University of AL-Tahadi Faculty of Agriculture Department of Plant production

Effect of some aqueous phytoextracts on the fusarial wilt in tomato seedlings

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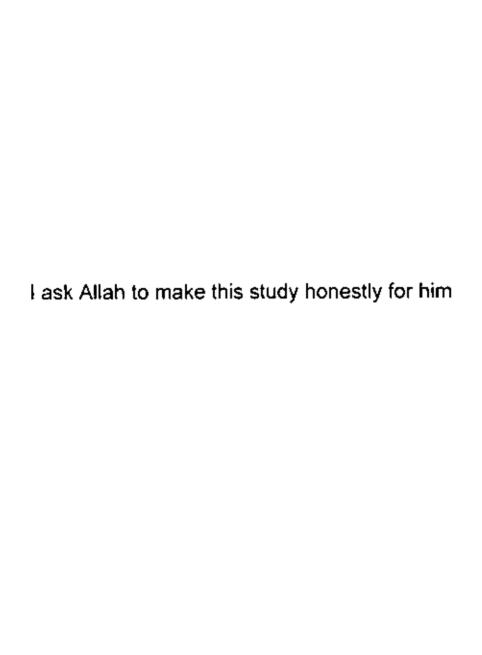


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INTRODUCTION

Tomato plant (*Lycopersicon esculantum* Mill) is subtropical plant require at least six hours of direct sun light to flower. It is quite sensitive to low light, It grows well when temperatures between 18.5 – 29 °C. Tomatoes grow on many soil types but they need well drained water retentive soil. They need a high water requirement during the vegetative growth. Tomatoes are heavy feeders due to their rapid growth and long production season.

Tomato is mainly grown as indoor and outdoor along coastal regions and in the southern parts as, Sebha, Jallo, Aljoufra, and other localities.

One of the most important limiting factors of tomato production is the spread of diseases. Tomato plants are susceptible to many diseases such as :Alternaria stem canker, anthracnose, Septoria spotting, late blight, early blight, bacterial wilt, bacterial spotting, and tomato mosaic virus, The most prevalent and devastating disease wherever tomato plants are grown is the fusarial vascular wilt, caused by *Fusarium oxysporum* f.sp *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans., which also causing foot rot, root rot, and damping off. The fungus is a Soilborne plant pathogen, lives as saprophyte in soil and organic matter, and remain for up to 10 years in infested soil without the host (Nelson, 1981). The disease causes great loss in particular on

susceptible varieties under favorable weather conditions. Infected plants become stunted soon wilt and finally die.

Fusarial wilt disease was first described by G.E. Massee in England in 1895. The disease is most destructive in warm climates and warm, sandy soils of temperate regions, (Agrios, 1997), the disease severity is associated with the optimum temperature of the pathogen (Fry, 1982). In general, factors favoring wilt disease development are, soil and air temperature at 28 °C, soil optimum moisture for tomato plant growth, plant preconditioned with low nitrogen and phosphorus and high potassium, high soil pH, short day length, and low light intensity. The pathogen disseminated via seeds, tomato stakes, infested soil adhering to transplants, wind, and farm machinery. The pathogen could be disseminated for long distance through seed and transplants, (Walker, 1969). The pathogen enters the plant through the root tip, through wounds of roots, or at the formation point of lateral roots, (Agrios, 1997). Mycelia of Fusarium grow intercellulary in root cortex and reaches to xylem through pits. The first indication of disease appears as slight vein clearing on the outer portion of younger leaves, followed by epinasty of the older leaves, at the seedling stage, infected plants may wilt and die soon after these symptoms become visible. But in older plants these symptoms are often followed by stunting, yellowing of the lower leaves, formation of adventious roots, wilting of leaves and young stems, defoliation, marginal necrosis of remaining leaves, and finally death of entire plant. The symptoms occur on one side of the plant. The browning of the vascular tissues of the stem near the base is characteristic symptom of Fusarium wilt disease; this discoloration often extends far up the stem and into petioles. When the soil moisture is high and the temperature relatively low, infected plants may produce good yield, however, the fungus reaches tomato fruits and penetrates or contaminates their seeds, usually the infected fruits decay and drop. These symptoms generally become more apparent during the period between blossoming and fruit maturation (Horst, 1971).

In Libya, vascular wilt of *Fusarium* on tomato was first reported in 1960 in Tripoli vicinity by Pucci, the disease occurs in the major tomato producing areas and is most severe during August and September on irrigated and non-irrigated plants. The fungus spread in some costal regions in Libya including, Ain zara, Zawia, Garabouli, Benghazi, and Maraj (Alarabi,1985)

Disease control using chemicals has limitations such as their high cost, and their adversely affects including many risks to man healthy, chemical residues in crops and environment, their risks to fishes and aquatic life, and finally the appearance of resistance races to these chemicals. Soil sterilization is too expensive for field application, and crop rotation are of limit value to control the disease. The usage of resistant varieties is the chief mean of control of Fusarium wilt in tomato, but infestation by nematodes may predispose plants to infection (Erwin,1981). More usually the pathogen developed new strains which breakdown the resistance of resistant verities often quite quickly and suddenly. Resistance can also be broken down by external factors, (Green, 1977).

New interesting and biologically approaches are being explored to suppress the disease through natural means and to reduce the use of the synthetic fungicides, they are gaining increased attention as crop protection tool for the control of

diseases foliary and as inoculants to restore or enhance soil microflora. Suppression of soil-borne plant pathogens by organic matters operates through a wide range of mechanisms, directly through the fungicidal effect, or indirectly (fungistatic effect), the improving of soil physical properties, inducing plant resistance as by increasing the activation of certain plant enzymes, and the releasing of nutrients from these amendments

Aiming to minimize the use of fungicides to control fusarium wilt of tomato in Libya, this work was proposed to:

- 1. Prove the pathogenicity of *Fusarium oxysporum* f. sp. *lycopersici* isolates in tomato seedlings and to determine the most virulent isolate and the most susceptible tomato cultivar.
- Test the effect of different plant extracts on growth and sporulation of the Fusarium isolates.
- 3. Test the role of the most effective two extracts in controlling the fusarium wilt in seedling stage in comparison to the effect of two common fungicides.

LITERATURE REVIEW

Urban and Filipowicz, (2004), observed that there were large variations among tested isolates of *Fusarium oxysporum* that cause crown- and root rot or wilt, and some of them that did not infect tomato.

Elarabi, (1985) reported that there were significant differences among indoor tomato cultivars and their susceptibility to the infection by *Fusarium oxysporum* f. sp. *lycopersici*, that were ranged from moderate to high.

Green, (1977) reported that the presence of antimicrobial compounds in higher plants has been known since antiquity as an important factor for disease control, Several higher plants and their constituents were successed in plant disease control, and proved that they are harmless and non-phytotoxic unlike chemical fungicides.

Kumran and his group, (2003) on their work on anthracnose and ripe fruit rot of chilli pepper (Capsicium annauum L.), caused by Colletotrichum capsici (Syd), stated that the ethanolic and water extracts of roots of Abrus precatorius, Rauvolfia tetraphylla, and Croton bonplanianus were shown higher percentages of mycelial inhibition than the standard chemical fungicide mancozeb, the ethanolic root extract of A. precatorius and R. tetraphylla, showed significant inhibitory effects

on both conidial germination and radial growth. They also found that ethanolic root extracts of some herbaceous non-agricultural plants showed completely inhibition of conidial germination.

The effects of aqueous extracts of some common weed species against *F. oxysporum* f.sp. *lycopersici* were investigated under laboratory conditions. *Anagallis foemina, Cerastium dicotomum, Falcaria vulgaris, Ranunculus asiaticus, Scorpiurus muricatus* and *Solanum nigrum* extracts were more toxic to the fungus. Fresh shoot extract of (*R. asiaticus*) added to a liquid medium reduced mycelial dry weight and incorporation of some amount of dried shoot or dried roots in the media strongly inhibited the growth of fungus. (Qasem, 1996).

Ouf, et al., (1994), pointed out that the aqueous methanolic root and aerial shoot extract of of *Zygophyllum*. coccineum, *Z. album* and *Z. dumosum* (wild-plants) showed a high inhibitory effect (fungistatics) toward spore germination of *Verticillium albo-atrum* and *Fusarium oxysporum* f.sp. *lycopersici*, while those of water or ethyl acetate root extracts of *Z. album* caused a marked suppression of spore germination of the tested fungi.

Cheah and Cox, (1995), determined the effects of some water plant extracts on the incidence of powdery mildew

(Sphaerotheca fuliginea) on squash and reported that Reynoutria extracts, olive oil and rapeseed oil reduced powdery mildew.

Carabet and Buchenauer, (2004) found that the ethanolic leaf extracts of *Hedera helix* and *Paeonia suffruticosa* exhibited a high degree of activity against *Phytophthora infestans* on tomato plants, the extracts induced systemic resistance in plants. In vitro studies showed that *Paeonia suffruticosa* extracts effectively retarded mycelium growth of this pathogen, while *H. helix* extracts had no effect.

Mahmoud *et al.* (2004). evaluated the efficiency of methanolic extracts of *Eucalyptus citriodora* Hook, *Allium sativum* L., in controlling *Botrytes fabae*, the causal of chocolate spot disease in the faba bean. Their results indicated that the mixture of *E. ciriodora* with *A. satvum* leaf extract was the most effective one on mycelium growth. Laboratory studies supported the use of leaf extracts of *E. citriodora*, and they documented that the releasing of nutrients from decomposing organic matter this in turn improved the vigor of the plants. The addition of leaves crude extract of *E. citriodora* to faba bean leaves infected by *Botrytis fabae* applied plant by major minerals like N, P,K, and Mg.

Sathyanarayana et al., (2006) found that *Dravya* at concentration 0.3%, (a sea weed extract) when used as seed treatment of paddy seeds against *Alternaria padwickii*, the causal agent of seedling stack burn showed the same results that achieved by Dithane M 45% of seed germination, runner length,

and shoot length. They also documented that extract might induce host resistance through activation of peroxidases enzymes which used in different physiological functions like, lignification, suberization, and xylem wall thickening.

Bium and Rodriguez-Kabana, (2004), noted that Amendments of Benzaldehyde [(purified benzaldehyde, aromatic compound extracted from bitter almond (*Prunus amygdalus*)] and dried powders of kudzu (*Pueraria lobata*), velvetbean (*Mucuna deeringiana*), and pine-bark (*Pinus elliottii* and *P. taeda*) when added to soil to investigate the fungicidal effect on germination of sclerotia of *Sclerotium rolfsii* they stimulated the sclerotia germination. Also they observed that the inhibitory effects of aqueous extracts of *Allium spp*, diminished with the increase of temperature during their extraction, allowing the decomposition of antifungal compounds.

Ferreira et al., (2005). Observed in vitro that hexane crude extract from spiny holdback (Caesalpinia spinosa) leaflets extract promoted partial inhibition of mycelial growth of Fusarium solani and Phoma tarda, on potato-dextrose-agar (PDA) at several concentrations, whereas the inhibition was directly proportional to the dosage tested.

The organic amendments containing high nitrogen, significantly reduced populations of a wide spectrum of soil-borne plant pathogens. Pathogen control was shown to arise directly from the ammonia which is quickly released after application and

(or) nitrous acid (more toxic) which needed more time. The changes in soil fauna may last for months if not years, also liquid swine manure was more effective when added to dry than moist soil, apparently because the active components were diluted, as application of some liquid manures leading to an increase in soil microorganisms populations by up to 1000-fold following amendment application, (Lazarovits, 2001). Tenuta and Lazarovits, (2002), observed that the addition of meat and bone meal (2.5%) to an acidic loamy sand resulted in the accumulation of ammonia and temporary death of microsclerotia of *Verticillium dahliae* within 2 weeks.

