FORMULATION, SPECIFICATIONS, EFFECTIVENES AND EVALUATION OF TESTED PLANT OILS AGAINST CERTAIN PLANT PATHOGIN FUNGI

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Abstract

A laboratory experiment had been conducted to throw light on the fungicidal activities of plant oils camphor oil, citronella oil and cotton seed oil against four species of fungi: *F. solani, R. solani, S. rolfsii* and *T. viride* These oils were prepared as emulsifiable concentrates (EC) using different surface active agents, namely: Sodium dodecyl sulphate, PEG 600 monolaurate, PEG 600 dioleate, Tween80 and Tween20, and solvents the xylene, isopropyl alcohol and dimethyl formamide. The physico – chemical properties for surface active agents, solvents, crude oils and prepared oils were measured. The bioassay experiments were designed to evaluate the EC_{50} for the plant oils against the fungi under investigation. Each plant oil was prepared in serial dilutions: 125, 250, 500, 1000 and 2000 µg/ml in PDA media. The results of bioassay showed that the response of the fungus *S. rolfsii* for all plant oils used was higher than other fungi under investigation. Also, the study showed that the fungus *F. solani* had more response to the plant oil citronella than for the other plant oils. On the other hand, the response of the fungi *R. solani* and *T. viride* to the plant oil camphor was higher compared to the other plant oils under investigation.

INTRODUCTION

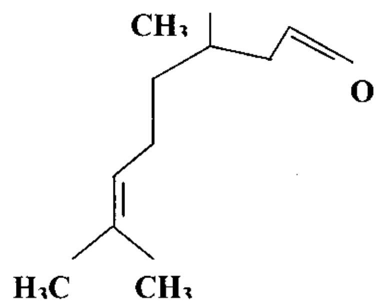
Among pesticides used to protect corps, fungicides were perceived until recently as relatively safe. A1987 National Academy of Sciences (NAS) report (Research Council, Board if Agriculture.1987) on pesticide residues on food indicated that fungicides pose more of a carcinogenic risk than insecticides and herbicides together. Therefore, Synthetic fungicides are suspect in our food chain, and pressure is increasing to find safer alternatives (Cutler and Hill 1994). Additionally, resistance by pathogens to fungicides has rendered certain fungicides ineffective, creating a need for new ones with alternative modes of action. Present activities to find both natural and synthetic fungicides focus on finding compounds that are safe to humans and the environment. (Wilson *et al.,* 1997)

Essential oils show antifungal activity against a wide range of fungi (Singh *et al.,*1980, Kurita *et al.,*1981). In this study the plant oils camphor, citronella and cotton seed against some of plant pathogen *F. solani, R. solani, S. rolfsii* and *T. viride* as the alternatives of the fungicides were evaluaited.

MATERIALS AND METHODS

A -Plant oils

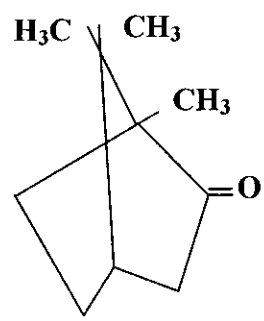
i – Camphor oil



Chemical structure of camphor oil

The main chemical components are a-pinene, camphene, b-pinene, sabinene, phellandrene, limonene, 1,8-cineole, y-terpinene, p-cymene, terpinolene, furfural,camphor, linalool, bornyl acetate, terpinen-4-ol, caryophyllene, borneol, piperitone,geraniol, safrole, cinnamaldehyde, methyl cinnamate and eugenol

ii - Citronella oil



Chemical structure of citronella oil

The main chemical components of citronella oil are citronellic acid, borneol, citronellol, geraniol, nerol, citral, citronellal, camphene, dipentene and limonene.

iii - Cotton seed oil

Cotton seed oil is rich in palmitic acid (22-26%), oleic acid (15-20%), linoleic acid (49-58%) in the crude oil. It also contains gossypol, a naturally occurring toxin that protects the cotton plant from insect damage. Therefore, unrefined cotton seed oil is sometimes as a pesticide.

