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EXTRACTION, FORMULATION AND EVALUATION OF ANTIFUNGAL ACTIVITY OF TOBACCO (NICOTIANA TABACUM LINN.) WASTE AGAINST SOME PATHOGENIC FUNGI

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ABSTRACT

Tobacco powders (*Nicotiana tabacum* Linn.) (Cigarette factor waste), was extracted in sequences with three solvents namely hexane, chloroform, and methanol. Qualitative phytochemical analysis for the crude extracts was done. The residual crude extracts were formulated as soluble concentrate 10 % SL in case of hexane and methanol extracts, and emulsifable concentrate 20 % EC in case of chloroform extract.

All tested extracts possessed phytochemical components. Saponins, flavonoids, tannins (pyrogalol), terpenes, and alkaloids were detected with hexane extract, while flavonoids, steroids and terpenoids were detected with chloroform extract; however tannins (pyrogalol), alkaloids, steroids and terpenoids were detected with methanol extract.

Antifungal activity of formulated extracts were evaluated against some pathogenic fungi, *Alternaria solani* and *Fusarium oxysporum* under laboratory conditions and the results showed that, *A. solani* was more sensitive than *F. oxysporum* to all the tobacco extracts at the different tested concentrations, where the EC_{50} values were 0.53, 0.82

and 1.7 mg/ml for hexane, chloroform and methanol extracts formulations respectively. However the EC $_{50}$ values for *F. oxysporium* were 0.76, 1.67 and 3.07 mg/ml for hexane, chloroform and methanol extracts formulations respectively.

Key words: tobacco waste, recycling, phytochemicals, formulation, antifungal activity.

INTRODUCTION

Fungal plant pathogens can cause enormous losses in yield and quality of field crops, fruits, and other edible plant materials and this becomes increasingly a more important issue to human health and the global economy in this century.

Early blight is caused by *Alternaria solani* and *A. alternata*, which is also the causal agent for brown spot. Early mainly affects potato foliage and leads to leaf necrosis and premature defoliation **Aqleem (2017).**

Healthy plants can become infected by *F. oxysporum* if the soil is infected with the Pathogen. *Fusarium* sp. this is the causal agents of tomato wilt cause root and basal stem deterioration and result in the wilting of vegetable plants. Browning the vascular tissue is strong evidence of *Fusarium* wilt. (**Ignjatov** *et al.*, **2012**)

Pests and insects are main problem of agriculture that damage many crop plants. Various methods have been used to protect the crops from these natural enemies. Although using of pesticides is recognized as the most widely used method to solve this problem, however the health risks and environmental effects from their uses should be concerned (**Feola** *et al.*, **2011**). Therefore it has become an important issue to find alternative control strategies are effective as synthetic pesticides. (**Javed** *et al.*, **2006**).

One such possibility relies on the role of tobacco as a traditional plant- derived pesticide, as nicotine in tobacco is toxic to most herbivore insects. Nicotine pesticides have been regarded as "green pesticides" with high activity and low toxicity (**Tomizawa and Casida 2003**). Tobacco extracts prepared with solvents of weaker polarity had higher fungicidal activity, and the inhibitory activity of tobacco extracts against *Valsa mali* was also cultivar dependent. Furthermore, the fungicidal effects of tobacco flower extracts were higher than those of the leaf extracts (**Duan, 2016**).

Phytochemicals are the chemicals produces by various parts of the plants. These bioactive constituents of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides. These compounds have various activities such as antimicrobial and antibacterial, some have been reported to exhibit heamolytic and foaming activity reported by (Feroz et al, 1993). Saponins are secondary metabolites synthesized by many different plant species and marine animals. They are large molecules and contain a hydrophobic part, composed of triterpenoid (30 carbon atoms) or steroid backbone (27 carbon atoms with 6-ring spirostone or a 5-ring furostane skeleton) and hydrophilic part consisting of several saccharide residues linked to the hydrophobic scaffold through glucose bonds. They have many medical uses including microbial, antitumor, antinsect, hepatoprotective, heamolytic and anti-inflammatory activities (Barbosa, 2014).