Zidan, et al., (2000) mentioned that the phytochemical constituents of *Eucalyptus camaldulensis* (river red gum) and *E. citriodora* (lemon scented spotted gum) were extracted in different organic solvents, separated and identified. Phytochemical components were found in different amounts according to plant species, plant parts and solvents used. In general, sterols, triterpenes, alkaloids, carbohydrates and glycosides were found in higher amounts, followed by tannins. Phenolic glycosides, cardiac glycosides and flavonoids were detected in trace amounts. While spotted gum was free of saponins.

El-Refai and Moustafa, (2004) indicated that Raphanus sativus, Brassica, napus and B. oleracea var. capitata contained higher amount of phenolic compounds, lipoidal matters and unsaturated fatty acids, respectively. The allelopathic effect of aqueous and ethanolic extracts of seeds on Rhizoctonia solani

were tested *in vitro*, tested extracts have a variable inhibitory effect on *R. solani*. The effect depends on the concentration of the extracts, plant species and the active constituents. Results also indicated that application of powdered seeds of *R. sativus, Eruca. sativa* and *Sinapis*. *alba*, to the soil infested with *R. solani* increased the germination percentage of cotton, reduced damping off percentage and improved the growth criteria of the cotton seedlings.

A study was undertaken to explore the effect of volatile oils from *Eucalyptus citriodora* fresh mature leaves and its major constituent monoterpene citronellal against two well-known rice pathogens, *Rhizoctonia solani* and *Helminthosporium oryzeae*. The radial growth and dry weight of both tested fungi were drastically reduced in response to the volatile oils. A complete inhibition of *R. solani* and *H. oryzeae* was observed, (Ramezani, et al., 2002).

Ke-Qiang and van Bruggen, (2001), demonstrated that there were inhibiting effects on the sporangia, zoospores germination, and hyphal growth of on *Phytophthora infestans* in petri dishes by non autoclaved garlic bulbs (*Allium sativum* L.) and scouring rush stems (*Equisetum hyemale* L.). They also found that the inhibiting effect of garlic bulbs was effective when it was not autoclaved, they also showed that garlic extracts from fresh bulbs had stronger inhibiting effect against the germination than dry bulbs, the sporangia and zoospores germination was totally stopped.

In this respect, another study reported that allicin and ajoene in garlic had antibacterial, antifungal, antiparasitic and antiviral activities, (Ankri and Mirelman, 1999)

Bowers and Locke, (2004), found that extract treatments of soil did not eliminate *Phytophthora nicotianae* totally, but inhibited its ability to germinate in soil dilution plates for period of time. They attributed this to the breakdown of the extract in soil. The pathogen population still survived in low or undetectable numbers, but it was able to infect and colonize roots and cause disease in the greenhouse over time. They also demonstrated in another study that some of essential oils inhibited pathogen growth *in vitro* when interacted with the pathogen. While others, only inhibit the pathogen when in direct contact, (Bowers and Locke, 2000).

Zacchino, et al., (1998), found that the dichloromethane extracts from aerial parts of *Polygonum punctatum*, *Polygonum ferrugineum* and the bark of *Luehea divaricata* inhibited polymer biosynthesis or assembly of the cell wall of various yeasts, and filamentous fungi.

JaChoon, et al., (1998), showed that Pn-AMPs peptides (hevein-like proteins) have similar fungicidal effects as thionins isolated from the seed of *Pharbitis nil* L. They exhibited potent antifungal activity against both chitin-containing and non-chitin-containing fungi in the cell wall the Pn-AMPs penetrated very rapidly into fungal hyphae and localized at septa and hyphal tips

of fungi, which caused burst of hyphal tips. Burst of hyphae resulted in disruption of the fungal membrane and leakage of the cytoplasmic materials.

Ouf, et al., (1999), showed that using of hot water pine needles extract in vitro, was inhibited the dry weight gain, polysaccharide content, insoluble proteins, total prtein content and nucleic acids content of *Rhizoctonia solani*. Also the same result was obtained in addittion of *Penicillium oxalicum* to the amended soil. They suggested that, antagonistic filtrates and pine needle extract contain inhibitory substances that are directly toxic to *R. solani*, the causal of damping-off of cucumber seedlings due to their interference with carbohydrate and nitrogen metabolism of the target pathogen.

Padmodaya and Reddy, (1999), showed that the percent of healthy tomato seedling from wilt disease, increased with the increase of amendment concentration of neem cake, pongamia green and dry leaves, eucalyptus dry leaves and farmyard manure amendments. They also found that two weeks of decomposition recorded higher healthy seedling stand (60%) more than four weeks (59%). They also reported that, organic amendments reduced the seedling wilt disease in tomato due to either the antibiosis or increasing population of selective microflora that reduced pathogen population or its activity.

Wurms, et al., (1999), explained the mode of action and efficacy both of prophylactic compound, and Milsana (leaf extract of Reynoutria sachalinensis), against powdery mildew

(Sphaerotheca fuliginea) development on long English cucumber (cv. Corona). Milsana application reduced disease incidence through induction of localized resistance. Microscopic observations showed most haustoria had collapsed (within 4 days of treatment application).

Srivastava and Lal, (1997), found that aqueous fresh leaf extracts of *Azadirachta indica* completely inhibited the mycelium growth and spore germination of *Curvularia tuberculata*.

Mert-Turk, (2006), demonstrated that number of tomato pathogens, including Septoria lycopersici, Botrytis cinerea, Fusarium oxysporium f.sp. lycopersici, Verticillium aboatrum, and Alternaria solani, are able to produce an detoxifing enzymes of saponine compounds like (α-tomatine and avenacins).

Anjum, et al. (2006) reported that the aqueous extract of fresh aerial parts of chickpea (Cicer arietinum L.), was relatively more effective in decreasing the mycelial growth of Drechslera hawaiiensis while D. tetramerain exhibited great resistance to this extract. In addition, the highest tested concentration of crude extract caused a persistent positive impact on growth of both fungal species.

Ekreem, (2002), found that, pigweed (Chenopodium murale) leaves, throne apple (Datura stramnium) stems, and eucalyptus (Eucalyptus camaldulensis) bark affected in vitro the mycelial growth of Sclerotinia sclerotiorum. While Eucalyptus leaves, pigweed stems, and throne apple leaves did not affect the

mycelial growth, extracts of throne apple leaves and pigweed stems in two weeks incubation inhibited the formation of sclertia.

Abou-Jawdah, et al, (2002), observed that methanolic extract of *Inula viscose* had no effect on the mycelial growth of *Fusarium oxysporum* f.sp.*melonis*, *Penicillium sp.*, and *Verticillium dahlia*, while the inhibition of spore germination was high.

It was proved that the olive leaves water extract has antimicrobial effect against bacteria and fungi at various concentrations. Olive leaves contain around 60~90 mg per gram (dry weight) oleuropein, plus significant levels of several types of other esters, flavonoids, and multiple iridoids,(www.mdidea.com).

Nwachukwu and Umechuruba, (2001), reported that when the crude and aqueous leaf extracts of plants of basil (*Ocimum basilicum*), bitter leaf (*Vernonia amygdalina*), lemon grass (*Cymbopogen citratus*), neem (*Azadirachta indica*), and paw-paw (*Carica papaya*) were tested *in vitro* against the incidence of seed borne fungi of *Fusarium moniliforme*, *Botrydiplodia theobromae*, *Aspergillus niger*, and *A. flavus*, it was found that the crude extracts were more effective than the aqueous leaf extracts in reducing seed-borne fungi and in increasing seed germination and seedling emergence. It was also observed that the incidence percentage of seed-borne fungi of *A. niger*, and *A. flavus* in presence of crude extract of neem (*Azadirachta indica*) were completely controlled, while *F. moniliformae* and *B. theobromae* gave 5.3 and 4% of incidence respectively.

Zhou et al., (2004), observed that, the addition of hairy vetch against wilt causal agent of watermelon, *Fusarium oxysporum* L sp. *niveum*, reduced the disease incidence, the fungus population, and increased the length of primary runners, aboveground biomass, and fruit weight more than the improvement achieved by the soil fumigant, (methyl bromide or 1,3-dicloropropene plus 35% chloropicrin). In addition amended soil also increased fruit sugar content. It was suggested that, the efficacy of organic amendments might vary with soil type, source and production of amendment, and host-pathogen system. They also demonstrated that lower rates of amendments reduced the potential phytotoxicity to host plant.

Gilani, et al. (2003), studied the allelopathic effect of Eucalyptus microtheca. in laboratory experiments. The aqueous extracts from different dried plant parts, soaked for 48 hours, inhibited radicle growth, plumule growth and seed germination of Pennisetum glaucum cv. bari-Hairy. The toxicity varied from part to part and related to concentration and soaking duration. Roots were highly toxic to the radicle growth followed by leaves, stem and whole plant material. For the growth of plumule, leaves were found to be highly toxic followed by whole plant, roots and stem.

Joshi, et al., (1998), observed that germination and root and shoot development of wheat, (*Triticum aestivum*) growing under the canopy of *Cassia fistula* have been shown to be inhibited. The effect of organic and aqueous extracts of stem bark,

leaves and pods of *C. fistula* on seed germination and seedling growth of *Triticum aestivum* was examined. All the aqueous extracts tested inhibited seed germination and seedling growth of *Triticum aestivum*, and the inhibitory effect increased with the increase of concentration. Bark extracts tended to be more inhibitory than those of leaves and pods. Bioassays of chemical compenents proved that the flavonoids showed maximum inhibition on germination and seedling growth of *Triticum aestivum* than anthraquinones.

Kurucheve et al., (1997), showed that cold water of fresh leaf extract of *Eucalyptus globules, Caesalpinia pulcherrima*, *Lawssonia inermis*, completely inhibited the sclerotial production of *Rhizoctonia solani*, while Eucalyptus hot water extract recorded variable effects on sclerotial production at the tested concentrations. The hot extract of *Prosopis juliflora* was completely inhibited the sclerotial production, while the cold water extract of *Prosopis juliflora* inhibited sclerotial production at the highest concentration.

Okigbo and Ogbonnaya, (2006), found that the reduction of mycelial growth by extract varied with, plant species extraction solvent and period of incubation. They found that ethanolic extracts of *Ocimum gratissimum* and *Afromomum meleguta* were more effective than hot and cold water extracts, when tested *in vitro* against *Fusarium oxyspoum*, *Aspergillus niger*, and *Botryodiplodia theobroae* causal agents of soft rot of yam tuber. The ethanolic extract from the *O. gratissimum* was more effective than

Afromomum meleguta in mycelial reduction of Fusarium oxysporum. They also found that the ethanolic extract of O. gratissimum having the highest mycelial reduction of Botryodiplodia theobroae, followed by cold water extract. The hot water extract was more effective than cold extract of O. gratissimum against Fusarium oxyspoum. In storage, the tubers dipped in the extract for longer time were more effective against these rot pathogens.

Bharathimatha, et al., (2002), reported that proteins extracted from seeds of Harpullia cupanioides (Roxb.) which exhibited strong inhibition of the growth of Rhizoctonia solani, Curvularia lunata, Cochliobolus lunatus, Colletotrichum musae and Alternaria alternate, they retained their antifungal activity even after heating at 100 °C for 10 minutes or autoclaving at 121 °C for 20 minutes.

Kishore, et al., (2001), mentioned that the aqueous and ethanol leaf extracts of Datura. metel and ethanol leaf extract of Azadirachta indica were highly inhibitory to Phaeoisariopsis personata, the causal organism of late leaf spot of groundnut even at 0.01% (100 ppm) concentration. Ethanol leaf extract of A. indica retained its activity up to 80 °C, while aqueous leaf extracts of D. metel and S. indicus up to 100 °C.