B -Surface active agents used

a. Sodium dodecyl sulphate (SDS): $CH_3\ (CH_2)_{11}\ OSO_3\ Na$ it was supplied from El-Nasr pharmaceutical, chemicals co. (ADWIC).

b. Poly ethylene glycol 600 mono-Laurate:

$CH_3\ (CH_2)_{10}\ COO\ (CH_2\ CH_2O)_\eta\ H$ $\eta = 600$

c. Poly ethylene glycol 600 di-oleate.

$CH_3\ (CH_2)_7\ CH=CH\text{-}(CH_2)_7\ COO\text{-}\ [CH_2\text{-}CH_2O]_\eta\ \text{-}CO\text{-}\ (CH_2)_7\ \text{-}\ CH=CH\text{-}(CH_2)_7\ \text{-}CH_3$.

They were produced by the National co. for yeast and detergent, Alexandria.

d. Tween80: Polyoxyethlene (20) sorbitan monooleate.

e. Tween20: Polyoxyethylene (20) sorbitan monolaurate.

They were supplied from El-Gomhouria co. for chemicals.

C – Solvents used

The following solvents were supplied from El-Nasr pharmaceutical, chemicals co. (ADWIC).

a. Xylene (dimethyl benzene): $C_6H_4\ (CH_3)_2$.

b. Isopropyl alcohol: $CH_3\ CHOHCH_3$.

c. N, N- dimethil formamide (DMF) $HCON\ (CH_3)_2$

D -Tested fungi

Fusarium solani (the causal agent of fusarium wilt) isolated from tomato root, *Rhizoctonia solani* (the causal agent of damping off) isolated from cotton root, *Sclerotium rolfsii* (the causal agent of southern blight) isolated from tomato stem and *Trichoderma viride*. The cultures of the all tested fungi were maintained on potato dextrose agar (PDA).

Determination of the physico – chemical properties of the constituents of prepared formulations

a- Plant oils

Solubility and free acidity or alkalinity they were determined according to WHO specifications (1979).

b- Surface active agents

1- Critical micelle concentration (CMC): It determined according to Osipow (1964).

2- Free acidity or alkalinity: It was determined according to WHO specifications (1979).

3- Hydrophilic – Lipophilic Balance (HLB): It was determined according to (Lynch and Griffin, 1974).

c- Solvents

Flash point, surface tension and free acidity or alkalinity was determined according to WHO specifications (1973).

Preparation of tested plant oils as emulsifiable concentrate (EC)

Plant oils: Cotton seed oil, citronella oil and camphor oil were prepared as emulsifiable concentrate (EC) using suitable solvents (e.g. xylene, isopropyl alcohol, and dimethyl formamide) and suitable emulsifiers (e.g. SDS,600 DO, 600 ML, Tween80, and Tween20) at different concentrations.

Determination the physico-chemical properties for the formulated emulsifiable concentrate

1- **Emulsion stability test.**
   It was conducted according to WHO specifications (1979)
2- **Free acidity or alkalinity.**
   It was determined according to WHO specifications (1979)
3- **Persistent foam.**
   It was determined according to (Dobrat and Martijn, MT 47.1, 1995)
4- **Storage stability.**
   It was determined according to (Dobrat Martijn, MT 39.1, and MT46.1.3, 1995)

***In vitro* plant oils assay**

The efficacy of the three tested plant oils on, *F. solani, R. solani, S. rolfsii and T. viride* was tested *in vitro* on PDA. Mycelial plugs of the pathogen (5mm in diameter removed from the margins of 7-day-old culture) were transferred to Petri dishes (90-mm) containing PDA amended with the tested plant oils at tested concentrations, 0, 125, 250, 500, 1000 and 2000 µg/ml. All concentrations were expressed as active ingredient. All plates were incubated at room temperature (25 ± 2°C). The radial growth of the mycelium was measured after 3 days for *R. solani, S. rolfsii and T. viride* and after 7 days for *F. solani* and the inhibition of growth was calculated when compared with the untreated check. Each treatment had four replications. Results are expressed as percentage of inhibition and as effective concentration (50% EC_{50})

