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Isolation and Identification of Mycotoxins Produced by Fusarium spp. and Alternaria solani

Zeinab H. Kheiralla, Mona I. Fahd and Amany A. Youssry
Botany Department, Women's College,
Ain Shams University, Cairo, Egypt

Abstract:

Fusarium oxysporum f. sp. lycopersici, Fusarium solani and Alternaria solani were isolated from tomato and pepper seeds and diseased plants. Fusarium spp. and Alternaria solani were grown on artificial media produced toxic substances which increased in concentration with the age of culture. These toxic substances inhibited growth of radicles or (hypocotyl, cotyledonary leaf and plumules) when seeds were germinated in the filtrate of the fungus. Inhibitory effect of extract appeared to be due primarily to phytotoxicity.

Studies were extended to test the biological activity of fungal toxins. Toxins produced by Fusarium spp. and Alternaria solani, at different temperatures, pHs, carbon and nitrogen sources, inhibited growth of Bacillus megaterium and Bacillus mycoides which differed in its toxicity to bacterial growth.

Fusarium spp. secreted zearalenone and trichothecenes in liquid media. Maximum amounts of zearalenone and trichothecenes were produced by Fusarium oxysporum and Fusarium solani at 25-28°C and pH 5.

Growing Fusarium oxysporum on different carbon sources, amounts of zearalenone were in the order of xylose > sucrose > glucose > maltose > lactose > mannose. While, trichothecene amounts were in the order of glucose > maltose > sucrose > xylose > lactose > mannose. In case of F. solani the efficiency for toxin production differ from F. oxysporum using the same carbon sources.

Ammonium phosphate gave high levels of zearalenone and trichothecene (24.73 and 92.35 μg/ml). In case of F. solani, zearalenone was high in presence of casein (319 μg/ml). On the other hand, ammonium molybdate gave a high concentrations of trichothecene.

Introduction:

The genus Fusarium comprised large complex group of fungi with ascomycete teleomorphs and contains numerous species that produce noxious secondary metabolites and/or cause serious plant diseases (Nelson el al., 1983 and Marasas el al., 1984). Members of the genus have wide geographic and host ranges. Several species of Fusarium are associated with stalk rots (El-Meleigi el al., 1983 and Gilbertson el al., 1985), leaf spots (Schieber and Muller, 1968) and Fusarium wilt.

Several investigators have reported the natural occurrence of Fusarium mycotoxins, including Deoxynivalenol (DON), Nivalenol (NIV), Zearalenone (ZEA, Zearalenol, T-2 and Trichothecenes, secondary metabolites of several Fusarium species, as one of the causative agents of toxification of plants (Ueno el al., 1986; Lu el al., 1988; Luo, 1988 and Plattner el al., 1989). In addition, some kinds of Fusarium mycotoxins have been suspected to be involved in human chronic mycotoxicoses, such as esophageal cancer and Kashin-Beck disease (Hsia el al., 1983; Sokoloff, 1985; Jone el al., 1987 and Yang, 1989). Ueno el al., (1975) and Bacon el al., (1977) and Krivobok el al., (1987). They developed rapid and sensitive methods for identifying toxin production by toxicogenic strains of Fusarium cultivated in liquid medium.

Nemec el al., (1991) concluded that Fusarium solani (Mart) Sacc. produced at least 11 structural related "naphthazarin" toxins, but did not produce detectable levels of fusaric acid or trichothecenes. In culture naphthazarin toxins may be synthesized in diseased roots.

A metabolic product of Alternaria solani, has been isolated, and identified as alternaric acid and zinniol, which possessed marked phytotoxic. Alternaric acid produced symptoms characteristic of early blight on tomatoes with leaf lesions,chlorosis and necrosis (Brian el al., 1951 and 1952; Pound and Stahmann, 1951 and Maiero el al., 1991).

Siler and Gilchrist, (1983) isolated phytotoxic fractions from extracts of necrotic leaves of tomato plants infected with A. alternata f. sp. lycopersici (AAL); these fractions were indistinguishable with those isolated from the pathogen Alternaria.