The aim of this study is recycling the tobacco waste (powder) from cigarette factors through: 1- Extraction the active phytochemicals using different polarity solvents. 2- Qualitative the phytochemical analysis of each extract component. 3- Formulation the crude extracts in appropriate formula. 4- Evaluation of antifungal activity against some pathogenic fungi.

MATERIALS AND METHODS

1. Chemicals.

- **Solvents**: Hexane, chloroform, methanol, xylene, acetone and dimethyl formamide were supplied by EL-Gomhoria Co., Cairo, Egypt.
- **Surface active agents**: Tween 20, tween 80 and toximol were supplied by EL-Gomhoria Co., Cairo, Egypt.
- Poly ethylene glycol 600 di-oleate, produced by the National Co., for yeast and detergent, Alexandria, Egypt.

2. Plant use.

- Tobacco (*Nicotiana tabacum* Linn.) waste powder supplied by Eastern Company for tobacco, Giza, Egypt.

3. Fungal strains used.

Pure cultures of *Alternaria solani* and *Fusarium oxysporium* were supplied from the department of Fungicides, Bactericides and Nematicides, Central Agricultural Pesticides Laboratory, A. R. C.

4. Preparation of crude plant extract.

Tobacco waste (powder) supplied by eastern company for tobacco was extracted with different polarity solvents in sequence as follow, hexane, chloroform and methanol by maceration method according to (**Handa** *et al.*, **2008**) 100 gram of tobacco powder macerated in 500 ml of hexane solvent for 3 days then filtered and evaporated by rotary to get the residual crude extract, then the same powder let to dry well then macerated with 500 ml of chloroform solvent for three days, filtered and evaporated. The same powder also let to dry well and macerated with 500 ml of methanol solvent for three days, filtered and evaporated to get the residual crude extract.

5. Qualitative phytochemical analysis of crude tobacco extracts.

Preliminary phytochemical analysis was carried out for the extract as per standard methods described by **Peach and Tracey** (1955), Harborne (1998).

6. The physico-chemical properties of basic formulation constituents.

6.1. Active ingredient:

a. Solubility: It was determined by measuring the volume of distilled water, acetone, xylene and dimethyl formamide for complete solubility or miscibility of one gram of active ingredient (residual of crude extract) at 20 °C (**Nelson and Fiero, 1954**). The solubility% was calculated according to the following equation:

% solubility =
$$W/V \times 100$$

[Where; W= active ingredient weight, V= volume of solvent required for complete solubility].

b. Free acidity or alkalinity: It was determined according to the method described by **WHO specification (1979)**.

6.2. Surface active agents:

a. Critical micelle concentration (CMC):

The concentration in which the surface tension of solution doesn't decrease with further increase in surfactant concentration, (CMC) of the tested surfactants was determined according to the method described by (Osipow, 1964).

b. Hydrophilic-lipophilic balance (HLB):

The solubility of surfactant in water is considered as approximate guide to its hydrophilic-lipophilic balance (Lynch and Griffin, 1974).

c. Free acidity or alkalinity:

It was determined by the same method described before.

d. Surface tension:

Surface tension: It was determined by using Du-Nouy tensiometer for solutions containing 0.5 % (w/v) surfactant according to **ASTM D-1331 (2001)**.

7. Preparation of tobacco crude extracts with hexane and methanol as 10% SL.

Crude extracts of tobacco with hexane and methanol were prepared as 10 % SL using different wetting agents added to crude extracts after dissolved in distilled water by serial concentrations, and then the physico-chemical properties were measured. Depending on surface tension for the prepared formulas. The best one was chosen to complete other formulation and bioassay testes.

