Possible antifungal effects of aqueous extracts and residues of some common wild plant species on certain soil borne plant pathogenic fungi

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Tawfiq

TO MY FAMILY

English Summary

This study was conducted during the period from 1988-1991 in order to investigate the antifungal effect of aqueous extracts and residues of some common uncultivated plant species on certain soil borne plant pathogenic fungi prevalent in Jordan.

The study started by screening forty plant species belonging to 21 botanical families against five plant pathogenic fungi. These were : *Fusarium oxysporum* Snyd & Hans. Schlecht, *Helminthosporium sativam* (Pam.,) King and Bakke, *Alternaria solani* (Ell & Mart.) Jones and Grout, *Cladosporium herbarum* LK. ex FC. and *Botrytis cinerea* Press ex Fr.,.

Results showed that plant species were highly variable in their effects on the fungal species. Differences were also found in the effects of extracts obtained from different plant parts or prepared from plants at different growth stages.

Anagallis arvensis exhibited strong inhibitory effects on *H. sativum* and moderate effect on *F. oxysporum.* The dried materials of this weed reduced significantly growth and development of both fungal species.

Shoot extract of *Inula viscosa* inhibited strongly growth of *H. sativum* and *F. oxysporum* while its dried shoot showed a moderate effect.

Studies on extracts of *I. viscosa* and *A. arvensis* showed that both species exhibited a fungistatic activity against the tested fungi. The toxic effects of the inhibitory materials gradually increased with plant age from seedling to flowering .

Inula viscosa was also found to possess a strong volatile antifungal effect against *H. sativum* and *F. oxysporum.* The inhibitors were more in shoots than in roots. Autoclaving or boiling shoots drastically reduced their antifungal effects. On the other hand, the antifungal effects of *A. arvensis* extract was not affected by autoclaving and boiling treatments. Shoot and root extracts of this species were

equally effective against the tested fungi.

A glasshouse experiment was conducted to detect any value for *I. viscosa* extract for controlling *Fusarium* wilt of tomato under glasshouse conditions. Results showed that *I. viscosa* shoot extract caused a partial reduction in tomato wilt infection caused by *F. oxysporum*. The extract proved to be non-phytotoxic to tomato plants. The exploitation of this species in the control of *Fusarium* wilt of tomato merits further studies.

Introduction

The past few decads have shown very exciting developments in the chemical control of plant diseases . Tremendous advances have been made in fighting the myriad of disease organisms responsible for great yield losses anually.

Recently, there have been many developments which may have significant effect on the future of agriculture and the pesticide industry . We have experienced shortages of agriculture commodities. However, the most important characteristic of agricultural resources is their renewability .

Fungi cause major destruction of various food commodities during production and storage. Furthermore, the production of mycotoxins by these fungi constitutes a serious threat to animal and human life .

There are many synthetic pesticides presentley marketed to control the fungal growth on food and feed commodites . It is now well established that many of these chemicals are carcinogenic and teratogenic. Also they are non-biodegradable, persistent and hence accumulate in food chain (48) . Therefore, a constant and intensive search for new effective and harmless pesticides is urgently needed .

During recent years, higher plants played a major role in controlling a number of fungi species . Accordingly, plants and their constituents are being promoted to provide us with fungicides which are less phytotoxic, easily bio-degradable and non-carcinogenic (17) .

The increase in environmental problems associated with the use of pesticides

has encouraged interest in using natural chemicals which are present in plants to control plant pathogens.

The main objectives of the present study were to : -

1 - Examine the effect of extracts of a number of uncultivated plant species , including some of the most common weed species occuring in Jordan on certain soil borne plant pathogens .

2 - Study the effect of extracts obtained from different plant parts in order to have an idea about the differences in the effectiveness of these extracts and their concentrations .

3 - Study the effect of extract of the most promising plant species at different growth stages .

4 - Study the effect of dried plant materials incorporated in the media on the growth and development of fungal species .

5 - Study the role of temperature and length of incubation period on the effectiveness and fungitoxicity of extracts of these plant species .

6 - Compare the efficiency of plant extracts with certain synthetic fungicides in controlling plant diseases .

Literature Review

The present literature review was divided into the following parts : -

1. General .
2. Screening plants for their antifungal activities .
3. Antifungal activity of different plant parts .
4. Antifungal properties of plant extracts.
5. Antifungal compounds .
6. Antifungal effects of *Anagallis arvensis* L. and *Inula viscosa* (L.) Ait.

1. General : -

The Egyptians, Greeks and Romans made use of materials of plant origin in human chemotherapy (17) . However, studies on utilization of plant parts and plant extract for their antifungal and antibacterial properties were very limited. Some workers observed the presence of fungitoxic substances in plants and plant extracts which have protective and therapeutic values and also contain antibiotic agents (19) .

Fawcett and Spencer (17) mentioned that in 1802 William Forsyth had recommended using a decoction containing tobacco and elder buds with lime and sulphur to wash the young and tender shoots of trees that are severely infected with mildew .

Carlson *et al* as cited by Rafiq *et al* (37) were first to determine the

antimicrobial activity of extracts of certain higher plants in 1948 . Later, Nene and Thapliyal (31) reported the antifungal activity of extracts of *Anagallis arvensis* against *Colletotrichum papayae* (Sydow) .

With increase awareness towards toxic hazards of fungicides, importance of indigenous products in plants for disease control substantial work has been done and encouraging results have been reported . Tripathi *et al* (48) found that the essential oil from the fruits of *Trachyspermum ammi* L. exhibited a strong toxixity against *Aspergillus flavus* Link and *A. niger* Van Tieghen, while Khune and Patil (24) found that neem (*Azadirachta indica* Juss.) leaf decoction was very effective in givning more than 75% inhibition of growth of sooty mold disease caused by *Capnodium citri* . On the other hand, El-Doksh *et al* (16) examined the contact insecticidal toxicity of 33 plant extracts against *Spodoptera littoralis* Boisd and *Tribolium confusum* Duv . They found that light petroleum extract of black pepper *(Piper nigrum* L. *)* with LD 50 13 Ug extract / larva and *Datura stramonium* with LD 50 17.5 Ug extract / Larva using topical application method , were the most toxic extracts to *S. littoralis* larvae . They also observed that black pepper oil (*Piper nigrum* L.) showed a highly toxic effect against the fungus *Rhizoctonia solani* Kuhn . Chesne *et al* (9) studied the antifungal effect of 49 indigenous plants, positive results against 10 phyto-pathogenic fungi were achieved with 12 crude extracts .

Misra and Dixit (30) observed that crude extract obtained from the aerial leaves

effects(48) . However, recent research on the use of constituents of higher plants as a possible source of alternative pesticides has been advocated on account of their non-phytotoxic , more systematic and easily biodagradable (2 and 17) . Therefore, the screening process of higher plants for their antifungal properties continues on a large scale .

The most extensive survey of higher plants for their antibacterial substances carried out by Osborn (33) who investigated water extract of 2300 different plant species and varieties against two species of bacteria ; which were *Staphaylococcus aureus and Escherichia coli*.

Dixit and Tripathi (12) screened 29 plant species against two plant pathogenic fungi , which were *Fusarium nivale* (Fries .) Cesati . and *Cephalosporium saccherri* Butl. Out of the taxa screened extracts of four plants showed strong fungistatic activity against both fungi while the extract of six other species were partially active in inhibiting spore germination of either of the two tested fungi .

Franje (19) reported that extract of 36 plant species out of 147 species screened , exhibited fungicidal properties against the tested organisms. Among the 36 plant species, 21 were found active against *Colletotrichum lindemuthianum* (Sacc.and Magn.) Bri. and 12 against *Cercospora cruenta* Sacc. Lapis and Dumancas (26) studied the antifungal activity of extract from 93 plant species against *Helminthosporium oryzae* Breda de Haan .(the causal organism of brown spot of rice) , and found that 24 of these species have inhibited the growth of this

fungus . Guesin and Reveillere (23) examined the effect of 41 plant extracts against 9 fungal species. They observed that the extracts of *Ruscus aculeatus* L., *Hibiscus sabdariffa* L. and *Tamarinds indica* L. had inhibited spore germination of many fungi. Other extracts (especially of *Zingiber officinal* Roscoe) had more restricted range of activity.The extract of *Viola tricolor* L. was only effective against*Trichophyton mentagrophytes* (Robin.) .

3 . Antifugal activity of different plant parts

In all screening programmes, leaf extracts were used to study the antifungal properties of plant species . Chaturvedi *et al* (7) screened leaf extracts of 150 plant species and found that *Adenocalymma allicea* which belongs to the family Bignoniaceae exhibited the strongest antifungal activity against *Drechslera oryzae* (Berda de Haan) Subram and Jain. Different parts of this species were tested for their toxicity to ascertain the distribution of toxic materials and they found that extracts of the leaves were the most toxic .

The fungitoxicity was found to be variable in different plant parts. Gilliver (20) tested different parts of some of the active plants and observed that few roots , seeds, flowers and less frequently fruits showed antifungal activity while the leaves were always the most active .

It has been observed that certain plant parts contain more antifungal activity as compared to other parts of the same plants . Tripathy *et al* (47) found that *Iberis*

amara (which belongs to Cruciferae family) showed more antifungal activity in seeds and flowers than roots , leaves and stem . On the other hand, Rizki *et al* (39) showed that *Withania coaqulans* Dunal. fruits had more antifungal activity than roots and there were more antifungal activities in *Withania somnifera* (Linn.) Dunal. twigs than with leaves.

4. Antifungal properties of plant extracts.

In many screening studies for the antifungal activities of plant extracts, the inverted Petri-plate method was used in order to detect the volatile antifungal effect of the plant extract against the tested fungi. This method was always used to dectect the volatile fungitoxic substances in plant extracts as described in the modified Bocher method (7) . So volatility of the plant extract was studied and there was a large number of reports showing that many plants posses volatile fungi toxic substances.

Dubey *et al* (13) observed that out of twenty one plant species screened, only leaf extract of *Chenopodium ambrosiodes* L. showed absolute volatile toxicity against *Rhizoctonia soloni* . In screening leaf extracts of some higher plants for their volatile antifungal activity against *Aspergillus flavus* Link. , extracts of *Ocimum adscendens* Willd exhibited the strongest fungitoxitity (2) .

Pandey *et al* (34) during screening of the leaves of 25 plant species for their volatile toxicity against *Funsarium lateritium* Nees f. sp. cajaini (Padwick) , found

that *Aegle marmelos* (L.) correa, *Citrus aurantifolia* (Christm) and *Mentha arvensis* L. exhibited strong toxicity and inhibited the mycelial growth completely .

The effect of certain physical factors on plant extracts such as drying , temperature and autoclaving were studied. In most cases, they found that toxic materials were destroyed by heating at temperatures above 50°C or after autoclaving.Chaturvedi *et al* (7) found that the toxic substances of *Adenocalymma allicea* were destroyed by drying at room temperature for 7 days or heating to 50°C or autoclaving . The active components of plant extraxts were isolated in a form of an essential oil through hydro-distillation using the Celvenger's apparatus (7,13,14 and 48) in order to obtain the fungitoxic constituents in an active state and at the maximum strength. (1)

Dubey and Tripathi (14) found that the essential oil of *Piper betle* L. exhibited fungistatic effect against *Aspergillus flavus* Link. The toxic properties were not affected by autoclaving , heating or strong and heavy inoculum density. The oil also showed no adverse effect on percent seed germination and seedling growth of *Pisum sativum* L. Asthana *et al* (2) reported that when the active materials were isolated in the form of oil , it becames stable to high temperature and for a long period of time. Food poisoned technique (21) and liquid culture technique (21 and 27) were among the most familiar methods used to study the antifungal properties of higher plants. By using these two techniques, some physical

properties of plant extracts were studied such as thermostability, drying of leaves , dilution and aging in order to formulate an idea aboat the stability of the active ingredient of plant extracts .

