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ANTIFUNGAL ACTION OF SOME ESSENTIAL OILS AGAINST FUNGI CAUSING COTTON SEEDLING DAMPING – OFF DISEASE

[63]

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ABSTRACT

Antifungal activity of essential oils , from 8 plant namely, Dill (*Anethum graveolens*), Geranium (*Pelargonium graveolens*) , Coriander (*Coriandram sativum*), Sweet basil (*Ocimum basilicum*) , Thyme (*Thymus vulgaris*) , Marjoram (*Majorana horensis*), Mint (*Mentha peperita*) and Cumin (*Cuminum cyminum*) were evaluated against certain pathogenic fungi: *M.phaseolina* , *S.rolfsii* , *F.solani* , *R.solani* and *Pythium sp.* which causing cotton seedling damping – off disease. *In vitro* tests essential oils revealed antifungal activity against the tested fungi. Higher antifungal activities were recorded with thyme, cumin and mint essential oils .The mycelial growth of certain fungi were completely inhibited with cumin (*M.phaseolina* , *S.rolfsii* and *R.solani*) and mint (*S.rolfsii and Pythium sp.*) essential oils , whereas thyme caused complete inhibition of all the tested fungi . The vapours of the tested essential oils exhibited almost similar influence after different periods from incubation.Vapours of thyme, cumin and mint essential oils completely suppressed the mycelial growth of tested fungi, except with mint oil against *F. solani* which caused only 75.52% of inhibition. The influence of these oil vapours were continuously persisted until 10 days , however the influence of the others decreased gradually by time . The effective concentration (EC_{50} values) of thyme , cumin and mint oils as compared with rizolex –T50 indicated more efficiency of the fungicide than the three oils . On the other hand, they showed fungistatic action at all tested concentration against certain fungi according the applied method. However, the nature of action to the other fungi were fungistatic at lower concentration and fungicidal at higher concentrations. The evaluation of the efficiency of thyme, mint and cumin oil in controlling cotton seeding damping – off disease as compared with rizolex –T50 under green house conditions , revealed almost similar reduction effect in disease incidence , but lower than rizolex - T50 .

Key words: Essential oils, Antifungal activity, Cotton seeding, Damping– off disease

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INTRODUCTION

Fugitoxicants from higher plants possibility may have a better future over synthetic fungicides due to their largely non–phytotoxic, systemic and easily biodegradable nature. So, the presence of naturally occurring substances in plants with antifungal properties have been recognized and tested against a wide range of fungi infecting many crops and commercially important plants (i.e. **Ross *et al* 1980; Tapas & Chitreshwar, 1982; Shetty *et al* 1989; Snigh & Dwivedi, 1990; Chauhan & Singh, 1991; Zedan, 1993; Baiuomy, 1997; Wilson *et al* 1997; Bowers & Locke, 2000 a & b and Abdel Ghafour, 2000**).

Attempts have been made by several research groups to isolate and identify the individual constituents present in the plant and relate the chemical composition to fungal activity. (**McDowell *et al* 1988**). In many plants exhibiting biological activities, the greatest antimicrobial power lies with the essential or volatile oil fraction (**Singh *et al* 1983; Shukla & Tripathi, 1987; Deans, 1991 and El-Shazly, 2000**).

Use of volatile oils as fungicides against field crop pathogens has not received widespread acceptance. Despite that laboratory tests, inhibited growth of many phytopathogenic fungi. Hence, the present investigation aimed to evaluate the antifungal activity of 8 essential oils against 5 fungi causing damping- off of cotton seedlings. The nature of toxicity and the efficiency of thyme, mint and cumin oils against those fungi was studied.

MATERIAL AND METHODS

Pathogenic fungi

Diseased cotton seedlings, showing damping- off symptoms were collected from Qaluobia governorate, accompanied fungi were isolated. Pathogenicity tests were carried out using cotton variety, "Giza- 80". The pathogenic fungi were reisolated and identified, *M.phaseolina*, *S.rolfsii*, *F.solani*, *R.solani* and *Pythium sp.*, according to **Clements & Shear, 1957; Barnett, 1960 and Nelson *et al* 1983**. Pure isolates were kept on PDA slant till needed.