RESULTS AND DISCUSSION

1-The physico-chemical properties of the constituents of prepared EC formulations

A- Plant oils

i- Solubility

The most important requirement in formulating a pesticide is the solubility of the toxicant in the used solvent. The solubility was determined at 25°C. Data in Table (1) showed clearly that the tested plant oils (camphor oil, citronella oil and cotton seed oil) were insoluble in water but they were miscible in xylene, acetone, and dimethyl formamide, except citronella oil was immiscible in dimethyl formamide.

ii- Acidity or alkalinity

Results obtained in Table (1) indicated that camphor oil, citronella oil and cotton seed oil were slightly acidic and their acidity values were 0.0588, 0.0196 and 0.049, respectively.

Table 1. Physico- chemical properties of the plant oils

Materials	% solubility at 25°C (wt/v)				Free	
	Water	Xylene	Acetone	DMF	Acidity % as H_2So_4	Alkalinity % as NaoH
Camphor oil	Non	100	100	solable	0.0588	-
Citronella oil	Non	Miscible	Miscible	Immiscible	0.0196	-
Cotton seed oil	Non	Miscible	Miscible	Miscible	0.049	-

B- Physical properties of the used surfactants

i- Solubility

Sodium dodecyl sulfate was soluble in water but it was insoluble in both acetone and xylene. Polyethylene glycol 600 mono- laurate (PEG-600 ml) was miscible in water and soluble in acetone and xylene, also polyethylene glycol 600 di-oleate (PEG 600-DO) soluble in acetone and xylene, while give emulsion in water. Tween80 soluble in water, acetone, and xylene. Where the solubility values were 25.0, 60.7 and 55.7%, respectively. Also tween20 was soluble in water, acetone and xylene and the solubility values were 40.0, 65.4 and 60.7%.

Surfactant which solublize in water such as (SDS, PEG 600 ml, tween80, and tween20) should be use as wetting and spreading agent for the preparation soluble powder or soluble concentrate formulations, while those give emulsion in water and soluble in xylene should be use as emulsifiers for preparation emulsifiable concentrates.

ii- HLB value

Since the HLB of a surfactant is related to its solubility, a surfactant having a high HLB range (13+) will tend to be water soluble (El – Attal *et al.,* 1974). As indicated in table (2) sodium dodecyl sulfate, PEG – 600 ml, tween80 and tween20 had a high HLB value suitable to be used as dispersing and suspending agent. Their HLB values were 18, 14, 16 and 16, respectively. While polyethylene glycol 600 dioleate had HLB value (10 – 12) suitable to be used as emulsifying agent.

iii- Free acidity or alkalinity

Data in Table (2) showed that polyethylene glycol 600 mono – laurate, tween80 and tween20 were slightly acidic and the acidity % as H_2SO_4 was 0.630, 0.061 and 0.49, respectively. While, sodium dodeyl sulfate and polyethylene glycol 600 dioleate were alkaline and the alkalinity % as NaoH were 0.029 and 0.25, respectively.

iv- Critical micelle concentration (CMC)

Data in Table (2) showed that polyethylene glycol 600 dioleate had the highest CMC value, followed by tween80, polyethylene glycol 600 mono- laurate, sodium dodecyl sulfate and tween20. Where their CMC values were 0.9, 0.5, 0.4, 0.3 and 0.2% (w/v), respectively. It could be stated that local surfactant proved effectiveness in stabilizing the formed emulsions and diminishing the high surface tension of water, thus facilitating wetting and spreading on the treated surfaces of foliages. CMC was determined to make sure that the applied concentration is far less than CMC to avoid any micelle formation. (El-Attal and Moustafa.1979), reported that: demulsification of the ECs was observed to occur at a concentration little greater than the CMC indicating that this value is important in the choice of emulsifiers to be used in very concentrated emulsions.

v- Surface tension at 0.5%

Data in Table (2) indicated clearly that polyethylene glycol 600 mono - laurate was the most effective surfactant in reducing surface tension of water, followed by sodium dodecyl sulfate, tween20, Polyethylene glycol 600 di-oleate, and tween80, where the surface tension values were: 29.1, 30.3, 37.02, 40.2 and 43.2, respectively.