Eksteen, et al., (2001), found that the inhibition of *Rhizoctonia solani* by *Eucomis autumnalis* methanolic crude extract was similar to that with the standard fungicide (carbendazim / difenoconazole).

Pretorius, et al., (2002), found that the root extract of Eriosema psoraleoides. showed more growth inhibition of Rhizoctonia solani than the synthetic fungicide carbendazim/difenoconazole , and inhibited completely the mycelial growth of Pythium ultimum. Aristea ecklonii was completely inhibited the mycelial growth of all tested pathogens while Agapanthus inapertus completely inhibited the growth of B. cinerea, R. solani, V.dahliae, and B. dothidea and showed relatively high inhibition of the remaining pathogens.

Babu, et al., (2000), tested the fungitoxic effects of several plant extracts from various plant species on the mycelial growth and spore germination of Alternaria solani, the causal agent of tomato leaf blight disease. Among them, 10% extracts of Acacia concinna pod and Basia latifolia oil cake were effective followed by Allium sativum bulb, Annona squamosa seed, Cissus quadrangularis stem extracts and neem products. The fungicide check mancozeb (0.2%) remained superior to all the plant products tested.

Leandro, et al., (2003), reported that when the symptomiess leaves of strawberry sprayed with conidial suspension of Colletotrichum, acutatum, the causal agent of strawberry anthracnose in presence of flower water extract of strawberry (cv. Tristar) resulted in increasing the number of conidia on these leaves compared with water. Also they observed that the numbers of conidia increased more when exposed to flower extract than leaf extract.

Babadoost and Johnston, (1998), observed the sporulation of some isolates of *Drechslera graminea* gathered from different countries. Sporulation of the fungus was induced by using straw extract from the barley cultivars Arta, Bowman, Bracken, Clark, Gallatin, and Salmas. There were significant differences in sporulation among isolates of the different areas as well as among isolates originated from the same barley field.

Yoshida and Shirata, (2004), mentioned that compounds of mulberry leaves inducing sporulation of *Colletotrichum dematium* (mulberry anthracnose fungus) were detected. Sporulation was induced only when a biotin solution applied to fungal colony on PSA plate at a concentration of at least 0.01 ppm. This result suggests that biotin, which occurs in mulberry leaves, has a role in inducing sporulation of *C. dematium*.

Wyss, et al., (2001), found that addition of plant extract may had no stimulatory effect on mycelial growth or sporulation but can promote the spores efficacy like germability, durability, and pathogen virulence. The conidial production of Dactylaria higginsii was low on hot water extract of brown rice grains compared with hot water extract of white rice grains but germination rate of conidia produced on white rice alone was 50% compared with the near 100% germination of conidia produced on PDA or on potato dextrose broth (PDB) amended with white rice. Conidia produced on white rice or PDA were less virulent on the fungal host, Cyperus rotundus than conidia from white rice amended with PDB. After

four washings, conidia from all three media produced the same level of disease severity. White rice supplemented with PDB and PDA in trays was suitable for mass production of conidia of Dactylaria higginsii.

Sporulation is often improved by the addition of sterilized host leaf material (e.g. wheat straw, maize leaves, carnation leaves) to 'weak' media, such as tap water agar. Many fungi can be induced to sporulate under or near ultraviolet light. A black light box can be constructed to induce sporulation in those fungi that require near ultraviolet light, (www.daff.gov.au)

Tuite, (1969), showed that there were some fungal species require or sporulate more abundantly on special nutritive requirements such as amino acids, calcium, and pH.

MATERIALS AND METHODS

1- Collection of infected plants

Fresh diseased tomato plants were collected from different regions in Libya including; El-Garabouli, Aujla, El-Zawia, Misurata, Sirte, Tripoli, and Jallo in January 2005. They well washed under running tap water then shoots were separated and roots were well washed to remove soil particles to obtain isolates from them.

2- Isolation of fungi

Root-stem plant parts were cut with sterilized sharp blade into thin wedges (2-5 mm thick) of one side of the plant included xylem just below the epidermis. The pieces were surface disinfected for 2 minutes in 0.1% sodium hypochlorite and rinsed in sterile distilled water. 3 wedges of the infected tissues were picked with a flamed sterilized forceps and inoculated on solid potato dextrose agar (PDA) medium contained in Petri plates of 9-cm-diameter. The plates were incubated at room temperature (25-28 °C). Once the fungi were grown sufficiently, they were subcultured onto fresh PDA solid medium to obtain pure cultures for each isolate separately. Plates were incubated at the same standard conditions of temperatures (25-28°C) for two weeks. The stock cultures were saved at 10°C until needed.

3- Identification of isolates

The fungus (*Fusarium oxysporum* f.sp. *lycopersici*) isolates were identified using light microscope. The microscopic characteristics, and cultural characteristics were compared to the description in Booth, (1971).

3-1- Conidial measurements

Conidia were obtained by flooding 7-days old culture of each isolate with distilled water. A slide of spores was prepared and observed under light microscope. Using the ocular micrometer (after calibration lay stage micrometer), one hundred macroconidia were randomly measured.

4- Pathogenicity test

Pathogenicity test aimed to determine the highly susceptible tomato variety to fusarium wilt disease and the most virulent *Fusarium oxysporum* f.sp. *lycopersici* isolate.

4-1- Emergence of tomato seedlings

The available tomato cultivars (Table,1) in Libyan market were used in this test with 7 fungal isolates at 3replicates arranged in split plot design. Seeds were washed by running tap water for 20 minutes to remove the seed-coat chemicals and after that rinsed in sterile distilled water for 5 minutes, the seeds then were sown in sterilized soil (5 soil:1 organic matter, hand mixed thoroughly and autoclaved together in 1.5 Kg cm⁻² pressure and 121°C for 20 minutes) in plastic pots of 9-cm-diameter and of 6 cm height. Each pot was sown with 5 seeds, and then thinned to 3

plants at 15 days after sowing. Pots were watered daily with 15 ml of sterilized water and maintained in the greenhouse (3x1.5x1.2m in high), where the air temperature varied between 22-35°C during different periods of experiment.

Table 1. List of tested tomato cultivars used in pathogenicity test combined with producing companies.

no.	Cultivar	Producer
1	Al-jood	Zagrit seed
2	Boushra	Zagrit seed
3	Call.j.	De ruiter
4	Falcato	De ruiter
5	Formula	Ergon
6	Haliem	Ergon
7	Midi A	Enzazaden
8	Rio grande	Zasco by
9	<u>Zahrah</u>	Enzazaden
		

4-2- Preparation of inoculum

Conidial suspensions were obtained from sporulating cultures of 2-weeks old of *Fusarium oxysporium* f.sp *lycopersici*. Isolates thoroughly mixed with 10 ml of sterile distilled water, then filtered through 4-layers of sterile cheese cloth to remove the mycelial fragments. The obtained conidial suspension were counted with the aid of a hemacytometer and adjusted to the required concentration (2.11x10⁵ conidia ml ⁻¹) for each isolate by dilution with sterile distilled water.

4-3- Plants inoculation

When tomato seedlings reached 4-leaflets stage, they were inoculated by using standard root-dip method. Seedlings were removed from soil, then were washed by tap water to remove the soil particles followed by sterile distilled water and air dried. Then dipped in the inoculum suspension for 5 minutes for each isolate separately. The inoculated seedlings were transplanted into plastic pots, three seedlings in each pot of 9-cm-diameter and 6 cm height. The pots were maintained in the greenhouse, and watered daily. After 3 weeks of inoculation, visual disease symptoms (fig. 1) were recorded at 5 classes according to the following scale.

- 0 = No visual symptoms (healthy).
- 1 = Clearing of veinlets.
- 2 = Epinasty of petioles.
- 3 = Yellowing of lower leaflets and partial wilting.
- 4 = wilting and death of plants.

The trial was established in three replications. The data were analyzed using ANOVA test ($\alpha = 0.05$).

5- Preparation of leaf extracts

Fresh green mature leaves were collected from different plants (Table, 2) growing in man made river authority camp, Sirt, Libya, during June, 2005. Fresh leaves from each plant were carefully washed thoroughly under tap water followed by sterile distilled water, then they were air dried and then kept in oven at 50 °C for 48h. Leaves were chopped, a cold water extracts of leaves were prepared by plunged tiny pieces in sterile distilled water at 3:7 (w/v) in a beaker for 48hrs, then they were reserved at room

temperature, Resultant suspensions were filtered through 4-folds of sterile cheese cloth, autoclaved and kept in the fridge at 4°C as stock extract until needed.

Table 2. List of plants, their common names, scientific names and families used in the preliminary screening of antifungal properties

Common name	Scientific name	family
Athel, Tamarisk salt tree	Tamrix articulata	Tamaraceae
Butter leaves	Atriplex nummularia	Chenopodiaceae
Eucalyptus	Eucalyptus gomphocephala	Myrtaceae
Garlic	Allium sativum	Liliaceae
Oleander:rosebay	Nerium oleander	Apocynaceae
Olive tree	Olea europaea	Oleaceae
Retama	Retama raetum	Fabaceae
Weeping fig	Ficus nitida	Moraceae
Zahtur	Thymus sp	Labiatoe

6- Laboratory experiment

In vitro evaluation of efficiency of leaf extracts on the culture of Sirte isolate

The objective of this trial was to investigate the most effective extracts. The stock solutions were diluted with distilled water to obtain various concentrations (5.00, 10.00 and 20.00%) according to food poison technique,(Ramezani et al,2002). The concentrations of each plate were obtained for 48h soaking period. 5ml of each concentration of each extract, and distilled water to PDA plates served as control treatment were dispensed into 9cm diameter Petri dishes followed by 20ml of melted PDA medium then they were shaken together and allowed to solidify.

Each plate was inoculated with one mycelial disk of 7mm-diameter cut from 2-weeks-old culture of virulent isolate (Sirte isolate), discs were separated by using 7mm sterilized cork borer. One disc was placed upside down on the center of plate contained PDA medium, all of these treatments were replicated four times, laid out in RCB design, and incubated at room temperature (25-28°C). The first evaluation of mycelium radial growth was measured after one week of incubation, the second one was recorded after 2 weeks; the growth average was obtained in two orthogonal axes. Fungitoxicity was expressed in terms of percentage of mycelial growth inhibition and calculated according to the formula of Pandey (cited after Ramezani et al., 2002.):

% of mycelial growth inhibition = (dc-dt) / dc x 100

Where dc= average diameter of fungal colony with control, dt = average diameter of fungal colony with treatment.

The sporulation was also counted by using a hemacytometer blood counting chamber. The number of spores per milliliter was obtained with the aid of the formula: number of spores above 1 large square x 250,000. The inhibition percentage of sporulation was calculated according to Abbott's formula;

% of sporulation inhibition = (% spores in control - % spores in treatment) / % spores in control x 100.

Calculations were performed after one and two weeks of inoculation.

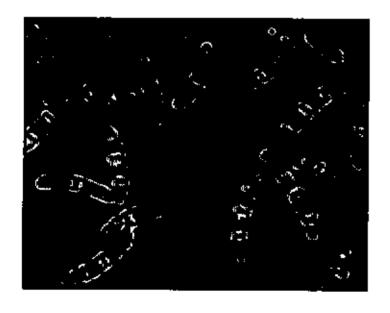
7- In vivo experiment

In accordance to the laboratory experiment, tested concentrations of two extracts (Eucalyptus and olive) were diluted to, (1.00%), (2.00%), and (3.00%) for eucalyptus and (6.00%), (9.00%), and (12.00%) for olive. Pots with 3 seedlings in each pot of highly susceptible tomato cv. Riogrande grown in greenhouse were inoculated with highly virulent isolate (Fusarium oxysporum f.sp. lycopersici) and treated with each of designed concentrations of the two extracts by the same way mentioned above. In order to compare their efficacy to fungicides, for this, two broad-spectrum fungicides were applied at high (0.75 g/l), medium (0.50 g/l), and low (0.25 g/l) of labeled rates of Benlate^R; (Benomyl, 50% g/l WP), and Tachigazole^R (hymexazol; Potasium salt, 30% EC), at rates of high (2.50 ml/l), medium (1.50 ml/l), and, low (0.50 ml/l). Pots were divided into three groups; first treated with one dose, and the second treated twice of each concentration of two extracts and two fungicides, pots were laid out employing completely randomized design (CRD) with three replicates, checked with 3 pots of inoculated-untreated and 3 pots of uninoculated-untreated. Pots watered daily with 20 ml of sterile-distilled water. Disease evaluation was recorded according to the scale from 0-4 after one and two weeks of inoculation as described above.