Cotty and Misaghi (1984) tested thirty one isolates of 10 pathogenic Alternaria spp., for production of Zinniol, a non selective phytotoxin. Whereas, Clouse el al., (1985), developed simplified rapid procedure for the purification of 2 phytotoxic metabolites (TA, TB) from cell free culture filtrates of the tomato pathogen Alternaria alternata.

The study presented here has directed to investigate toxin production by the three fungi, carbon and nitrogen sources on mycotoxins produced by the investigated fungi.

Material and Methods:

1- Isolation of fungi:

Fusarium oxysporum, Fusarium solani and Alternaria solani were isolated and identified (Kheiralla el al., 1994).

2- Effect of toxins produced by F. oxysporum, F. solani and A. solani on germination of tomato and pepper seeds:

The three isolated fungi were grown on medium containing 200 g glucose, 0.5 g KCl, 0.5 g $MgSO_4$, 1.0 g KH_2PO_4 and 1.0 g yeast extract, and 1000 ml distilled water.

The liquid medium (100 ml) was dispensed in 250 ml Erlenmyer flasks, inoculated with 1 ml fresh prepared spore suspension (10^5-10^6) and incubated for one and two weeks at 28°C for the two Fusarium spp. and at 25°C for Alternaria solani. Fungal filtrates were used instead of water, to germinate tomato seeds (Kastle rock) and pepper seeds (California Wonder). Seeds were placed on surface of sterile filter papers wetted with fungi filtrates in sterile petri-dish. For control treatment, filter paper was wetted with sterile water. Percentages of germination, length of radicle and (hypocotyl, cotyledonary leaf and plumules) were recorded.

3- Standards toxins:

Standards of Zearalenone, Trichothecenes, Alterniol (AOH), Alternuene (ALT) and Alterniol mobomethyl ether (AME)were obtained from Sigma Chemical Co., P.O. BOX 14508, St., Louis, Mo 63178, USA and were used as reference standards.

4- Extraction and toxin analysis:

One ml of spore suspension from cultures of the two Fusarium spp. and Alternaria solani (10^6 /ml) were added to 100 ml of liquid medium in 250 ml Erlenmeyer flasks. The flasks were incubated at different temperatures or fungi grown on medium with different pH, carbon or nitrogen sources. Mycotoxins extraction and quantification were carried out using the method of A.O.A.C. (1984).

The culture filtrate or whole culture was extracted twice in separatory funnel with an equal volume 50 ml of ethyl acetate and the second extraction with 50 ml chloroform. Sodium chloride was added during shaking. The combined extracts were filtered over an hydrous sodium sulphate. The extracts were combined and evaporated to dryness on a rotary evaporator or under nitrogen to a small volume (1-2 ml). Purification of mycotoxins were carried out using SEP-PAC silica cartridge. Water Associates, Inc., Milford, MA, 01757. The final extract was then evaporated and stored at 3-5°C, to be used for microbial assay and chromatographic analysis.

All residues were analyzed by dissolving in 0.2 ml acetone or ethyl acetate or chloroform-acetone (9:1 v/v), spotting on thin-layer chromatography (TLC) sheet 13179 silica gel without a fluorescent indicator, activated at 110°C for 30 min, and development in unlined, unequilibrated tanks containing 100 ml solvent. Zearalenone and trichothecenes were determined on plate according to the method of Gimeno (1979). Initially, the plate was developed with hexane-ethyl ether-acetic acid (70:30:2 v/v/v) subsequently the plate was developed with hexane-ethylacetate (1:3 v/v). Plate was detected under UV light (365 and 254 nm) which appeared as a greenish blue fluorescent spot under shortwave, and confirmed by spraying with a fresh solution of 50% sulphuric acid in methanol and then heated for 10-20 min at 120°C. Zearalenone turns yellow and then brown, trichothecenes gave a blue colour.

Alerniol (AOH), Alter monomethyl ether (AME) and Alternune (ALT) were determined on plate using developing solvent which include chloroform-acetone (88-12 v/v) and toluene-ethyl acetate-formic acid (5:4:1 v/v/v). The compounds were visible as blue spots under long, and short wave UV light, AME and AOH being brighter under the latter. AME and AOH spots remained fluorescent blue after spraying with 50% ethanolic sulphuric acid, but changed to greenish fluorescent-after-spraying and heating for 5 min at 100°C.