Table 2. Physico – chemical properties of used surfactants

Surfactants used	% Solubility at 25°C (wt/v) in			HLB	Free		CMC at 25°C % (w/v)	Surface tension at 0.5%
	Water	Acetone	Xylene		Acidity % as H_2SO_4	Alkalinity % as NaOH		
Sodium dodecyl benzene sulfate	39	Insol.	Insol.	18	-	0.029	0.3	30.3
PEG 600 monolaurate	Miscible	29.4	50	14	0.63	-	0.4	29.1
PEG 600 dioleate	Give emulsion	25	22.7	10 -12	-	0.25	0.9	40.2
Tween80	25	60.7	55.7	16	0.061	-	0.5	43.2
Tween20	40	65.4	60.7	16	0.49	-	0.2	37.02

PEG = polyethylene glycol

C- Physical properties of the solvents

i – Free acidity or alkalinity

As shown in Table (3) all the tested solvents were slightly acidic. Dimethyl formamide had the highest acidity value (0.0392) followed by isopropyl alcohol, but xylene had the lowest acidity value 0.0064.

ii – Surface tension

Surface tension was estimated using Du-nouy tensiometer. Dimethyl formamide had the highest value of surface tension followed by isopropyl alcohol and xylene. Where their surface tension values were 59.47, 57.0 and 32.0 respectively, table (3).

iii– Flash point

As shown in Table (3) Dimethyl formamide had the most flash point value (57°C) followed by xylene 43°C while isopropyl alcohol had the lowest flash point value 23°C it estimated using Cleveland open taster.

These properties show clearly that the tested solvents had moderate flash point and slightly acidic which agree with FAO/ WHO (2002) recommendation. And xylene was considered as a suitable solvent for local formulations of emulsifiable concentrates.

Table 3. Physico – chemical properties of used solvents.

Solvents used	Flash point	Surface tension at 25°C dyne/cm	Free acidity % as H_2SO_4
Xylene	43°C	32	0.0064
Isopropyl alcohol	23°C	57	0.0294
Dimethyl formamide	57°C	59.47	0.0392

iv– Physico – chemical properties of the prepared tested plant oils as emulsifiable concentrates

a– Emulsion stability

The local prepared plant oils formulated as emulsifiable concentrates passed successfully through emulsion stability test, since no oily separation or sedimentation was observed when it added at 5% in both soft and hard water. The results in Table (4) showed that, the formed creamy layer was not exceeded than FAO/ WHO (2002) recommendations.

b– Free acidity or alkalinity

The results obtained in Table (4) indicated that the successful formulated plant oils (EC) showed slight acidity comply with WHO (1979) specifications. Cotton seed oil showed the highest value (0.0686), followed by citronella oil (0.0392), and camphor oil (0.0294).

c– Cold storage

As shown in Table (4) all prepared plant oil EC'S passed successfully cold test comply with WHO (1979) specifications.

d– Accelerated storage

All tested prepared plant oils (EC) passed successfully heat storage at 54°C for 14 days. Emulsion stability test since no oily.

Table 4. Physico – chemical properties of prepared tested plant oils as emulsifiable concentrates (ECs).

Materials used	Emulsion stability (ml, cream sep)		Foam Cm	Free acidity % as H_2SO_4	Cold storage at 0°C	Accelerated storage			
						Emulsion stability (ml, creamy sep)		Foam cm	Acidity % as H_2SO_4
	S.W.	H.W.				S.W.	H.W.		
Camphor oil	0.4	0.7	2	0.0294	Passed	0.5	0.7	2	0.0294
Citronella oil	0.5	0.6	3	0.0392	Passed	0.6	0.7	2	0.049
Cotton seed oil	0.6	0.8	-	0.0686	Passed	0.7	0.9	1	0.0686

Separation or sedimentation was observed, results in table (4) showed that the formed creamy layer was not exceeded than WHO (1979) recommendations. Also all the stored plant oils (EC) was acidic, where camphor oil (EC) and cotton seed oil had no change in their acidity values after heat storage, while citronella oil had increased in acidity value . While foam didn't exceed about 2 cm with all plant oils (EC).

