et al*., 2021 and Min *et al*., 2024). *Fusarium* wilt diseases can inhibit photosynthetic capacity, chlorophyll content, and photosynthesis-related enzyme activity (Fernandez-Martinez *et al*., 2013). Excessive use of chemical fungicides puts the environment, and animal and human health at risk. Additionally, many of these chemicals are too costly for low-income farmers. (Shabana *et al*., 2004). In recent years, the trend has been to use safe alternative compounds such as organic acids that use fungicidal, bactericidal and nematicidal activities (Shemshura *et al*.,2016), and they can control plant diseases such as Pythium root, Phytophthora root rot and Rhioctonia attenuation diseasess (Bonanomi *et al*., 2010).

Malic acid is an organic acid that contains two carboxyl groups that have been shown to improve the stability of medicinal formulations. (Schiff, 2006). When malic acid was administered simultaneously with copper sulfate, the two displayed synergistic nematicidal activity (Eloh *et al*., 2016, Yeon *et al*., 2019).

Malic acid stimulates plant growth by boosting chlorophyll content, reducing stress-induced damage to photosynthetic structures, and increasing plant biomass (Chen *et al*., 2020). Additionally, fungal cytoplasmic pH can be directly lowered by organic acid, which similarly inhibits cell viability. The antifungal properties of malic, lactic, and citric acids considerably reduced the growth of *F. oxysporum* mycelium. (El-Abeid, *et al*., 2020).

Thiourea, a molecule that contains sulfur and nitrogen and has improved water solubility and absorption potential, is known to provide tolerance against common biotic stressors (Dhikwal, *et al*., 2012).

Thiourea has strong antifungal activities that are comparable to the activity observed for the common antifungal Moreover, they have antibacterial and insecticidal properties (zhimei, 2008).

The majority of technical pesticides are prepared in advance of usage by combining active compounds with adjuvants, diluents, preservatives, and inert to create a product that is safe, easy to handle and apply, has a good shelf life, and doesn't have any unfavorable side effects (Dipak and Purkait 2019).

The present study aimed to prepare malic acid; thiourea and its mixture as soluble powder formulations and evaluate their efficacy against *F. oxysporum* which causes wilt disease in cucumber plants.

MATERIALS AND METHODS

1. Chemicals.

- Malic acid, (dicarboxylic acid with a molecular formula $C_4H_6O_5$) and thiourea, an organosulfur compound with the formula ($SC(NH_2)_2$).

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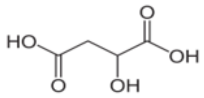
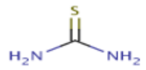


Malic acid



Thiourea

- Surface active agents: Tween 20, and 80, sodium dodecyl sulfate (SDS), polyethylene glycol 600 mono-laurate (P.E.G.600 ml). Solvents: acetone, xylene, ethanol, and dimethylformamide (DMF), all chemicals were supplied by EL-Gomhoria Co., Cairo, Egypt.

Fungicide used.

Uniform 390 SE (azoxystrobin, and mefenoxam) was used at 650 cm^3/ Feddan.

Source of Plant.

Cucumber (*Cucumis sativus* cultivar Madrid hybrid) seedlings were obtained from the Unit of Vegetable Crops, Horticulture Research Institute, Agricultural Research Centre (ARC), Giza, Egypt

Physico-chemical properties of all formulation components.

Active ingredient.

- Solubility: It was determined according to (Nelson & Fiero, 1954).
- Free acidity or alkalinity: This was determined by using the same methods outlined in the WHO recommendation (1979).

Surface active agents.

- Surface tension: According to ASTMD-1331 (2001), it was ascertained by employing a Du-Nouy tensiometer for solutions containing 0.5% (W/V) surfactant.
- Hydrophilic-lipophilic balance (HLB): A surfactant's water solubility is thought to be a rough indicator of its hydrophilic-lipophilic balance (Lynch and Griffin, 1974).
- Critical micelle concentration (CMC): The method outlined by (Osipow, 1964) was used to determine the critical micelle concentration (CMC) of the tested surfactants at which the surface tension of the solution doesn't decrease with further increase in surfactant concentration.
- Free acidity or alkalinity: It was determined as mentioned before.

Locally prepared malic acid, thiourea, and its mixture as 90 % (SP) soluble powder formulation.