The intensity of the mycotoxins were measured with DESAGA CD_{60} fluirodensitometer at an excitation wave length of 365 um and emission wave length of 443 nm. The amount of mycotoxins extraction was the mean of three replicate samples on one TLC plate, and each spot was scanned twice.

5- Toxicity test and Biological assay:

Biological assay for toxicity was carried out using Bacillus megaterium strain 1057 and Bacillus mycoides EMCC 1084 (Ain Shams University, Microbiological Resource Centre, Cairo, Egypt). The organism was grown in 10 ml tryptone yeast glucose broth (TYG) Stott and Bullerman, 1975) for 24 h at 37° and then transferred to saline (0.85% w/v NaCl), diluted to a cell density of approx 10^6 ml. Aliquots 0.1 ml were inoculated on (TYGA) plates, B. megaterium for Fusaria toxins, while B. mycoides for Alternaria toxins. The plates were dried at 30°C for 60 min. To sterile filter-paper discs (5 mm diameter) 25 μl toxin extract was applied, (25 μl chloroform in control) After drying, the discs were placed on the inoculated plates which were incubated at 10-15°C for 1 h and then at 37°. After 24 h the width of the zone of inhibition round the edge of the disc was measured in duplicate experiments with 2 replicates for each extract concentration.

Statistical analysis was done using the complete randomized design in factorial arrangement (F-test). The least significant difference (L.S.D.) was used for comparing treatment means (Snedecor and Cochran, 1980).

Results and Discussion:

Fusarium spp. and Alternaria solani, when grown under artificial conditions, produced toxic diffusible substances which increased in concentration with the age of culture. These toxic substances inhibited growth of the radicels or (hypocotyl, cotyledonary leaf and plumules) when seeds were placed in the filtrate of the fungus.

Data in Tables (1 &2) show the effect of toxins produced by F. oxysporum and A. solani on germination of tomato seeds. It is clear that percentage of germination was lower when tomato seeds were treated with filtrates of these fungi as compared with control; (water). Also the length of seedlings radicels and (hypocotyl, cotyledonary leaf and plumules) were less than control after the different periods of germination. Filtrates produced by these fungi were more toxic when fungi were incubated for two weeks compared with one week period. Percentage of germination decreased from 60 to 50 and from 70 to 60 incase of F. oxysporum and A. solani respectively.

The toxic effects of toxins produced by F. oxysporum and A. solani at different incubation periods when tested on the growth of pepper (hypocotyl, cotyledonary leaf and plumules) and radicle length decreased with increasing the incubation period till two weeks which were sensitive to phytotoxins Table (3 & 4). Also, percentage of germination decreased from 70 to 60 and from 90 to 80 when pepper seeds were treated with the two weeks old filtrates of F. solani and A. solani compared with the filtrates from one week old cultures.

Growth of hypocotyl, cotyledonary leaf and plumules was retarded and did not appear till after 6 days from the start of germination, i.e. reached 0.3 mm on the 6th day compared with the length hypocotyl, cotyledonary leaf and plumules incase of control (15.6 mm) Table (3), using filtrate of one week old culture of F. solani and A. solani. Where as no hypocotyl, cotyledonary leaf and plumules appeared till th 8th day i.e. length was 2-3 mm and 3-3 mm incase of F. solani and A. solani, using the filtrate of two week old culture, length of pepper radicle was affected by the fungal filtrate. The reduction in radicle length of the treated seeds reached approximately half that of the control.

These data show that all fungi isolates has the potential to produce phytotoxins which caused deformation of hypocotyls, chlorosis of cotyledons and stunting of seedlings. These toxins could be absorbed by the root after formation in the rhizosphere, or could be produced within the root cortex by invading pathogenic fungi, causing wilt or vessel plugging.

Results are in agreement with those obtained by Nemec el al., (1977) and Nemec, (1978) who reported that toxin produced by F. solani, was readily spread through the cortex and stele of rough lemon fibrous roots, causing growth reductions and chlorosis. Only inorganic salts and glucose, which would be available in the root cortex are required for the elaboration of these toxins. F. solani are known to vary widely in toxin production (Kern and Naef-Roth 1965).