7.1. physico- chemical properties of extracts formulated as 10%SL:

a. Free acidity or alkalinity:

It was measured as mentioned before.

b. Surface tension:

Surface tension: It was determined by using surface tensiomate for solutions containing 0.5 % (w/v) surfactant according to **ASTM D-1331 (2001)**.

c. Emulsion stability:

It was measured according to CIPAC (2002).

d. Foam:

It was measured according to CIPAC (2002).

e. Stability at 0 °C. (Cold storage):

It was measured according to CIPAC (2002).

f. Stability at elevated temperature 54 ± 2 °C (accelerated storage)

It was measured according to CIPAC (2002).

8. Preparation the tobacco crude extract with chloroform as 20% EC.

Crude extract with chloroform was not soluble in water, xylene and acetone while, soluble in dimethyl formamide so it suitable to formulate as emulsifable concentrate. The crude extracts was dissolved with suitable solvent then added the emulsifier at different rates then tested the emulsion stability test for all formula's and the best one which no oily or creamy separation formed transferred to complete the rest of physical properties then to bioassay test.

8.1. physico- chemical properties of extract formulated as 20% EC:

a. Free acidity or alkalinity:

It was measured as mentioned before.

b. Emulsion stability:

It was measured according to CIPAC (2002).

c. Foam:

It was measured according to CIPAC (2002).

d. Stability at 0 °C. (Cold storage):

It was measured according to CIPAC (2002).

e. Stability at elevated temperature 54 \pm 2 $^{\rm o}C$ (accelerated storage)

It was measured according to CIPAC (2002).

9. The physico- chemical properties of spray solution at field dilution rate (0.5%) for tested formulated tobacco extracts.

a. Surface tension:

It was determined as mentioned before.

b. pH:

It was determined by using Jenway pH meter according to **Dobrat and Martijn** (1995).

c. Viscosity:

It was determined by using Brookfield viscometer Model DVII+Pro, where centipoise is the unit of measurement according to **ASTM D-2196 (2005).**

d. Electrical Conductivity:

It was determined by using Cole-Parmer PH/Conductivity meter 1484-44, where μ mhos is the unit of electrical conductivity measurements according to **Dobrat and Martijn (1995).**

10. Effect of formulated tobacco extracts on pathogenic fungi.

Antifungal activity of formulated tobacco extracts were determined by food poisoned technique (**Mohanty** *et al.*, **2012**). Added separately to get the required concentrations, 4, 2, 1, 0.5 and 0.25 mg/ml were mixed with 50ml of sterilized PDA medium and transferred equally into three Petri dishes. The media was allowed to solidify. Then seven day old fungal culture disk of 5-mm diameter was taken and inoculated to the center of Petri dishes containing formulated tobacco extracts. Instead of PDA medium without formulated tobacco extracts served as control. All dishes were incubated at 27±2°C and radial growth of colony was measured when the mycelia of control had almost filled the Petri dishes. Each test was performed in triplicate

The fungal growth inhibition which was calculated due to treatment against control using the following formula: (Satya et al., 2014).

Inhibition of growth (%) = R-r/R *100

R is the radial growth of fungal mycelia in the control plate.

r is the radial growth of fungal mycelia in the treated plate.

11. Statistical analysis.

The concentration inhibition regression lines were drawn according to the method of **Finney** (1971).

RESULTS AND DISCUSSION

Effect of different solvent on the yield of tobacco waste extracts.

Data in **Table** (1) presented the yield of crude extracts using n-hexane, chloroform and methanol from dry powder of *Nicotiana tabacum* waste where n hexane gave the highest yield followed by chloroform and methanol.

Table (1). The yield of tobacco waste extraction with different solvents (w/w %).

Solvent	Weight in (gm.)Tobacco waste (powder)	% yield		
n-hexane	100	24.9		
Chloroform	100	16.9		
Methanol	100	10.4		

Qualitative Screening for Phytochemical Constituents of different extracts.