Soluble powder formulations are the simplest formulation form, where it is cheap, easy to produce and no solvents are used so it is less hazardous to the environment, after determining the physico-chemical characteristics of the active ingredient and surface-active agents. Several trials were conducted by mixing different ratios of the active ingredients (malic acid, thiourea, and its mixture) and surface active agents, then, the solubility, surface tension, foam, pH, and free alkalinity or acidity were measured to determine which formulas passed the reported tests and were appropriate for use.(Hamouda, *et al.,* 2022).

Physico-chemical properties of prepared malic acid, thiourea, and its mixture as 90 %(SP) before and after accelerated storage at 54±2 ºC.

Solubility, Foam, pH, and Free acidity/ alkalinity were determined as mentioned before.

Physico-chemical characteristics of locally prepared formulations spray solution at a field dilution rate (0.5 %).

- Surface tension and viscosity were determined as mentioned before.
- Electrical Conductivity and Salinity: The Cole-Parmer H/Conductivity meter 1484-44 was utilized to determine it, with µmhos serving as the unit of measurement for electrical conductivity, as per Dobrat and Martijn (1995).

Inoculum preparation.

An old culture of *F. oxysporum* growing for 7 days using Petri dishes with a diameter of 9 cm containing a potato dextrose agar (PDA) medium at 25± 2 °C was used to make the inoculums. It was supplied from the Bactericide & Fungicide and Nematicide Research Department, Central Agricultural Pesticide Laboratory (CAPL), Agricultural Research Centre (ARC), Dokki, Giza, Egypt. Five-mm discs were cut from a seven days old active growing culture of *F. oxysporum* using a sterile cork borer, and inoculated into a flask containing sterilized a corn sand meal medium (50 g sand + 100 g corn + 100 ml dist. water) then incubated and kept for 15 days at 25± 2 ºC (Abd El-Gahny, 2001).

Evaluation of antifungal activity *in vitro* test.

Evaluation of the antifungal activity of malic acid, thiourea, and a mixture of malic acid with thiourea before and after formulations at concentrations (250, 500, 1000, and 2000 ppm) against *F. oxysporum* was determined by food poisoned technique according to (Mohanty *et al*., 2012 and Hassanin *et al*., 2017). Added separately concentrations were adjusted at 50 ml of potato dextrose agar (PDA) medium before solidification, concerning the control PDA medium (without treatment). Then, the samples were inoculated at the center with a mycelial disc (5-mm diameter) taken from the margins of 7-day-old *F. oxysporum.* Three replicates were conserved for each treatment and untreated control. All Petri-dishes were incubated at 25±2 °C. The colony diameter was measured until the mycelia were fully covered in the control. The percentage of inhibition mycelial growth was calculated as following equation: (Satya *et al*., 2014).

$$\mathrm{I}\ \% = \frac{T - t}{T} \times 100$$

Where T is the diameter of the mycelia growth in the control, and t is the diameter of the mycelia growth in the treatment.

LdP line program was used to calculate median effective EC_{50} and nightly effective EC_{90} concentration values according to Finney (1971).

Experiment under greenhouse conditions.

Evaluation of antifungal activity and plant promotion of the malic acid, thiourea, and malic acid with thiourea mixture formulations at three concentrations of 1000, 2000, and 4000 ppm were applied against *F. oxysporum.* Uniform fungicide 390 SE was used as a reference control. This experiment was conducted under the greenhouse of the Central Agricultural Pesticide Laboratory (CAPL), Agricultural Research Center (A.R.C.) according to the method described by (Haggag and Nadia, 2012) with some modifications.

Plastic pots (20 cm diameter) containing sterilized sandy-clay soil (1:1w/w) and this soil was infested with an inoculum of *F. oxysporum* at the rate of 3 % (w/w) of soil weight then irrigated and incubated seven days under greenhouse condition. The cucumber seedlings (two weeks old) were transplanted into plastic pots. 5 pots/treatment, using two seedlings per each pot. Added separately 50 ml of spray solution of the prepared formulations to each pot as a drench application. Five pots infected with *F. oxysporum* were served as infected untreated control and five pots as treated control with uniform fungicide, all pots were arranged in a randomized block design, and irrigated as needed. Then result observations were recorded after 30 days of planting such as the percentages of disease incidence (%), survival of plants, plant height (cm), root length (cm), plant fresh and dry weight (g), root fresh and dry weight (g) were recorded. The percentages of disease incidence were calculated as the following equation: (Song *et al*., 2004).

$$\mathbf{DI}\ \% = \frac{\textbf{Number of infected plants}}{\textbf{Total plant numbers}} \times \mathbf{100}$$

The percentages of survival plants were calculated as the following equation:

$$\mathbf{Survival}\ \% = \frac{\textbf{Number of living plants}}{\textbf{Total numbers of plant cultivated}} \times \mathbf{100}$$

Statistical analysis.