RESULTS

1- Isolation of Fusarium oxysporum f.sp. lycopersici

Fusarium oxysporum f.sp. lycopersici was isolated from the different samples of infected tomato plants that collected from different regions. Each isolate was identified according to the shape and length of macroconidia (Figure, 1 and Figure, 2) (Table, 3). Later it was confirmed by disease symptoms appearance (Figure, 3).



Figure, 1. Macroconidia (boated shape) of the fungus.

lso.lo	Faced plate	Reversed plate	Isolat	Faced plate	Reversed plate
Al-Zawia	0		Tripoli		
Garabouli			Misurata		
Au		Sirte			
Aujla			Jallo		

Figure, 2. Cultures of *Fusarium oxysporum* f.sp. *Lycpersici* isolates growing on Petri dishes contained with PDA solid media Isolated from infected tomato plants collected from the different localities.

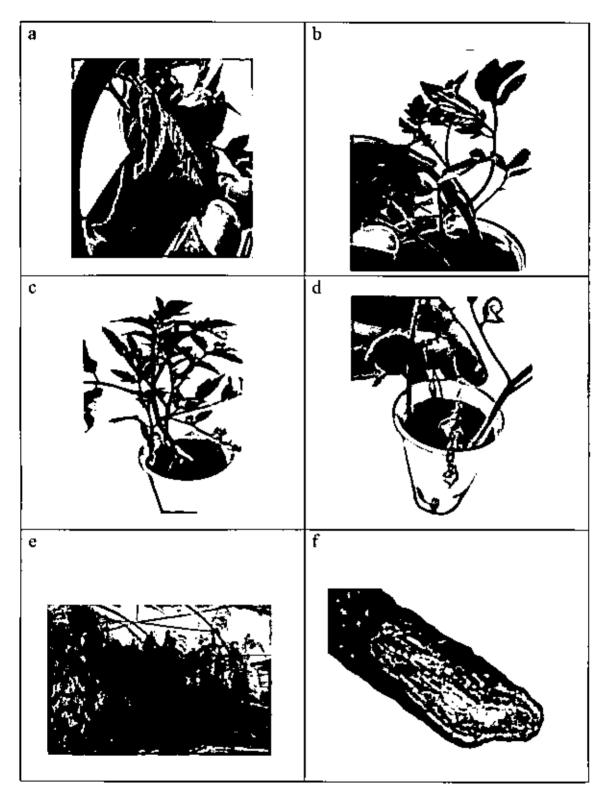


Figure 3. Symptoms of wilt disease, vein clearing (a), epinasty (b), yellowing of lower leaves (c) of lower leaves, death of plant (d), wilting of plants in greenhouse (e), and xylem browning (f).

Table 3. Geographical origin of tested isolates with macroconidial length (μm)

Isolate. no	Isolate locality	Length of macroconidia
1	AL-Zawia	(um) 31.35
2	Al-Garabouli	29.2
3	Al-Aujla	36.4
4	Sirt	33.7
5	Tripoli	33
6	Misurata	24.75
7	Jallo	30.25

Isolate, No. = Isolate number.

2-Pathogenicity test

Firstly, after 2 weeks of inoculation results indicated that all isolates had the same virulence effect except the isolate of Jallo. Records of disease severity 3 weeks after inoculation showed variations among the tested isolates. The highest disease index (2.667) caused by the Sirte isolate and the lowest was that of Jallo (1.648) (Table, 4). Sirte isolate was chosen to be the tested isolate for subsequent experiments.

Table 4. Virulence of isolates after 2 weeks and 3 weeks of inoculation

Geographic origin	Disease index after 2 weeks	Disease index after 3 weeks
Sirte	2.407 ^A	2.667 ^A
Al-Aujla	2.259 ^A	2.370 ^{AB}
Tripoli	2.457 ^A	2.358 ^{AB}
Al-Zawia	2.457 ^A	2.167 ^B
Misurata	2.395 ^A	2.117 ⁸⁰
Garabouli	2.222 ^A	2.025 ^{BC}
Jailo	0.086 ^B	1.648 ^C

Values with the same letters are not significantly different at α = 0.05.* LSD= 0.3474**LSD= 0.4819

Results of inoculation of tomato cultivars with Sirte isolate showed that all of them were susceptible. The highest disease severity (2.356) was recorded on Riogrande (Table, 5). It was selected for green house experiment.

Table 5. List of susceptibility levels of tested cultivars evaluated by disease index.

cultivar	Disease index
Riogrande	2.365 ^A
Zahra	2.222 ^A
halieem	2.175 ^{AB}
Formula	2.111 ^{ABC}
Boushra	2.095 ^{ABC}
Aljoud	1.905 ^{BCD}
Falcato	1.873 ^{BCD}
Call J.	1.841 ^{CD}
Midi A	1.778 ^D

Values followed by the same letter do not differ significantly, at $\alpha\text{=}\ 0.050.05\text{=}0.3033.$

3- Laboratory results

3-1- Effect of leaf extracts on the radial growth and sporulation of the fungus.

Results indicated that the effects of tested leaf extracts on the radial growth of Fusarium oxysporum f.sp. lycopersici were ranged from highly to slightly suppression. Olive (Olea europaea) extract completely inhibited the mycelial growth followed by eucalyptus (Eucalyptus gomphocephala) and butter leaves (Atriplex nummularia) extracts. Lower inhibition was obtained by Garlic (Allium sativum), Ratema (Retama raetum), Oleander (Nerium oleander), and Athel (Tamrix articulata) extracts, while Weeping fig (Ficus nitida) was gave the lowest suppression,

Olive (Olea europaea) extract recorded the highest of sporulation inhibition (89.09%) followed by eucalyptus (Eucalyptus gomphocephala) of 74.24% sporulation inhibition. Other extracts stimulated the sporulation of the fungus from +1.2% for ratema (Retama raetum) to 1311.63% for athel (Tamrix articulata). (Figure, 4)(Table, 6.)

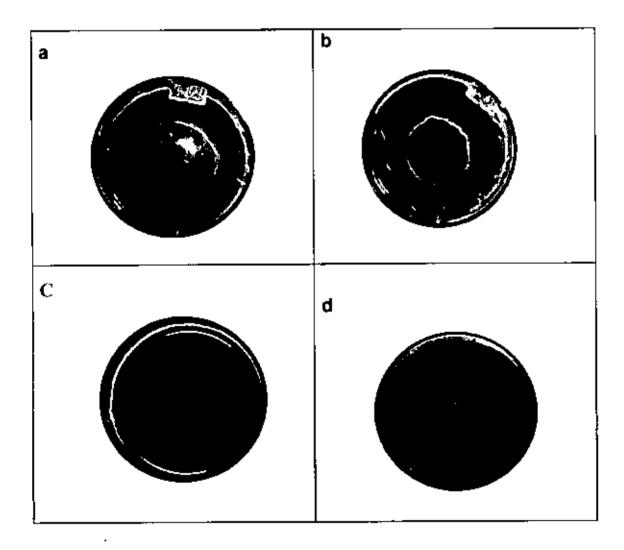


Figure 4. Effect of different Eucalyptus extract concentrations on the radial growth of the fungus, 0.10 w/v (a), 0.20(b), Olive extract(c), and control (d)

Table 6. Effect of leaf extracts on the radial growth and sporulation of the fungus isolate.

	Radial growth		Sporulation	
Extract	Cultural diameter (cm)	Mycelial Inhibition.(%)	Spores No ml	Sporulation Inhibition(%)
Olea europea	0.00 ^F	100.000	7500 ^D	89.091
Eucalyptus gmphocephala	5.917 ^E	28.279	17 707 ^D	74.244
Retama raetum	7.333 ^{CD}	11.115	69575 °	+01,200
Neriuum oleander	7.167 ^{co}	13.127	226675 ^C	+229.709
Ficus nitida	7.875 ^{AB}	4.545	276500 ^C	+302.182
Allium sativum	7.042 ^D	14.642	309000 ^{BC}	+349.455
Tamrix articulata	7.583 ^{BC}	8.085	422750 ^B	+514.909
Atriplex питтиlaria	5.958 ^E	27.782	970500 ^A	+1311.636
Control	8.250 ^A	-	68750 ^D	-

^{(+):} Stimulation.

Values followed with the same letter were not significantly different at α =0.05.

3-2- Effect of different concentrations of leaf extracts on the radial growth and sporulation of cultures

Generally, results showed that the highest concentration (0.20 w/v) was not effective, while the concentrations of (0.10 w/v) and (0.05 w/v) was the most effective while (0.10 w/v) concentration was the best one. The efficiency obtained from isolate cultures, submitted to different concentrations of extracts are shown in Table, 7.

Results showed that all Olive concentrations were gave the highest suppression level followed by Eucalyptus (0.20), whereas Weeping fig at (0.10), Garlic at (0.05), Ratema (0.5) where the effect increased by concentration increasing, and Tamarix (0.20) were at the same level of control, Table,7.

As in table 7, all concentrations of Eucalyptus and Olive extracts were gave the lowest sporulation level, while butter leaves extract at 0.10 stimulated the sporulation and was gave the highest level.

Table 7. Effects of different extract concentrations on mycelial growth and sporulation of Sirte isolate.

Extract	Rac	Radial growth (cm)	cm)	Sporu	Sporulation (spores No/ ml ⁻¹)	No/ ml ⁻¹)
	0.05	0.10	0.20	0.05	0.10	0.20
Olea europea	0.000 P	0.000 P	0.0000 P	6250 ^J	12500 ^{LJ}	3750 1
Eucalyptus gmphocephala	6.500 ^{HLJ}	6.375 ™	4.875 L	28125 HU	17500 "	7500 ^J
Atriplex nummularia	6.375 FGHI	6.000 JK	5.500 KL	1065750 ⁸	1473750^	372000 ^{CDE}
Allium sativum	8.000 ABCD	6.625 GHIJ	6.500 HIJ	355000 ^{CDE}	251250 ^{DEFGHI}	320750 ^{0EF}
Neriuum oleander	7.250 DEFGH	7.000 ^{DFGH}	7.250 ^{DEFGH}	275750 ^{DEFG}	138125 ^{ЕГСНІЈ}	266250 ^{0EFGH}
Retama raetum	7.625 ABCDEF	7.500BCDEF	6.875 ^{гдні}	81250 FGHIJ	57500 GHIJ	70000 GHIJ
Tamrix articulata	7.500 BCDEF	7.375 ^{CDEFG}	7.875ABCD	460750 ^{CD}	585750 ^c	221875 ^{DEFGHIJ}
Ficus nitida	7.750 ABCDE	8.250 AB	7.625 ABCDEF	280000 ^{DEFG}	273750 DEFG	275750 DEFG
Control	8.250 AB	8.250 AB	8.250 AB	71250 GHIJ	71250 GHIJ	71250 GHIJ

Values Followed with the same letter were not significantly different at $\alpha = 0.05$

4- Greenhouse experiment

4-1- In vivo evaluation of the efficiency of leaf extracts

The Eucalyptus extract showed above average of potently (2.111) (Figure, 5) followed by olive extract while both of tested fungicides gave the lowest effect as shown in, Table 9.