Kumar and Nene (25) found that leaf extracts of *Cleom isocandra* L. were inhibitory up to a dilution of 1 : 2000 against *Helminthosporium maydis* Nishikado and Niyake. The inhibition was not adversly affected by boiling the leaf extracts for five minutes on direct heat . Extracts prepared from oven dried leaves were as effective as fresh extracts and the leaf extracts did not lose their inhibition when stored at room temperature for seven days .

Tripathi *et al* (47) observed that the fungitoxicity of *Iberis amara* L. seed extracts was retained up to 35 days but was lost within 15 days in leaf extract of *Allium sativum* Linn and *Clematis gouriana* Roxbs ex Dc. The same authors (47) reported that the active materials of *Anagallis arvensis* L. have been found to be too phytotoxic for practical use while seed extracts of *Iberis amara* had no adverse effect on *Oryza sativa* L..

There is also evidence that compounds with high or moderate antifungal activity in vitro (or on spores) often posses negligible systemic fungicidal activity in vivo . On the other hand, certain compounds with negligible antifungal activity in vitro were markedly active as chemotherapeutants (17).

Many experiments were conducted in order to compare the efficacy of some plant extracts with certain sythetic fungicides . Misra and Dixit (29) found that the

crude extracts of *Clematis gouriana* Roxb, ex Dc. exhibited marked toxicity against many fungi . Its activity was compared with four commercial fungicides against *Alternaria tenuis* Neen , *Curvularia lunata* (Wakker) Boedign , *Fusarium nivale* (Fries) Cesati and *Helminthosporium gramineum* Rab. ex Schlecht . The active component of plant extracts was 27 times more active than Diathane Z-78 (Zineb) and 55 times more active than Ziram (Milbam) on all tested Fungi .

Chaturvedi *et al* (7) compared the efficiency of *Adencalymma allicea* oil with some synthetic fungicides against *Drechslera oryzae* and found that the oil was ten times more active than Dinocap (Karathane) , and four times more active than Diathane M-45 (Mancozeb) , Ceresan (Organomercury compound) and Hinosan-50 (Edifenphos) .

Some studies were conducted in the field to compare the efficiency of some commercial fungicides and plant extracts for the control of some plant diseases. Singh *et al* (41 and 42) compared the efficacy of garlic (*Allium sativum* L.) leaf extract neem (*Azadirachta indica* Juss) , ginger (*Zingiber officinale* Rosc.) and oil of garlic with some synthetic fungicides [80% Wettable sulphar (Sulfex), Carbendazim (Bavistin) and Dinocap (Karathane)] for the control of powdery mildew (*Erysiphe polygoni* Dc.) of peas (*Pisum sativum* L.) . They found that neem leaf and garlic bulb extracts gave consistently good control of the disease but were not effective as fungicides compered with , ginger extracts or garlic oil .

On the other hand, some plant extracts were reported to stimulate the growth of

some plant pathogens . Tewari and Dath (46) reported that leaf extract of *Oryza sative* encouraged the growth as well as sporulation of *Drechslera oryzae* , while Dixit and Tripathi (12) observed that extracts of *Brassica juncea* L. and *Brassica pekinensis* Lour stimulated the spore germintion of *Cephalosporium sacchari* and *Fusarium nivale* . Rizki *et al* (39) found that the extract of *Allium sativum* bulb , *Bridelia montana* (Dc.) Willd fruits, *Cassia angustifolia* Vahl. leaves and fruits and *Capparis decidua* Edgew leaves have shown stimulatory activities on *Aspergillus niger, A. flavus* and *Penicilliuim citrinum* Thom. Thus these reports strongly suggest the presence of growth promoting substances in the extracts of some plant species. Such factors are worth exploiting for developing simple , cheap and effective media in culturing the Pathegens . (42) .

5 . Antifungal compounds: -

Perhaps the presence of fungicidal substances in the host plant make these plants resistant to invading pathagens. There are two groups of fungitoxic substances present in plants. One of these is called phytoalexins. In this group chemical compounds accumulate in the plants and limit the development of infectious agent (44 and 45) . The other group is composed of substances which are inhernt and / or present in the plant with or without pathogens. The main concern of this study is the later group . There are several examples of these chemicals which give positive results against different phytopathogenic fungi

including Gibberellic acid, Amino acids, Phenolic compounds, Lactons, Coumarins, Acetylenic Compounds . Quinons, Tropolones and many others which can be founds in different plants (1 and 35) .

Bosa and Voros as cited by Fawcett and Spencer (17) reported that wheat seed infected with bunt (*Tilletia foetida*) soaked in gibberellic acid solution before planting gave increased yield and less disease .

Sivanathan and Adikman (43)isolated antifungal compounds from peel of unripe avocado fruits. They identified them as 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12.15-dien.These compounds had previously been isolated from avocado leaves.They inhibited vegetative growth of *Colletotrichum gloeosporiodes* (Penz.) Penz. & Sacc in vitro and totally inhibited spore germination. On the other hand, Zehavi *et al* (50) found that Saponin A when isolated from *Styrax officinatis* L. fruits exhibited a strong activity against *Trichoderma viride* , *Fusarium oxysporum* . *Aspergillus niger* .

The nature and the concentration of antifungal chemical compounds of each plant extract determine the effectiveness of these extracts against different fungi(1).

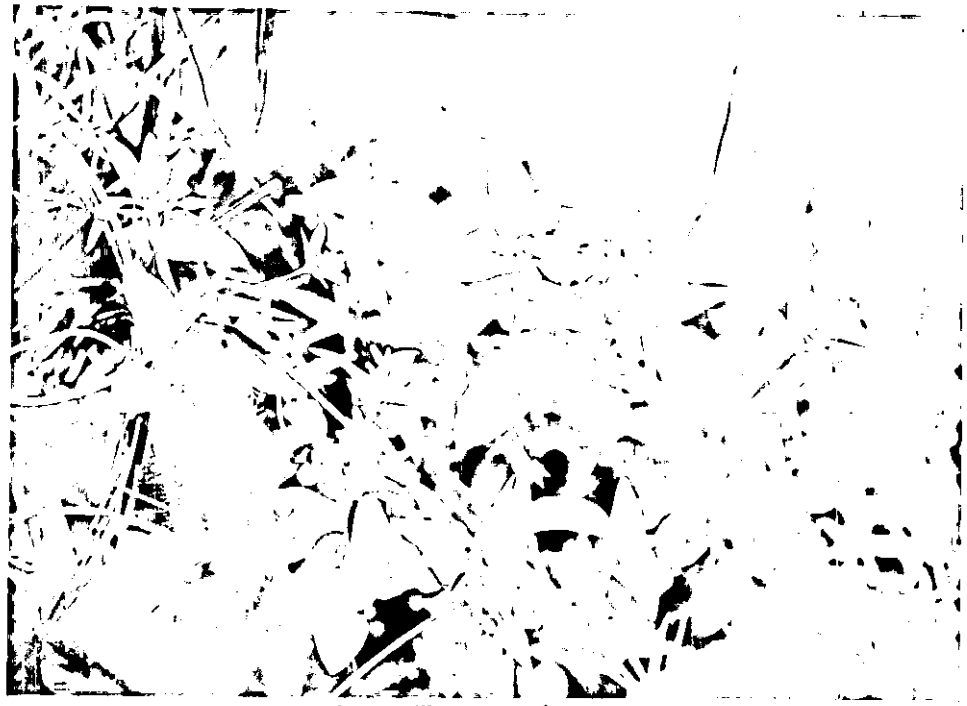
6- Antifungal activity of *Anagallis arvensis* L.and *Inula viscosa* (L.)Ait.

There are many studies on the antifungal activity of *Anagallis arvensis.* Nene and Thapliyal (31) reported the antifungal activity of extracts of *Anagallis arvensis* against *Colletotrichum papayae* . They also noted that the antifungal principle

gradually increased with the growth of plants from seedling to flowering stage . Rafiq *et al* (37) reported that leaf extract of *Anagallis arvensis* was found to inhibit the growth of *Helminthosporium oryzae* , *H. carbonum* and *H. turcicum*, while its stem extracts had restricted the growth of the first two pathogens . Leaf extracts of *Anagallis arvensis* were also found inhibitory to the growth of *Pythium aphanidermatum* Edson (Fitz) in a liquid culture technique (31). *Anagallis arvensis* is one of the most common annual weeds which can be found in different parts of Jordan (Plate 1 a) . It belongs to Primulaceae family and flowers from February to June under local conditions (51).

The other species *Inula viscosa* is a perennial shrub which belongs to Compositae family (Plate 1 b). It is used medicinally (18) and can be found in different parts of Jordan. It flowers from June to December under local conditions . As far as can be detected, these two weeds have not been studied before in Jordan .

a



Anagallis arvensis

b

Inula viscosa

Plate 1 : a) *Anagallis arvensis*

b) *Inula viscosa*

Materials and Methods

1- Preparation of plant extracts.

Forty plant species belong to 21 plant families including some of the most common weeds which can be found in Jordan were collected at pre-flowering and flowering stages from different parts of the country (Table 1). Shoots were washed with running tap water, then rinsed several times with distilled water. One Kilogram of fresh shoots was added to a liter of distilled water, blended and homogonized using a Waring Blender for five minutes. The mixture was allowed to stand for half an hour. Then it was filtered through a Whatman No-1 filter paper, and passed through a membrane filter to rid it from any bacterial or fungal contamination. The filtrate was considered a full strength and it was stored in a deep freezer.

2. Fungal species and their source of inoculum:

The selected soil borne plant pathogenic fungi for the screening experiment were :

a) *Fusarium oxysporum* (Snyd & Hans). Schlecht from tomato roots.

b) *Helminthosporium sativum* (Pam.,) King and Bakke from wheat leaves .

c) *Alternaria solani* (Ell & Mart.) Jones and Grout from tomato fruits.

d) *Cladosporium herbarum* LK. ex FC. from cucumber leaves

e) *Botrytis cinerea* press ex Fr., from tomato fruits .

The selected fungi were maintained on potato dextrose agar (PDA).

The following laboratory and glassshouse experiments were carried out during this study :

Table 1 Family and scientific names, common names and stages of plant species screened for their extract effect on the tested fungi .

Family and Scientific name	Common name	Growth stage
1. Amaranthaceae		
Amaranthus blitoidis S.Wats	Prostrate pigweed	Flowering
Amaranthus retroflexus L.	Redroot pigweed	Flowering
2. Asclepiadacae		
Calotropis procera (Ait) . Fil	Sodom-apple	Pre-flowering
3. Boraginaceae		
Heliotropium europaeum L.	European heliotrope	Pre-flowering
4. Chenopodiaceae		
Chenopodium album L.	Common goose-foot	Pre-flowering
Chenopodium murale L.	Nettle-leaved goose-foot.	Pre-flowering
Suaeda asphaltica (Boiss)Boiss,Fl.	Sea blight	Pre-flowering
5. Compositae		
Anthemis palestina Rent.	Palestine chammomile	Flowering
Calendula arvensis L.	Field marigold	Flowering
Centaurea iberica Trev. ex spring	Iberian star thistle	Flowering
Conyza bonariensis L.	Fleabane	Flowering
Inula viscosa (L.) Ait	Clammy inula	Pre-flowering
Notobasis syriaca L.	Syrian plumed thistle	Flowering
Sonchus oleraceus L.	Common sow thistle	Flowering
Xanthium spinosum L.	Sping cocklebur	Flowering
6. Cruciferae		
Capsella bursa -pastoris L.	Shepherd's purse	Flowering

Cont. Table 1.