Data were analyzed by (ANOVA) with SAS. The least significant difference (LSD) was 0.05 confidence level, according to (SAS, 1988).

RESULTS AND DISCUSSION

Physico -chemical properties of the active ingredient:

A pesticide formulation is a process by which the pesticide is put into a form that can be easily produced, stored, transported, and applied by the practical method to achieve a safe, convenient, and effective method of pest control economically (Dipak and Purkait 2019). To prepare any active ingredient must measure its physical characteristics; data illustrated in Table (1) display that malic acid and thiourea have acidic value (2.66) while thiourea showed higher value (6.03). Both materials are soluble in water and DMF, but non-soluble in xylene, however, malic acid is soluble in ethanol while thiourea is non-soluble.

Table 1. Physico - chemical properties of the tested active ingredient.

Compound	Solubility % (w/v)				pH	Free acidity as % H_2SO_4
	Water	Ethanol	DMF	Xylene		
Malic acid	soluble	soluble	soluble	N.S	2.66	88.21
Thiourea	soluble	N.S	soluble	N.S	6.03	0.074

N.S*: non- soluble

Physico-chemical characteristics of surfactants:

Physical SDS displayed alkaline properties and its value was (0.03), while PEG 600ml, Tween 80, and Tween 20 displayed acidic properties and their values were: 0.63, 0.58, and 0.41 respectively, Tween 80 displays the highest CMC value (0.5) while tween 20 showed the lowest value (0.2). PEG 600 ml showed the lowest surface tension value (29.2) while tween 80 showed the highest value (37.9). These results agreed with (Janku *et al*., 2012) who characteristics of the suggested surface active agent measured and results displayed in Table (2) indicated that all tested surfactants showed nearly the same HLB value (>13) so all of them could be suitable as wetting and spreading agents. reported that by accumulating its molecules near the air-water interface, where it is saturated, the adjuvant lowers surface tension. Critical micelle concentration (CMC) is the concentration of tank-mix adjuvant at this moment. From these results, the suggested surfactant could be used for preparing the needed formulations.

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

Surface active agents	Surface tension (Dyne/cm)	CMC* %	HLB*	Free acidity as H_2SO_4 %	Free alkalinity as NaOH %
SDS*	33.1	0.3	>13	-	0.03
Tween 80	37.9	0.5	>13	0.58	-
P.E.G. 600 ml*	29.2	0.4	>13	0.63	-
Tween 20	36.7	0.2	>13	0.41	-

P.E.G.600 ml *: poly ethylene glycol 600 monolurate. SDS*: Sodium dodecyl sulfate.
CMC*: Critical micelle concentration. HLB*: Hydrophilic-lipophilic balance

Physico-chemical properties of prepared (SP) formulations before and after accelerated storage:

After studying the physical characteristics of the active ingredient and the suggested surfactant, the results illustrated that the tested materials could be prepared as soluble powder formulation by mixing the different ratios of active ingredients and surfactants and then measuring the solubility, foam, pH, and free acidity for each formula, the formula which passes all specified tests it will be suitable for use and applied. Results in Table (3) illustrated the physical properties of locally prepared formulation before and after accelerated storage at 54±2 ºC for 14 days. All prepared formulations displayed 100% solubility, and slightly foam formed in hard and soft water before and after storage. Malic acid (SP) showed the lowest pH value (2.8), before storage but slightly decreased after storage and its value was (1.97) while thiourea (SP) showed the highest value (6.15) and (6.82) before and after storage respectively, followed by the mixture of malic acid and thiourea (SP) where its value (2.87) and decreased to (2.1) after storage. All prepared formulations displayed acidic properties and their values were: 78.85, 0.054, and 40.60 for malic acid, thiourea, and a mixture of malic acid with thiourea respectively, however, slightly increased with malic acid (SP). Its value was (80.26), while slightly decreased with thiourea (SP) and a mixture of malic acid with thiourea (SP) their values were 0.052 and 40.16 respectively. These results confirm the suitability of prepared soluble powder formulations for production and application in pest control.

