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Induced Resistance in Cucumber Plant Against Cucumber Mosaic Virus by Fungal Isolates

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The induction of resistance of Cucumber plants (*Cucumis sativa*) against cucumber mosaic virus (CMV) by either ethyl alcohol extracts or cultural filtrates of twenty fungal isolates from Al-Madinah AL-Monawarah date palm farms, Kingdom of Saudi Arabia have been investigated(*Aspergillus clavatus, A. fumigatus, A. glaucus, A. nidulans, A. niger, A. terreus, A. versicolor, Penicillium expansum, P. funiculosum, P. griseofulvum, P. janczewskii, P. janthinellum, P. nigricans, P. notatum, P. rubrum, P. chrysogenum, Botrytis squamosa, B. byssoidea, Fusarium solani* and *F. oxysporum*). Seeds of cucumber were soaked with either the filterates of cultivated fungi or the ethyl alcohol extracts of these fungi at different periods. An inhibitory effect on the infectivity of CMV was recorded. *In vitro* and *in vivo* studies on the antiviral activities of different fungal agents were applied. The purification of the most potent fungal extract (*F. oxysporum*) was done using DEAE- cellulose column chromatography and the active substance with high antiviral activity was found to be protienacious in structure.

Key words: Fungal extracts, cucumber mosaic virus, induction of resistance.

In contrast to bacterial infectious diseases, viral diseases cannot be treated by common antibiotics and specific drugs are urgently needed. Antiviral effects are described not only for whole fungal extracts but also for isolated compounds. They could be caused directly by inhibition of viral enzymes, synthesis of viral nucleic acids or adsorption and uptake of viruses into living cells[1].

Certain microorganisms are of great importance in controlling viral diseases of crops. So great works were done to use the metabolites of these organisms as a source of antiviral agents or virus-induced resistance. Harpaz *et al.,*[2] have concluded that an inhibitor of tobacco mosaic virus (TMV) was demonstrated in extracts of *Nicotiana glutinosa* infected with the fungus *Thielaviopsis basicola*. Also a similar inhibitor was extracted from the fungal mycelium, differing only slightly from the one extracted from fungus-infected plant tissue. The mycelial extracts of *Trichoderma roseum* were capable of inhibiting TMV infection in *Nicotiana glutinosa* plants[3]. Mannan sulphates (MS) synthesized on the basis of extracellular

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linear mannan (LM) of *Rhodotorula rubra* induced resistance of immune-580 tobacco and thorn apple (*Datura stramonium*) to tobacco mosaic virus[4]. Furthermore, inhibitors of plant virus infection with systemic effects were found in the culture filtrates of Basidiomycetes such as *Fomes fomentarius* and *Schizophyllum commune*. These inhibitors were highly active against the mechanical transmission of TMV[5]. The incidence of Barley yellow dwarf virus (BYDV)was lower in *Lolium pretense* infected by *Neotyphodium* than endophyte free plants[6,7].

The presented work aimed to study the induction of resistance of certain fungal isolates from Almadinah Almonawarah date palm farms, Kingdom of Saudi Arabia against cucumber mosaic virus on cucumber plants and different methods of applications. Also, the partial purification of the most potent fungal extract.

MATERLAL AND METHODS

Fungal isolates

The tested fungi were isolated from date palm leaves from Al- Madinah Al- Monawarah region, Kingdom of Saudi Arabia**.** The isolates were grown on potato –dextrose agar medium . The common isolates were identified according to Raper and Fennel[8], John[9], Camicheal *et al.* [10], Hanlin[11], John and Summerell[12], Elad[13] and Marazaei *et al.,*[14]. The most common fungal isolates were presented in Table 1.

Virus

Identified strain of cucumber mosaic virus (CMV) on cucumber leaves was used for this study. Mosaic and yellowing symptoms were recorded on the second and third leaves generations.

Sources of induced-resistance substances

Culture filtrate

The culture filtrate of each fungus containing metabolites was collected after 15 days-growth of the fungus. The filtrate was concentrated to certain volume (50ml) and then used for applying or soaking the seeds of cucumber (Beit-alpha, obtained from Al-Madinah Al-Monawarah, Kingdom of Saudi Arabia).