	Eruca sativa (Mill.)	Garden rocket	Flowering
	Sinapis arvensis L.	Wild mustard	Flowering
	Sisymbrium irio L.	London rocket	Flowering
7.	Cucurbitaceae		
	Ecballium elaterium L.	Squirting cucumber	Fruiting
8.	Euphorbiaceae		
	Chrozophora obliqua (Vahi) ad. Juss	Mullen-leaved croton	Flowering
	Euphorbia helioscopia L.	Sun spurge	Pre-flowering
	Euphorbia peplus L.	Petty spurge	pre-flowering
	Mercurialis annua L.	Annual mercury	Pre-flowering
9.	Fumariaceae		
	Fumaria densiflora DC.	Dense-flowered fumitory	Flowering
10.	Gramineae		
	Cynodon dactylon (L.) pers	Bermuda grass	Pre-flowering
	Echinochloa colonum L. (Link)	Water grass	Pre-flowering
	Sorghum halepense L. (Pers)	Johnson grass	Flowering
11.	Hypericaceae		
	Hypericum triquitrifolium Turra.	Curled-leaved st. Johntwort	Pre-flowering
12.	Labiatae		
	Lamium amplexicaule L.	Great henbit	Flowering
13.	Malvaceae		
	Malva sylvestris L.	Common mallow	Pre-flowering
14.	Mimosaceae		
	Prosopis fracta (Banks et sol) Macbride	Mesquite	Fruiting
15.	papaveraceae		
	Papaver rhoeas L.	Corn poppy	Flowering

Cont. Table 1.

16. Papilionaceae		
Scorpiurus muricatus L.	Two-flowered caterpillar	Pre-flowering
17. Polygonaceae		
Polygonum aviculare L.	Knot grass	Flowering
Rumex obtusifolius L.	Broad leaved dock	Pre-flowering
18. Primulaceae		
Anagallis arvensis L.	Scarlet pimpernel	Flowering
19. Scrophulariaceae		
Verbascum sinaiticum Benth.	Figwort	Pre-flowering
20. Solonaceae		
Solanum nigrum L.	Black nightshade	Pre-flowering
21. Urticaceae		
Urtica urens L.	Small nettle	Pre-flowering

* Nomenclature is that of Flora Palaestina (51) and common names were selected arbitrarily.

I. Laboratory experiments :

1. Aqueous extract effect of plant species on the tested fungi.

This experiment was carried out to investigate the possible antifungal activity of plant extracts against the fungi under study.

The PDA- medium was autoclaved for 15 minutes at 120°C and 15 PSI. Streptomycin (lg/liter) was added to the medium as a bacterial disinfectant when the medium temperature was about 40°C (4 , 7). Twenty ml of PDA medium were added to each of four sterilized Petri-dishes. Four mycelial discs, each of one cm in diameter were cut from the periphery of a seven-day old culture of each tested fungus and placed in each Perti-dish. Three ml of fresh shoot extract were added to each Petri-dish on the discs.

In another treamtment three ml of sterilized distilled water were added to each Perti-dish and considered as a control. Petri-dishes were incubated at 24°C for 16 days. Each treatment was replicated four times. Data on the growth of the tested pathogens were taken at 4,8 and 16 days after incubation.

2. Antifungal effects of *Anagallis arvensis* L. and *Inula viscosa* (L.) Ait.

Results obtained from experiment 1 showed that extracts of *Anagallis arvensis* and *Inula viscosa* were the most effective extracts on *Helminthosporium sativum* and *Fusarium oxysporum.* Thus this work was concentrated on these two plant species in all subsequent tests.

This experiment was conducted in order to confirm the results obtained from experiment 1 using different experimental techniques.

One cm diameter fungal discs taken from a seven-day old culture of *Fusarium oxysporum* or *Helminthosporium sativum* were soaked for one and ten minutes in the extracts. One disc of the fungus was then transfered to the center of each sterilized Pertri-dish with PDA medium.

In another treatment fungal discs were soaked in sterilized distilled water, Placed in Petri-dishes and considered as a control. Petri-dishes were incubated at 24°C for 16 days. Colony daimeter and percentage of mycelial growth inhibition (MGI%) were determined using the formula given by Misra and Dixit (29).

$$(MGI\%) = 100\ (dc - dt)/dc.$$

Where

dc : Colony diameter in the control.

dt : Colony diameter in the treatment.

3. Antifungal activity of different plant parts.

This experiment was conducted in order to investigate the antifungal activity of shoots and roots of *Anagallis arvensis* and *Inula viscosa* on *Helminthosporium sativum* and *Fusarium oxysporum* using the liquid culture technique (21 and 27).

Six ml of plant extract and a fungal disc of 1 cm in diameter of the tested fungi were placed in each of four flasks containing 40 ml of Potato Dextrose Broth.

In another treatment one fungal disc was placed per each flask with six ml of sterilized distilled water added to the medium and considered as a control. Flasks were incubated at 24°C. The experiment was terminated at one week after incubation. Mycelium was filtrated through a Whatman No-1 filter paper, washed with distilled water, oven dried at 70° C for 24 hours and its dry weight was determined.

4. Antifungal activity of dried plant material.

The objective of this experiment was to study the antifungal activity of dried shoots and roots of *Anagallis arvensis* and *Inula viscosa* against all tested fungi using " the food poisoned technique " (22). Plant shoots and roots were oven dried separately at 70° C for 48 hours, ground into a fine powder then 0.4 g of dried shoots or roots were added to each flask containing 80 ml of PDA. The PDA-Plant material mixture of one flask was used for four Petri-dishes as replicates.

In another treatment Petri-dishes containing PDA without any dried material were included and considered as a control. One cm in diameter fungal disc of a seven day old culture of the tested fungi was placed in the center of each Petri-dish. Petri-dishes were incubated at 24° C for 16 days. Data on the growth and colony diameter were taken at 4 , 8 and 16 days after incubation. Percentage of mycelial growth inhibition (MGI%) was determined based on the procedure followed by Misra and Dixit (29).

5. Effect of extract concentration:

The objective of this experiment was to determine the minimum concentration of the extracts of *Inula viscosa* or *Anagallis arvensis* which inhibit the growth of *Fusarium oxysporum* or *Helminthosporium sativum*.

Fresh shoot extracts of *Anagallis arvensis* and *Inula viscosa* were used separately at 0, 0.1 , 0.2, 0.3, 0.4, 0.5, 1.0 , 1.5, 2.0 , 2.5 and 3.0 ml per Petri-dish. The final volume was made up to 3.0 ml by adding distilled water.

The effect of different extract concentrations on the growth of the tested fungi was investigated using two different techniques which were :

a) Liquid culture technique (21 and 27) ,and,

b) Food poisoned technique (22).

In the first technique, flasks were filled with 20 ml of Potato Dextrose Broth, three ml of plant extract and 1 cm diameter mycelial disc taken from a seven day old culture of *Fusarium oxysporum* or *Helminthosporium sativum*. In another treatment three ml of sterilized distilled water and 1 cm diameter mycelial disc of the tested fungi were added to the medium and considered as a control.

Flasks were incubated at 24°C. The experiment was terminated one week after incubation. Mycelia were filterated through a Whatman No.1 filter paper, washed with distilled water, oven dried at 70° C for 24 hours and their dry weight was determined.

When the food poisoned technique was used twenty ml of sterilized PDA was

poured in each of 11 cm diameter Petri-dishes. Three ml of shoot extract was added to each Petri-dish when the temperature was about 45° C. In another treatment three ml of sterilized distilled weter were added and considered as a control.

One cm diameter fungal disc taken from a seven-day -old culture of the tested fungi was transferred to each Petri-dish. The Petri-dishes were incubated at 24°C for 16 days.

Data on colony diameter was taken at 4,8 and 16 days after incubation, then percentage of mycelial growth inhibition was calculated.

6. Effect of plant stage on extract activity.

This experiment was carried out in order to study the effect of extract prepared from plants at different growth stages, on the examined fungi. Shoots of *Anagallis arvensis* were collected at seedling, vegetative and flowering stages, while *Inula viscosa* shoots were harvested at early vegetative, full vegetative and flowering stages. In this experiment both liquid culture technique (21 and 27) and food poisoned technique (22) were used as described earlier.

7. Effect of autoclaving and boiling on the effectiveness of plant extract.

This experiment was conducted to investigate the effect of autoclaving and boiling on the effectiveness of *Inula viscosa* and *Anagallis arvensis* shoot extracts against *Fusarium oxysporum* and *Helminthosporium sativum*.

8. Possible volatile effect of the extract.

This experiment was carried out to determine any possible volatile effect of the extract on the fungi under study. Extracts of *Anagallis arvensis* and *Inula viscosa* were tested separately for their volatile effects on *Fusarium oxysporum* and *Helminthosporium sativum* using the inverted Perti-dish technique (2 and 7).

Twenty ml of sterilized PDA were poured into each of four Petri-dishes. A mycelial disc of one cm in diameter was taken from a seven-day-old culture of the tested funig and placed in the center of the Petri-dish. The dishes were inverted up side down. Three ml of fresh shoot extract were added to the bottom side of each dish. In another treatment, 3 ml of sterilized distilled water were added to each Petri-dish and considered as a control. Petri-dishes were incubated at 24° C for 16 days . Data on colony diameter were taken at 4, 8 and 16 days after incubation . Percente inhibition of mycelial growth was calculated.

II. Glasshouse experiment :

One experiment was carried out under glasshouse conditions in order to :

1- Investigate any importance of *Inula viscosa* shoot extract effects on *Fusarium oxysporum.*

2- Investigate the possibility of using *I. viscosa* shoot extract for controlling *Fusarium* wilt of tomato.

3- Evaluate the effectiveness of plant extracts compared with some commercial

fungicides in controlling tomato wilt disease caused by *Fusarium oxysporum*.

Plastic pots of 15 cm in diameter were filled with autoclaved soil peatmoss mixture (3:1). Pots were planted with one month old seedlings of tomato (*Lycopersicon esculentum* Mill. c.v *Acora*), using one seedling per pot .The tomato cultivar used was known for its susceptibility to *Fusarium oxysporum.*

The inoculum of *Fusarium* was obtained by growing the pathogen on PDA. Six day after incubation at 25° C, the fungal growth with the agar were blended using a Waring Blender with sufficient amount of sterile distilled water to make a thin slurry (10). Microspore suspensions were filtered through cheese cloth and the final concentration was adjusted to 1 x 10^6 spore/ml. The inoculation was accomplished by pouring twenty ml of a microspore suspension into each pot without disturbing the plants (11). The following treatments were included:

1- Tomato inoculated with *Fusarium oxysporum* (Control)

2- Tomato inoculated with *Fusarium oxysporum* and treated with *Inula viscosa* shoot extract.