Regarding to phytochemical screening for different extracts, data in **Table (2)** showed clearly that n-hexane extract contains the biggest number of phytochemicals like (saponins, tannins (pyrogalol), alkaloids, flavonoids and terpenoids) followed by methanol extracts which contains (tannins (pyrogalol), alkaloids, steroids and terpenoids. However chloroform extract contains (flavonoids, steroids and terpenoids).

Table (2). Phytochemical screening of (*Nicotiana tabacum*) extracted with different solvents.

Phytochemicals	Tobacco (Nicotiana tabacum) extracted with:							
r nytochemicais	Hexane Chloroform		Methanol					
Saponins	+	-	-					
Tannins (pyrogalol)	+	-	+					
Alkaloids	+	-	+					
Flavonoids	+	+	-					
Steroids	-	+	+					
Terpenoids	+	+	+					

(+): found (-): disappear

Physico- chemical properties of formulation constituents. Active ingredient:

Data presented in **Table** (3) indicated that tobacco (*N. tabacum*) extracted with n-hexane, chloroform and methanol were different in its physical properties where, hexane extract was soluble in water and non-soluble in xylene, acetone and dimethyl formamide, also methanol extract was soluble in water and dimethyl formamide but non-soluble in xylene and acetone. While chloroform extracts was soluble in dimethyl formamide but non-soluble in water, xylene and acetone. All tobacco extracts were acidic and hexane extract showed the highest value 5.88 followed by methanol extract which showed acidity value 4.9, while chloroform extract showed the lowest acidic and its acidity value were 0.49.

Table (3). The physico- chemical properties of tobacco crude extracts.

	%	4 %			
Materials	Water	Water acetone Xyle		DMF	Free acidity as H ₂ SO ₄
Crude extract with Hexane	Sol.	N.S	N.S	N.S	5.88
Crude extract with Chloroform	N.S	N.S	N.S	Sol.	0.49
Crude extract with Methanol	Sol.	N.S	N.S	Sol.	4.9

(N.S): No soluble, (Sol.): Soluble, DMF: dimethyl formamide

Physico- chemical properties of surfactants used in formulation.

Data presented in **Table** (4) indicated the physical properties of the surfactants used in preparing and formulating the tested tobacco extracts. Toximol 500 has the lowest HLB value (8-10), but PEG 600 DO was (10-12), so they suitable for used as emulsifying agent to prepare the emulsifiable concentrate. However tween 80 and tween 20 have HLB values more than 13 so they suitable as wetting agent used to prepare the soluble concentrate formulations. Tween 80, tween 20 and toximol 500 were acidic and the acidity values were: 0.6, 0.42 and 0.39 respectively. However, PEG 600 DO was alkaline and the alkalinity value was 0.3. PEG 600 DO gave the highest value in case

of CMC (0.9) followed by tween 80, toximol 500 and tween 20 and their values were 0.5, 0.3and 0.2 respectively. Surface tension was measured and tween 80 gave the highest value 40.3 followed by tween 20, PEG 600 DO, and toximol 500 and their values were 39.2, 37.1, and 27.6 respectively.

Table (4). The physico- chemical properties of the suggested surface active agent.

		Fre	ee		at
Surfactant	нгв	Acidity % as H ₂ SO ₄	Alkalinity %as NaOH	CMC at 25 °C %(w/v)	Surface tension a 0.5% dyne/cm
Toximol 500	8- 10	0.39	-	0.3	27.6
PEG 600 DO	10- 12	-	0.3	0.9	37.1
Tween 80	13<	0.6	-	0.5	40.3
Tween 20	13<	0.42	-	0.2	39.2

HLB: hydrophilic-lipophilic balance, CMC: critical micelle concentration

Physico- chemical properties of tobacco extracts formulated as $10\% \ SL$.

Data presented in **Table (5)** showed clearly the physical properties of tobacco extracts formulated as 10% soluble concentrate where both the n-hexane and methanol extracts were acidic and soluble in water showed clear solutions and no separation or sedimentation. After accelerated storage at 54 °C for 3 days the formulated extracts showed slightly increasing in acidity, surface tension and pH values. Both prepared formulas passed successfully from cold storage at 0 °C for 24 hours. Where no separation, sedimentation or solubility after storage.

