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VARIATION OF THREE BLACK CUMIN CULTIVARS IN HERITABILITY; CHEMICAL AND ANTIVIRAL ACTIVITY

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ABSTRACT

Black cumin is one of the important medicinal plant and well known to flok remedy. Balady, Sori and Turki cultivars belong to black cumin are variated in chemical composition and antiviral activity. The results reflected significant heritability between the three cultivars in length and number of capsule per plant and number of branches per plant, while non-significant values of plant height, number of locules/ capsule, and seed yield per plant. Eight fatty acids methyl esters were detected in seeds of three cultivars by GLC. It was found change quantitatively of fatty acid between three cultivars. SDS-PAGA showed change qualitatively of poly peptides content accompanying three cultivars. A similarity of about 90% was found between Balady and Sori cultivars in relation to heritability. Water seed extracts, *in vitro* reduced ToMV infectivity to 6.73; 6.78 and 5.08% of Balady, Sori and Turki respectively. Four hours post and pre ToMV inoculation were most sensitive period to ToMV replication for three cultivars. On the other hand, the antiviral event changed in conformation and chemical structure of virion, coat protein and nucleic acid of ToMV with black cumin seed extract by spectroscopy. Balady, Sori and Turki; black cumin cultivars were varieted based on heritiability, chemical composition according to fatty acid GLC and polypeptide fraction SDS-PAGE analysis as well as antiviral activities against tomato mosaic tobamovirus.

Keywords: Black cumin, Fatty acids, Polypeptide fraction, SDS-PAGE, Antiviral, Tomato mosaic virus (ToMV)

INTRODUCTION

Black cumin *Nigella sativa* L is an herbaceous plant belong to family Ranunculuceae. The plant has a long history of flok medicine. The seeds are used as a carminative, diuretic and useful for asthma.Many authers studied the genetic

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variability of black cumin cultivars (**Banafar *et al* 2002 and D'Antuono *et al* 2002**) and heritability, variation and correlation coefficient among nigella characters (**Salem *et al* 2001**). Morever, fatty acids methyl esters and sterols olated from nigella seeds were studied by **Perifanova *et al* (2002) and Atta, (2003)**. It was previously reported that extracts of mature leaves, roots, stems and seeds of plants inhibited to varying degrees, however, this is true only when they were mixed with the virus prior to inoculation or applied to leaves within a short time before or after virus inoculation (**El-Dougdoug, 1997**). The effect of plants extracts was attributed to their chemical contents as antiviral agent such as phenolic components (**Woods and Agrios, 1973**); Steroids, (**Menzel, 1987**) alkaloids (**Attaur-Rahaman *et al* 1985**) and protein (**Othman *et al* 1991**).

The present study aim to evaluate the heritability, chemical composition among black cumin cultivars and to investigate the antiviral activity of their cultivars extracts against tomato mosaic tobamovirus.

MATERIAL AND METHODS

Plant material

The plant material used in this study is seed of three cultivars of Nigella (Balady, Sori, and Turki) were obtained from Genetic and Breeding of Medicinal and Aromatic plants Group, Genetic and Cytology Department National Research Center.

Virus source

Tomato mosaic tobamovirus (ToMV) was obtained from virology lab. Microbiology Department, Faculy of Agriculture. Ain Shams Univ. maintenance on *Nicotina tabacum* cv. Samson as well purified ToMV virus particles.

Experimental form

Seeds were sown in hill 30 cm space of lines 5m long 50 cm wide. Five replicates of each cultivar were designed in three complete blocks at farm faculty of Agriculture, Ain Shams Univ. All culture practices were carried out. At maturity, the plants were harvested, separately. The plant height, number of branches and capsule, seed yield per plant, capsule length, and diameter as well number of locules per capsule were recorded. The obtained data were statistically analyzed according to **Steel and Torrie (1980)**.