Table 5. The linear growth, the percent of inhibition of investigated fungi, estimated EC_{50} and slope for the plant oil

	Camphor oil				Citronella oil				Cotton seed oil			
	Fusarium solani											
Concentration ppm	Linear growth in mm	% of inhibition	Estimated EC_{50} ppm	Slope value	Linear growth in mm	% of inhibition	Estimated EC_{50} ppm	Slope value	Linear growth in mm	% of inhibition	Estimated EC_{50} ppm	Slope value
2000	16.00	82.22			7.75	91.39			66.25	26.39		
1000	34.50	61.67			22.75	74.72			72.50	19.44		
500	57.50	36.11			36.75	59.17			81.75	9.17		
250	75.00	16.67			44.25	50.83			86.50	3.89		
125	83.75	6.94	720.673	2.036	49.25	45.28	214.913	1.121	90.00	0.00	5955.324	1.319
Untreated check	90.00	0.00			90.00	0.00			90.00	0.00		
	Rhizoctonia solani											
2000	0.00	100.00			20.75	76.94			70.00	22.22		
1000	0.00	100.00			34.25	61.94			77.50	13.89		
500	25.25	71.94			53.00	41.11			83.50	7.22		
250	52.00	44.22			62.25	30.83			88.50	1.67		
125	71.25	20.83	266.156	2.25	81.00	10.00	654.297	1.572	90.00	0.00	6857.404	1.343
Untreated check	90.00	0.00			90.00	0.00			90.00	0.00		
	Sclerotium rolfsii											
2000	0.00	100.00			14.50	83.89			60.25	33.06		
1000	0.00	100.00			22.25	75.28			66.50	26.11		
500	11.75	86.94			30.50	66.11			72.25	19.72		
250	21.25	76.39			38.25	57.50			80.50	10.56		
125	45.50	49.44	128.86	2.31	45.25	49.72	139.946	0.818	87.25	3.06	2626.086	1.044
Untreated check	90.00	0.00			90.00	0.00			90.00	0.00		
	Trichoderma viride											
2000	0.00	100.00			66.25	26.39			74.25	17.50		
1000	16.75	81.39			74.50	17.22			78.00	13.33		
500	51.25	43..06			82.00	8.89			84.50	6.67		
250	65.00	27.78			90.00	0.00			90.00	0.00		
125	81.75	9.17	6732.39	2.342	90.00	0.00	4488.446	1.581	90.00	0.00	20004.95	1.321
Untreated check	90.00	0.00			90.00	0.00			90.00	0.00		

Effects of the plant oil on the linear growth of the tested fungi

The purpose of this study was to examine the inhibitory effect of plant oils against certain fungi. Data in Table (5) and Figs 1, 2 and 3 showed on unamended that, the mycelium of all check pathogens under investigation covered the 90-mm Petri dishes within 3 days for *R. solani, S. rolfsii* and *T. viride* and after 7 days for *F. solani*. The higher concentrations of the investigated plant oil showed the highest inhibition of the fungal growth and vice-versa. *S. rolfsii* showed the highest response to the all investigated plant oils. The EC_{50} values were 128.86, 139.946 and 2626.086 µg / ml for camphor oil, citronella oil and cotton seed oil, respectively. *F. solani* was more sensitive to citronella oil than camphor oil and cotton seed oil. The EC_{50} values were 214.913, 720.673 and 5955.324 µg / ml for citronella oil, camphor oil and cotton seed oil, respectively.

R. solani was more sensitive to camphor oil than the other two plant oils. The EC_{50} values were 266.156, 654.297 and 6857.404 µg / ml for camphor oil, citronella oil and cotton seed oil, respectively. These results agree with the results of Dhingra *et al.,* 2004.