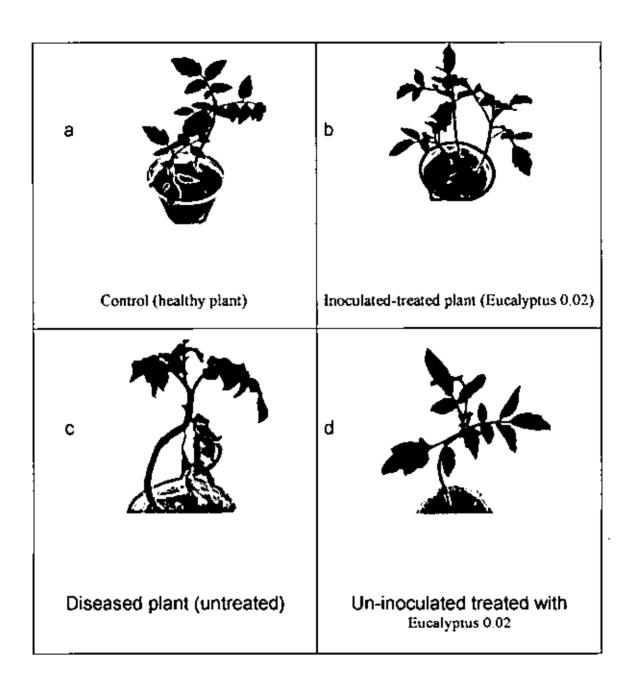


Figure 5. plant appearance treated with Eucalyptus 0.02 w/v compared to inoculated control,control (healthy plant)

Table, 8. Values of disease index of tested substances

No.	Substance	Disease index
i i	Olive extract	2.Ĝ67 ^{AB}
2	Eucalyptus	2.111 ^B
3	Benlate ^R	2.889 ^A
4	Hymexazol ^R	2,833 ^A

Values followed with the same letter were not significantly different at alpha 0.05,

Also results indicated that there were significant differences among concentrations used. 2%, 9%, 0.5 g/l, and 1.5ml/l of olive, eucalyptus, Benlate, and Hymexazol respectively were the best concentrations, they were recorded the lowest disease index value (2.208), while the first and third concentrations were gave (2.75 and 2.917) respectively, Table, 10.

Table, 9. Values of disease index of tested substances with their concentrations.

Concentration/ _substance	(1)	(2)	(3)
(1) Olive	2.5000	1.8333	3.6667
(2) Eucalyptus	2.5000	1.5000	2.3333
(3) Benlate ^R	2.8333	2.6667	3.1667
(4) Hymexazol ^R	3.1667	2.8333	2.5000
Control (water)		2.625	

Visually we had noted that the action of the extracts was slow more than fungicide did, and also the water holding capacity was affected positively by Olive extract application.

Discussion

Pathogenicity test

Variations in virulence among fusarium isolates infecting tomato were also noticed by Urban and Filipowicz, (2004) that may be attributed to presence of differential pathogenic response, also the variations were exhibited among tested tomato cultivars.

Results showed that cultivars of Riogrande and Zahra were highly susceptible to the infection while cultivar of Midi A. was the lowest susceptible one this variation among other tomato cultivars was also noticed by (Elarabi, 1985).

Effect of extract and extract concentration on the radial growth and sporulation

The results of this study showed generally the increase of mycelial inhibition of *Fusarium oxysporum* f. sp. *lycopersici* by the increase of extract concentration. Similar results had been reported for *Rhizoctonia solani* (El-Refai and Moustafa,2004); *Fusarium solani* and *Phoma tarada* (Ferreira et al. ,2005), and *Drechslera hawaiiensis* (Anjum et al. ,2006).

Different extracts found to be of different inhibitory effect on the Fusarium oxysporum f, sp. lycopersici such variations depends on the nature of the plant, plant part and type of the solvent used (Zidan et al., 2000; Ekreem, 2002; Okigbo, and Ogbonnaya, 2006; and Ouf et al., 1994)

the olive leaves extract was completely inhibited the mycelial growth and recorded 100% inhibition regardless of concentration and this

may be attributed to the strongly effect of oleuropein, the major constituent of the extract and (or) its hydrolysis products, elenolic acid and its salt of calcium (www.mdidea.com).

Besides to olive *Eucalyptus* extract was also effective on the fungal growth. According to (Ramezani, et al., 2002), the fungicidal activity of eucalyptus was related to citronellal oil and eucalyptus oil. Mahmoud *et al.*, (2004) mentioned that eucalyptus work to decrease the activity of some pathogen enzymes that used in the host invasion

Both olive and eucalyptus extracts recorded the best effect on spores inhibition among all while others decreased the inhibition percent, these results agreed with Srivastava, and Lal, (1997). While others were increased the sporulation compared to control with variable degrees this may be due to the nature of plants used, (El-Refai and Moustafa, 2004).

Although gartic is consider one of the oldest components in folk medicine and has been proved to be an inhibitory factor to many fungi, other microbes and even animals. Many investigations have revealed that garlic has antimicrobial and antiviral activities and was ascribed mainly due to the presence of allicin and ajoene (Ankri and Mirelman, 1999, and Cowan ,1999), In this study, garlic leaf extract did not show the expected inhibitory effect on the growth of the fungal mycelium, this might due to the lower content of these active constituents in the leaves than bulbs, or the completely lost of its efficacy when it was autoclaved, (Ke-Qiang and van Bruggen ,2001), or at least due to the decreasing of its antifungal activity by temperature, (Blum and Rodriguez-Kabana, 2004). In the study of Bharathimatha *et al.*, (2002) different extracts retained their antifungal activities even at autoclaving degrees. In other extracts which exhibited variable inhibition activities were found to retain their antifungal activities

even at autoclaving degrees. In terms of Ratema extract the mycelial inhibition effect was clearly increased proportionally to the increase of the extract concentration.

Weeping fig was gave the lowest extent effect on mycelial inhibition this might be due to the plentiful of favorable nutritive agent(s), or might be to the limited ability of fungus to detoxifying of these toxic substances; (Mert-Turk, 2006 & Anjum et al. 2006).

Greenhouse experiment

Because of the mostly effect in laboratory test of olive and eucalyptus therefore they were selected for this trial, and because that the high concentration of extracts cause phytotoxicity on seedlings; (Zahou and Events,2004 & Joshi et al.,1998),and to the relation especially of eucalyptus water extract phytotoxicity to extract concentration and soaking period,(Gilani et al., 2003), for this reason preliminary experiment was conducted to achieve suitable concentrations without detrimental effects which obtained from lower rates especially in case of eucalyptus. In that preliminary study it was found that the addition of these treatments two times was equal to that of three times and was better than one application. This is why twice application of treatments with one week interval was applied in this experiment, and relied on this point, and because the efficacy of extract decreased by time, (Padmodaya and Reddy, 1999). It was determined one week to be the best interval between the two applied doses, but here the doses were showed a similar affect.

It was shown in the greenhouse experiment that the two commercially fungicides and olive extract were at the same level of low efficacy while the eucalyptus was the potent one. The effect of extracts and fungicides on pathogens varied with the target pathogen, and the plant part used in extract; (Eksteen et al.,2001& Pretorius et al.,2002), but in addition to the fungicidal affect of extract it is also improved crop production by many ways; (Zhou et al., 2004 and Sathyanarayana et al., 2006). The effect of extracts on plants was delayed more than fungicides did, this due to that the active constituents released from the extract consumed a period of time to release and to act, this observation was agreed with that reported by Lazarovits, (2001).

Cheah and Cox (1995), proved that the watery extract of olive oil was effective against squash downy mildew. But our results revealed that the olive extract principles (oleuropein and other active compounds) seems to be leached readily by irrigation water or might be broke down by one or more environmental factor(s).

Summary

This study was conducted in Ras Ali, Misurata, Libya to test the effect of watery leaf extracts of nine different plant species, belonging to different plant families, against seedling disease in tomato caused by *Fusarium oxyspor*um f.sp. *lycopersici* in pot culture under greenhouse conditions.

Initially, the pathogenicity test was carried out to test the most virulent isolate among seven isolates collected from different regions in Libya including; El-Garabouli, El-Aujla, El-Zawia, Misurata, Sirte, Tripoli, and Jallo, on common locally cultivated cultivars involving, Al-Jood, Boushra, Call J., Falcato, Formula, Haliem, Midi A., Riogrande, and Zahra to obtain the most susceptible variety to the disease. The results showed that these isolates were almost at moderate level of virulece but Sirte isolate was the most effective one. The outdoor cultivar Riogrande was recorded the most susceptible one therefore it was selected with Sirte isolate for subsequent trails.

In vitro test was aimed to determine the most two potent extracts with suitable concentrations for the inhibition of both mycelial growth and sporulation by leaf watery extracts of plants of

Athel (Tamrix articulate), Butter leaves (Atriplex nummularia), eucalyptus

(Eucalyptus gomphocephala), garlic (Allium sativum), oleander (Nerium aleander), olive tree (Olea europaea), retama (Retama raetum), and weeping fig (Ficus nitida). The obtained results indicated that both leaf extracts of olive and eucalyptus were the most effective ones of the tested group. Olive extract was the most superior and recorded 100% of mycelial inhibition and 89.1% of sporulation inhibition while eucalyptus recorded 28% mycelial inhibition and inhibited the sporulation with 74%. Butter leaves gave the similar effect of eucalyptus in respect of mycelial inhibition, but in contrary recorded the highest sporulation level, but the other extracts were showed variable results.

Greenhouse experiment was undertaken to confirm the efficacy of the two selected extracts (olive and eucalyptus) and compared them to two locally common fungicides viz, Benomyl and Hemexazol. The results showed that Eucalyptus treatment at 2% was the best obtained one.

Literature cited

- Abou-Jawdah, Y., Sobh, H.,and Salameh, A. (2002). Antimycotic activities of selected plant flora, growing wild in Lebanon, against phytopathogenic fungi. J. Agric. Food chem.; 50: 3208-3213.
- Agrios, G. N.(1997). Plant pathology. 4th edition. Academic Press, New York.
- Babadoust, M., and Johnston M. R. (1998). Sporulation of *Drechslera graminea* on barley straw extract agar. Mycologia: 90(1): 63-68.
- Eksteen, D., Pretorius, J.C., Nieuwoudt, T.D., and Zietsman, P. C. (2001). Mycelial growth inhibition of plant pathogenic fungi by extracts of South African plant species. Ann. Appl. Biol.139:243-249.
- Anjum,T., Bajwa, R., and Shafique, S. (2006). Evaluation of antifungal activity of Cicer arietinum L. fourth world congress on allelopathy. 3-6 March 2006, Beechworth, Victoria.
- Ankri, S. and Mirelman, D., (1999). Antimicrobial properties of allicin from garlic. Microbes and Infection. 1(2): 125-129 (abstract)
 Babadoost, M., and Johnston, M. R. (1998). Sporulation of Drechslera graminea on barley straw extract agar. Mycologia: Vol. 90 (1): 63-68.