Table (5). The physico- chemical properties of tobacco extracts formulated as $10\% \ SL$

	Before accelerated storage at 54 °C				After accelerated storage at 54 °C					
Materials	Acidity % as H ₂ SO ₄	Surface tension dyne/cm	% Solubility	Sed.	Hd	Acidity % as H ₂ SO ₄	Surface tension dyne/cm	% Solubility	Sed.	ЬН
n-Hexane extract 10% SL	0.49	38.6	Sol.	0	6.55	0.54	39.4	Sol.	0	6.72
Methanol extract 10% SL	0.59	37.8	Sol.	0	6.46	0.68	38.2	Sol.	0	6.62

(Sol.): Soluble (sed.): sedimentation

Physico- chemical properties of tobacco extract formulated as 20% EC.

Data in **Table** (6) indicated the physical properties of tobacco extracted with chloroform and formulated as 20 % EC, where it showed slightly acidic, reach 0.49 and pH value 6.75. Also, no oily or creamy separation formed when emulsion stability test was done at 5% and no foam formed before accelerated storage, while showed slightly increasing in acidity and pH values after accelerated storage. Also the prepared formula passed successfully from cold storage at 0 °C for 24 hours.

Before accelerated After accelerated storage at 54 °C storage at 54 °C Materials **Emulsion** Emulsion Acidity % as Acidity % as stability stability H2SO4 Foam H₂SO₄ Foam Ħ 펖 H.W H.W S.W S.W Chloro form 0 0.049 0 0 6.75 0.1 0 6.94

Table (6). Physico- chemical properties of tobacco extracts formulated as 20% EC

H.W = hard water, S.W= soft water

Physico-chemical properties of spray solution at field dilution rate (0.5 %) of formulated tobacco extracts.

Data in **Table** (7) indicated the physical properties of spray solution at 0.5% of formulated to bacco extracts, where the chloroform extract 20% EC gave the highest viscosity value 2.05 centipoise, followed by hexane extract 10% SL and methanol extract. Methanol extract 10% SL showed the highest electrical conductivity value 508 μ mhos followed by hexane extract 10% SL 507 μ mhos, while chloroform extract 20% EC showed the lowest value 424 μ mhos. Also surface tension was measured and its values were, 38.6, 37.8 and 34.4 dyne/cm for hexane extract, methanol extract and chloroform extract respectively. The pH values were 6.75, 6.55 and 6.46 for chloroform extract hexane extract and methanol extract respectively. While all the tobacco extracts formulated had the same salinity value 0.2.

Viscosity
Centipolise
Surface tension
dyne/cm

1.94

1.89

2.05

Hexane extract 10% SL

Methanol extract 10% SL

Chloroform extract 20% EC

Table (7). Physico-chemical properties of spray solution at field dilution rate (0.5 %).of formulated tobacco extracts.

Effect of n-Hexane extract formulation 10% SL of N. tabacum on fungi.

507

508

424

38.6

37.8

34.4

6.55

6.46

6.75

0.2

0.2

0.2

Tobacco extracts formulated after passed all physical properties assessed the antifungal activity against some pathogenic fungi under laboratory condition using the poisoned food (PF) technique. Data in **Table (8)** showed clearly the percent of growth inhibition, EC_{50} and EC_{90} values for the tested fungi *A. solani* and *F. oxysporium*. The results showed that *A. solani* was more sensitive to the n-hexane extract 10% SL than *F. oxysporium*. Where the EC_{50} and EC_{90} values were 0.53 and 8.6 mg/ml for *A. solani* while were 0.76 and 17.57 mg/ml for *F. oxysporium*.

Effect of chloroform extract formulation 20% EC of *N. tabacum* on fungi.