Crude water extract

Water extracts of three cultivars of (*Nigella sativa* L.) were reported by macerating, one gram fine powder seeds with 9ml distilled water at water bath, then distilled water was add at reach 1/10 (w/v). Petroleum ether (40-60°C) used for seed extraction according to the procedure carried out **Ottai, (1994)**. The fatty acid methyl esters were prepared from Petroleum ether extracts by the method of **Vogel, (1975)**. Then quantitative analysis of the fatty acid esters for three cutivars were performed with Gas liquid chromatography (GLC)using Sp. 2310 Column 55% Cyanopropyl phenyl silicon dimensions 1.5 x 4 mm. The temperature propram was 70°C (initial temperatures) at a role of 8°C / min. to 190°C (final temperature). Injector and detector temperatures were mentained at 250 and 300°C respectively. Nitrogen was used as carrier gas at a rate of 30 ml /min. The relative percentage of each compound was deter-

mined on the base of the peak area. The qualitative identification of fatty acids were achieved by comparing the retention time (Rt) of their expected authentics chromatographed under the same conditions.

Electrophoretic analysis

Sodium dodecyl sulphate-polyacreylamide gel electrophoresis (SDS-PAGA) was performed in 12.5% acrylamide (w/v) slab gel containing sodium dodecyl sulphate (SDS) **(Laemmli, 1970)**. The gels were cross linked with 0.3% (w/v) N, N-methylene bis-acrylamide at pH 8.8 and stacking gels were made 5.0% (w/v) polyacreylamide at pH 6.8. Samples (50µl) were denaturated by boiling buffer at 100°C for 10min. in 1% SDS containing 2-mercaptoethanol. Molecular weights of the protein fractions were estimated from a low molecular weight standard (Pharmacia Motreal) electrophoreses under identical condition. Marker protein for molecular weight measurement were: 97.400; 58.100; 39.800; 29.000; 20.100; and 14.300 KDa.

Assay of antiviral activity

In vitro Mixing 1.0 ml of infected crude sap with 1.0 ml crude extracts of three cultivars. The mixtures were inoculated at lab. temperature (25°C±2°C) for 0; 1 and 24 hr. The control was carried out with distilled water. The inhibitory effect on virus infectivity was assayed by local lesion assay (applied 100 µl inoculum) on *N. glutinosa.*

In vivo This experiment was carried by rubbing of *N.glutinosa* leaves (1ml/leave) with crude extract of three varieties pre and post virus inoculation (50µl/leaves) using glass spatula. The experiment control was carried out with distilled water. The inhibitory effect was calculated according to the formula I = (1- C/Co) x 100, where I= percentage of infection C= number of local lesions of treatment and Co = number of local lesions of the control. The relative inhibition was calculated as the difference in number of local lesions produced between treatment and control. Multiplied by 100 and divided by number of local lesions of control.

Effect of black cumin water seed extract on virus particles and protein preparation

The RNA was prepared from ToMV particles by repeated chloroform-phenol extraction. The pellet containing RNA was resuspended in 0.1M Tris pH 8.0 and 0.01M EDTA and stored at -20°C. the supernatant containing protein was separated by the acetic acid **(Sambrook, 1989)**.

The effect of seed extracts on RNA, protein, and virion was spectrophotomatically determined by mixing 50µl of each them with 50µl of seeds extracts for three cultivars. The three mixtures were determined using UV-Vis spectrophotometer, Shimadizo 1201 program Photometric 2 at 260 nm for RNA and virion and 280 nm for protein at different times intervals 5min. through 45 mins.