Table 3. physico-chemical properties of the prepared (SP) formulations before and after accelerated storage at 54±2ºC.

Formulation	Before storage						After storage					
	Solubility		Foam		pH	Free acidity as H_2SO_4	Solubility		Foam		pH	Free acidity as H_2SO_4
	S. W	H. W	S. W	H.W			S. W	H. W	S.W	H. W		
Malic acid	100	100	0	2	2.8	78.85	100	100	0	3	1.97	80.26
Thiourea	100	100	1	3	6.15	0.054	100	100	2	4	6.82	0.052
Malic acid+ Thiourea	100	100	0	2	2.87	40.60	100	100	0	2	2.1	40.16

S.W.: Soft water, H.W.: Hard water

Physico-chemical properties of the spray solution of prepared (SP) formulations at a field dilution rate (0.5 %).

Physical characteristics of spray solution of locally prepared formulation at field dilution rate 0.5 % were measured using the specified methods and the results in Table (4) revealed that. All prepared formulations displayed lower surface tension values with spray solution at 0.5% as follows: 24.26, 25.84, and 32.53 dyne/cm for malic acid and thiourea mixture, malic acid and thiourea respectively, The efficiency of pesticides is increased when an adjuvant that can quickly lower the dynamic surface tension of the solution lowers the surface tension of pesticide droplets below the critical surface tension of plant stems and leaves. This allows the droplets to moisten and disseminate readily on the plant surface. Agricultural tank-mix adjuvants are frequently employed in pesticide application processes to improve disease, insect, and weed control effectiveness (Evandro, *et al*., 2016). Also, thiourea and malic acid mixture (SP) showed the highest viscosity value (1.26) centipoise followed by thiourea (SP) (1.14) and malic acid (SP) displayed viscosity value (1.06) centipoise these results caused lowering the droplet size of spray solution and agreed with (Dorr *et al*., 2015) who reported that Pesticide formulation affects parameters including spray solution's physical and chemical properties and droplet size. Changes in these factors could affect several things, including target coverage, active component adherence to leaves, and droplet bounce. The droplet size spectra, which are the consequence of the interaction between the spray nozzle and the spray solution, also affect spray drift; the potential for drift increases as the droplet size decreases. (Hilz and Vermeer, 2013). However malic acid revealed the highest conductivity value (1234) µ mhos followed by the mixture of malic acid and thiourea (SP) (925) µ mhos and thiourea (SP) (114.7) µ mhos. Also, malic acid (SP) displayed a salinity percent higher than malic acid and thiourea mixture (SP) and thiourea (SP), and its values were: 0.6, 0.5 and 0.1% respectively, these results increased the biological efficacy for the tested formulas against the target, that agreed with (Molin and Hirase, 2004) they reported that The decrease of pH with an increase in electrical conductivity is a necessary additional feature because the former would increase the attraction between the treated plant and the spray solution, increasing penetration and deposition on the tested surface and, ultimately, increasing effectiveness.

Table 4. Physico-chemical properties of spray solution of the prepared (SP) formulations at field dilution rate (0.5 %).

Formulation	Viscosity centipoise	Surface tension (Dyne/cm)	Conductivity (µ mhos)	Salinity %
Malic acid	1.06	25.84	1234	0.6
Thiourea	1.14	32.53	114.7	0.1
Malic acid + Thiourea	1.26	24.26	925	0.5

Evaluation of tested materials as active ingredient and its (SP) formulations on *F. oxysporium* under laboratory conditions.

Evaluation of the efficacy of malic acid, thiourea, and its mixture as active ingredient and its (SP) formulations against *F. oxysporium* under laboratory conditions. The results illustrated in Table (5) and Fig (1) revealed that the malic acid with thiourea mixture (a.i) displayed the highest mycelial growth inhibition 75.7% for *F. oxysporium* followed by thiourea (a.i) 72.4 % at concentration 2000 ppm. While malic acid (a.i) gave the inhibition percent (49.6%) at the same concentration.

Table 5. The efficacy of malic acid, thiourea, and their mixture as active ingredients and its (SP) formulations against *F. oxysporium* under laboratory conditions.