Fungal extracts

The fungal mats were washed several times with distilled water and dried through Whatman filter paper No. 1. The fungal extract was extracted by homogenizing each culture in 100 ml of 70% ethyl alcohol solution (v/v distilled water). The homogenized culture was filtered through (W. No. I) . The supernatant was evaporated and the crude ethyl extract was collected and stored until used for testing its antiviral effect.

Application method

***In vitro* application**

Equal volumes of viral sap or crude ethanol fungal extract were mixed together in a test tube for ten minutes, then inoculated on the cotyledonary leaves of cucumber seedlings (50ul/ cotyledonary leaf). Twenty plants were inoculated for each fungal extract. Inoculated plants with CMV sap were done as well as healthy cucumber plants as control.The percentage of induced resistance against viral infectivity was calculated for each fungal extract and the most potent effects were chosen for further studies.

In Vivo application

Treatment before viral inoculation

Fifty ul of each highly potent crude fungal extract or cultural filtrate were applied on each cotyledonary cucumber leaf ,then inoculated with viral sap after 2,6,12and 24 hours of treatment .

$$\% \text{ induced resistance} = \frac{\text{Control-Treatment}}{\text{Control}} \times 100$$

where

Control

The mean number of L.L. on plants of seeds soaked with distilled water.

Treatment

No of L.L. on plants of seeds soaked with either culture filtrate or fungal extract.

Treatment after viral inoculation

The above mentioned method of application was repeated but after viral inoculation by 2,6,12 ,24 and 48 hours.

Effect of seed soaking

Fifty cucumber seeds (*Cucumis sativa*) were soaked with either the cultural filtrate or the fungal extract at 1, 2, 3, 4 and 5 hours and then planted in sterilized clay soil in pots of 40 cc in diameter(10 plants per pot). Control groups of seeds were prepared by soaking each group in

sterilized potato- dextrose medium as well as in distilled water or in phosphate buffer solution at the above mentioned time intervals. A group of dried seeds was planted simultaneously. Irrigation of cultivated seeds was taken place regularly, until the end of each experiment.

Partilly purification of the most potent antiviral activity of fungal isolates

Ethyl alcohol extract of Fusarium oxysporum which showed the most inhibitory effects against cucumber mosaic virus (CMV) was partially purified by column chromatography according to Wood[15]. The column was filled with diethyl amino-ethyl cellulose(DEAE-cellulose) and 0.1, 0.2 and 0.3 M Na Cl, respectively. Eluents (5 ml volume) were collected at room temperature. The total protein content of each tube was determined by absorbance measurements at 750nm. The resulted optical densities were drawn against the tube number on millimeter paper.Tubes representing each fraction were pooled together then dailzed against distilled water and lyophilized to complete dryness. The different lyophilized fractions were tested against CMV.

RESULTS AND DISCUSSION

The data presented in Table 1 showed that twenty fungal isolates were screened for their antiviral activities against cucumber mosaic virus (CMV) using ethanol extracts or cultural filtrates-containing metabolites. It's clear that an inhibitory effects were recorded with all treatments and the most potent antiviral activity was reached with ethanol extract or cultural filtrate of *Fusarium oxysporum* (92,56%), respectively followed by ethanol extract of Botrytis squamosa (81%), *Penicillium chrysogenum* extract (74%), *Penicillium griseofulvum* extract (72 %) and *Aspergillus terreus* (62 %). These isolates were chosen for further studies.However, the induction

Table 1. Screening of antiviral activity of either ethanol extracts (E) or cultural filtrates (C) of fungal isolates against cucumber mosaic virus on cucumber plants

Fungal isolate	%I	
	E	C
Aspergillus clavatus	22	19
A. fumigatus	23	10
A. glaucus	56	00
A. nidulans	42	32
A. niger	56	14
A. terreus	62	26
A. versicolor	36	11
Penicillium expansum	50	30
P. funiculosum	42	18
P. griseofulvum	72	52
P. janczewskii	65	21
P. janthinellum	27	32
P. nigricans	19	24
P. notatum	56	11
P. rubrum	25	42
P. chrysogenum	74	30
Botrytis squamosa	81	29
B. byssoidea	56	23
Fusarium solani	34	45
F. oxysporum	92	56

E= ethanol extract
C = cultural filtrate
%I= percentage of inhibition of CMV

Table 2. *In vivo* studies of the most potent fungal isolates before CMV inoculation at different periods (hours)