Appearance and spread of toxin-generating strains may be random, or may be favoured by changes in nutritional status or stress of the plants. Toxins produced by F. solani are known to disrupt plant metabolism by inhibiting anaerobic and oxidative decarboxylation reactions, (Kern et al., 1970).

Nedelnik, (1993) reported that 6 purified toxic substances, belonging to the trichothecenes, zearalenone and fusaric acid groups, at concentrations of 100, 10 and 1 g/ml, were phytotoxic on seeds of 12 varieties of Medicago sativa (Lucerne) and Trifolium pretense. Fusaric acid and acetoscirpenol at 100 g/ml had the most inhibitory effect on germination, followed by deoxynivalenol and H 1-2 toxins. Trichothecenes were significantly more phytotoxic than zearalenones. These toxins also caused deformation of hypocotyls, chlorosis of cotyledons and stunting of seedlings.

Results in Fig. (1-4) indicate tbe biological effect of different fungal extracts produced at different temperatures, pH, carbon and nitrogen sources on the growth of Bacillus megaterium 1057 and Bacillus mycoides EMCC 1084 as a possible biological confirmation for detecting toxins.

Toxins produced by F. oxysporum, F. solani showed the highest inhibition zones, at 28°C and pH 5, while toxins produced in media of pH 3 and 9 had lower effect on both bacteria. Also, sucrose and glucose were the best carbon sources, and led to a higher inhibition zones in case of F. oxysporum and F. solani respectively. Bacillus megaterium showed more inhibition by toxins produced by the two Fusarium spp. when using casein and ammonium chloride as nitrogen sources.

In case of A. solani, the highest inhibition zone for B. mycoides was detected at 25°C and at pH 7, while toxins were not detected when the investigated fungi were incubated at 40°C. Also, glucose was the best carbon source for growth and production of toxins by A. solani and led to higher inhibition zones Fig. (1-4). On the other hand, toxins produced by A. solani showed inhibitory effects on B. mycoides and was more pronounced in case of ammonium molybdate and ammonium chloride.

Statistical analysis of the previous data showed significant differences among the different factors under study. The biological effects of zearalenone and the F_2 series were widely discussed by Mirocha et al., (1971). Zearalenone was non-mutagenic to Salmonella typhimurium in the Ames test. Zearalenone showed a narrow range of antibacterial activity limited to some Gram-positive aerobic spore-forming bacteria. Alternaria toxins (AME, AOH and ALT) were toxic to Bacillus mycoides and Hela cells, and weakly toxic to mice when administrated as a single dose (Betina, 1984).

Fusarium spp. secreted zearalenone and trichothecenes into the medium when grown in liquid cultures at different incubation temperatures, pH, carbon and nitrogen sources. The results in Table (5) indicate that the optimal temperature for both zearalenone and trichothecenes production by F. oxysporum was at 25°C (15,27 and 169.02 μg/ml), while the total toxin levels was 184.29 μg/ml. In case of F. solani, maximum amount of trichothecenes. (32.19 μg/ml) was detected at temperature 28°C while the greatest levels of zearalenone 10.43 μg/ml was observed at 25°C. At 25°C, maximum production of total toxins was 39.34 μg/ml. Data in Table (5) show that trichothecenes were the most frequently detected mycotoxins while zearalenone was present in low levels.

The effect of pH on toxin production by both F. oxysporum and F. solani is summarized shown in Table (6). The greatest amounts of both trichothecenes and zearalenone were achieved at pH 5. No toxins were detected for both fungi at pH 3.

Results in Table (7)show the effect of different carbon sources on the production of zearalenone and trichothecenes. The amounts of zearalenone produced by F. oxysporum were in the order of xylose> sucrose> glucose> maltose> lactose> mannose, while for the other toxin, trichothecene, the amounts were in the order of; glucose> maltose> sucrose> xylose> lactose> mannose. Generally, the best carbon source for total toxins production (72.64 μg/ml) was glucose as compared with the other carbon sources.