3- Tomato inocalated with *Fusarium oxysporum* and treated with Benomyl (Benlate(R)) fungicide.

4- Tomato free of *Fusarium oxysporum* and *Inula viscosa* extract (Control).

5- Tomato treated with *Inula viscosa* shoot extract only.

Eight weeks after the start of the experiment, observation on the experiment

and evaluation of the treatments were made based on the leaf symptoms and vascular browning using a scale ranged from 0 - 2 (10) where

0 = Healthy plant with no external symptoms and no vascular browning .

1= Plants with or without slight external symptoms but with slight internal symptoms.

2= Dead plants or plants with severe external symptoms and extensive vascular browning.

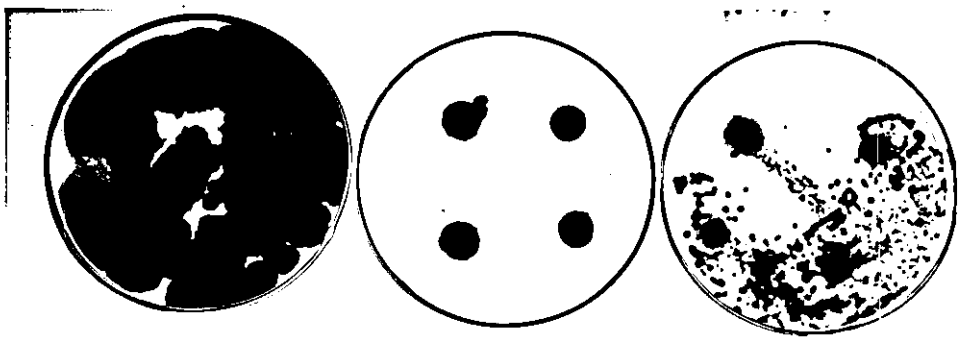
The experiment was terminated after eight weeks from the start. Plant height from the soil surface to the apex was measured, shoots and roots were harvested, oven dried at 70 °C for 48 hours and their dry weights were recorded .

Treatments in all experiments were laid out in a complete randomized design with four replicates. Data were statistically analysed and treatments means were compared using least significant differences (LSD) at 5% propability.

Cont . Table 2

Plant species	*F. oxysporum*			*H. salivum*			*A. solani*			*C. herbarum*			*B. cinerea*		
	Days after incubation			Days after incubation			Days after incubation			Days after incubation			Days after incubation		
	4	8	16	4	8	16	4	8	16	4	8	16	4	8	16
Inula viscosa	2	3	6	2	3	6	4	7	8	4	7	8	4	7	8
Lamium amplexicaule	5	7	9	7	9	10	8	8	10	5	8	10	5	8	9
Malva sylvestris	7	8	9	7	8	9	5	6	9	4	6	8	6	8	9
Mercurialis annua	9	10	10	9	10	10	6	8	10	5	7	8	7	9	9
Notobasis syriaca	3	6	9	7	9	10	8	9	10	5	8	8	5	7	9
Papaver rhoeas	4	7	9	4	7	8	7	10	10	4	7	9	6	9	10
Polygonum aviculare	6	8	9	6	7	10	6	8	9	6	8	9	5	7	9
Prosopis fracta	5	7	10	6	8	9	5	7	8	5	7	8	7	8	10
Rumex obtusifolius	4	8	9	5	6	9	7	9	9	4	7	8	5	7	9
Scorpiurus muricatus	5	8	9	8	9	9	7	8	9	5	7	8	5	8	9
Sinapis arvensis	8	9	9	5	6	8	6	8	9	6	8	9	5	8	9
Sisymbrium irio	6	9	10	8	8	9	8	9	10	5	8	10	6	8	9
Solanum nigrum	6	9	9	9	10	10	8	9	10	7	8	9	5	7	8
Sonchus oleraceus	8	10	10	7	8	10	6	7	8	6	9	10	5	7	9
Sorghum halepense	4	7	10	5	6	7	5	6	8	4	7	8	5	7	9
Suaeda asphaltica	4	8	10	6	8	9	6	7	8	6	8	8	7	8	9
Urtica urens	5	8	9	8	9	9	8	9	10	5	8	9	6	8	9
Verbascum sinaiticum	7	8	9	6	7	8	5	7	8	5	7	8	5	7	9
Xanthium spinosum	7	9	10	4	8	9	4	6	8	4	7	8	5	7	9
LSD (P = 0.05)	1.0	1.0	0.7	1.2	1.1	1.0	1.0	1.0	1.0	1.0	0.9	1.0	0.7	1.0	1.0

* (1-10) Scale where the lower score (1) denots no fungal growth while the higher score (10) denotes that the Petri-dish full of fungal growth.



Centaurea iberica *Inula viscosa* *Papaver rhoeas*

Plate 3 : Growth of *Helminthosporium sativum* treated with fresh shoot extract of *Centaurea iberica*, *Inula viscosa* and *Papaver rhoeas*.

Antifungal effect of *Anagallis arvensis* and *Inula viscosa* on *Helminthosporium sativum* lasted for up to 16 days after incubation, while the effect of extracts of other plant species decreased or completely disappeared with time.

C- *Alternaria solani*

Water extracts of sixteen species showed different strength of antifungal effect against *Alternaria solani* four days after incubation. These were ; *Chenopodium album, Calendula arevensis, Centuarea iberica , Inula viscosa, Eruca sativa, Capsella bursa-pastoris, Xanthium spinosum, Euphorbia helioscopia, Chrozophora obliqua, Echinochloa colonum, Cynodon dactylon, Hypericum triquitrifolium, Sorghum halepense, Malva sylvestris, Prosopis facta and Verbascum sinaiticum* . The antifungal activities of these species decreased or completely lost at 8 days after incubation. Extracts of *Sisymbrium irio* and *Notobasis syrica* stimulated growth of *Alternaria solani.*

D- *Cladosporium herbarum*

Twelve species from all plants tested for their antifungal activity against *Cladosporium herbarum* showed different levels of antifungal effects after four days of incubation (Table 2). These included *Chenopodium murale, Calendula arvensis, Inula viscosa, Xanthium spinosum, Chrozophora obliqua, Cynodon dactylon, Echinochloa colonum , Sorghum halepense, Hypericum triquitrifolium, Malva sylvestris, Papaver rhoeas* and *Rumex obtusifolius.* Extract effect of all these species were greatly reduced after 8 days of incubaton. On the other hand,

water extract of *Sonchus oleraceus* seemed to stimulate growth of this fungus.

E. *Botrytis cinerea.*

Extracts of eighteen plant species exhibited partial antifungal activities against *Botrytis cinerea* four days after incubation (Table 2) . These were , *Calotropis procera, Centaurea iberica, Inula viscosa, Sonchus oleraceus, Notobasis syriaca, Xanthium spinosum, Sinapis arvensis, Echinochloa colonum, Ecballium elaterium, Sorghum halepense, Hypericum triquitrifolium, Lamium amplexicaule, Scorporius muricatus, polygonum aviculare, Rumex obtusifolius , Anagallis arvensis,Verbascum sinaiticum* and *Solanum nigrum* . Extracts of non of the above mentioned species remained active for longer period of incubation . Antifungl activities of these species decreased or completely vanished after 16 days of incubation.

2. Antifungal effects of *Anagallis arvensis* and *Inula viscosa* on *Helminthosporium sativum* and *Fusarium oxysporum.*

The previous results (Table 2) showed that fresh shoot extract of *Inula viscosa* and *Anagallis arvensis* exhibited a strong antifungal effect on *Helminthosporium sativum.* Also shoot extract of *Inula viscosa* showed a significant effect on *Fusarium oxysporum.* Accordingly, the study was concentrated on *Anagallis arvensis* and *Inula viscosa* in the follow up experiments in order to investigate their antifungal activities on *Helminthosporium sativum* and *Fusarium oxysporum.*

Table 4. Antifungal effect of shoot and root extracts of *Anagallis arvensis* and *Inula viscosa* on mycelial dry weight of *Helminthosporium sativum* and *Fusarium oxysporum*.

Plant species	Source of extract	Mycelial dry weight (mg)	
		H. sativum	*F. oxysporum*
	dH2O (control)	129.9	128.7
I. viscosa	Shoot	78.1	56.0
	Root	161.0	122.0
A. arvensis	Shoot	73.2	103.8
	Root	79.4	98.0
LSD (p = 0.05)		8.2	23.8

compared with the control. Root extract of the same species showed no any antifungal effect on the same fungus. Shoot and root extract of *Anagallis arvensis* had significant antifungal effect on *Fusarium oxysporum* compared with the control.

4. Antifungal activity of dried plant materials:

A. *Effect on Fusarium oxysporum*

Dried shoots or roots of *Anagallis arvensis* incorporated in the medium significantly reduced growth of *Fusarium oxysporum* compared with the control (Table 5). At four days after incubation mycelial growth inhibition was 66 and 70% when treated with dried shoots or roots of *Anagallis arvensis* respectively (Plate 4) . However, the antifungal effect was gradually decreased with time .

Incorporation of dried shoot of *Inula viscosa* in the medium resulted in 61% growth inhibition after four days of incubation. The effect was drastically reduced with time. In comparision, incorporated dried root material showed less antifungal effect . Mycelial growth inhibition was 25% at four days after incubation, but this effect has completely disappeared after 16 days of incubation.

B. Effect on *Helminthosporium sativum*

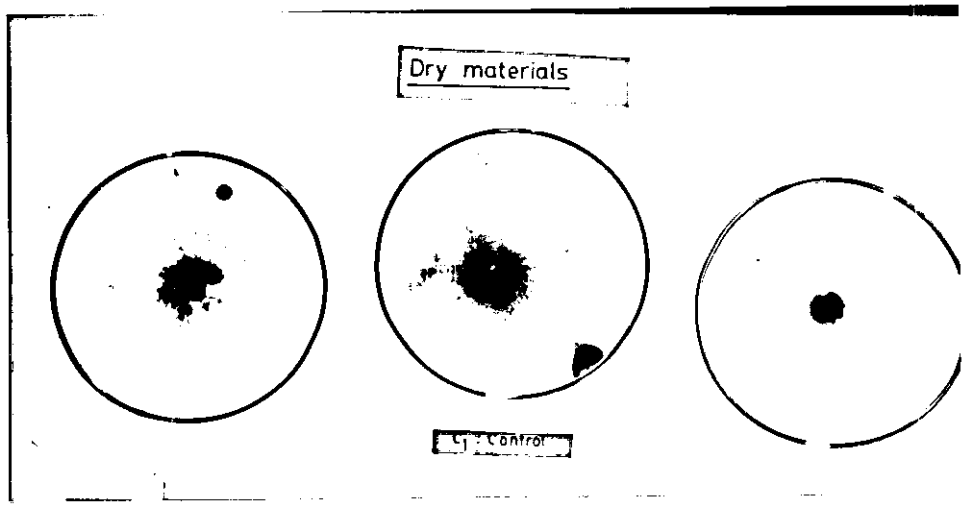
Dried shoots and dried roots of *Anagallis arvensis* strongly inhibited growth of *Helminthosporium sativum* (Plate 5 a) . At eight days after incubation, mycelial growth inhibition was 76 and 77% with dried shoot and dried root , respectively (Table 5). The effect continued up to 16 days .