- Babu, S., Seetharaman, K., Nandakumar, R. and Johnson, I. (2000). Fungitoxic properties of some plant extracts against Alternaria solani, the tomato leaf blight pathogen. Journal-of-Ecotoxicology-and-Environmental-Monitoring.10(2):157-159
- Bharathimatha, C., Doraiswamy S.and Velazhahan, R.,(2002). Inhibition of fungal plant pathogens by seed proteins of Harpullia cupanioides (Roxb.). Acta Phytopathologica and Entomologica-Hungarica.; 37(1/3): 75-82.
- Blok, W. J., Lamers, J. G., Termorshuizen, A. J., and Bollen, G. J., (2000). Control of soil borne plant pathogens by incorporating fresh organic amendments followed by tarping. Phytopathology 90:253-259.
- Blum, L., and Rodriguez-Kabana, R. (2004). Effect of organic amendments on sclerotial germination, mycelial growth, and Sclerotium rolfsii- induced disease. Fitopathol. Bras.; 29 (1):1-15.
- Booth, C. (1977). The genus Fusarium. CMI, Kew, Surrey.
- Bowers, J. H., and Locke, J. C. (2004). Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of Phytophthora blight in the green house. Plant Dis. 88:11-16.
- Bowers, J. H., and Locke, J. C.(2000). Effect of botanical extractson the population density of Fusarium oxysporum in

soiland control of Fusarium wilt in the greenhouse. Plant Dis. 84:300-305.

- Candole, B. L., and Rothrock, C. S. (1997). Characterization of the suppressiveness of hairy vetch-amended soils to *Thielaviopsis* basicola. Phytopathology87:197-202.
- Carabet, R. E. A., and Buchenauer, H. (2004). Effectiveness of plant extracts of *Paeonia suffruticosa* and *Hedera helix* against diseases caused by *Phytophthora infestans* in tomato and *Pseudoperonospora cubensis* in cucumber. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz.: 111(1): 83-95.
- Cheah, L. H., and Cox, J. K. (1995). Screening of plant extracts for control of powdery mildew in squash._Proceedings of the Forty eighth New-Zealand Plant Protection Conference; New-Zealand, August 8-10: 340-342.
- Ekreem, M. (2002). Effect of phytoextracts on growth and sclerotia production of of *Sclerotinia spp.* causing cottony rot disease. MSc. Thesis Faculty of Agriculture. El-Fatah University. 85 pp (in Arabic)
- Elarabi, K. F. (1985). Distribution and pathogenicity of *Fusarium* oxysporum isolates on tomato crop in some costal regions of the Libyan Jamahiriya. MSc. Thesis Faculty of Agriculture. El-Fatah University. 55 pp.

- El-Refai, I. M. and Moustafa S. M. (2004). Allelopathic effect of some cruciferous seeds on *Rhizoctonia solani* kuhn and *Gossypium barbadense* L. Pakistan Journal of Biological Sciences; 7(4): 550-558.
- Erwin, D. C.(1981).Life cycle and epidemiology of *Fusarium* oxysporum, P.51-80. In: M. E. Mace, C. H. Beckman, and A. A. Bell, (ed). Fungal wilt diseases of plants. Academic Press, Inc., New York.
- Ferreira, J. C., Cardoso, M., de Souza, P.E., Miranda, J. C., and da Silva Barreto, S.(2005). Inhibitory effect of *Caesalpinia spinosa* leaflets crude extract on *Fusarium solani*_and *Phoma tarda*. Acta. Sci. Biol. Sci. 27:185-188.
- Fry, W. E. (1982). Principels of plant disease management. Academic Press, Inc. New York.
- Gilani, S. S., Chaghtai, S. M., and Khan, U. (2003). Phytotoxicity of *Eucalyptus microtheca* F. Muell. on *Pennisetum glaucum* cv. BARI-Hairy. Pakistan Journal of Forestry. 53(1): 87-97.
- Green, M. B.,et al. (1997). Chemicals for crop protection and pest control. Peramon Press, Oxford.
- Horst, R. K.(1971). Westcott's plant disease handbook. 4th edition. Van Nostrand Reinhold Co., New York
- http://www.daff.gov.au/planthealth.

- http\\ www. mdidea. com\ products\ proper\ proper062. html# title.
- JaChoon K., SoYoung L., HyunJin C., YongHwa C., JaeSu C., Kawabata S., Miyagi, M., Tsunasawa, S., KwonSoo H., DongWon B., ChangDeok H., Boki.uel L., and MooJe C. (1998). Two hevein homologs isolated from the seed of Pharbitis nil L. exhibit potent antifungal activity. Protein Structure and Molecular Enzymology.1382(1): 80-90.
- Joshi, R. K., Prasad, D., Nautiyal, R., Pant, G., Rawat, M. S. M. (1998). Phytotoxicity of Cassia fistula on *Triticum aestivum* seedlings.: Journal of Hill Research. 11(1): 43-48.
- Ke-Qiang C., and van Bruggen A. H. (2001). Inhibitory efficacy of several plant extracts and plant products on *Phytophthora infestans*. Journal of Agricultural University of Hebei.
- Kishore, G. K., Pande, S., and Rao, J. N. (2001). Control of late leaf spot of groundnut (*Arachis hypogaea*) by extracts from non-host plant species. Plant-Pathology-Journal. 2001; 17(5): 264-270
- Kumran, R. S., Gomathi, V., and Kannabiran, B. (2003). Fungitoxic effects of root extracts of certain plant species on *Colletotrichum capsici* causing anthracnose in *Capsicum annum*. Indian Phytopath. 56 (1): 114-116.
- Kurucheve, V., Ezhilan, J. G., and Jayaraj, J. (1997). Screening of higher plants for fungitoxicity against *Rhizoctonia solani* in vitro. Indian Phytopath. 50 (2): 235-241.

- Lazarovits, G. (2001). Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. Can. J. Plant Pathol. 23;1-7.
- Leandro, L. F. S., Gleason, M. L., Nutter, F. W., Jr., Wegulo, S. N., and Dixon, P. M. (2003). Strawberry plant extracts stimulate secondary conidiation by *Colletotrichum acutatum* on symptomless leaves. Phytopathology 93:1285-1291.
- Mahmoud, Y. A. G., Ebrahim, M. K. H., and Aly, M. M. (2004). Influence of some plant extracts and microbioagents on some physiological traits of faba bean infected with Botrytis fabae. Turk. J. Bot. 28: 519 528.
- Mert-Turk, F. (2006). Saponins versus plant fungal pathogens. Journal of Cell and Molecular Biology 5: 13-17.
- Nelson, P.(1981).Life cycle and epidemiology of *Fusarium oxysporum*, P.51-80. In: M. E. Mace, C. H. Beckman, and A. A. Bell, (ed). Fungal wilt diseases of plants. Academic Press, Inc., New York.
- Nwachukwu, E. O., and Umechuruba, C. I. (2001). Antifungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. J. Appl. Sci. Environ. Mgt. 5 (1),29-32.
- Okigbo, R. N., and Ogbonnaya, U. O. (2006). Antifungal effects of two tropical plant leaf extracts (Ocimum gratissimum and

Aframomum melegueta) on postharvest yam (Dioscorea spp.) rot.African Journal Biotechnology.5 (9): 721-731.

- Ouf, S. A., Hady, F. K. A., El-Gamal, M. H., and Shaker, K. H. (1994). Isolation of antifungal compounds from some *Zygophyllum* species and their bioassay against two soil-borne plant pathogens. Folia-Microbiologica.; 39(3): 215-221.
- Ouf, S. A., Sharaf, E. F., and Sumner, D. R. (1999). Interaction of pine needle soil amendment with microbial antagonists on pathogenicity and some metabolic activities of *Rhizoctonia solani*. Acta Microbiologica Polonica.48(2): 173-183.
- Padmodaya, B., and Reddy, H. R.(1999). Effect of organic amendments on seedling disease of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. J. Mycol. Pl. Pathol. 29(1):38-41.
- Pretorius, J. C., Zietsman, P. C., and Eksteen, D. (2002). Fungitoxic properties of selected south African plant species against plant pathogens of economic importance in agriculture. Ann. Appl. Biol. 141; 117-124,
- Qasem, J. R.(1996). fungicidal activity of Ranunculus asiaticus and other weeds against Fusarium oxysporum f. sp. lycopersici.
 Ann. Appl. Biol. 128: 533-540.
- Ramezani, H., Singh, H. P., Batish, D. R., Kohli, R. K., and Dargan, J. S. (2002). Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronellal. New Zealand Plant Protection . 55 : 327-330.

- Sathyanarayana, S. L., Kumar, T. V., and Shetty, H. S. (2006). A putative organic treatment against *Alternaria padwickii* infection in paddy. Integrative Biosciences 10: 21-25.
- Srivastava, A. K., and Lal, B.(1997). Studies on biofungicidal properties of leaf extract of some plants. Indian Phytopath.50(3):408-411.
- Tenuta, M., and Lazarovits, G. (2002). Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. Phytopathology. 92:255-264.
- Tuite, J.(1969). Plant pathological methods- fungi and bacteria.
 Burgess Publ. Co., Minneapolis, Minnesota.
- Urban, L., and Filipowicz, A. (2004). pathogencity test of *Fusarium oxysporum* isolates originated from tomato. *Agronomus vestis* (Latvian Journal of Agronomy), (7), 161-164.
- Walker, J. C.(1969). Plant pathology. McGraw-Hill, New York.
- Wurms, K., Labbe, C., Benhamou, N., and Belanger, R. R.(1999). Effects of Milsana and benzothiadiazole on the ultrastructure of powdery mildew haustoria on cucumber. Phytopathology. 89(9): 728-736.
- Wyss, G. S., Charudattan, R., and DeValerio, J. T. (2001). Evaluation of agar and grain media for mass production of conidia of *Dactylaria higginsii*. Plant Dis. 85:1165-1170.

- Yoshida, S., and Shirata, A. (2004). Biotin Induces Sporulation of Mulberry Anthracnose Fungus. Journal of General Plant Pathology. 66: 117-122.
- Zacchino, S., Santecchia, Lopez, C., Gattuso, S., Munoz, J., Cruanes, A., Vivot, E., Cruanes, M., Salinas, A., Ruiz, R., and Ruiz, S.(1998). In vitro antifungal evaluation and studies on mode of action of eight selected species from the Argentine flora. Phytomedicine. 5(5): 389-395.
- Zhou, X. G., and Events, K. L. (2004). Suppression of *Fusarium* wilt of watermelon by soil amendment with hairy vetch. Plant Dis. 88: 1357- 1365.
- Zidan, Z. H., El-Hammady, A. A., Abdel-All, S. M., and Aly, M. M. (2000). Preliminary screening, separation and identified of photochemical constituents of Eucalyptus plant extract. Annals of Agricultural Science, Cairo.: 45(2): 757-767.

الخلاصة

أجريت هذه الدراسة لاختبار تأثير المستخلصات المائية لاوراق تسعة من الانواع النباتية والتي تنتمي الى عائلات نباتية مختلفة على مرض الذبول الفيوزارمي في باذرات نبات الطماطم النامية تحث الاغطية المتسبب عن الفطر .lycopersici.

أجرى إختبار المرضية لسبعة من العزلات جمعت من مناطق مختلفة شملت مناطق القربولى و الوجلة والزاوية و مصراته وسرت و طرابلس و جالو وعلى تسعة اصناف شائعة الزراعة محليا وهى الجود وبشرى وكال جى وقالكاتو وقور مولا وحليم وميدى إى وريوقراندى ،وقد اظهرت النتائج أن العزلات كانت على مستوى عام متوسط من الشدة المرضية الا أن عزلة سرت كانت قد تقوقت فى شدة المرضية على بقية المجموعة كما اظهر الصنف ربوقراندى اعلى مستوى للقابلية بالإصابة .