Data in **Table (9)** indicated the effect of tobacco extracted with chloroform and formulated as 20% EC and evaluated against the tested fungi *A. solani* and *F. oxysporium* under laboratory conditions. The results showed that the chloroform extract formulation 20% EC was more effective against the fungus *A. solani* than the fungus *F. oxysporium* as showed in **Table (8)** Where, the EC₅₀ and EC₉₀ values were 0.82 and 17.85 mg/ml for *A. solani* while, were 1.67 and 57.46 mg/ml for *F. oxysporium*.

Table (8). The inhibition percent, EC₅₀, EC₉₀ and slope values for A. solani and F. oxysporium with n-Hexane extract formulation (10% SL)

	n-Hexane extract formulation 10% SL										
1	_ A. solani					F. oxysporium					
Conc. mg/ml	%of inhibition	ECso mg/ml	EC ₉₀ mg/ml	Slope	%of inhibition	EC ₅₀ mg/ml	EC ₉₀ mg/ml	Slope			
4	82.35	0.53	8.6	1.06±	75.12	0.76	17.57	0.9387±			
2	72.89			0.1097	65.4			0.1071			
1	61.4				54.5						
0.5	48.84				43.27						
0.25	36.37				32.56						
0.125	25.2				23.13						
Control	0.0				0.0						

Table (9). The inhibition percent, EC₅₀, EC₉₀ and slope values for A. solani and F. oxysporium with chloroform extract formulation (20% EC)

	Chloroform extract formulation(20% EC)									
-	_ A. solani					F. oxysporium				
Conc. mg/ml	%of inhibition	ECso mg/ml	EC90 mg/ml	Slope	%of inhibition	ECso mg/ml	EC ₉₀ mg/ml	Slope		
4	74.54	0.82	17.85	0.9565±	62.4	1.67	57.46	0.8341±		
2	64.51			0.1075	52.59			0.1075		
1	53.36				42.6					
0.5	41.94				33.1					
0.25	31.15				24.57					
0.125	21.8				17.38					
control	0.0				0.0					

Effect of methanol extract formulation 10% SL of N. tabacum on fungi.

Tobacco waste was extracted with methanol and formulated as 10% SL and assessed its antifungal activity against pathogenic fungi A. solani and F. oxysporium under laboratory conditions. The obtained results in **Table (10)** indicated that the effect as mycelial growth inhibition on A. solani was better than F. oxysporium. Where the EC₅₀ and EC₉₀ values were 1.7 and 52.5 mg/ml for A. solani while were 3.07 and 120.3 mg/ml for F. oxysporium.

Table (10). The inhibition percent, EC_{50} , EC_{90} and slope values for *A. solani* and *F. oxysporium* with methanol extract formulation (10% SL)

	Methanol extract formulation(10% SL)										
=		A. s	solani	F. oxysporium							
Conc. mg/ml	%of inhibition	EC ₅₀ mg/ml	EC90 mg/ml	Slope	%of inhibition	ECso mg/ml	EC90 mg/ml	Slope			
4	62.32	1.7	52.5	0.8655±	53.67	3.07	120.30	0.8047±			
2	52.12			0.1083	44.03			0.1106			
1	41.8				34.74						
0.5	31.99				26.28						
0.25	23.32				19.03						
0.125	16.14				13.16	1					
control	0.0				0.0						

Generally there are positive relationships between tested concentrations and percentages of inhibition with all tested formulated extracts against tested fungi. From above results could be discussed that the antifungal effect of these extracts may be due to the role of their secondary products (saponins, tannins (pyrogalol), alkaloids, flavonoids and terpenoids) these products have many mechanisms to inhibition the pathogenic fungi growth like, Flavonoids are very important in plant resistance against pathogenic bacteria and fungi. The antifungal activity is often based on the inhibition of spore development and mycelium hyphae elongation. Flavonoid antipathogenic activity can also be more specific. It is suggested that

the mechanism of flavonoid antibacterial activity is based on their ability to inactivate microbial adhesion, and cell envelope transport proteins. Fat-soluble flavonoids may also disrupt microbial membranes, change their fluidity and may disrupt the respiratory chain. (Mierziak, et al, 2014). Also (Glauert et al, 1962) reported that the first mode for the mechanism of action of saponins, cause an increase in membrane permeability. On the other hand from data in Tables 8, 9 and 10 could be concluded that, tobacco waste extracted by hexane, chloroform and methanol had antifungal activity against the tested fungi; however hexane extract formulated showed the best effect against both tested fungi followed by chloroform and methanol extracts.