RESULTS AND DISCUSSION

The analysis of variation between the cultivars of black cumin (Balady, Sori and Turki) is presented in Table (1). Highly significant variability were noted

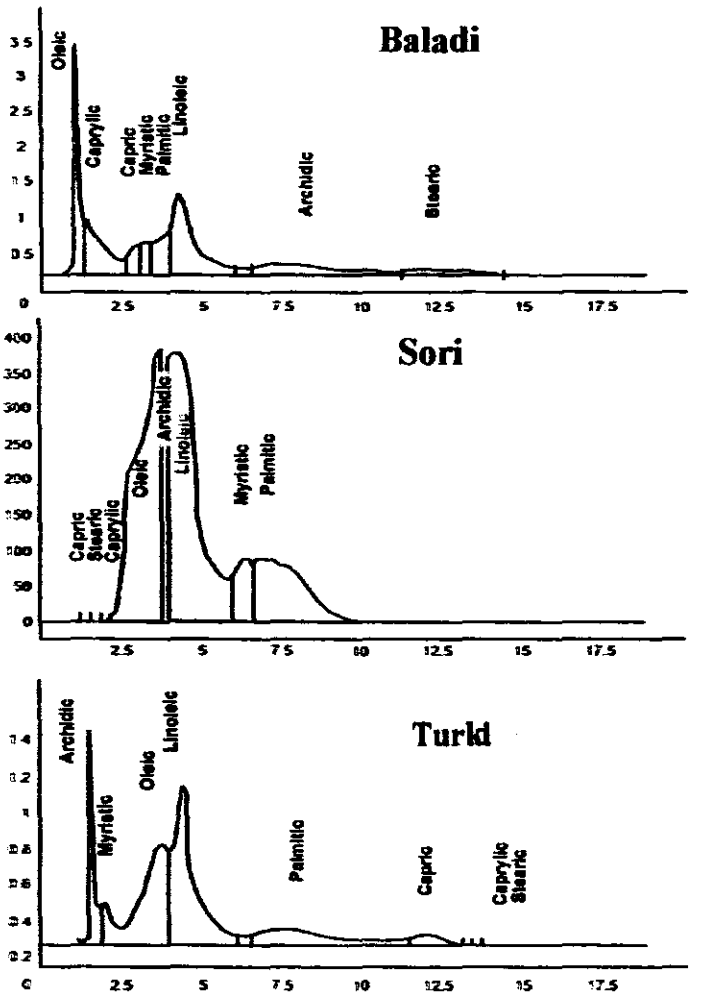

Figure 1. GLC Qualitative and Quantitative anaylsis of methyl esters fatty acids in black cumin seeds

Table 1.Variation of morphological characters for three black cumin cultivars

Morphological character \ Cultivars	Balady	Sori	Turki	L.S.D (1%)
Plant height (cm)	65.50	52.50	40.25	10.5
No. of branches (cm)	13.75	15.25	10.50	2.4
Capsules length (cm)	1.50	1.25	1.35	0.25
Capsules diameter (cm)	1.00	0.92	0.89	Ns
No. of capsule/plant	75.0	45.25	37.25	11.2
No. of locules/capsule	6.50	7.21	5.75	0.75
Seed yield/plant (g)	8.00	3.50	4.00	2.5

Average from 10 replicates
Ns = non significance

for plant height, number of branches, capsules and seed yield per plant, as well as capsule length addition to, variations in capsules diameter and number of locules per capsule. These results are agreement with those of **Salem *et al* (2001). Banafar *et al* (2002)**. There are found that high heritability values for capsule length, number of branches and capsules per plant, while moderate values for other characters. Balady was found to be the best cultivar in the most of trails and it had homogentical plants. Different pattern of phenotypic correlation were noted between each cultivar characters. Figure (1) refers to the peaks obtained using GLC which resulted qualitative and quantitative analysis of methyl esters fatty acids based on the area under peak. The relative percentages of the detected fatty acids extracted from the petroleum ether are shown in Table (2) and Figure (1) three cultivars. Eight fatty acid were determined in the three cultivars. Unsaturated fatty acids were found with major amounts 52.84, 61.95 and 69.67 as well saturated fatty acids were found with minor amount 47.16; 38.05 and 29.83 in Balady; Sori and Turki cultivars respectively.Fatty acid quantitative were differed between the three cultivars. Caprylic and Capric acids were found high relative percentage in Balady 10.40, 5.70 followed by Sori 4.24, 2.85, while trace and 1.16 in Turki cutivars respectively. Stearic was found high relative percentage in Sori 2.78 followed by Balday 1.92 and trace in Turki cultivars. Other fatty acids were found with relative percentage differential between three cultivars. These results were in good agreement with those of **Mona Ahmed (1991); Ozguven *et al* (2001); Ramadan and Morsel (2002)**. The polypeptides from various subcellullar fractions of black cumin seeds were analyzed by SDS- PAGE under reducing conditions (Figure 2). The polypeptide patterns of cultivars varied in number and molecular