In case of F. solani, the efficiency of toxin production differed for F. oxysporum, using the same carbon sources. The amounts of zearalenone were in the order of xylose> glucose> mannose> maltose> lactose> sucrose. While for the other toxin, trichothecene, the amounts were in order of maltose> xylose> sucrose> mannose> glucose> lactose. The lowest amounts toxins, produced by F. solani, were detected using lactose as a carbon source.

Table (8) shows the effect of concentrations of the two toxins produced by F. oxysporum in presence of different nitrogen sources. Ammonium phosphate gave high levels of zearalenone and trichothecenes, 24.73 and 92.35 μg/ml respectively. While casein resulted in high levels of trichothecenes were lower than ammonium phosphate. In case of F. solani, there was a high concentration of zearalenone in presence of casein, 319.03 μg/ml. On the other hand, using ammonium molybdate, a high concentration of trichothecenes was detected, 183.68 μg/ml.

Much attention should be devoted to the natural occurrence of trichothecenes together with zearalenone and the combined effects of these toxin on human health. Thrane (1986), detected, zearalenone, T-2, HT-2, diacetoxyscripenol, neosolaniol, deoxnivalenol, fusaranon, produced by 114 Fusarium isolates. Also Krivobok el al., (1987) detected citrinin and zearalenone, 4 toxigenic from Fusarium spp. grown in liquid medium.

Data obtained in Table (9 & 10) show the effect of temperature and pH on quantities of Alternoil monomethyl ether (AME), Alternoil, (AOH) and Altenune (ALT) toxins produced by Alternaria solani. The higher quantities of AME, AOH and ALT were produced at 28°C. Also, large amounts of total toxins were produced at 28°C, white, the lowest amounts of toxins were detected at 35°C. Meanwhile, at pH 7 higher amounts of total toxins were produced, 184.38 μg/ml Table (8).

The production of the various mycotoxins by A. solani in the different carbon sources are given in (Table 11). It was observed that the high amounts of AME were detected in the presence of the order xylose> sucrose> mannose> lactose> maltose> glucose. while ALT was detected in the order glucose> xylose> sucrose> mannose> maltose> lactose. A high concentration of AOH was recorded in the presence of glucose followed by xylose.

The results in Table (12) indicate that the greatest amounts of (AME) were observed in ammonium molybdate and ammonium sulphate 0.8 μg/ml, while the maximum (ALT) was observed in the presence of casein, (AOH) production followed a similar pattern. Generally, casein and ammonium molybdate were the most favourable nitrogen sources for mycotoxins production by A. solani. Where as, ammonium chloride, ammonium phosphate and ammonium sulphate, showed lower efficiency for toxin production.

Toxin production by a given fungius has been shown to depend on different environmental factors. Inadequate storage conditions, such as high moisture and warm temperature (25-30°C), can create conditions favourable for the growth of a fungus and production of mycotoxins (CAST, 1989).

The trichothecenes mycotoxins are potent inhibitors of protein and DNA synthesis in eukaryotic cells, and the bone marrow, thymus and intestinal epithelia are the target organs Ueno, (1987). Only limited mutagenic activity of the trichothecenes has been demonstrated in several short-term tests, such as the Ames test.

Zearalenone (ZEA) [6-(10-hydroxy-6-oxo-trans-1 undecenyl)-Bresorcylic acid μ-lactone] is an oestrogenic secondary metabolite produced by various species of Fusarium, which causes hyperestrogenism in swine as well as in other mammals (Schuh and Glawisching, 1980).

Alternaria is one of the most commonly occurring postharvest fungi in the decay of plants including many fruits and vegetables. In human foods, studies indicated that some of the mycotoxins produced by Alternaria, such as alternariol methyl ether (AME) and tenuzonic acid (TA), could be high in apple and tomato products, (Jelinek el al., 1989). Mycotoxins produced by Alternaria include dibemz [a] pyrone-type toxins, such as alternariol (AOH), AME, altenunene, tetramic acid types of metabolites, such as TA and perylene derivatives, such as altertoxins I, II and III. Crude extracts of A. alternata have been shown to be positive in the Ames test, Scott and Stoltz, (1980).