وفى التجربة المعملية والتي كانت تهدف الى تحديد اقوى مستخلصين لتثبيط نمو الميسليم والتجربة المعملية والتي كانت تهدف الى تحديد اقوى مستخلصين لتثبيط نمو الميسليم والتجربة لمستخلصات اوراق نباتات كلا من : الزيتون Petama raetum واليوكالبتس والدفل Eucalyptus gomphocephala والرئم Retama raetum والنفل Nerium oleander والقطت Ficus nitida والأثل والثوم Allium sativum والاثل والأثل Tamrix articulata وقد سجل مستخلص أوراق الزيتون تثبيطا كاملا لهذا النمو كما سجل تثبيطا للتجرثم قدره 9.18%، اما مستخلص اوراق اليوكالبتس فقد ادى الى تثبيط التجرثم حيث ثبط مانسبته 74%، بينما كانت نسبة تثبيطه لنمو الميسيليم 28%، ، اما مستخلص اوراق القطف فانه أعطى نفس مستوى الفاعلية لمستخلص

اوراق اليوكالبنس غير انه أدى الى تحفيز التجرثم والذى وصل الى اعلى معدلاته عند التركيز 0.01 وضوعف عنده التجرثم الى 21 ضعف ما انتجه الشاهد، اما بقية المستخلصات فقد سجلت نتائج متفاوتة.

وأخيرا أجريت التجربة الحقلية وذلك لتأكيد فاعلية مستخلصى الزيتون واليوكالبنس حقليا مع مقارنة فاعليتهما بمبيدي البونوميل والهيمكسازول الشانعي الاستعمال محليا وقد أظهرت النتائج تغوق مستخلص أوراق اليوكالبنس عند التركيز 2%.

Appendices

Title: Pathoscale

Function: FACTOR

Experiment Model Number 9: Randomized Complete Block Design for Factor A, with Factor B a Split Plot on A Data case no. 1 to 189.

Factorial ANOVA for the factors:

Replication (Var 1: REPLICATION) with values from 1 to 3

Factor A (Var 3: var) with values from 1 to 9 Factor B (Var 4: isos) with values from 1 to 7

Variable 5: pathoscale1

Grand Mean = 2.041 Grand Sum = 385.659 Total Count = 189

TABLE OF MEANS

1 3 4	5	Total
1 * * 2 * *	1.810 2.127	113.999 133.997
3 * *	2.185	137.663
* 1 *	1.905	40.000
* 2 *	2.095	43.999
* 3 *	1.841	38.665
* 4 *	1.873	39.334
* 5 *	2.111	44.332
* 6 *	2.175	45.665
* 7 *	1.778	37.332
* 8 *	2.365	49.666
* 9 *	2.222	46.666
* * 1	2.457	66.332
* * 2	2.222	59.9 99
* * 3	2.259	61.000
* * 4	2.407	64.998
* * 5	2.457	66.330
* * 6	2.395	64.667
* * 7	0.086	2.333
* 1 1	2.445	7.334
* 1 2	2,111	6.333
* 1 3	2.111	6.334
* 1 4	2.222	6.666

*	1	5	2.444	7.333
*	i	6	2.000	6.000
*				
	1	7	0.000	0.000
*	2	1 2	2.555	7.666
*	2	2	2.111	6.333
*	2	3	2.333	7.000
*		4	2.667	8.000
*	2	5	2.444	7.333
*	2 2 2	6	2.556	7.667
*				
	2	7	0.000	0.000
*	3	1	2.111	6.333
•	3	2	2.000	6.000
*	3	3	2.000	6.000
*	3	4	2.556	7.667
*	3	5	2.333	6.999
*	3	6	1.889	5.666
*	3	7	0.000	0.000
*				
	4	1	1.889	5.667
*	4	2	2.333	7.000
*	4	3	2.000	6.000
*	4	4	2.111	6.333
*	4	5	2.333	7.000
*	4	6	2,445	7.334
*	4	7	0.000	0.000
*	5	1	2.667	8.000
*	5	2		
*			2.444	7.333
*	5	3	2.667	8.000
	5	4	2.333	6.999
*	5	5	2.667	8.000
*	5	6	2.000	6.000
*	5	7	0.000	0.000
*	6	1	2.555	7.666
*	6	2	2.444	7.333
*	6	3	2.667	8.000
*		4		8.333
*	6		2.778	
	6	5	2.444	7.333
*	6	6	2.333	7.000
*	6	7	0.000	0.000
•	7	1	2.333	7.000
*	7	2	2.111	6.333
*	7	3	1.444	4.333
•	7	4	2.000	6.000
*	7	5	2.111	6.333
*	7	6	2.444	7.333
*	7	7	0.000	0.000
*				
	8	1	2.889	8.666
*	8	2	2.556	7.667
*	8	3	2.444	7.333
*	8	4	3.000	9.000
•	8	5	2.778	8.333

*	8	6	2.889	8.667
•	8	7	0.000	0.000
*	9	i	2.667	8.000
*	9	2	1.889	5.667
*	9	3	2.667	8.000
*	9	4	2.000	6.000
•	9	5	2.555	7.666
*	9	6	3.000	9.000
*	9	7	0.778	2.333

ANALYSIS OF VARIANCE TABLE

K Valu	ie Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
]	Replication	2	5.150	2.575	12,0039 0	.0007
2	Factor A	8	6.710	0.839	3.9100 0.0	0098
-3	Error	16	3.432	0.215		
4	Factor B	6	121.664	20.277	85.6556	0000.0
6	AB	48	10.163	0.212	0.8944	
-7	Error	108	25,567	0.237		
	Total	188	172.686			

Coefficient of Variation: 23.84%

s_ for means group 1: y	0.0584	Number of Observations: 63
s_ for means group 2: y	0.1011	Number of Observations: 21
s_ for means group 4: y	0.0936	Number of Observations: 27
s_ for means group 6: y	0.2809	Number of Observations: 3

Case Range: 206 - 212 Variable 5: pathoscale1 Function: RANGEI

Error Mean Square = 0.2370 Error Degrees of Freedom = 108

No. of observations to calculate a mean = 27

Least Significant Difference Test LSD value = 0.2626 at alpha = 0.050

Original Order Ranked Order

Mean 1 = 2.457 A Mean 1 = 2.457 A

Mean Mean 2 = 2.222 A 5 = 2.457 A 3 = 2.259 AMean Mean 4 = 2.407 A2.407 A Mean 6 = 2.395 AMean 4 = Mean 5 = 2.457 AMean 3 = 2.259 A2.395 A Mean 2 = 2.222 AMean 6 =Mean 7 = 0.08641 B Mean 7 = 0.08641 B

Case Range: 195 - 203 Variable 5: pathoscale1 Function: RANGE

Error Mean Square = 0.2150 Error Degrees of Freedom = 16 No. of observations to calculate a mean = 21

Least Significant Difference Test LSD value = 0.3033 at alpha = 0.050

Original Order Ranked Order

Mean 1 = 1.905 BCD Mean 8 = 2.365 AMean 2 = 2.095 ABC Mean 9 = 2.222 A Mean 3 = 1.84) CD Mean 6 = 2.175 ABMean 5 = 2.111 ABCMean 4 =1.873 BCD Mean 5 = 2.111 ABC Mean 2 = 2.095 ABC Mean 6 =2.175 AB Mean I = 1.905 BCD Mean 7 =1.778 D Mean 4 = 1.873 BCD Mean 8 = 2.365 A Mean 3 = 1.841CD Mean 9 = 2.222 AMean 7 = 1.778 D

Title: sporulation/2500

Function: FACTOR

Experiment Model Number 9:

Randomized Complete Block Design for Factor A, with

Factor B a Split Plot on A

Data case no. 1 to 120.

Factorial ANOVA for the factors:

Replication (Var 1: rep) with values from 1 to 4 Factor A (Var 2: ext) with values from 1 to 10 Factor B (Var 3: conc) with values from 1 to 3

Variable 6: csum

Grand Mean = 95.974 Grand Sum = 11516.880 Total Count = 120

TABLE OF MEANS

1 2 3	6	Total
1 * *	125.247	3757.400
2 * *	84.237	2527.120
3 * *	93.313	2799.400
4 * *	81.099	2432.960
* 1 *	169.077	2028.920
* 2 *	123.547	1482.560
* 3 *	90.633	1087.600
* 4 *	110.580	1326.960
* 5 *	388.147	4657.760
* 6 *	27.840	334.080
* 7 *	12.333	148.000
* 8 *	3.000	36.000
* 9 *	7.083	85.000
* 10 *	27.500	330.000
* * 1	64.375	2575.000
* * 2	116.658	4666.320
* * 3	106.889	4275.560
* 1 1	88.790	355.160
* 1 2	234.190	936.760
* 1 3	184.250	737.000
* 2 1	128.190	512.760
* 2 2	100.450	401.800
* 2 3	142.000	568.000
* 3 1	106.520	426.080
* 3 2	55.190	220.760

* 3 3	110.190	440.760
* 4 1	110.250	441.000
* 4 2	109.500	438.000
* 4 3	111.990	447.960
* 5 1	148.750	595.000
* 5 2	589.500	2358,000
* 5 3	426.190	1704.760
* 6 1	28.000	112.000
* 6 2	23.000	92.000
* 6 3	32.520	130.080
* 7 1	6.750	27.000
* 7 2	14.250	57.000
* 7 3	16.000	64.000
* 8 1	1.500	6.000
* 8 2	5.000	20.000
* 8 3	2.500	10.000
* 9 1	3.000	12.000
* 9 2	7.000	28.000
* 9 3	11.250	45.000
* 10 1	22,000	88.000
* 10 2	28.500	114.000
* 10 3	32,000	128,000

ANALYSIS OF VARIANCE TABLE

K Valı	ue Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	3	36689.791	12229,930	3.0595	0.0452
2	Factor A	9	1495000.613	166111.179	41.5557	0.0000
-3	Error	27	107927.531	3997.316		
4	Factor B	2	61818.475	30909,237	12.4153	0.0000
6	AB	18	390952.408	21719.578	8.7241	0.0000
-7	Error	60	149376.546	2489.609		
	Total	119	2241765,363	3		

Coefficient of Variation: 51.99%

s_ for means group 1: 11.5431 Number of Observations: 30 y

s_ for means group 2: 18.2513 y Number of Observations: 12

```
Number of Observations: 40
                       7.8892
s_ for means group 4:
                      24.9480
                                  Number of Observations: 4
s_ for means group 6:
```

Ranked Order

Effect of extract type on sporulation

```
Case Range: 188 - 197
Variable 6 : esum
Function RANGEI
Error Mean Square = 2490.
Error Degrees of Freedom = 60
No. of observations to calculate a mean = 12
Least Significant Difference Test
LSD value = 40.75
                     at alpha = 0.050
```

Original Order

Mean] = 169.1 B Mean 5 = 388.1 A Mean 2 =123.5 C Mean 1 = 169.1 BMean 3 =90.63 C Mean 2 =123.5 \mathbf{C} Mean 4 = 110.6 C Mean 4 = 110.6 \mathbf{C} Mean 5 = 388.1 AMean 3 = 90.63Mean 6 = 27.84 D Mean 6 = 27.84D Mean 7 = 12.33D Mean 10 = 27.50D D Mean 7 = 12.33Mean 8 = 3.000D

Mean 9 = 7.083D Mean 9 = 7.083D

Mean 10 = 27.50D Mean 8 = 3.000D

Effect of extract type on the radial growth

Case Range: 129 - 138 Variable 4: diam Function RANGE Error Mean Square = 0.1190Error Degrees of Freedom = 60No. of observations to calculate a mean = 12Least Significant Difference Test LSD value = 0.2817 at alpha = 0.050

0 Original Order Ranked Order

Original Order

Ranked Order

Mean	1 =	7.583	C	Mean	10 =	8.250	Α
Mean	2 =	7.042	Ε	Mean	4 =	7.875	В
Mean	3 =	7.167	DE	Mean	1 =	7.583	С
Mean	4 =	7.875	В	Mean	6=	7.333	CD
Mean	5 =	5.958	F	Mean	3 =	7.167	DE
Mean	6 =	7.333	CD	Mean	2 =	7.042	Ε
Mean	7 =	4.125	G	Mean	5 =	5.958	F
Mean	8 =	0.0000	Н	Mean	9 =	5.917	F
Mean	9 =	5.917	F	Mean	7 =	4.125	G
Mean	10 =	8.250	Α	Mean	8 =	0.0000	Н

Effect of extract concentration on the radial growth

Case Range: 141 - 143 Variable 4: diam Function RANGEI

Error Mean Square = 0.1190 Error Degrees of Freedom = 60

No. of observations to calculate a mean = 40

Least Significant Difference Test

LSD value = 0.1543 at alpha = 0.050

Original Order

Ranked Order

Mean	1 =	5.713	C	Mean	3 =	6.525	Α
Mean	2 =	6.137	В	Меап	2 =	6.137	В
Mean	3 =	6.525	Λ	Меап	1 =	5.713	C

Effect of extract concentration on the sporulation

Case Range : 200 - 202

Variable 6 : csum Function RANGEI

Error Mean Square = 2490.