Table 2. GIC anaylsis of fatty acids for three black cumin cultivars

Fatty acids component \ Cultivars	Balady	Sori	Turki
Saturated fatty acids			
palmitic	13.60	14.15	12.40
Arachidic	11.12	8.20	11.54
Myristic	4.42	5.83	4.75
Capric	5.70	2.85	1.16
Stearic	1.92	2.78	Trace
Caprylic	10.40	4.24	Trace
Total	47.16	38.05	29.83
Unsaturated fatty acids			
Linoleic	32.42	34.83	38.42
Oleic	20.42	27.12	31.25
Total	52.84	61.95	69.67
Total fatty acids	100	100	99.50

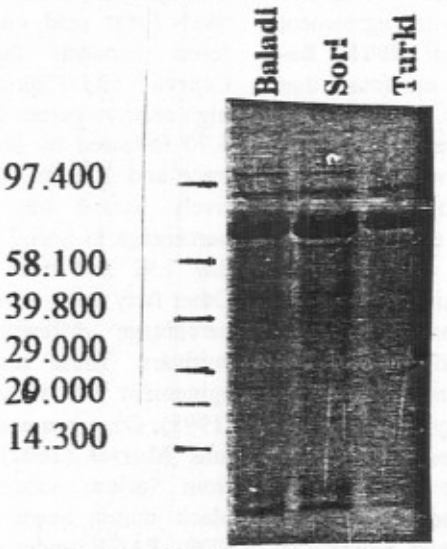

Figure 2. SDS-polyacrylamide gel (12.5%) electrophoresis of black cumin seeds polypeptides from three culivars Balady, Sori and Turki.

weight, the alteration detected the polypeptide patterns as judged by staining appears in the enhancement 87.50 and 39.3 KDa in Balady and Sori than Turki cultivars. However 77.75, 58.10, 14.30 and 12.0 KDa common polypeptides were detected in three cultivars. **Babayan *et al* (1978) and Aly *et al* (1994)** reported that the analysis of Nigella sativa L. seeds contained 21% protein.

Effect of seed extracts on ToMV infectivity

In vitro It was found that the seed extracts at dilution 10^{-1}had the high effect on ToMV infectivity where as reduced the number of local lesions *N. glutinosa*, Table (3). The reduction reached to maximum after 24 hrs inoculation. Sori var. had the least effect on virus infectivity and inhibitory effect, while Balady and Turki had highest effects.

In vivo In post virus inoculation, It was clear that the seed extracts were effective virus infectivity after ½, 1 and 4 hrs. These periods gave the best results concerning the infectivity and inhibition i.e. it gave the lowest number of local lesions.

On the other hand the infectivity was increased again by increasing the period. up to 24 hr (Table 4). Within each period the seed extracts of var. Balady dose not regularity reduce the ToMV infectivity and increases the inhibitory effect. From the result in Table (4), it was found that, the seed extract have inhibitory effect on ToMV through eclipse period (1/2hr) and multiplication (1-4hr, from ToMV penetration to translocation in *N. glutinosa*).