Generally all tested formulations showed the nearest slope values with all tested fungi these indications may be due to the all formulation possessed the same active ingredient. On the other hand slope values showed slight differences as a result to type of formulation. Also changed from tested pathogen to other.

Conclusions

Tobacco waste (powder) was extracted with different polarity solvents, n-hexane, chloroform and methanol. Qualitative phytochemical analyses were assessed and the results detected a lot of phytochemicals.

The tobacco waste extracts were formulated in appropriate formulations form and passed successfully from all physico- chemical testes.

The formulated tobacco extracts were evaluated its antifungal activity and the results showed good efficacy against both tested fungi A. solani and F. oxysporium whereas A. solani was more sensitive to tested formulations than F. oxysporium. On the other hand formulated hexane extract showed the highest activity against both tested fungi followed by chloroform and methanol. This indication may be due to the number of phytochemicals detected in each crude extract. These results gave and throw the light on reuse of wastes as control agent for diseases as natural alternatives of synthetic pesticides, however it needs further studies to prove their safety for environmental application and effectiveness in field.

REFERENCES

- American Society of Testing Materials. ASTM. (2005). Standard Test Method for Rheological Properties of Non Newtonian Materials by Rotational (Brookfield type) Viscometer, D-2196.
- American Society of Testing Materials. ASTM. (2001). Standard Test Method for Surface and Interfacial Tension Solution D-1331.
- **Aqleem, Abbas (2017).** First Report of *Alternaria* Blight of Potatoes in Nomal Valley, Gilgit-Baltistan Pakistan 3:3, 0.4172/2471-9315.1000137
- **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. Review article.
- **CIPAC** (2002). Collaborative International Pesticides Analytical Council Limits. Hand book. Vol. F, Physico- chemical Methods for technical and formulated pesticides.
- **Dobrat, W. and A.Martijn,** (1995). CIPAC Hand Book, vol. F, Collaborative International Pesticides Analytical Council Limited.
- **Duan, S., D.Yongmei; H.Xiaodong; Y.Ning; D.Weije; M. Xinxin** and **Z.Zhongfeng (2016).** Chemical basis of the fungicidal activity of tobacco extracts against *Valsa mali*. Molecules, 21, 1743.
- **Feola, G.; E.Rahn, and CR.Binder (2011)**. Suitability of pesticides risk indicators for less developed countries: A comparison. Agriculture, Ecosystems and Environment; 142: 238-245.
- **Finney, D. J. (1971).** Probit Analysis Statistical, 2nd Ed, Cambridge University.
- Feroz, M.; R.Ahmad; S. T. A. K.Sindhu, and A. M. Shahbaz, (1993). Antifungal activity of saponin from indigenous plant roots. Pak. Vet. J., 13: 44.
- Glauert, A. M.; J. A.Dingle, and J. A. Lucy (1962). Action of saponin on biological cell membranes. J Nature, 196: 953-955.
- **Harbone, J. B.** (1998). Phytochemical methods: a guide to modern techniques of plant analysis. 3ed end. Chapman and Hill Int. Ed., New York.
- Handa, S. S.; S. P. S.Khanuja; G.Longo, and D. D.Rakesh, (2008) Extraction Technologies for Medicinal and Aromatic Plants, (1stedn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology. Herbicides. Weed Sci. 49: 290-297.