Antifungal activity of the tested essential oils

Samples of 8 plant essential oils namely Dill (*Anethum graveolens*), Geranium (*Pelargonium graveolens*), Coriander (*Coriandram sativum*), Sweet basil (*Ocimum basilicum*), Thyme (*Thymus vulgaris*), Marjoram (*Majorana horensis*), Mint (*Mentha peperita*) and Cumin (*Cuminum cyminum*) were obtained from Sector of Perfume and Additives, EL-Hawamdia Sugar Company. Evaluation tests of the oils and their vapours, as natural inhibitors for the mycelial growth of 5 fungi causing damping- off of cotton seedlings were carried out under laboratory conditions. Paper disc technique was used as follows:

Plates containing PDA medium were inoculated with discs (5mm) in diameter of each fungus. Two impregnated paper discs (6mm) with 10 ul of each essential oil were placed at the two sides of fungal disc, and were incubated at 25±2°C. Plates without essential oils were served as control. Following incubation for the

time of completely full growth of the control the percentage inhibitions were calculated. For determination the inhibition activity of the tested essential oils vapours, three impregnated paper discs (6mm) with 20 ul of each essential oil were placed on center lid of the inoculated Petri dishes. The two bases of Petri dishes were taped together with parafilm and inverted, then incubated at 25 ± 2°C. The extent of fungal inhibition by vapours from the discs was determined after 3,6 and 10 days from inoculation. The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = A - B / A \cdot 100$$

Where: A= the linear growth of the fungus in the control.
B= the liner growth of the fungus in the treatment.

Determination of the effective concentration of the most efficient oils

Effective concentrations (EC_{50}) of the most efficient oils (i.e. thyme, mint and cumin) as will as the fungicide rizolex-T50 were determined against the 5 tested fungi. Adequate volumes of each essential oil solved in dimethyl sulfoxide were incorporated with liquid PDA medium to give alternate 5 concentrations (50, 75, 100, 150 and 200 ppm for thyme essential oil and 250, 300, 350.400 and 450 ppm for mint and cumin essential oils) The used concentrations of rizolex- T50 were 4, 8, 20, 50, 100 and 200 ppm. Discs (5mm) of each fungus taken from the margin of 5 days old cultures were used for inoculations of the treated and untreated plates, and were incubated at 25 ± 2°C. Five replicates were specified for each treatment. The percentage inhibitions were calculated at the time of completely full growth of the control. The standard probit method for analysis data of the bioassay experiments was adapted to represent the obtained results (**Finny, 1972**). Value of EC_{50} and the slope of inhibition line were calculated for each essential oil as will as rizolex –T50.

Nature of the toxicity action (fungistatic / fungicidal) of thyme, mint and cumin oils

The nature of toxicity – fugistatic / fungicidal of thyme, mint and cumin oils were tested against the 5 tested fungi. Groups of Petri – dishes containing incorporated PDA medium with three concentrations of each oil (thyme at 300, 500 and 1000 ppm & mint and cumin at 500, 1000 and 2000) were prepared. Discs (5mm) in diameter of each fungus were inoculated with treated and untreated (control) plates (4 replicates) and incubated at 25 ± 2°C for 7 days. The complete full growth (%100) of the treated plates were recorded and not growing discs (inhibited) were reinoculated with new plates of PDA medium and incubated at 25 ± 2°C for another 7 days. The nature of the toxicity was considered as fungistatic action (S) for the completely growing fungus and fungicidal action (C) for not growing fungus.

Effect of seed treatment with essential oils (thyme, mint and cumin) and fungicide rizolex-T50 on cotton damping – off disease

Essential oils, thyme, mint and cumin, as will as rizolex-T50 (recommended fungicide against cotton root – rot fungi) as a seed treatment was evaluated for the

control of pre and post emergence damping - off of cotton seedlings were evaluated under green house conditions. Each fungal inoculum was prepared by growing it on corn meal sterilized medium in bottles, then left for 15 days at lab temperature, 25 ± 2°C. Pots (25cm) with sterilized sandy loam soil (1:1) were infested with each fungus (3%w/w), mixed thoroughly with upper surface, irrigated and were left for 7 days to ensure even and distribution of the inoculum. Surface disinfected cotton seeds (by immersing in 5% sodium hypochlorite for 3 mints.) were treated with each essential oil, 5% powder at the rate of 15 g / kg seed and rizolex-T50 at the rate 3g/kg seeds. The required amount of seeds in a plastic container, were sprayed with sterilized water provided with few drops Arabic gum as sticker. The treated seeds were planted in artificially infested soil at the rate of 5 seeds / pot (4 replicates). Percentage of pre – and post emergence damping–off were estimated after 15 and 45 days from planting, respectively. The untreated seeds were planted as mentioned before. The obtained data Statistically analyzed using ANOVA method.

RESULTS AND DISCUSSION

Effect of essential oils on mycelia growth

Data in Table (1) indicate that all the tested essential oils had anti - fungal activity against all tested fungi. However the response of the tested fungi varied according to the nature of the essential oils. The highest inhibition activity against mycelial growth of the tested fungi were recorded with thyme, cumin and mint essential oils.