In pre- virus inoculation, the seed extracts of Balady cultivar had the inhibitory effect on ToMV infectivity. High reduction in number of local lesions on *N. glutinosa* leaves which sprayed with seed extracts of cultivar Balady before virus inoculation. The inhibitory effect tends to decrease by increasing time (1-24hr) (Table 4). Generally, the rubbing with water extracts of black cumin seeds before virus inoculation, was more effective in reducing ToMV infectivity than after ToMV inoculation.

The water extract of black cumin seeds included more virus inhibitors such as alkaloids, sterols, saponin, and nigellicine **(Attaur-Rohman *et al* 1985).** The inhibitory effect was attributed to chemical content of seed extracts as antivirals interfere with the host defense mechanism associated with the precipitation of the virus in bands such bands may not be infectious or may behave as one virus particles **(Mayer *et al* 1995).** Indeed, there results shown that following infection with ToMV, the microsomal RNA of the host is rapidly degraded and its degradation products are utilized in the virus synthesis. It was further demonstrated by authers **(Kiraly and Pazsar, 1964).** Similarly, the results suggested that, the protein of ToMV is formed at the expense of an electrophoretically homogenous protein of tomato leaves. This protein fraction is degraded after infection as virus protein is synthesized **(El-Dougdoug, 1997).**

Results in Table (5) illustrate the effect of black cumin on UV absorption rate of virion, genome (RNA) and coat protein, as well as effect on concentration and conformation of chemical composition of ToMV at 45 mins It was found that the maximum effect on virion and coat protein after 20 mins and genome(RNA) after 15 mins.

Table 3. Inhibitory effect of water seed extract (Black cumin cultivars) on ToMV infectivity in vitro

Parameters / Treatments	O-time			1-hr			24hr		
	No. of L.L	% of inf.	Inhibitory effect	No. of L.L	% of inf.	Inhibitory effect	No. of L.L	% of inf.	Inhibitory effect
ToMV crude sap	90	100	-	85	100	-	85	100	-
ToMV treated with black cumin cultivars:									
Balady	16.5	18.33	81.67	11.5	13.53	86.47	5.72	6.73	93.27
Sori	20.7	23.00	77.00	12.7	14.94	85.06	5.25	6.18	93.82
Turki	15.8	17.55	82.44	10.8	12.71	87.29	4.35	5.08	94.92

No. of local lesions (L.L) calculated from five replicates of *N. glutinosa*
% of virus infectivity calculated (base on control 100%)

Table 4. Inhibitory effect of water seed extract (Black cumin cultivar Balady) on ToMV infectivity *in vivo*

Parameter / Treatment	Post- inoculation			Pre- inoculation		
	No. of local lesions	% of infectivity	Inhibitory effect	No. of local lesions	% of infectivity	Inhibitory effect
Inoculated plants and sprayed with water (control)	95	100	-	80	100	-
Inoculated plants and sprayed with black cumin (Balady):						
0-time	35.12	36.97	63.03	7.25	9.06	90.94
1/2hr	10.15	10.68	89.32	9.00	11.25	88.75
1hr	12.31	12.95	87.64	10.23	12.79	87.21
4hr	10.25	10.80	89.21	15.45	19.31	80.69
24hr	30.75	32.37	67.63	22.73	28.41	71.59
48hr	25.72	27.07	72.93	30.82	38.53	61.48

Table 5. UV absorption of ToMV virion, coat protein and nucleic acid treated with water seed extract of Black cumin cultivar Balady