Table 5. Effect of dried shoots or roots residues of *Anagallis arvensis* and *Inula viscosa* incorporated in the medium on the growth of *Fusarium oxysporum* ,*Helminthosporium sativum* ,*Alternaria solani* , *Cladosporium herbarum* and *Botrytis cineriea* at three periods after incubation at 24° C (The values in brackets are the percentage of mycélial growth inhibition)

Conoly diameter

(cm)

Plant species	Plant part used	*F. oxysporum*			*H. sativum*			*A. solani*			*C. herbarum*			*B. cinerea*		
		Days after incubation			Days after incubation			Days after incubation			Days after incubation			Days after incubation		
		4	8	16	4	8	16	4	8	16	4	8	16	4	8	16
A. arvensis	dH2O	6.7	10.6	11.0	7.0	10.5	11.0	6.6	9.7	10.5	2.6	4.8	5.8	9.2	11.0	11.0
	Shoots	2.3 (66)	4.6 (57)	6.7 (39)	1.8 (74)	2.5 (76)	3.8 (65)	5.1 (23)	8.0 (18)	9.6 (9)	2.2 (15)	4.8 (0)	5.3 (9)	5.4 (41)	11.0 (0)	11.0 (0)
	Roots	2.0 (70	4.4 (58)	6.6 (40)	1.7 (76)	2.4 (77)	3.4 (69)	6.0 (9)	9.0 (7)	10.0 (5)	2.4 (8)	4.6 (4)	5.5 (5)	8.2 (11)	11.0 (0)	11.0 (0)
I. viscosa	Shoots	2.6 (61)	5.5 (48)	10.3 (6)	4.3 (39)	6.6 (37)	11.0 (0)	4.2 (36)	8.3 (14)	9.7 (8)	2.3 (12)	4.1 (15)	4.9 (16)	6.3 (32)	11.0 (0)	11.0 (0)
	Roots	5.0 (25)	9.1 (14)	11.0 (0)	6.9 (1)	11.0 (-)	11.0 (0)	6.5 (2)	9.4 (3)	10.2 (3)	2.6 (0)	4.1 (15)	5.2 (10)	8.3 (10)	11.0 (0)	11.0 (0)
LSD (p = 0.05)		0.4	0.6	0.3	0.3	0.8	0.2	0.1	0.5	0.3	0.6	0.6	0.4	0.7	0.0	0.0



A. arvensis shoot . *A. arvensis* root

Plate 4 : Effect of dried shoot and root of *Anagallis arvensis* incorporated at 0.5% in the medium on the growth of *Fusarium oxysporum* at 16 days after incubation .

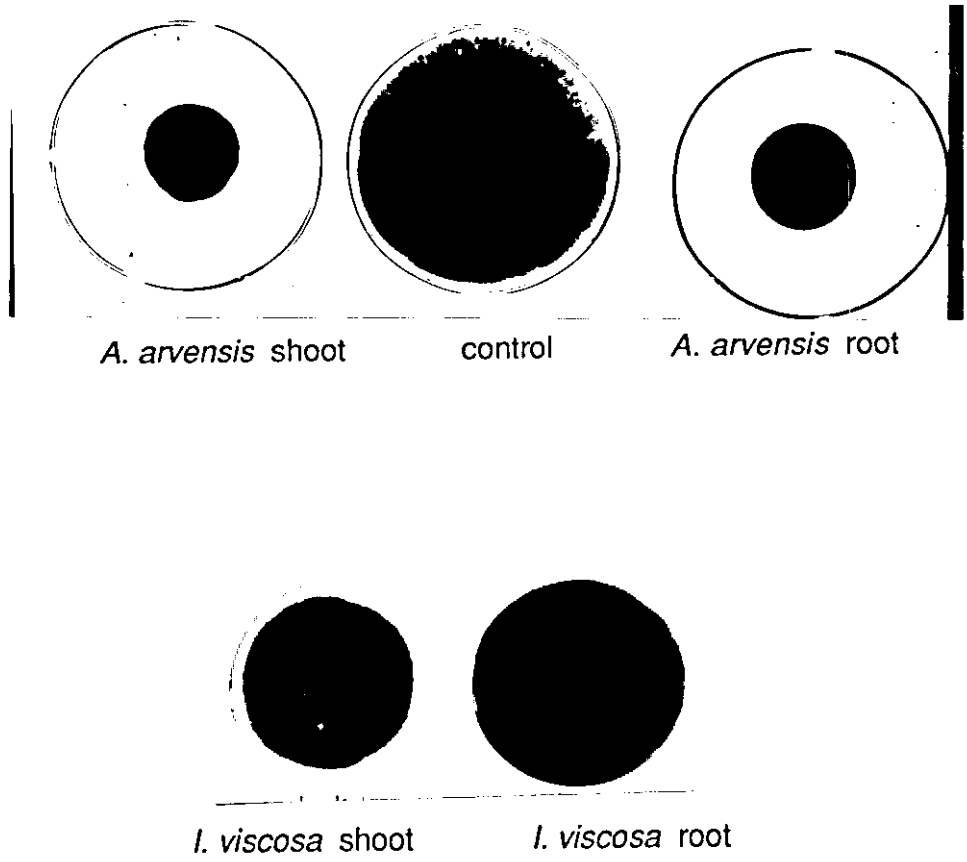


Plate 5 : Effect of dried shoot and root of *Anagallis arvensis* and *Inula viscosa* incorporated at 0.5 % in the medium on the growth of *Helminthosporium sativum* at 16 days after incubation .

Dried shoot of *Inula viscosa* caused 37% inhibition of mycelial growth after eight days of incubation, but this effect has completely disappeared at longer incubation periods, (Plate 5 b)

C. Effect on *Alternaria solani*

Data presented in (Table 5) show that dried shoot of *Anagallis arvensis* and *Inula viscosa* had some antifungal effect on *Alternaria solani* and caused 23 and 36% growth inhibition when dried shoots of *Anagallis arvensis* and *Inula viscosa* were added to the medium after four days of incubation, respectively. At longer incubation periods the effect was gradually decreased. Dried root of *Anagallis arvensis* showed little effect on the fungus , while dried root of *Inula viscosa* had no significant effect on the fungus growth compared with the control .

D. Effect on *Cladosporium herbarum*

After eight days of incubation, dried shoots and roots of *Inula viscosa* when incorporated in the medium caused 15% growth inhibition of *Cladosporium herbarum* , respectively (Table 5). The effect of dried materials lasted up to 16 days after incubation. Incorporation of dried shoot of *Anagallis arvensis* exhibeted slight antifungal effects at 16 days after incubation and resulted in a 9% mycelial growth inhibition.

E- Effect on *Botrytis cinerea*

Results of this experiment (Table 5) indicated that dried shoot of *Anagallis arvensis* and *Inula viscosa* had some antifungal activities on *Botrytis cinerea.* At four days of incubation, 32 and 41% mycelial growth inhibitions were detected for both species, respectively. The antifungal effect of dried shoots disappeared after eight days of incubation.

5. Effect of extract concentration :

Results in Table 6 showed that as the concentration of *Inula viscosa* and *Anagallis arvensis* shoot extract was increased, the antifungal effect of these extracts on *Helminthosporium sativum* was increased. This effect was more pronounced at earlier incubation periods. All concentrations of *Anagallis arvensis* caused significant mycelial growth inhibition when compared with the control except the lowest concentration after 8 days of incubation.

Shoot extract of *Inula viscosa* did significantly reduce mycelium growth of *H. sativum* compared with the control (Plate 6 a) . The effect was more pronounced at concentrations greater than 0.2 ml /Petri-dish. The antifungal effects of both species on *H. sativum* were concentration dependent. Antifungal effect of shoot extract of *I. viscosa* against *Fusarium oxysporum* increased with extract concentration . All used concentrations remained effective and showed significant effect on mycelial growth, after 16 days of incubation.

Shoot extract of *Anagallis arvensis* exhibited slight antifungal effect against

Table 6. Effect of different concentrations of shoot extract of *Anagallis arvensis* and *Inula viscosa* on the growth of *Fusarium oxysporum* and *Helminthosporium sativum* at three periods after incubation at 24°C (The values in brackets are the percentage of mycelial growth inhibition)

Plant species	Extract conc.(ml)	Colony diameter (cm) *Fusarium oxysporum* Days after incubation			*Helminthosporium sativum* Days after incubation		
		4	8	16	4	8	16
Anagallis arvensis	dH2O	2.7	6.6	10.1	5.7	7.3	10.8
	0.1	2.1 (22)	5.3 (20)	8.3 (18)	4.3 (25)	6.7 (8)	10.8 (0)
	0.2	2.2 (19)	5.2 (21)	8.1 (20)	2.9 (49)	4.9 (33)	7.3 (32)
	0.3	2.1 (22)	4.8 (27)	8.0 (21)	2.9 (49)	3.7 (49)	5.9 (45)
	0.4	2.1 (22)	5.1 (23)	7.7 (24)	2.2 (51)	3.2 (56)	4.4 (59)
	0.5	2.1 (22)	4.8 (27)	7.8 (23)	1.8 (68)	3.1 (58)	4.4 (59)
	1.0	2.0 (26)	4.7 (29)	7.8 (23)	1.5 (74)	2.1 (71)	4.3 (60)
	1.5	2.1 (22)	4.8 (27)	7.7 (24)	1.4 (75)	2.2 (70)	3.9 (64)
	2.0	2.0 (26)	4.7 (29)	7.7 (24)	1.4 (75)	1.7 (77)	2.6 (76)
	2.5	2.0 (26)	4.5 (32)	7.7 (24)	1.2 (79)	1.6 (78)	2.5 (77)
	3.0 (Full)	1.9 (30)	4.7 (29)	7.6 (25)	1.0 (82)	1.5 (79)	2.3 (79)
LSD (p = 0.05)		0.2	0.4	1.1	0.4	0.6	0.6

Cont. Table 6.