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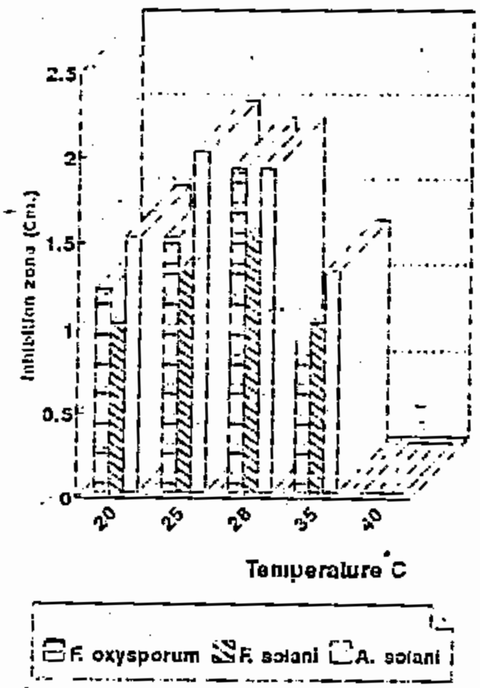

Fig. (1): Effect of toxin produced by three fungi at different temperatures on growth of Bacillus megaterium and Bacillus mycoides.

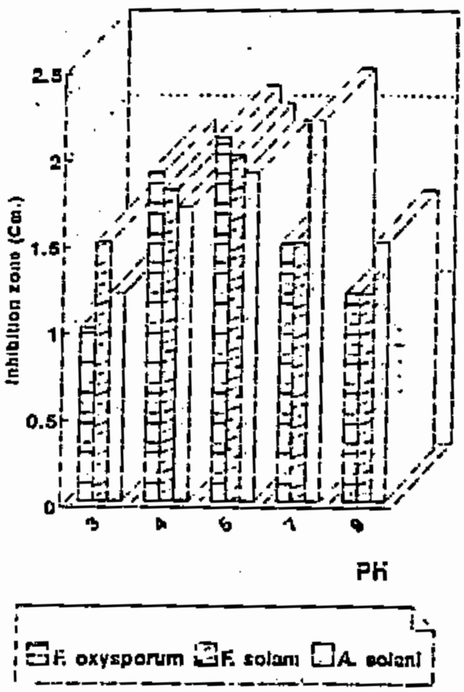

Fig. (2): Effect of toxin produced by three fungi at different pH's on growth of Bacillus megaterium and Bacillus mycoides.

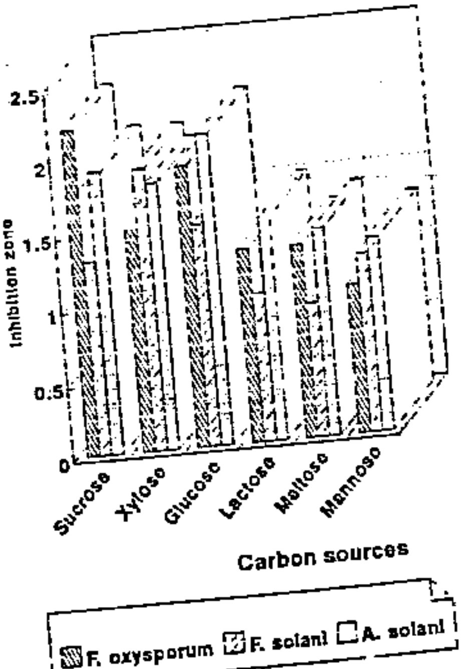

Fig. (3): Effect of toxin produced by three fungi using different carbon sources on growth of Bacillus megaterium and Bacillus mycoides.