The same trend of results was found with *T. viride*, the EC_{50} values were 482.85, 6732.39 and 20004.95 µg / ml for camphor oil, citronella oil and cotton seed oil. respectively.

Data also indicated that the cotton seed oil had the lowest effect on the all fungi under investigation. The EC_{50} values were 5327.134, 6951.62, 4388.773 and 8638.328 µg / ml for *F. solani, R. solani, S. rolfsii* and *T. viride*, respectively.

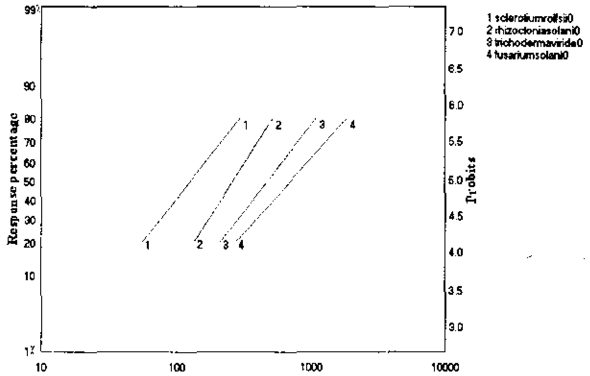


Fig. 1 Inhibition growth regression lines of camphor oil against *F. solani, R. solani, S. rolfsii* and *T. viride*

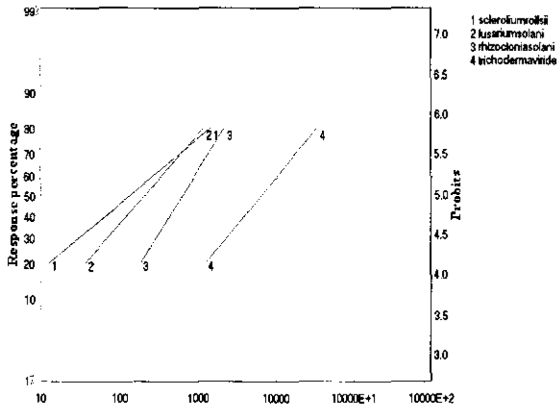


Fig. 2 Inhibition growth regression lines of cironella oil against F. solani, R. solani, S. rolfsii and T. viride

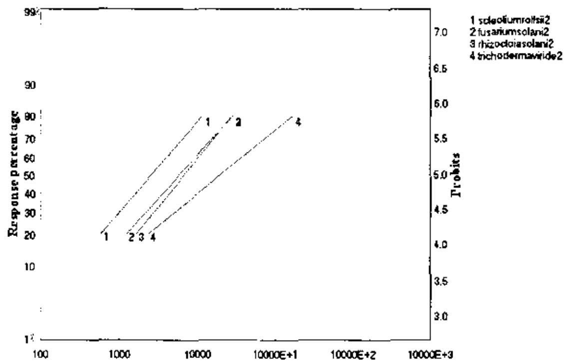


Fig. 3 Inhibition growth regression lines of cotton seed oil against F. solani, R. solani, S. rolfsii and T. viride