Colony diameter (cm)

Plant species	Extract conc.(ml)	*Fusarium oxysporum*			*Helminthosporium sativum*		
		Days after incubation			Days after incubation		
		4	8	16	4	8	16
Inula viscosa	dH2O	2.7	6.6	10.1	5.5	7.1	10.5
	0.1	2.1 (22)	3.8 (42)	6.8 (33)	4.3 (22)	5.9 (17)	10.3 (2)
	0.2	2.0 (26)	3.7 (44)	6.1 (39)	3.9 (29)	5.5 (23)	10.2 (3)
	0.3	2.1 (22)	3.3 (50)	5.9 (42)	3.8 (31)	4.1 (42)	9.4 (10)
	0.4	2.0 (26)	3.2 (52)	5.6 (45)	3.5 (36)	4.1 (42)	7.1 (32)
	0.5	1.6 (41)	3.2 (52)	5.4 (47)	2.1 (62)	3.8 (46)	5.5 (48)
	1.0	1.5 (44)	3.0 (55)	4.9 (51)	2.0 (64)	3.5 (51)	4.7 (55)
	1.5	1.5 (44)	3.0 (55)	4.9 (51)	1.7 (69)	2.8 (61)	3.8 (64)
	2.0	1.5 (44)	3.0 (55)	4.7 (53)	1.4 (75)	2.6 (63)	3.5 (67)
	2.5	1.3 (52)	2.9 (56)	4.5 (55)	1.2 (78)	1.8 (75)	3.2 (69)
	3.0 (Full)	1.3 (52)	2.7 (59)	4.5 (55)	1.2 (78)	1.8 (75)	2.6 (75)
LSD (p = 0.05)		0.3	1.4	0.6	0.4	0.6	0.5

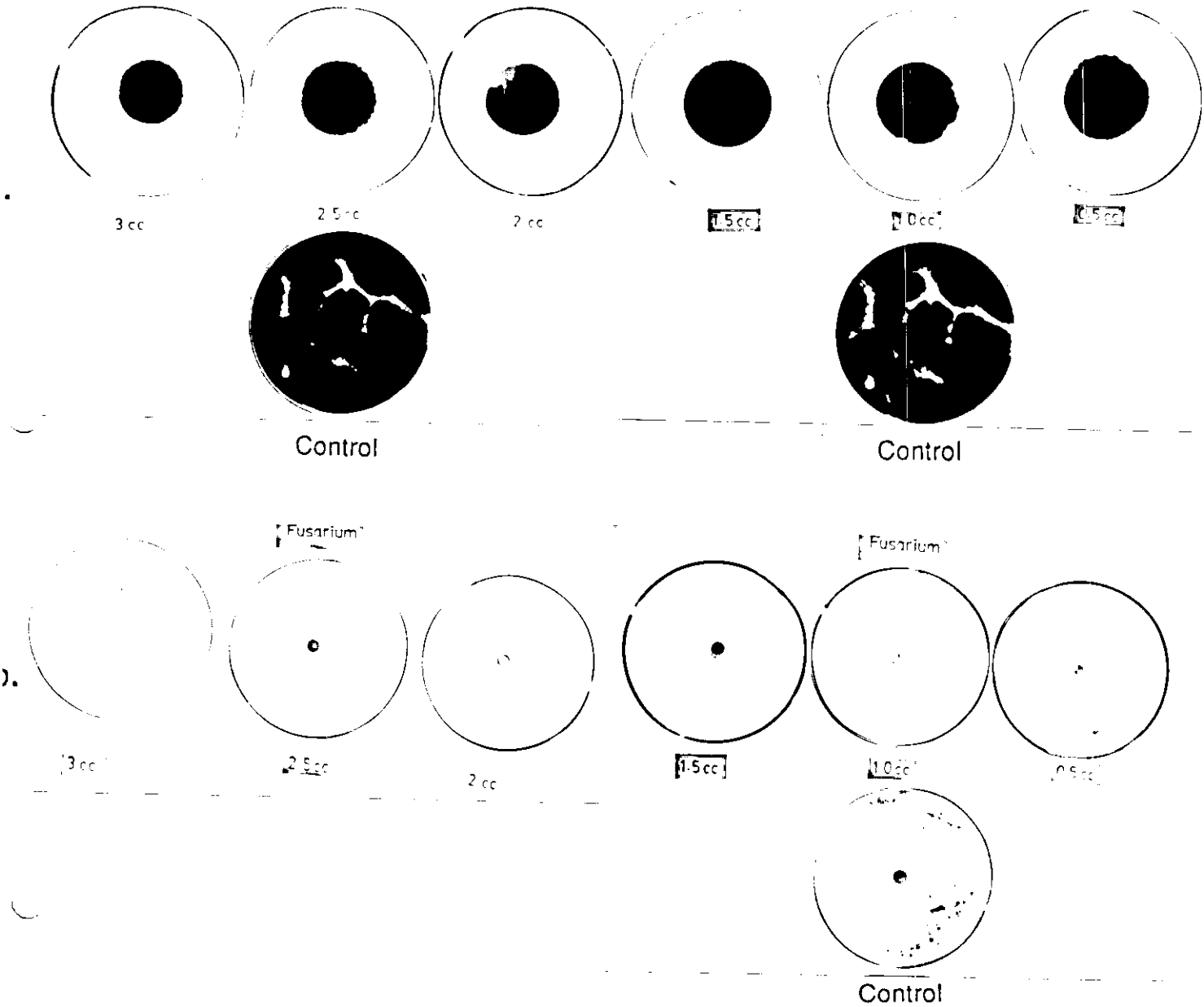


Plate 6 : Effect of different concentrations of *Inula viscosa* fresh shoot extracts

(a) On *Helminthosporium sativum* (b) On *Fusarium oxysporum*

at 16 days after incubation .

viscosa showed 79 % growth inhibition [illegible]

arvensis and *I. viscosa* at early vegetative growth exhibited 39 and 37% mycelial growth inhibition respectively.

The antifungal activity of *I. viscosa* shoot extract on *Fusarium oxysporum*

Table 7. Effect of different concentrations of shoot extract of *Anagallis arvensis* and *Inula viscosa* on the growth of *Fusarium oxysporum* and *Helminthosporium sativum* using liquid culture technique

Extract concentration (ml)	*Inula viscosa*		*Anagallis arvensis*	
	Mycelial dry weight (mg)		Mycelial dry weight (mg)	
	H. sativum	*F. oxysporum*	*H. sativum*	*F.oxysporum*
dH2O (control)	82.5	71.6	80.5	71.6
0.5	69.0	62.5	69.7	68.8
1.0	68.7	47.1	66.7	64.1
1.5	67.7	43.4	63.6	62.9
2.0	67.7	36.6	60.0	60.3
2.5	61.9	33.6	53.9	61.4
3.0 (Full)	52.8	30.7	44.1	62.3
LSD (p = 0.05)	7.4	11.4	11.8	8.2

Table 8. Effect of growth stage on the effectiveness of *Inula viscosa* and *Anagallis arvensis* shoot extract on the growth of *Helminthosporium sativum* and *Fusarium oxysporum* (The values in brackets are the percentage of mycelial growth inhibition)

Plant species	Growth stage	Colony diamter (cm)					
		Helminthosporium sativum			*Fusarium oxysporum*		
		Days after incubation			Days after incubation		
		4	8	16	4	8	16
dH2O (control)		6.2	10.3	10.8	3.7	7.7	10.1
A. arevensis	Seedling	5.2	6.3	6.7	3.5	6.5	8.8
		(16)	(39)	(38)	(5)	(16)	(13)
	Vegetative	1.2	2.3	3.3	3.5	6.3	8.2
		(80)	(78)	(69)	(5)	(18)	(19)
	Flowering	1.1	2.0	3.2	3.1	5.7	8.1
		(82)	(81)	(70)	(16)	(29)	(20)
I. viscosa	Early vegetative	5.2	6.5	10.8	2.0	4.0	6.5
		(16)	(37)	(0)	(46)	(48)	(37)
	Full vegetative	2.0	2.3	5.9	1.4	2.7	4.7
		(68)	(78)	(45)	(62)	(65)	(53)
	Flowering	1.7	2.2	5.2	1.3	2.4	4.4
		(73)	(79)	(52)	(65)	(69)	(56)
LSD (p = 0.05)		0.6	1.0	0.6	0.5	0.6	0.5

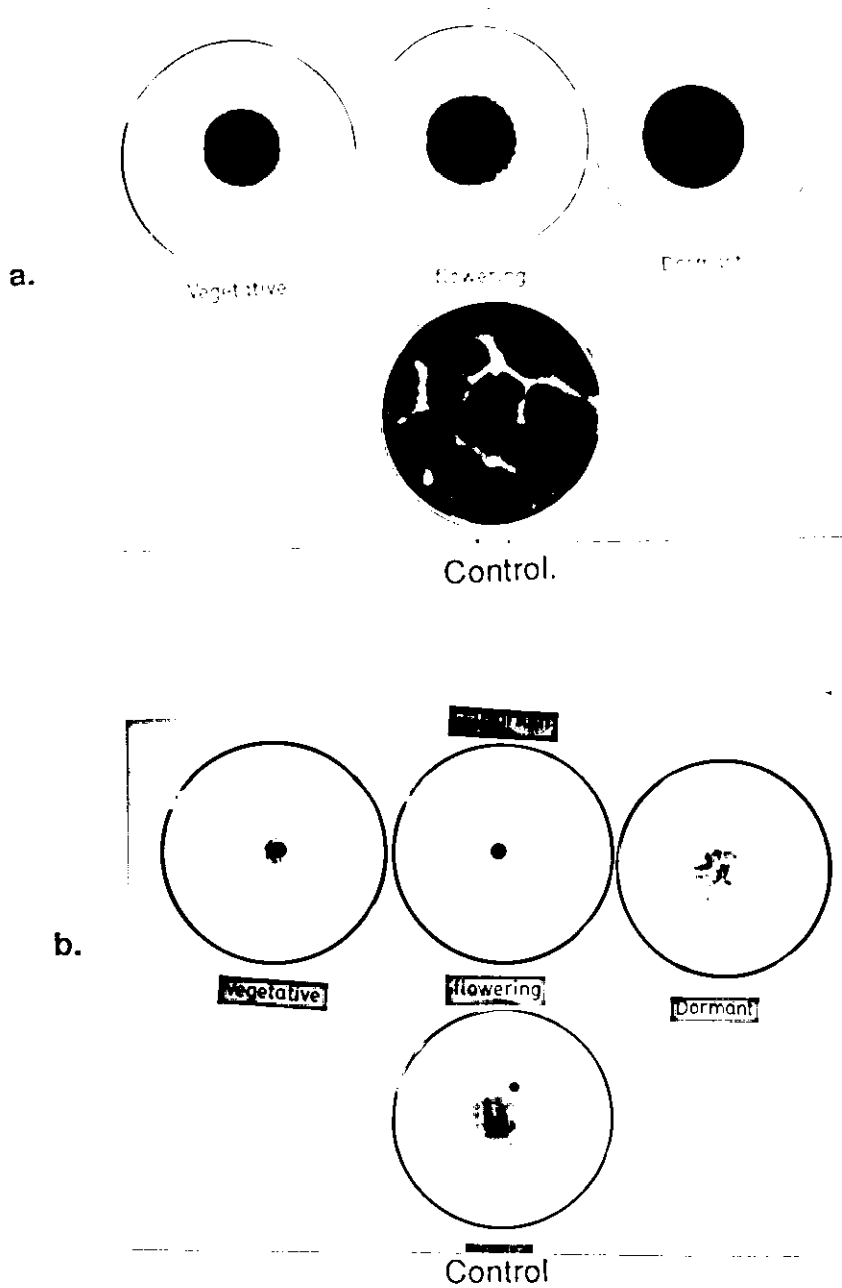


Plate 7 : Effect of fresh shoot extract of *Inula viscosa* collected at different growth stages

(a) On *Helminthosporium sativum.*

(b) On *Fusarium oxysporum.*

Table 9. Effect of growth stage on the effectiveness of *Inula viscosa* and *Anagallis arvensis* shoot extracts on the growth of *Helminthosporium sativum* and *Fusarium oxysporum* using liquid culture technique

Plant species	Growth stage	Mycelial dry weight (mg)	
		H. sativum	*F. oxysporum*
dH2O (control)	- - - -	114.3	104.7
A. arvensis	Seedling	75.5	100.0
	Vegetative	54.0	92.3
	Flowering	53.0	91.3
I.viscosa	Early vegetative	80.8	60.3
	Full vegetative	57.5	54.0
	Flowering	56.5	53.5
LSD (p = 0.05)		7.8	6.9