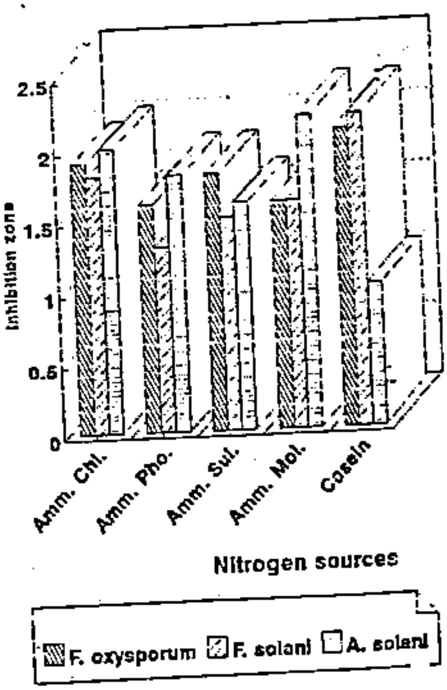

Fig. (4): Effect of toxin produced by three fungi using different nitrogen sources on growth of Bacillus megaterium and Bacillus mycoides.

Table (1): Effect of toxin produced by <u>Fusarium</u> <u>oxysporum</u> f.sp. lycopersici and <u>Alternaria</u> <u>solani</u> from one week old culture. on growth of tomato seedlings .

Treatment	Length of radicle (mm.)				Length of plumule (mm.)				Germination
	Days after germination				Days after germination				%
	2	4	6	8	2	4	6	8	
1	2.0	4.6	10.0	19.3	5.0	0.0	5.6	9.3	60.00
2	1.6	11.3	17.3	28.0	0.0	4.3	16.0	27.6	70.00
Control	6.0	18.6	24.3	32.6	5.3	12.3	29.6	40.0	100.00

*1- Seeds watered with F. oxysporum filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

Table (2): Effect of toxin produced by <u>Fusarium</u> <u>oxysporum</u> f. sp. lycopersici and <u>Alternaria</u> <u>solani</u> from two weeks old culture, on growth of tomato seedlings.

Treatment	Length of radicle (mm.)				Length of plumule (mm.)				Germination
	Days after germination				Days after germination				%
	2	4	6	8	2	4	6	8	
1	2.6	3.6	5.0	11.0	0.0	0.6	5.0	16.6	50.00
2	2.6	10.0	13.3	19.0	6.0	7.6	9.0	15.0	50.00
Control	6.0	18.0	24.3	32.6	5.3	12.3	29.6	40.0	100.00

*1- Seeds watered with F. oxysporum filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

Table (3): Effect of toxin produced by *Fusarium solani* and *Alternaria solani* from one week old cultures. on growth of pepper seedlings.

Treatment	Length of radicle (mm.)				Length of plumule (mm.)				Germination %
	Days after germination				Days after germination				
	2	4	6	8	2	4	6	8	
1	1.3	5.0	9.6	16.0	0.0	0.0	0.3	3.3	70.00
2	3.3	7.0	11.0	17.3	0.0	1.3	5.0	6.0	90.00
Control	4.3	12.3	17.0	22.3	0.6	3.3	15.6	21.0	90.00

*1- Seeds watered with F. solani filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

Table (4): Effect of filtrates produced by *Fusarium solani* and *Alternaria solani* from two weeks old culture, on growth of pepper seedlings.

Treatment	Length of radicle (mm.)				Length of plumule (mm.)				Germination %
	Days after germination				Days after germination				
	2	4	6	8	2	4	6	8	
1	2.0	3.6	8.3	14.0	0.0	0.0	0.0	2.3	60.00
2	2.3	5.0	9.0	16.0	0.0	0.6	0.0	3.3	50.00
Control	4.3	12.3	17.0	22.3	0.6	3.3	15.6	21.0	90.00

*1- Seeds watered with F. solani filtrate.

*2- Seeds watered with A. solani filtrate.

* Control seeds watered with sterile water

able (5): Effect of temperature on concentration of toxins produced by two Fusarium species .

Fungus	Temperature C	Toxin production ug./ml.		Total toxin ug./ml.
		Zearalenone	Trichothecenes	
F. oxysporum	20	1.012	58.940	59.952
	25	15.265	169.020	184.285
	28	12.749	61.675	74.424
	35	1.803	45.631	47.434
F. solani	20	8.685	10.719	19.404
	25	10.429	12.347	22.776
	28	7.152	32.190	39.340
	35	1.333	6.098	7.431

Table (6): Effect of pH on concentration toxins produced by two Fusarium species.

