

## 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 folk remedy. Balady, Sori and Turki cultivars belong to black cumin are varied 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-PAGE 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 varied based on heritability, 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 Ranun-

culuceae. The plant has a long history of folk medicine. The seeds are used as a carminative, diuretic and useful for asthma. Many authors studied the genetic var-

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iability 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**). Moreover, fatty acids methyl esters and sterols isolated 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, Faculty of Agriculture, Ain Shams Univ. maintenance on *Nico-*

*tina 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 cultivars were performed with Gas liquid chromatography (GLC) using Sp. 2310 Column 55% Cyanopropyl phenyl silicon dimensions 1.5 x 4 mm. The temperature program was 70°C (initial temperatures) at a rate of 8°C / min. to 190°C (final temperature). Injector and detector temperatures were maintained 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 determined on the base of the peak area. The

qualitative identification of fatty acids were achieved by comparing the retention time (Rt) of their expected authentic chromatographed under the same conditions.

### Electrophoretic analysis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) 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) polyacrylamide at pH 6.8. Samples (50µl) were denatured 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  $I = (1 - C/Co) \times 100$ , 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 spectrophotometrically 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



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

Morphological character	Cultivars			L.S.D (1%)
	Balady	Sori	Turki	
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 cultivars respectively. Stearic was found high relative percentage in Sori 2.78 followed by Balady 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 subcellular 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 analysis of fatty acids for three black cumin cultivars

Fatty acids component \ Cultivars	Balady	Sori	Turki
<b>Saturated fatty acids</b>			
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
<b>Unsaturated fatty acids</b>			
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

97.400

58.100

39.800

29.000

20.000

14.300

Figure 2. SDS-polyacrylamide gel (12.5%) electrophoresis of black cumin seeds polypeptides from three cultivars 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. **Babayán 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 regularly 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 re-

duction 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 nigelline (**Attar-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 authors (**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



**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 الوراثي والكيماوي والنشاط المضاد للفيروس لأصناف حبة البركة

[ ٤ ]

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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 للبدور 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 الفيروسي المعالج بالمستخلص المائى لبدور حبة البركة اختلاف ناتج عن التباين الوراثى والتركيب الكيماوى للأحماض الدهنية كمضاد للفيروس .

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

### وتتلخص النتائج الدراسة فيما يلى

**اولاً:** 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 الكبسولة وعدد الغرف فيها وكمية البذور الناتجة . حيث أظهرت

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