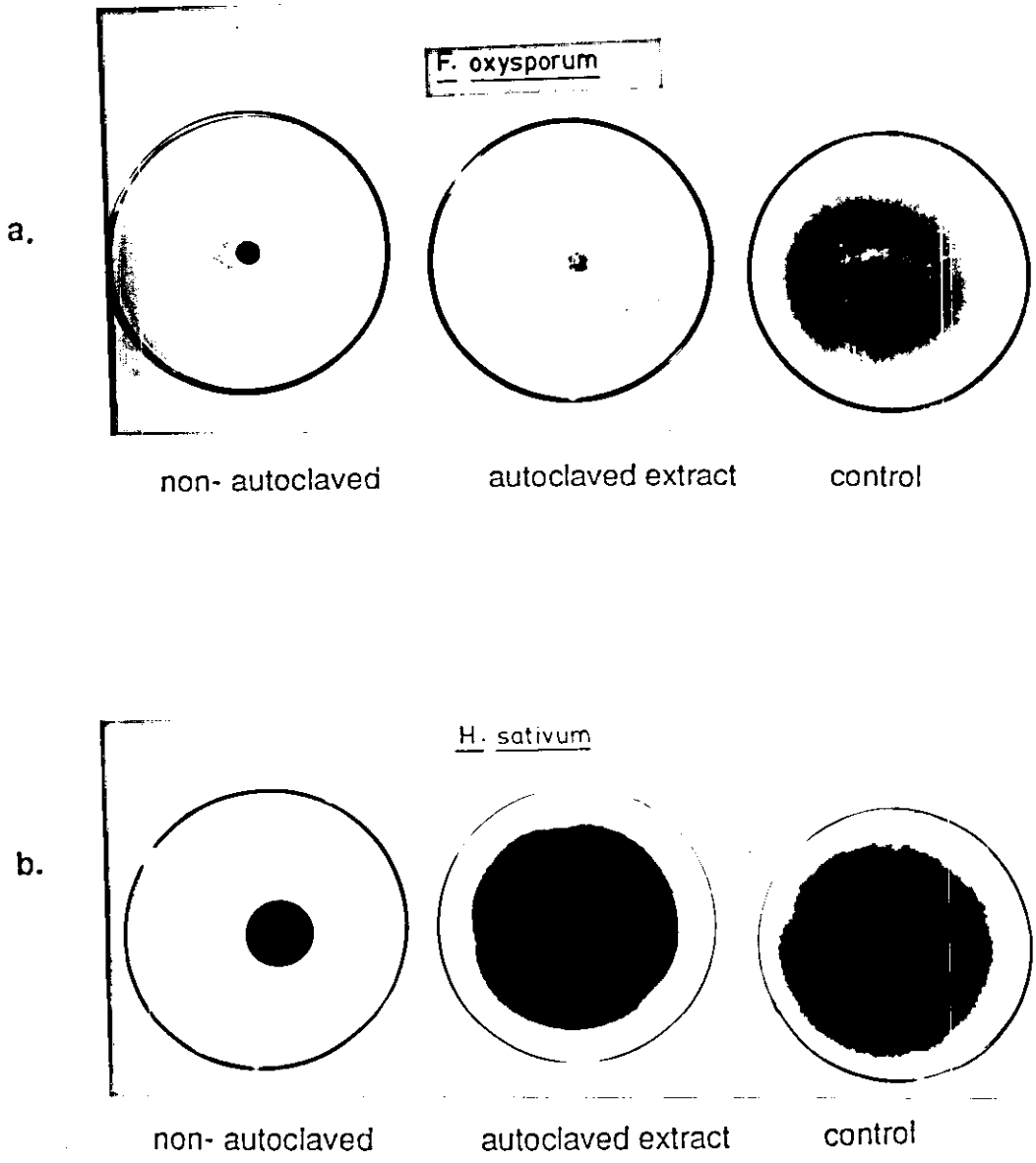


Plate 8 : a) *Fusarium oxysporum* treated with autoclaved and non- autoclaved extract of *Inula viscosa*.

b) *Helninthosporium sativum* treated with autoclaved and non - autoclaved extracts of *Inula viscosa*.

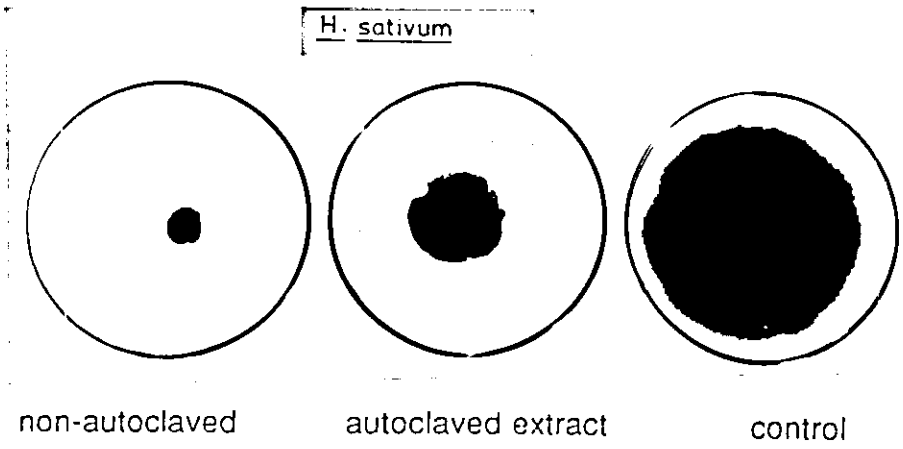


Plate 9 : *Helminthosporium sativum* treated with autoclaved and non-autoclaved extracts of *Anagallis arvensis*

Table 10. Effect of autoclaved and not autoclaved extract of *Inula viscosa* and *Anagallis arvensis* on *Helminthosporium sativum* and *Fusarium oxysporum* , at three dates after incubation. (The values in brackets are the percentage of mycelial growth inhibition)

Colony diameter (cm)

Plant species	Extract treatment	*Helminthosporium sativum*			*Fusarium oxysporum*		
		Days after incubation			Days after incubation		
		4	8	16	4	8	16
dH2O (control)		5.1	9.4	11.0	4.1	8.1	11.0
I. viscosa	Autoclaved	4.9	7.9	11.0	3.1	5.7	10.7
		(4)	(16)	(0)	(32)	(30)	(3)
	Not Autoclaved	1.8	2.4	6.0	1.6	3.0	7.8
		(65)	(74)	(45)	(61)	(63)	(29)
A. arvensis	Autoclaved	1.9	3.2	4.6	3.6	6.6	9.5
		(63)	(66)	(58)	(12)	(19)	(14)
	Not autoclaved	1.4	1.7	4.5	3.4	6.6	9.4
		(73)	(82)	(59)	(17)	(19)	(15)
LSD (p = 0.05)		0.3	0.9	1.1	0.4	0.7	0.7

Table 11. Effect of boiling treatment on the effectiveness of *Inula viscosa* and *Anagallis arvensis* shoot extract on the growth of *Helminthosporium sativum* and *Fusarium oxysporum*, at three dates after incubation. (The values in brackets are the percentage of mycelial growth inhibition)

Colony diameter (cm)

Plant species	Extract treatment	*Helminthosporium sativum*			*Fusarium oxysporum*		
		Days after incubation			Days after incubation		
		4	8	16	4	8	16
	dH2O (control)	5.2	9.9	11.0	3.8	7.2	10.3
I. viscosa	Boiled	6.0	10.2	10.6	3.8	6.9	9.8
		(-)	(-)	(4)	(0)	(4)	(5)
	Not boiled	1.7	2.5	5.4	1.5	2.7	4.7
		(67)	(75)	(51)	(61)	(63)	(54)
A. arvensis	Boiled	1.4	2.7	4.6	3.5	7.0	9.8
		(73)	(73)	(58)	(8)	(3)	(5)
	Not boiled	1.2	2.2	3.3	3.5	7.0	9.8
		(77)	(78)	(70)	(8)	(3)	(5)
LSD (p = 0.05)		0.5	0.6	0.5	0.5	0.4	0.4

Table 13. Volatile effect of *Inula viscosa* and *Anagallis arvensis* extracts on the growth of *Helminthosporium sativum* and *Fusarium oxysporum.* (The values in brackets are the percentage of mycelial growth inhibition)

Colony diameter (cm)

Treatment	*Helminthosporium sativum*			*Fusarium oxysporum*		
	Days after incubation			Days after incubation		
	4	8	16	4	8	16
dH2O (Control)	3.2	6.3	10.4	3.2	5.0	9.1
I. viscosa extract	1.8	2.1	3.2	1.8	1.9	3.1
	(44)	(66)	(69)	(44)	(62)	(66)
A. arvensis extract	3.2	6.2	10.1	2.9	4.8	8.9
	(0)	(2)	(3)	(9)	(4)	(2)
LSD (p = 0.05)	0.5	0.9	0.5	0.5	0.8	0.5

II. Glasshouse experiment:

Significant differences in plant height, shoot dry weight and root dry weight were found between tomato free from *Fusarium oxysporum* and *Fusarium* inoculated tomato treated with *I. viscosa* shoot extract (Table 14). Shoot and root dry weight of inoculated tomato plants were significantly higher when treated with *I. viscosa* extracts compared with untreated inoculated plants. Shoot extract of *I. viscosa* significantly reduced the disease severity index of tomato wilt. Results showed that no significant differences between inoculated tomato treated with *I. viscosa* extract and tomato treated with Benomyl fungicide. In addition, no sign of any phytotoxic effect of the extract was detected on tomato plants since differences were found between tomato free of *Fusarium oxysporum* treated or untreated with *I. viscosa* extract were not significant .

Table 14. Effect of *Inula viscosa* shoot extract on disease severity caused by *Fusarium oxysporum* on tomato plants under glasshouse conditions.

Treatment	Tomato plant			
	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)	Disease index*
Fusarium free tomato (Control)	35.1	3.5	0.5	0.0
Fusarium inoculated tomato + *Inula* extract	21.4	2.3	0.3	0.8
Fusarium inoculated tomato without extract	14.1	1.3	0.2	1.5
Fusarium inoculated tomato + Benomyl fungicide	30.0	2.6	0.4	0.5
Fusarium free tomato + *Inula* extract	29.4	3.0	0.4	0.0
LSD (p = 0.05)	8.9	0.9	0.1	0.7

* = Disease severity index ranged from 0-2 where :

0 = Healthy plant, no external symptoms and no vascular browning

1 = Plants with or without slight external symptoms but with slight internal symptoms.

2 = Plants dead or with severe external symptoms and extensive vascular browning.

Discussion

Disease resistance in some plants is known to be due to the presence of certain chemical substances in the host tissues toxic to different microorganisms which serve as chemical protective barriers to infection (26).

With increase in awareness towards toxic hazards of fungicides, the importance of indigenous products in plant disease control has been emphasized. Encouraging results in this area have been reported by different researchers (1, 2, 7, 8, 9, 13, 15, 19, 26, 27 and 30). Also, recent researchs on the use of constituents of higher plants as a possible source of natural pesticides has been advocated on the account of their non-phytotoxic and easily biodegradable.

In vitro assesment of the potential of some plant extracts as fungitoxicants showed that plant species were different in their fungistatic effects, some either inhibited, others stimulated or had no apparent effects. Other workers (36 and 38) showed that plant extracts are different in their phytotoxic effects against different plant species. In this study results (Table2) indicated the presence of active fungistatic materials in the extract of certain plant species. These fungistatic materials are most probably water soluble materials.

Aqueous extracts of different plant species were found to contain allelochemicals (35 and 38). Screening plant extracts for their antifungal activity imply that higher plants are potential sources of fungitoxic compunds. Among 40 localy available

plant species tested in vitro. *I. viscosa* was found to show activity against *Fusarium oxysporum* and two species were effective against *Helminthosporium sativum* . These were *Inula viscosa* and *Anagallis arvensis*.