Table 1. Effect of certain essential oils on the inhibition (%) of mycelial growth of five fungi causing cotton seedling damping – off disease.

Tested oils	% inhibition				
	M. phaseolina	*S. rolfsii*	*F. solani*	*R. solani*	*Pythium sp.*
Dill	29.71	34.16	14.28	38.05	42.21
Geranium	34.16	61.94	38.90	57.77	53.60
Coriander	59.72	100.00	21.27	63.05	38.04
Sweet basil	39.71	76.38	24.92	76.10	53.60
Thyme	100.00	100.00	100.00	100.00	100.00
Marjoram	41.94	85.83	25.83	73.88	60.83
Mint	79.99	100.00	35.25	88.05	100.00
Cumin	100.00	100.00	50.27	100.00	81.94

Sig., at 0.05 level for essential oils = 0.019

Thyme essential oil completely inhibited mycelial growth of tested fungi. Cumin essential oil caused 100% inhibition for *M. phaseolina*, *S. rolfsii* and *R. solani*, 81% for *Pythium sp.*, and 50.27% for *F. solani*. Mint oil caused 100% inhibition for *S. rolfsii* and *Pythium sp.*, 88.05% for *R. solani*, 79.99% for *M. phaseolina* and 35.25% for *F. solani*. Some of the tested oils recorded considerable inhibition activity against certain fungi such as coriander essential oil against *S. rolfsii*, *R.solani* and *M. phasolina*, sweet basil oil against *S. rolfsii*, *R. solani* and *Pythim sp.* and marjoram oil against *S. rolfsii*, *R. solani* and *Pythium sp.* Considering the effect of the tested essential oil vapours after different incubation periods on mycelial growth.

Data in Table (2) clearly indicate again that the tested fungi were sensitive to the essential oils, but in a variable degrees due to the nature of the essential oil and the incubation period. Vapours of thyme and cumin essential oils completely suppressed mycelial growth (100% inhibition) of the tested fungi, after three days from incubation .Vapours of mint essential oil exhibited similar influence, except against *F. solani* (75.525% inhibition). The antifungal activity of thyme oil did not degrade by increasing time of incubation and stay persist until 10 days from incubation against the tested fungi. Cumin and mint essential oils showed similar activity against *S. rolfsii*. However, the antifungal activity for the two oils against *M. phasolina*, *F. solani* and *Pythim sp.* were decreased gradually. In general it is clearly observed that other essential oil vapours recorded sufficient activity against few fungi, especially sweet basil oil against *S. rolfsii*, *F. solani*, *R. solani* and *Pythim sp.* The other tested essential oil vapours caused different descending inhibition effect against the tested fungi by collapsing time.

Reviewing the obtained data it could be concluded that thyme, cumin and mint essential oils were the most efficient oil sources against the tested fungi. These results were in harmony with **Saksena and Tripathi, 1985; Wilson *et al* 1987; Agha, 1992; Zedan *et al* 1994; El-Shazly, 1996; Zambonelli *et al* 1996; Baiuomy, 1997; Jaspal & Tripathi, 1999 and El-Shazly, 2000.** The used essential oils differed in their action against the tested fungi. This may be due to the present of phenolic compounds and other inhibitory substances at different degrees in these oils. The antifungal activity of thyme essential oil was mainly attributed to carvacrol and thymol compounds **(Agarwal and Mathela, 1979 and Nachman *et al* 1994)**. The antifungal activity of mint essential oil attributed to menthol, isomenthol and sabinene, whereas the cumin aldehyde in cumin essential oil was the active antifungal agent **(Linskens and Jackson, 1991)**. The high antifungal activity of thyme oil against the tested pathogenic fungi is probably a result of chitin penetration of the hyphal wall which damage the lipoprotin cytoplasmic membrane, leading to escape of cytoplasm **(Zambonelli, *et al* 1996)**. On the other hand, **Zedan, *et al* 1994,** reported that the fungicidal activity of essential oil against pathogenic fungi may be due to permeability increase of the cell of pathogens or due to essential oil which inhibit the fungal detoxification enzymes of antifungal compounds in the essential oil.

Table 2. Effect of certain essential oils vapours on the inhbition (%) of the mycelial growth of five fungi causing cotton seedling amping–off disease .