Conc. (Ppm)	Mycelial growth inhibition (%)					
	Active ingredient (a.i)			Formulations (SP)		
	M	T	MT	M	T	MT
250	12.4	35.3	32.8	18.5	40.7	37.6
500	17.8	48.5	41.6	24.8	56.5	54.2
1000	31.2	59.2	68.6	40.6	67.4	72.4
2000	49.6	72.4	75.7	61.7	83.7	86.9
control	0.0	0.0	0.0	0.0	0.0	0.0
EC_{50}	2198.6	564.2	568.9	1353.6	387.7	410.7
EC_{90}	20810.9	9250.3	4844.9	1188.8	3804.3	1094.9
Slope	1.31±0.21	1.15±0.19	1.78±0.19	1.36±0.20	1.29±0.20	1.58±0.21

M: malic acid; T: thiourea; MT: malic acid+ thiourea mixture; SP: soluble powder; ppm: parts per million; Control was: (PDA medium without any treatment).

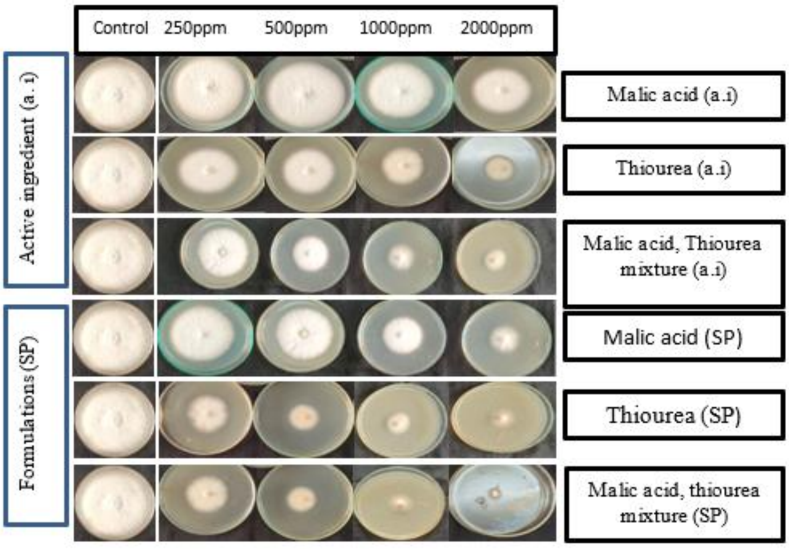


Fig. 1. Diameter of the mycelia growth for *F. oxysporium* after 7 days incubation at 25±2 ^{O}C with different concentrations of malic acid, thiourea, and its mixture as active ingredient (ai) and formulations (SP).

However, these materials were tested after being formulated as soluble powder (SP) formulations and the data in Table (5) revealed that the formulated malic acid thiourea mixture (1:1) displayed the highest mycelial growth inhibition at 86.9% followed by formulated thiourea (SP) and malic acid (SP) and their values were: 83.7 and 61.7% at 2000ppm respectively. The results indicated that the *F. oxysporium* showed the response to thiourea and malic acid with thiourea mixture formulations where their EC_{50} values were: 387.7ppm and 410.7ppm respectively, greater than malic acid formulation which displayed EC_{50} values of 1353.6ppm. These results showed clearly that the formulation increased the efficacy of the tested materials against the target pathogen may be due to adding adjuvants to an active ingredient, which was agreed with (Dipak and Purkait, 2019). They reported those additional additives that increase the efficacy of formulations by improving or enhancing an active ingredient's handling, storage, performance, application, and safety.

Effect of (SP) formulations on Disease Incidence and plant parameters under greenhouse conditions.

According to the previous *in vitro* results which showed excellent mycelial growth inhibition of *F. oxysporium*. A greenhouse experiment was done to evaluate the efficacy of malic acid, thiourea, and malic acid with a thiourea mixture formulated as soluble powder (SP) formulations against wilt disease on the artificial infected cucumber plants. Results illustrated in Table (6) and Fig. (2, 3 & 4) indicated clearly that malic acid (SP) at all tested concentrations decreased significantly the percentage of disease incidence and recorded higher percentages of plant survival in cucumber plant followed by malic acid with thiourea mixture (SP) and thiourea (SP) compared with infected untreated control (I.U.T.) which recorded 72 % disease incidence. Malic acid (SP) at 4000 ppm showed the highest efficacy against *F. oxysporium* and increased all plant parameters which confirm plant promotion like: plant height, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight and its values were: 95.1 cm, 27.4cm, 51.2g, 3.9g, 1.7g, and 0.14g respectively, and its values at 2000ppm were: 80.9 cm, 23.6cm, 44.2g, 3.4g, 1.4g, 0.13g respectively, which was comparable with the standard fungicide uniform at (1ml/L) which displayed values were: 81.2cm, 24.3cm, 44.5g, 3.6g, 1.5g, 0.14g respectively. These agreed with (Guo *et al.,* 2017) who reported that a biological phytohormone effect of malic acid encourages seedling development and resilience to cold. Gharib and Hegazi (2010) mentioned that increases in growth plants may be attributed to the elicitor's effect on ion uptake, cell elongation, cell division, enzyme activity, and protein synthesis. (Hanan *et al.,* 2020) Displayed that Individual applications of lactic and malic acids at 1300 ppm considerably increased the plant height, number of branches, and number of capsules/plant. These acids were also more effective than citric acid in controlling *F. oxysporium* and demonstrated the lowest percentage of disease severity.