Based on their strong antifungal effect and their stable activity in vitro against the tested fungi (Table 2), two plant speicies were selected, to follow up on this work. These were *Anagallis arvensis* and *Inula viscosa*.

The persistent effect of some plant extracts on different fungi indicate that the active principle present can remain effective for a long period of incubation. However this activity varied as shown by a significant decrease of the mycelial growth inhibition with time for some plant extracts. This suggested that the active principle of the extracts has dessipated with time which might be attributed to the decomposition of the active compounds or transformation of these compounds to some other inactive forms .

The antifungal activity of *Anagallis arvensis* has been reported earlier. Nene and Thapliyal (31) reported the antifungal activity of *Anagallis arvensis* extracts against *Colletotrichum papayae, Pythium aphanidermatum* , *Helminthosporium turcicum* and *H. maydis*. Rafiqe *et al.* (37) found that water extract of *Anagallis arvensis* inhibited the growth of *Helminthosporium oryzae, H. carbonum* and *H. turcicum* . The present data on the effect of *A. arvensis* on *H. sativum* were in agreement with results reported by Nene and Thapliyal (31) and Rafiqe *et al* (37). No reports were found concerning the Antifungal activities of *Inula viscosa* .

Results showed that the antifungal properties are neither a family character nor a generic one. It varied from family to family, genus to genus and species to species. Plant species which inhibited growth of one fungus was found to have no effect on others . These results were in agreetment with those reported by Dixit and Tripathi (12) who found that extracts of *Brassica campestris* L. and *B. oleracea* L. showed strong fungistatic activity against *Cephalosporium sacchari* and *Fusarium nivale* . The extract of *Brassica rapa* L. was partially active and only inhibited spore germination of *Cephalosporium sacchari* , while extracts of *B. pekinensis* Lour. and *B. juncea L. stimulated* germination of fungal spores.

The stimulatory activities caused by some plant extracts indicated that the growth promoting compounds in these extracts are present. Some plant extracts were reported to stimulate the growth of some plant pathogens (12, 34 and 46). These reports strongly suggested the presence of growth promoting substances in plants extracts. Such materials are important to exploit for developing simple, cheap and effective media in culturing the pathogen (3). The variations in the activity of different plant materials to different organisms may be due to differences in the nature of the inhibitory materials or differences in the solubility of inhibitory materials in water. At the same time, it is also possible that the failure of some plant extracts to completely inhibit growth of some fungi may be due to the inability of water to dissolve the active compounds in the plant material which may differ from one plant material to another.

The extract may inhibit the growth of fungi either temporarily (Fungistatic) or permenantly (Fungicidal) (7). Extracts of both *Anagallis arvensis* and *Inula viscosa* were found to possess fungistatic activities against the tested fungi species. However, the effect of extracts obtained from different plant parts were quite variable from the effect of water extracts of the whole plant (37). In case of *Anagallis arvensis* , shoot and root extracts had a strong antifungal activities while only shoot extract of *Inula viscosa* was found to possess a significant antifungal effect. This may indicate the presence of inhibitory materials at higher concentrations in shoots than in roots of this species. These results were in agreement with those reported by Nene and Thapliyal (31) who also found that antifungal activities of *Anagallis arvensis* was distributed in the stem and the leaves, but the effect of root extract had not been studied.

The present experiments demonestrate that the dried shoot and root materials of *A. arvensis* had a strong antifungal effect on *H. sativum* and *F. oxysporum* , while *I. viscosa* dried shoots were found to possess moderate effects on these two fungi. This might be due to the nature of the antifungal materials which may be volatile materials lost during drying period. The results showed that fresh shoot extract of *I. viscosa* possessed a volatile antifungal activities against the tested fungi. Chaturvedi *et al* (7) showed that *Adenocalymma allicea* exhibited a strong volatile antifungal activity against *Drechslera* oryzae. The toxic materials in the extracts were destroyed by drying at room temperature at 28° C for seven

days. Dried shoots and roots of *A. arvensis* showed similar inhibitory effect against *H. sativum* as well as fresh parts extract (Table 5). This may indciate that fungal inhibitory materials are non volatile and could be water soluble materials. Dried materials of *A. arvensis* exhibited stronger antifungal activities against *F. oxysporum* than fresh parts. This may be due to the changes or modification of the inactive compound to active antifungal form as a result of high temperature treatment during drying period. This agreed with data obtained by Nene and Thapliyal (31) who found that the effect of *A. arvensis* on *Pythium aphanidermatum* was more pronounced after drying the plants and studying its activity after 6 monthes. In addition, this plant was found to contain glycosidic saponine. Cavallito *et al* (6) demonestrated that the active principle of *Allium sativum* is not present as such in the plants but occures in the form of a thermostable precursor which when acted upon by an enzyme yields the antibiotic principle .The precursor and the enzyme are present in different cells of the garlic cloves. The active agent is produced only when the cells were crushed and their contents were mixed.

The effect of plant age on the efficiency of plant extract was studied. The results showed that such extract exhibited strong antifungal activities with the advances in plant age (Table 8). Nene and Thapliyal (31) reported that the antifungal activities of *A. arvensis* extract gradually increased with the advances in the growth of the plant from seedling to flowering stage. The concentration of antifungal compounds which was low at the very young stage increased gradually

with plant development and reached the maximum at flowering stage.

Effects of different concentrations of *A. arvensis* and *I. viscosa* shoot extracts was studied in order to determine the least inhibitory concentration against the tested fungi. Results showed that the antifungal activities were concentrations dependent. The effect was more pronounced at shorter than longer incubation period. This might be due to the reduction in the active materials with time which could be due to the influence of high incubation temperature for relatively long period. However, *I. viscosa* is well known for its strong oder which is mainly due to some volatile materials. These materials may be easily removed during incubation. This was confirmed through autoclaving and boiling the shoot experiment (Table 10 and 11) where the inhibitory effect of *I. viscosa* shoot extract was drastically reduced by both treatments, and through the effect of volatile experiment (Table13) where *I. viscosa* found to possess volatile antifungal activity.

Glasshouse experiment was performed in order to investigate the possible use of *I. viscosa* shoots extract in controlling *Fusarium* wilt of tomato. Results of this experiment showed that application of *I. viscosa* shoot extract to infected tomato plants with *F. oxysporum* caused a significant reduction in the disease serverity (Table 14). The important effect of *I. viscosa* as a control measure is that it was comparable to the activity of Benlate fungicide against *F. oxysporum* in tomato plants . This indicated that the active material had a protective and control powers to reduce the infection in the soil. These results are of ecological significance . The

significant reduction in disease severity caused by *I. viscosa* extract compared with the infected and extract untreated tomato indicated that plant material of this species was a potential source of fungicidal agent with fungistatic action. In the present study *I. viscosa* shoot extract was found to be non-phytotoxic to tomato plants since no significant differences were detected in plant height or shoot and root dry weights between *Fusarium* free plants treated or untreated with *I. viscosa* extract .

In general the active principle present in higher plants may be influenced by several factors such as age of the plant, plant parts, age of the tested organism, method of extraction and time of harvesting plant materials. Gillver (20) reported that age of plant portion and nitrogen content affected the inhibitory power of the extract. The active material presents in plants may be in greater amount in the younger stage of plant growth or plant parts. They may be reduced with age or *vice versa* and occurred in one or many plants parts (19). Walker *et al* (49) mentioned that the reduction in the viability of spores as the culture aged, makes the microorganisms generally more sensitive with age.

Different plants require different extracting solvent. Shekhawat and Pracada (40) mentioned the use of cold distilled water, boiled distilled water and acetone in the extraction of 41 plant species with antifungal activity. On the other hand, Nicolls (32) used ethyl acetone and n-butanol in the extraction and stability of active principle in *Passiflora* species. These findings of workers support the idea that the

active substances may be present in plants in inacative form and become active only if the plant is properly processed, and also indicated that the diversity in the methodology of extraction and the difference in the result obtained using different experimental techniques (36). This increased the need for instituting more efficient, convenient and cheaper method of extraction to facilitate more extensive utilization of fungicidal extracts especially if greater quantity of extracts must be prepared for large-scale production.

Some plant extracts showed a greater mycelial growth inhibition during the preliminary assay than that of the result in the preceeding assay. This is likely due to the effect of variation in time of the year when the plant materials were assayed (19).

Further studies on the detection, isolation, characterizaton, purification and evaluation of the active agents, coupled with studies on their mode of action, economic and dosage of the extract are suggested to be able to recommend the possibility of successful larger scale production of these extracts for crop protection.

Conclusions and Recommendations

1- Plant species are variable in the antifungal effect of thier shoots and roots extracts. Some were inhibitory, others stimulatory or have no apparent effect.

2- Results of the screening experiment indicated that heigher plants are potential source of fungitoxic compounds provided renewable sources of useful pesticides.

3- *Anagallis arvensis* was found to have strong antifungal activities against *H. sativum* and slight effect on *F. oxysporum*. Dried materials of this species exhibited a significant effect on both tested fungi.

4. *Inula viscosa* shoots exhibited a significant antifungal effect on both *F. oxysporum* and *H. sativum*, while dried shoots caused moderate effect on these fungi.

5. Both species exhibited fungistatic activities against the tested fungi .

6. Shoots extract of *I. viscosa* showed strong volatile antifungal activity.

7. *Anagallis arvensis* extract was thermostable and its toxicity remained unchanged even with autoclaving and boiling treatments,, while the inhibitory effects of *Inula viscosa* was drastically reduced by both treatments.

8. The concentration of antifungal compounds increased gradually with plant development and reached maximum at maturity stage.

9. In pot experiment, *Inula* shoot extract caused significant reduction in the

severity of tomato wilt disease caused by *Fusarium oxysporum* . From these results we can conclude that this plant has antifungal effect and its extracts can be used as a valuable sources of chemicals for the control of *Fusarium* wilt of tomato. However, extracts purification and identification of active components merits further investigations.

10. In light of these findings, it becomes clear that there exists a great potential in the search for new and more potent antifungal substances from the natural sources.

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للذبول الفيوزارمي ، والفطر المسبب لمرض التبقع الهلمنثوسبوري .

أدت عملية التعقيم والغلي للأجزاء الخضرية لنبات الطيون الى نقص شديد في التأثيرات المثبطة لمستخلصات هذه الأجزاء ، بينما لم تؤدي هذه المعاملات الى خفض في التأثيرات المثبطة لمستخلص نبات عين الجمل .

أظهرت النتائج المتحصل عليها في تجربة البيوت الزجاجية بأن المستخلص المائي للمجموع الخضري الطازج لنبات الطيون ذو قدرة تثبيطية لنمو فطر الفيوزاريوم عند اضافة هذا المستخلص الى التربة المعاملة بالفطر والمزروعة بنباتات البندورة . كما أشارت النتائج الى عدم وجود أي تأثيرات مثبطة لنمو البندورة نتيجة معاملتها بمستخلص هذا العشب .

بينت نتائج التجارب السابقة امكانية الاستفادة من مستخلصات أو مخلفات بعض النباتات في مكافحة بعض الأمراض النباتية الفطرية ، كما أن هذه النباتات البرية تعتبر مصدراً محتملاً للحصول على بعض المواد الكيماوية التي يمكن استعمالها في مكافحة بعض الآفات الزراعية .