REFERENCES

1. Anonymous. 1973. Specifications for pesticides used in public health. Geneva. WHO.
2. Anonymous.1979. Specifications for pesticides used in public health. Geneva. WHO.
3. Cutler, H.G. and R.A., Hill. 1994. Natural fungicides and their delivery systems as alternatives to synthetic. Pages 135 -152 in: Biological Control of Postharvest diseases.
4. EL-Attal, Z. M., M. S. S. Sawy and A. A. A. Said. 1974. Laboratory studies on some Egyptian granular carrier for lindane and endrin. Bull. Ent. Soc. Egypt, Econ. Ser., 8:7-11.
5. EL-Attal, Z. M. and O.K. Moustafa. 1979. Influence of dilution rate on the quality of the emulsion of pesticide concentrates, Proc.3rd Pesticide Conf., Tanta Univ., Sept. 1979, vol.1, p. 277 – 286.
6. FAO specifications are developed with the basic objective of promoting, as far as ... SC formulations comply with the requirements of the Manual (FAO/WHO, 2002).
7. Dhingra, O.D., M.L.N. Costa, JR. G.J Silva, & E.S.G. Miaubuti, Essential oil of mustard to control *Rhizoctona solani* seedling damping off and seedling blight in nursery. Fitopatologia brasileira 29:683-686. 2004.
8. Dobrat, W. and A. Martijn. 1995. CIPAC hand book, volumes F. Physico-chemical methods for technical and formulated. pesticides. Collaborative International Pesticides Analytical Counil Limited.
9. Kurita, N., M. Makoto, R. Kurane and Y. Takahra. 1981. Antifungal activity of components of essential oils. Agric. Biol. Chem. 45:945-952.
10. Lynch, M. J. and W. C. Griffin. 1974. Food Emulsion edited by Lissant, K. J. Volvi (Emulsion and Emulsion Technology). Marcel Dekker, Inc, New York, 250 – 289.
11. Osipow, L. I. (1964). Surface chemistry. Theory and industrial applications. Rienhold publishing Crop., New York, PP. 473.
12. Research Council, Board of Agriculture. 1987. Regulating Pesticides in Food – The Delaney Paradox. National Academy Press. Washington, DC.
13. Singh, A. K., A. Dickshit, M. L. Sharma and S. N. Dixit. 1980. Fungitoxic activity of some essential oils. Econ. Bot. 34:186-190.
14. Wilson, C. L., J. M. Solar, A., El Ghaouth and M. Wisniewski, E.1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea.* Plant Dis. 81:204-210.

تجهيز وتقدير خواص وكفاءة بعض الزيوت النباتية ضد بعض الفطريات الممرضة للنباتات

سامي شفيق رمسيس ، مجدي عدلي إسكندر

المعمل المركزي للمبيدات – مركز البحوث الزراعية – الدقي – جيزة.

أجريت عدد من التجارب المعملية لدراسة الآثرالأبادي لعدد من الزيوت النباتية (زيت الكافور – زيت السترونيلا- زيت بذرة القطن) علي أربعة أنواع من الفطريات (فطر الفيوزاريوم سولاني – فطر ريزوكتوينا سولاني – فطر أسكليروشيم رولفزياي – فطر الترايكودرما فيردي) حيث تم تجهيز هذه الزيوت في صورة مركزات قابلة للأستحلاب في الماء باستخدام بعض المواد النشطة سطحيا والمذيبات العضوية وقد تم قياس الخواص الطبيعية للزيوت النباتية موضع الدراسة قبل وبعد التجهيز (أختبار الذوبانية- الحموضة والقلوية- ثبات الإستحلاب – التخزين البارد والحار .....). أجريت بعد ذلك الأختبارات الحيوية لتوضيح الآثر الأبادي لهذه الزيوت وكذلك تقدير التركيز المثبط لنصف النمو الميسليومي للفطريات موضع الدراسة حيث أستخدمت هذه الزيوت في سلسلة من التركيزات 125، ٢٥٠، ٥٠٠، ١٠٠٠، ٢٠٠٠ جزء في المليون في بيئة بطاطس ديكستروز آجارضد الفطريات موضع الدراسة مع وجود أطباق بتري تحتوي علي بيئة بدون إضافة أي من الزيوت النباتية للمقارنة وقد أظهرت النتائج أن فطر سكليروشيم رولفزياي كان الأكثر إستجابة لجميع الزيوت النابتية المستخدمة كما أظهرت النتائج أن فطر فيوزاريوم سولاني كان أكثر إستجابة للزيت النباتي سيترونيلا عنه عن زيت الكافور وزيت بذرة القطن بينما كان فطر ريزوكتونيا سولاني وفطر ترايكودرما فيردي أكثر إستجابة للزيت النباتي كافور عنه عن باقي الزيوت النباتية المستخدمة في الدراسة.