Thiourea (SP) displayed moderate efficacy against fusarium wilt on cucumber plants, decreasing by decreasing the treated concentrations, while the plant promotions increased by decreasing the treated concentrations that showed clearly in all measured parameters, where thiourea at the lowest rate of 1000ppm showed lowest efficacy value and the highest parameters values compared by the high rate 4000 ppm, Where its parameters values were 71.5cm, 18.5cm, 34.2g, 3.2g, 1.2g, 0.11g for plant height, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight respectively at 1000ppm. These results agreed with (Premaradhya, *et al.,* 2018) they displayed that when thiourea was applied at 1000 ppm during pre-flowering and pod initiation, the number of pods per plant (73.8), total dry matter (19.52 g/plant), and shoot length (34.1 cm) all rose significantly. This increased grain, straw, and protein yields.

Malic acid and thiourea mixture (1:1) (SP) formulation showed good efficacy value against fusarium wilt on cucumber plants and its value was (92%) with 4000ppm. Also displayed good plant promotion in measured parameters plant height, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight, and its values were: 78.4cm, 19.3cm, 37.8g, 3.4g, 1.4g, 0.12g respectively, where the efficacy was decreased by decreasing the treated concentrations, these results agreed with (Yeon, *et al.,* 2022) they displayed that In pot trials, the application of a wettable powder formulation of copper sulfate 3.33% and malic acid 26.67% shown outstanding protective action with control values of 84% pythium blight on creeping bentgrass, 91% damping-off on cucumber, and 73% phytophthora blight on the red pepper.

Table 6. the effect of malic acid, thiourea, and their mixture formulations against *F. oxysporium* and some growth parameters of cucumber plants under greenhouse conditions.

Treatments/ Conc. Ppm	Disease incidence(%)	Survival (%)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
M1 (4000)	6.0	94	95.1^{a}	27.4^{a}	51.2^{a}	3.9^{a}	1.7^{a}	0.14^{a}
M2 (2000)	8.0	92	80.9^{b}	23.6^{b}	44.2^{b}	3.4^{bc}	1.4^{bcd}	0.13^{b}
M3 (1000)	12.0	88	77.5^{c}	18.6^{c}	36.8^{c}	3.2^{cd}	1.2^{cde}	0.11^{C}
T1 (4000)	16.0	84	64.5^{h}	13.5^{e}	22.5^{f}	1.5^{e}	0.8^{f}	0.08^{e}
T2 (2000)	20.0	80	69.6^{f}	16.3^{d}	26.7^{e}	3.1^{d}	0.9^{ef}	0.10^{f}
T3 (1000)	26.0	74	71.5^{e}	18.5^{c}	34.2^{d}	3.2^{cd}	1.2^{de}	0.11^{c}
MT1 (4000)	8.0	92	78.4^{c}	19.3^{c}	37.8^{c}	3.4^{bc}	1.4^{bc}	0.12^{d}
MT2 (2000)	14.0	86	74.3^{d}	17.7^{cd}	34.2^{d}	2.9^{d}	1.2^{de}	0.11^{c}
MT3 (1000)	18.0	82	66.6^{g}	14.7^{e}	21.4^{f}	1.5^{e}	0.8^{f}	0.089^{g}
I.T.	6.0	94	81.2^{b}	24.3^{b}	44.5^{b}	3.6^{ab}	1.5^{b}	0.14^{c}
I.U.T.	72.0	28	32.6^{i}	8.5^{f}	9.5^{g}	0.7^{f}	0.3^{g}	0.02^{c}
L.S.D. at 5%	1.01	--	1.80	1.52	1.75	0.33	0.22	0.20

M1, M2, M3: malic acid; T1, T2, T3: thiourea; MT1, MT2, MT3: malic acid and thiourea mixture, I.T.: Infected control treated with uniform pesticide (ml/L); IUT: Infected untreated control.