Error Degrees of Freedom = 60

No. of observations to calculate a mean = 40

Least Significant Difference Test

LSD value = 22.32 at alpha = 0.050

Original Order

Ranked Order

Mean 1 = 64.38 B Mean 2 = 116.7 A Mean 2 = 116.7 A Mean 3 = 106.9 A Mean 3 = 106.9 A Mean 1 = 64.38 B

The effect of the interaction of extract type with extract concentration on the radial growth

Case Range: 146 - 175
Variable 4: diam
Function RANGED
Error Mean Square = 0.1190
Error Degrees of Freedom = 60
No. of observations to calculate a mean = 4
Least Significant Difference Test
LSD value = 0.4879 at alpha = 0.050

Original Order

Ranked Order

Mean	=	7,875 AB	С	Mean	30 =	8.250	Α
Mean	2=	7.375 D	EF	Mean	28 =	8.250	A
Mean	3 =	7.500 CI	DΕ	Mean	11 =	8.250	A
Mean	4 =	6,500	IJ	Mean	29 =	8.250	Α
Mean	5 =	6.625	HIJ	Mean	6 =	8.000	AΒ
Mean	6 =	8.000 AB		Mean] =	7.875	ABC
Mean	7 =	7.250	FG	Mean	12 =	7.750	BCD
Mean	8 =	7.000 I	FGH .	Mean	18 =	7.625	BCDE
Меап	9 =	7.250 E	FG	Mean	10 =	7.625	BCDE
Mean	10 =	7.625 BC	CDE	Mean	17=	7.500	CDE
Mean	11 =	8.250 A		Mean	3 =	7.500	CDE
Mean	12 =	7.750 BC	CD	Mean	2 =	7.375	DEF
Mean	13 =	5.500	L	Mean	9 =	7.250	EFG
Mean	14 =	6.000	K	Mean	7 =	7.250	EFG
Mean	15 =	6.375	JK	Mean	8 =	7.000	FGH
Mean	16 🛎	6.875	GHI	Mean	16=	6.875	GHI
Mean	17=	7.500 C	DE	Mean	5 =	6.625	HII
Mean	18 =	7.625 BC	CDE	Mean	4 =	6.500	11
Mean	19 =	2.375	О	Mean	27 =	6.500	IJ
Mean	20 =	4.000	N	Mean	26=	6.375	JK
Mean	21 =	6.000	K	Mean	15 =	6.375	JK
Mean	22 =	0.0000	P	Mean	21 =	6.000	K
Mean	23 =	0.0000	P	Mean	14=	6.000	K
Mean	24 =	0.0000	P	Mean	13 =	5.500	L
Mean	25 =	4.875	M	Mean	25 =	4.875	M
Mean	26 =	6.375	JK	Mean	20 =	4.000	N
Mean	27 =	6.500	IJ		19=	2.375	0
Mean	28 =	8.250 A		Mean	24 =	0.0000	P
Mean	29 ≖	8,250 A		Mean	22 =	0.0000	P
Mean	30 -	8.250 A		Mean	23 =	0.0000	P

The effect of the interaction of extract type with extract concentration on the sporulation

Case Range: 205 - 234 Variable 6: csum Function RANGEI

Error Mean Square = 2490. Error Degrees of Freedom = 60

No. of observations to calculate a mean = 4

Least Significant Difference Test

LSD value = 70.57 at alpha = 0.050

Original Order Ranked Order

Mean] =	88.79	EFGH	Mean 14 = 589.5 A
Mean	2=	234.2	C	Mean $15 = 426.2 B$
Mean	3 =	184.3	CD	Mean 2 = 234.2 C
Mean	4 =	128.2	ÐE	Mean $3 = 184.3$ CD
Mean	5 =	100.4	EFG	Mean $13 = 148.8$ DE
Mean	6=	142.0	ÐE	Mean $6 = 142.0$ DE
Mean	7 =	106.5	EF	Mean $4 = 128.2$ DE
Mean	8 =	55.19	FGHI	Mean 12 = 112.0 EF
Mean	9=	110.2	EF	Mean 10 = 110.3 EF
Меап	10 =	110.3	EF	Mean 9 = 110.2 EF
Mean	11 =	109.5	EF	Mean $11 = 109.5$ EF
Mean	12 =	112.0	EF	Mean $7 = 106.5$ EF
Mean	13 =	148.8	DE	Mean $5 = 100.4$ EFG
Mean	14 =	589.5	Α	Mean 1 = 88.79 EFGH
Mean	15 =	426.2	В	Mean 8 = 55.19 FGH
Mean	16=	28.00	Hl	Mean 18 = 32.52 GHI
Mean	17=	23.00	Hl	Mean $30 = 32.00$ GHI
Mean	18 =	32.52	GHI	Mean $29 = 28.50$ HI
Mean	19=	6.750	l	Mean $16 = 28.00$ HI
Mean	20 =	14.25	I	Mean $17 = 23.00$ HI
Mean	21 =	16.00	I	Mean $28 = 22.00$ HI
Mean	22 =	1.500	I	Mean $21 = 16.00$ 1
Mean	23 =	5.000	1	Mean $20 = 14.25$ 1
Mean	24 =	2.500	1	Mean $27 = 11.25$ 1
Mean	25 =	3.000	1	Mean $26 = 7.000$ 1
Mean	26 =	7.000	I	Mean $19 = 6.750$ 1
Mean	27 =	11.25	1	Mean $23 = 5.000$!
Mean	28 =	22.00	HI	Mean $25 = 3.000$
Mean	29 =	28.50	HI	Mean $24 = 2.500$ I
Mean.	30 = 32	.00	GHI	Mean $22 = 1.500$

Title: field experiment

Function: FACTOR

Experiment Model Number 10:

Three Factor Randomized Complete Block Design

Data case no. 1 to 72.

Factorial ANOVA for the factors:

Replication (Var 1: REPLICATION) with values from 1to 3

Factor A (Var 3: extrac) with values from 1 to 4 Factor B (Var 4: conc) with values from 1 to 3 Factor C (Var 5: doses) with values from 1 to 2

Variable 2: pathoscale

Grand Mean = 2.625 Grand Sum = 189.000 Total Count = 72

TABLE OF MEANS

1	3	4	5	2	Total
1	•	•		2.667	64.000
2 3	+	•	4	2.625	63.000
3	*	*	*	2.583	62.000

*	1	*	*	2.667	48.000
ŧ	2	•	*	2.111	38.000
+	3	•	*	2.889	52.000
*	4	+	*	2.833	51.000
•	•	1	*	2.750	66.000
•	•	1 2	•	2.208	53.000
•	+	3	+	2.917	70.000
•	1	1	•	2.500	15.000
*	1	2	*	1.833	11.000
*	1	3 1	*	3.667	22.000
*	2	1	*	2.500	15.000
*	2	2	*	1.500	9.000
*	2	3	*	2.333	14.000
*	3	1	*	2.833	17.000
*	3	2	*	2.667	16.000
4	3	3	4	3.167	19.000
+	4	1	*	3.167	19.000
*	4	2	*	2.833	17.000
	4	3	*	2.500	15.000

	*	+	+	1	2.472	89.000
	+	*	٨	2	2.778	100.000
-						
	*	1		1	2.667	24.000
		i		2	2.667	24.000
		2	*	1	2.000	18.000
	*	2	4	2	2.222	20.000
	*	3	*	1	2.556	23.000
	*	3	+	2	3.222	29.000
	٠	4	*	1	2.667	24.000
	٠	4	•	2	3.000	27.000
-					·	
_			1	1	2.750	33.000
			1	2	2.750	33.000
	4		2	1	1.917	23.000
	*	+	2	2	2.500	30.000
	*	*	3	1	2.750	33,000
	*	*	3	2	3.083	37.000
_	*	1	1	1	3.333	10.000
	*	1	1	2	1.667	5.000
	*	1	2	1	1.333	4.000
	*	1	2	2	2.333	7.000
	*	ì	3	1	3,333	10.000
	*	1	3	2	4.000	12.000
	*	2	1	1	2.333	7.000
	*	2	l	2	2.667	8.000
	*	2	2	1	1.333	4.000
	٠	2	2	2	1.667	5.000
	•	2	3	1	2.333	7.000
	*	2	3 1 2 2 2	2	2.333	7.000
	T	3	ī	1	2.333	7.000
	T	3	ī	2	3.333	10.000
	•	3	2	1	2.333	7.000
	•	3		2	3.000	9.000
	•	3	3	1	3.000	9.000
	*	3	3	2 1	3.333	10.000
	•	4	1	1	3.000	9.000
	•	4	1	2	3.333	10.000
	•	4	2	1 2	2.667	8.000
	*	4	2	2	3.000	9.000
	*	4	3	i	2.333	7.000
		4	3	2	2.667	8.000

ANALYSIS OF VARIANCE TABLE

K Value Prob	Source			Mean Square	
					
	-				
1	Replication	2	0.083	0.042	
0.0524	-				
2	Factor A	3	6.819	2.273	
2.8583	0.0471				
4	Factor B	2	6.583	3.292	
4.1390	0.0222				
	AB	6	9.306	1.551	
	0.0927				
	Factor C	1	1.681	1.681	
	0.1528	_			
10		3	1.042	0.347	
0.4366		_			
12		2	1.028	0.514	
0.6462		_		0.050	
14		6	5.750	0.958	
	0.3209		36 503	A 30F	
-15	Error	40	36.583	0.795	
	Total	71	68.875		
					

S_ for means group 1: 0.1820 Number of Observations: 24

y

s_ for means group 2: 0.2102 Number of Observations: 18

y

s_ for means group 4: 0.1820 Number of Observations: 24

y

s_ for means group 4: 0.1820 Number of Observations: 24

y

s_ for means group 6: 0.3641 Number of Observations: 6

y

s_ for means group 8: 0.1486 Number of Observations: 36

s_ for means group 12: 0.2574 Number of Observations: 12

У

جامعة التحدي كلية الزراعة قسم الإنتاج النباتي

تأثير بعض المستخلصات النباتية المانية على مرض الذبول الفيوزارمي في بادرات الطماطم

مقدمة من: سالم أحمد محمد صافار 2007/7/26

	أعضاء اللجنة
	د.صلاح سعید العماری (مشرفا)
داختیا) ۴۰۰۰ (کیسید	د. عبد الناصر عبد العال جلال (ممتحناً
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	يعتمد
د. علطف سيد شرحاته	د. مديد الدراوي العانب
معدد الدراسات العليا بالعلية	أمين اللجنة الشعبية لكلية الزراعة
61,117	

جامعة التحدي كليعة الرزاعية قسم الإنتاج النباتي

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مقدمة من:

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إشراف:

ا.د. صلاح سعید العماری

قدمت هذه الأطروحة استكمالا لمتطلبات الإجازة العالية (الماجستير) في أمراض النبات