- **Ignjatov, M.; D.Milosevic; Z.NiKolic; Gvozdanovic- J.Varga; D.Jovicic, and G. Zdjelar (2012).** *Fusarium oxysporum* as causal agent of tomato wilt and fruit rot. Pestic. Phytomed. (Belgrade), 27(1), 25-31.
- Javed, N.; S.R.Gowen; Inam-V-Haq; M. K.Abdullah; and F.Shahina (2006). Systemic and persistent effect of neem (Azadirachta indica) formulation against root- knot nematodes Meloidogyne javanica and their storage life. Crop protect. 26(7), 911-916.
- 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.
- Mierziak, J.; K.Kostyn, and A.Kulma, (2014). Flavonoids as important molecules of plant interactions with the environment. Molecules 19, 16240-16265.
- **Mohanty, R. C.; P.Ray, and S. Rath** (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. C. and G. W. Fiero (1954). A selected Aromatic Fraction Naturally Occurring in Petroleum as Pesticides Solvents; *J. Agric. Food Chem.*, 14(2): 1737-1765.
- **Osipow, L. I.** (1964) Theory and Industrial Applications Reinhold publishing Crep., New York, 473: pp.
- **Peach, J. and M. V. Tracay, (1955).** Modern methods of plant analysis, vol. IV. Spring. Verlag, Berlin
- **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.
- **Tomizawa, M. and J. E. Casida (2003).** Selective toxicity neonicotinoids attributable to specificity of insect and mammalian nicotinic recrs. Annu. Rev. Entomol. 48, 339-364.
- World Health Organization (WHO) (1979) Specification of Pesticides used in public Health Sth ED., Geneva.

استخلاص وتجهيز وتقييم النشاط الإبادى لمخلفات نبات الدخان ضد بعض الفطريات الممرضة للنبات

أمجدي عدلي اسكندر - 2عزة رسمى عماره

1- قسم بحوث مستحضرات المبيدات - 2- قسم بحوث المبيدات الفطرية والبكتيرية والنيماتودية

المعمل المركزي للمبيدات مركز البحوث الزراعية. دقى - جيزة

تم الحصول علي عينة من مخلفات نبات الدخان من الشركة الشرقية للدخان- محافظه الجيزة لدراسة اعادة استخدامها والاستفادة من هذة المخلفات في مجال مكافحة الافات وخصوصا الفطريات الممرضة للنبات.

تم استخلاص مسحوق مخلفات نبات الدخان بثلاث مذيبات مختلفة القطبية بالتوالي وهي (الهكسان - الكلوروفورم - الميثانول).

تم الكشف علي المركبات الثانوية (بالأختبارات اللونية) الموجودة في كل مستحضر، حيث تم التعرف علي الصابونين، التانينات، القلويدات، الفلافونيدات و التربينات مع مستخلص الهكسان وكذلك تم التعرف علي التانينات، القلويدات ، الاستير ويدات و التربينات مع مستخلص الميثانول مع مستخلص الكلور وفورم فقد تم التعرف علي الفلافونيدات ، الاستير ويدات و التربينات .

تمت دراسة الخواص الطبيعية للمستخلصات وبناء عليها تم تحضير كلا من مستخلص الهكسان والميثانول في صورة مركزات قابلة للذوبان في الماء بتركيز 10% ، وكذلك تم تحضير مستخلص الكلوروفورم في صورة مركزات قابلة للاستحلاب في الماء بتركيز 20%.

تم دراسة الفعل الابادي للمستحضرات علي الفطريات الممرضة للنبات، حيث اظهرت النتائج تاثير قوي للمستحضرات المجهزة علي الفطريات تحت الدراسة (الترناريا سولاني و فيوزاريم اوكسيسبوريم). حيث اظهر مستحضر الهكسان أعلي تاثير علي كلا الفطريات تحت الدراسة، ثم مستحضر الكلوروفورم ثم مستحضر الميثانول.

كذلك اظهرت النتائج ان التاثير علي فطر الترناريا سولاني كان اكبر من فطر فيوزاريم اوكسيبوريم مع كل المستخلصات المختبرة.