Treatments		Time exposure (mins)	0-time	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min
ToMV virion	Untreated with black cumin	UV absorption	0.0090	0.095	0.099	0.103	0.113	0.115	0.125	0.125	0.125	0.125
		concentration	0.045	0.043	0.050	0.053	0.055	0.055	0.055	0.055	0.055	0.055
	Treated with black cumin	UV absorption	0.002	0.005	0.095	0.095	0.105	0.110	0.115	0.115	0.115	0.115
		concentration	0.0020	0.0017	0.045	0.049	0.090	0.0501	0.0501	0.0501	0.0501	0.0501
		% conformation	9.95	9.10	0.35	0.45	0.50	0.30	0.30	0.30	0.30	0.30
ToMV protein	Untreated with black cumin	UV absorption	0.301	0.205	0.182	0.185	0.182	0.181	0.180	0.180	0.180	0.180
		Concentration	0.165	0.150	0.141	0.192	0.140	0.139	0.139	0.139	0.139	0.138
	Treated with black cumin	UV absorption	0.095	0.093	0.115	0.114	0.112	0.112	0.112	0.112	0.112	0.112
		concentration	0.091	0.075	0.085	0.088	0.085	0.085	0.085	0.085	0.085	0.085
		% conformation	12.0	11.4	6.75	7.25	6.50	6.50	6.85	6.75	6.75	6.75
ToMV nucleic acid	Untreated with black cumin	UV absorption	0.095	0.091	0.099	0.097	0.098	0.102	0.105	0.105	0.105	0.105
		concentration	0.0045	0.0049	0.0048	0.0052	0.0052	0.0052	0.0052	0.0052	0.0052	0.0052
	Treated with black cumin	UV absorption	0.005	0.005	0.011	0.015	0.011	0.005	0.001	0.000	0.000	0.000
		concentration	0.0005	0.0003	0.0006	0.0002	0.0002	0.0001	0.000	0.000	0.000	0.000
		% conformation	8.50	5.40	8.2	8.2	9.010	10.000	0.000	0.000	0.000	0.000

Optical density for virion=260 nm, protein=280nnm and nucleic acid=260 nm ToMV virion E 0.1% 1cm 260 nm =2.3; protein E 0.1% 1cm 280=1.3 and nucleic acid E 0.1% 1cm 260 nm=20 (Noordam,1973)

El-Dougdoug, (1997) reported that, the seed extracts of Khella and black cumin event changing in conformation and chemical structure of ToMV virion, coat protein and RNA of ToMV by spectroscopy. Furthermore black cumin are much more affective against protein synthesis **(Menzel, *et al* 1987)** and nucleic acid **(Yordanova, *et al* 1996).**

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التباين الوراثى والكيماوى والنشاط المضاد للفيروس لأصناف حبة البركة

[٤]

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تعتبر حبة البركة من أهم النباتات الطبية المستخدمة في الطب الشعبي لإحتواءها على أحماض دهنية ومكونات غير متصبنة في البذور وتتضمن الدراسة ثلاث أصناف البلدي والسوري والتركي. وأظهرت النتائج اختلاف في الشكل الظاهري للنبات والتركيب الكيماوي للبذور وكذلك استخدام المستخلص المائي للبذور كمضاد للفيروس.

وتتلخص النتائج الدراسة فيما يلى

اولا: عكس التباين الوراثى بين الثلاث اصناف إختلاف ملحوظ فى الطول وعدد الكبسولات وعدد الافرع لكل نبات ولم تعطي النتائج اختلاف ملحوظ بالنسبة لارتفاع النبات وقطر الكبسوله وعدد الغرف فيها وكمية البذور الناتجة . حيث أظهرت النتائج تشابه بنسبة ٩٠% بين الصنفين البلدى والسورى .

ثانياً : أظهرت نتائج دراسة التباين الكيماوى اختلاف ملحوظ فى الأحماض الدهنية التى تم دراستها بالنسبة للثلاث أصناف .

ثالثاً : استخدام المستخلص المائى للبذور كمضاد للفيروس أحدث اختزال للإصابة إلى ٦,٧٣ ، ٦,٧٨ ، ٥,٠٨ % لكل من أصناف البلدى والسورى والتركى على التوالى وقد أظهرت نتائج دراسة خواص امتصاص الأشعة فوق البنفسجية للمستخلص الفيروسى المعالج بالمستخلص المائى لبذور حبة البركة اختلاف ناتج عن التباين الوراثى والتركيب الكيماوى للأحماض الدهنية كمضاد للفيروس .

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