Fungus	pH	Toxin production ug./ml.		Total toxin ug./ml.
		Zearalenone	Trichothecenes	
F. oxysporum	3	0.000	0.000	0.000
	5	18.230	88.030	106.260
	7	1.211	36.043	37.254
F. solani	3	0.000	0.000	0.000
	5	15.145	13.871	29.016
	7	8.780	9.147	17.927

70): Effect of different carbon sources on concentration of toxins produced by two Fusarium species .

Fungus	Carbon sources	Toxin production ug./ml.		Total toxins ug./ml.
		Zearalenone	Trichothecenes	
oxysporum	Sucrose	34.957	19.795	54.752
	Glucose	18.917	53.727	72.644
	Lactose	5.950	7.451	13.401
	Maltose	16.812	33.830	50.642
	Mannose	3.352	4.392	7.744
	Xylose	31.080	16.900	47.980
solani	Sucrose	1.379	62.272	63.651
	Glucose	8.416	43.702	52.118
	Lactose	1.860	22.283	24.143
	Maltose	2.301	88.682	90.983
	Mannose	2.440	53.78	56.220
	Xylose	8.500	71.180	79.680

Table (8): Effect of different nitrogen sources on concentration of toxin produced by two Fusarium species.

Fungus	Nitrogen sources	Toxin production ug./ml.		Total toxin ug./ml.
		Zearalenone	Trichothecenes	
F. oxysporum	Amm. chloride	19.750	2.172	21.922
	Amm. molybdate	10.789	7.024	17.813
	Casein	6.210	49.452	55.662
	Amm. phosphate	24.731	92.349	117.080
	Amm. Sulphate	5.013	4.564	9.577
F. solani	Amm. chloride	9.410	4.638	14.048
	Amm. molybdate	15.650	183.677	199.317
	Casein	319.028	53.549	372.580
	Amm. phosphate	12.200	35.261	47.461
	Amm. Sulphate	14.085	2.082	16.167

e('9): Effect of temperature on concentration of toxins produced by Alternaria solani.

Temperature C	Toxins (ug/ml.)			Total toxins ug./ml.
	AME	ALT	AOH	
20	79.200	0.880	36.552	116.632
25	57.600	2.550	126.734	186.885
28	144.800	2.497	163.480	310.777
35	48.000	0.900	48.000	96.900

* AME= Alternoil monomethyl ether
* ALT= Altenune
* AOH= Alternoil

(10): Effect of pH on concentration of toxins produced by Alternaria solani

pH	Toxins (ug/ml.)			Total toxins ug./ml.
	AME	ALT	AOH	
5	83.200	1.100	15.790	100.090
7	168.000	0.383	16.000	184.383
9	11.200	2.680	18.410	32.290

* AME= Alternoil monomethyl ether
* ALT= Altenune
* AOH= Alternoil

Table (11): Effect of different carbon sources on concentration of toxins produced by Alternaria solani.

Carbon sources	Toxin production ug./ml.			Total toxins ug./ml.
	AME	ALT	AOH	
Sucrose	261.096	10.440	37.556	309.092
Glucose	0.128	57.136	46.250	103.514
Lactose	160.450	0.136	18.945	179.531
Maltose	82.590	1.016	19.548	103.154
Mannose	164.240	2.208	5.355	171.803
Xylose	305.984	18.440	42.710	367.134

* AME = Alternoil monomethyl ether
* ALT = Altenune
* AOH = Alternoil

Table (12): Effect of different nitrogen sources on concentration of toxins produced by Alternaria solani.

Nitrogen sources	Toxin production ug./ml.			Total toxins ug./ml.
	AME	ALT	AOH	
Amm. chloride	0.080	4.880	7.832	12.792
Amm. molybdate	0.800	3.640	22.426	26.866
Casein	0.264	14.430	54.493	69.187
Amm. phosphate	0.560	0.056	15.531	16.147
Amm. Sulphate	0.800	1.580	11.380	13.760

* AME = Alternoil monomethyl ether
* ALT = Altenune
* AOH = Alternoil