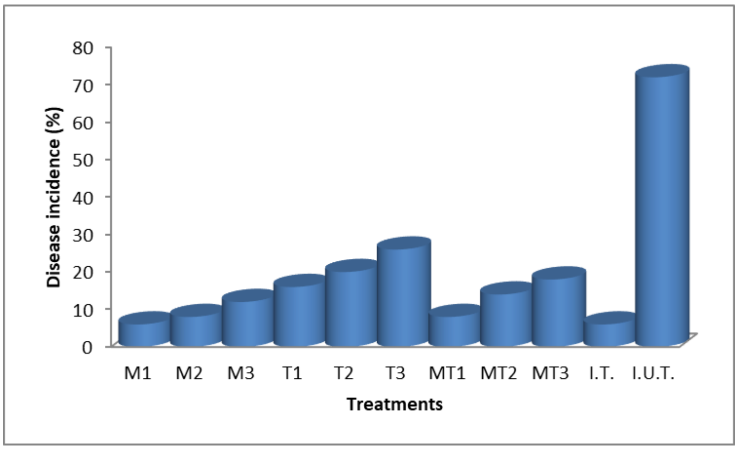


Fig.2. Effect of malic acid, thiourea, and malic acid with thiourea mixture formulations on wilt disease incidence (%) cucumber plants caused by *F. oxysporum* under greenhouse conditions.

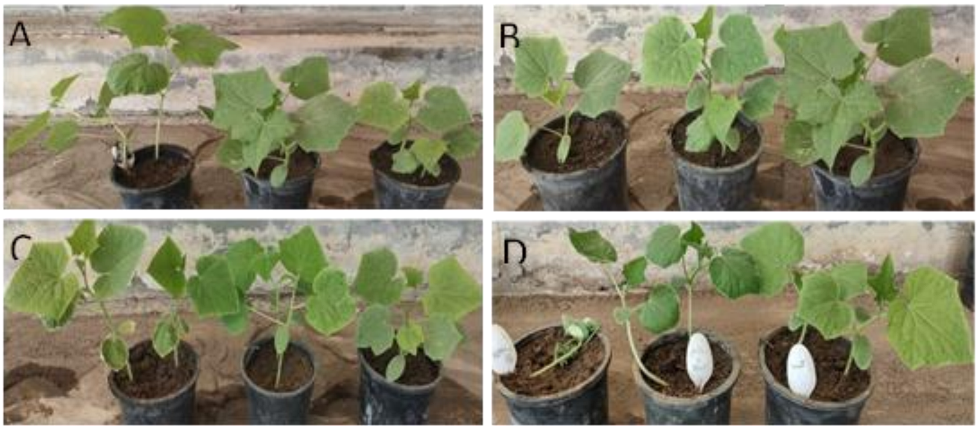


Fig. 3. Effect of treating cucumber seedlings infected with *F. oxysporium* with different concentration of its formulations:(A) malic acid at 4000, 2000, 1000ppm, (B) thiourea at 4000, 2000, 1000ppm, malic acid with thiourea mixture at 4000, 2000, 1000ppm, (D) infected untreated control (I.U.T); infected control treated with uniform pesticide ml/L (I.T.), healthy plant untreated (H.U.T.).