Alcoholic extract	% I / Fungal species				
	2	6	12	24	48
A. terreus	52	52	78	74	89
Penicillium griseofulvum	76	62	74	78	87
P. janczewskii	91	71	66	79	76
P. chrysogenum	65	86	77	82	92
Botrytis squamosa	64	64	77	82	90
Fusarium oxysporum	51	63	69	83	93

%I= percentage of inhibition

of resistance against CMV by ethanol extract or metabolites-containing filtrate may be due to presence of antiviral substances extracted externally from fungal isolates[16,17]. The rapid destruction of the virus by certain fungi is not necessarily indicative that the virus is a chemical substance as contrasted to a living organism [8]. In vivo studies of the selected isolates revealed that *A. terreus, P. Griseofulvum ,P.janczewskii, P. Chrysogenum, B. Squamosa* and *F. Oxysporum* led to induced resistance against CMV when their extracts were applied before viral inoculation at 2,6,12,24 and 48 hours Table 2. Application of ethanol extract of *P. chrysogenum* 48 hrs. before CMV inoculation led to 92% of induced resistance followed by *P. janczewskii* at 2 hrs (91%), *B. Squamosa* at 48 hrs. (90%) and *A. terreus* at 48 hrs. (89%).However, application of the selective fungal extracts showed induced resistance against CMV when applied at different

Table 3. *In vivo* studies of the most potent fungal isolates after CMV inoculation at different periods hours

Alcoholic extract	% I / Fungal species				
	2	6	12	24	48
Aspergillus terreus	48	68	36	37	25
Penicillium griseofulvum	49	55	42	47	17
P. janczewskii	60	59	40	36	13
P. chrysogenum	48	59	53	46	40
Botrytis squamosa	58	67	47	32	22
Fusarium oxysporum	69	69	54	45	42

Table 4. Effect of cucumber seed soaked in different ethanol extracts of fungal isolates at different periods (hours) on the infectivity of CMV

Seed soaking after (hrs)	%I / Fungal species				
	2	6	12	24	48
Aspergillus terreus	00	14	17	21	28
Penicillium griseofulvum	07	15	24	20	38
P. janczewskii	11	12	32	26	37
P. chrysogenum	08	17	15	43	49
Botrytis squamosa	12	19	28	47	48
Fusarium oxysporum	22	25	46	53	67

periods.This is due to the active substances-containing fungi which may act directly against the activities of the virus or may be induced the synthesis of new substances in the host that act on virus replication or transmission[19,20].The data in Table 3 concluded that application of different fungal extract on cucumber plants after CMV inoculation showed antiviral activities with various values. Application of *Fusarium oxysporum* 2 and 6 hrs after CMV inoculation

Table 5. Antiviral activity of different eluents of ethyl alcohol extract of *Fusarium oxysporum* against CMV

Peak No.	%I
2	62
3	54
4	58
5	95

%I= percentage of inhibition

led to 69 % inhibition of virus followed by A. Terreus extract at 6 hrs. (68 %I) . Similar results were obtained by Abou El- Hawa *et al.,* [21] who found that treatment of cucumber plants infected with Zucchini Yellow mosaic virus by bacterial suspension led to reduction of viral symptoms.

Seeds of cucumber were investigated for induction of resistance against CMV inoculation by soaking it with each fungal extract at different periods Table 4. It's worthy to mention that all fungal extracts led to different values of resistance when cucumber seeds were soaked in it at different period and the highest value was reached with *F .oxysporum* after 5 hrs. of soaking (67 %0) followed by *P. Chrysogenum* after 5 hrs (49 %) and B. Squamosa at 5 and 4 hrs. (48,47 %), respectively.These finding were in agreement with Galal[22] who stated that soaking of cucumber seeds for 2hr. In the actinomycetes filtrates resulted in the highest CMV inhibition.

The ethanol extract of Fusarium oxysporum was partially purified using DEAE-cellulose column chromatography Table 5. The data showed that the active compound in this extract was in protienacious structure (peak No.5) which showed high antiviral activity (95%) as compared with the other peaks (2,3 and 4). Nassar[23] could isolate a protienacious compound from Laurancia papillosa and Anabaena spherica which gave high antiviral activities against tobacco necrosis virus.

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