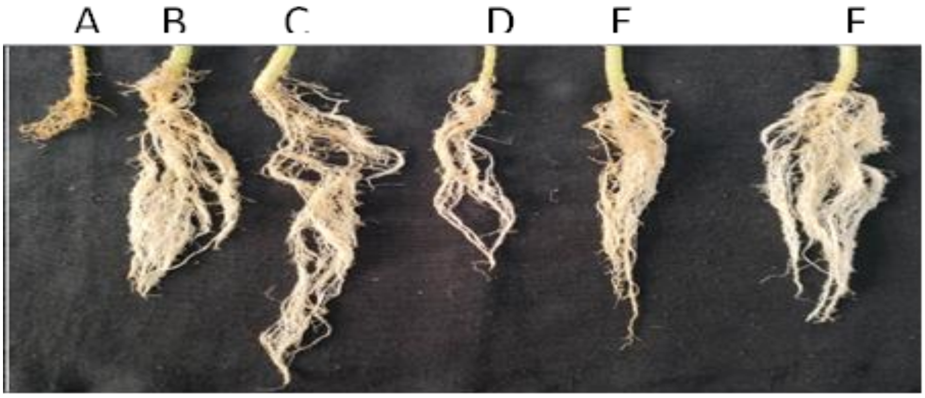


Fig. 4. Image of roots cucumber plant under different treatments: (A) plants were only inoculated with *F. oxysporium*, (B) infected plants and treated with uniform pesticide ml/L, (C) infected plants and treated with malic acid formulation, (D) infected plants and treated with thiourea formulation, (E) infected plants and treated with malic acid & thiourea mixture formulation, (F) healthy plants were irrigated with water.

CONCLUSION

Malic acid, thiourea, and malic acid with thiourea mixture were suggested and its physicochemical characteristics were measured, and evaluated against *F. oxysporum* in the laboratory, the results displayed good efficacy, so it prepared as soluble powder (SP) formulation. Malic acid (SP), thiourea (SP), and malic acid mixture with thiourea (SP) were tested against *F. oxysporum* under laboratory conditions, all tested materials displayed great inhibition of mycelial growth of *F. oxysporum* where malic acid and thiourea mixture (SP) displayed the highest value, followed by thiourea (SP) and malic acid (SP). A greenhouse experiment was done and the results obtained indicated that malic acid (SP) at 4000ppm showed the highest efficacy against wilt disease that was comparable with the standard fungicide Uniform 390 SE, and great effect as plant promotion that showed clearly in the results of plant growth parameters like plant height, root length, root fresh weight, root dry weight, shoot fresh weight and shoot dry weight. Malic acid mixture with thiourea (SP) showed good efficacy against wilt disease and as plant promotion, followed by thiourea (SP) which showed moderate efficacy against wilt. From the previous results, malic acid formulated (SP) and malic acid mixture with thiourea formulated (SP) could be used as safety alternatives to controlling the Fusarium wilt after completing the needed studies.

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دراسة فعالية مستحضر حمض الماليك منفردا و مخلوطا مع الثيويوريا ضد فطر *Fusarium oxysporium* في نباتات الخيار

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الملخص

حمض الماليك يعمل على خفض درجة الحموضة لسيتوبلازم خلية الفطريات ويمنع قدرتها على التكاثر. كما أنه يشجع نمو النباتات. تم قياس الخواص الفيزيائية والكيميائية لحمض الماليك والثيويوريا وخليط حمض الماليك مع الثيويوريا (كمواد فعالة) ثم تم تحضيرها على شكل مسحوق قابل للذوبان في الماء واجتازت بنجاح جميع الاختبارات المحددة. تم اختبار فعالية حمض الماليك والثيويوريا وخليط حمض الماليك مع الثيويوريا (كمواد فعالة) ضد *F. oxysporum* تحت ظروف المعمل، وكانت نسب تثبيط نمو الفطريات: ٤٩,٦ و٧٢,٤ و٧٥,٧٪ على التوالي عند ٢٠٠٠ جزء في المليون، بينما أظهرت المستحضرات تثبيطًا أعلى لنمو الفطريات وكانت قيمها: ٨٦,٩ و٨٣,٧ و٦١,٧٪ لخليط حمض الماليك مع الثيويوريا (SP)، والثيويوريا (SP)، وحمض الماليك (SP) عند ٢٠٠٠ جزء في المليون على التوالي. أجريت تجربة تحت ظروف الصوبة وأظهرت النتائج أن حمض الماليك بجميع التركيزات المختبرة أدى إلى انخفاض معنوي في نسبة الإصابة بالمرض وزيادة كفاءة خليط حمض الماليك مع الثيويوريا (SP) والثيويوريا (SP) حيث بلغت قيم كفائتة: ٩٤، ٩٢ و ٨٤٪ عند ٤٠٠٠ جزء في المليون على التوالي ضد الذبول الفيوزاريومي على نباتات الخيار. أظهر حمض الماليك (SP) عند ٤٠٠٠ جزء في المليون أعلى معدل نمو للنبات والذي ظهر بوضوح في النتائج المقاسة . وأخيرا من النتائج السابقة يمكن استخدام حمض الماليك كبديل آمن للمبيدات الفطرية الكيميائية ضد الذبول الفيوزاريومي على نباتات الخيار بعد استكمال الدراسات اللازمة.