Tested oils	% inhibition after different days														
	M. phaseolina			*S. rolfsii*			*F. solani*			*R. solani*			*Pythium sp.*		
	3	6	10	3	6	10	3	6	10	3	6	10	3	6	10
Dill	23.05	0.00	0.00	88.45	69.71	14.44	19.99	16.70	3.05	58.88	6.10	0.97	46.30	6.10	0.00
Geranium	1.24	0.00	0.00	100.00	100.00	100.00	57.69	60.45	61.94	89.58	50.55	1.94	76.13	35.69	8.88
Coriander	68.32	0.00	0.00	100.00	100.00	100.00	100.00	74.55	31.38	96.38	51.10	19.99	78.33	26.52	0.00
S. basil	87.49	13.60	0.00	100.00	100.00	100.00	100.00	84.31	26.49	100.00	100.00	100.00	100.00	100.00	100.00
Thyme	100.00	100.0	100.0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Marjoram	98.05	26.38	3.33	100.00	100.00	100.00	87.41	69.30	36.24	100.00	100.00	100.00	100.00	94.58	33.74
Mint	100.00	89.99	2.22	100.00	100.00	100.00	75.52	69.82	34.16	100.00	100.00	100.00	100.00	98.05	81.55
Cumin	100.00	90.55	10.41	100.00	100.00	100.00	100.00	91.89	78.33	100.00	100.00	64.99	100.00	89.44	33.05

Sig , at 0.05 level for:

Essential oils = 0.000

Tested fungi = 0.000

Time = 0.000

Essential oils* Fungi = 0.019

Effect of essential oils on cotton damping - off as compared by rizolex-T50

Data concerning the effect on the tested essential oils and rizolex-T50 against cotton seedling damping – off are summarized in Table (3). Comparison of the EC_{50} values clearly indicated that the sensitivity of all fungus to the tested essential oils were varied than rizolex–T50.In terms of figures, rizolex – T50 revealed the lowest EC_{50} values 12.00, 9.20, 55.40, 9.50 and 11.20 ppm against *M. phaseolina, S. rolfsii, F. solani, R. solani* and *Pythium sp.*, respectively.

Table 3. Efficiency of certain essential oils and rizolex-T50 against the growth of five fungi causing cotton seedling damping–off.

Essential oils	*M. phaseolina*		*S. rolfsii*		*F. solani*		*R. solani*		*Pythium* sp.	
	EC_{50} (ppm)	Slope (b)	EC_{50} (ppm)	Slope (b)	EC_{50} (ppm)	Slope (b)	EC_{50} (ppm)	Slope (b)	EC_{50} (ppm)	Slope (b)
Thyme	93.20	3.72	90.50	4.70	112.30	4.08	110.0	4.16	92.00	4.68
	4.02		4.00		3.31		3.38		3.81	
Cumin	352.00	12.33	355.00	12.80	372.00	15.00	357.00	16.20	351.00	12.56
	1.06		1.02		1.00		1.04		1.00	
Mint	375.00	17.20	362.00	15.20	352.00	21.10	372.00	15.67	351.00	16.96
	1.00		1.00		1.05		1.00		1.00	
Rizolex-T50	12.00	1.05	9.20	1.161	55.40	1.12	9.50	1.685	11.2	1.380
	31.25		39.34		6.71		39.12		31.33	

The underlined figure = Relative efficiency

These data proved that rizolex–T50 was more efficient against all fungi. The higher relative efficiency values of rizolex–T50 against the most tested fungi confirmed such finding. The relative efficiency values ranged from 31.25 to 39.34 times than mint essential oil against the tested fungi, except with *F. solani* (6.71 times) indicating more tolerance of the fungus to rizolex–T50. On the other hand, the effective concentrations of thyme essential oil against the same fungi ranged from 90.50 to 112.30 ppm, whereas they were 351-375 ppm for cumin and mint essential oils. These data showed more antifungal activity of thyme oil than mint and cumin oils, which revealed similar efficiency against the tested fungi.

Superiority of the antifungal effect of thyme essential oil were reported by **Singh *et al* 1983; Nachman *et al* 1994; Baiuomy, 1997 and Abdel Ghafor, Siham, 2000.**

Nature of the toxic action (fungistatic / fungicidal) of tested essential oils

Data in Table (4) clearly indicated that thyme, cumin and mint essential oils exhibited different nature of toxic action against the tested fungi due to the genera of fungi and the strength of concentration. The tested oils showed fungistatic action with all concentrations against *Pythium sp.*, whereas mint and cumin essential oils showed the same action against *M. phaseolina, F. solani*. The nature of the toxic action of the tested oils against the other fungi were changed from the fungistatic at lower concentrations to fungicidal action at higher concentrations. This finding is in agreement with **Singh *et al* 1983** who reported that mentha oil exhibited fungicidal nature of its minimum inhibitory concentration . Also , **Dube *et al* 1991** found that essential oils from seeds of *Apium graveolens, Cuminum cyminum* and *Zanthoxylum alatum* were fungistatic at low doses and fungicidal at high doses to aflatoxin -producing strains of *A. flavus and A. parasiticus.*

Table 4. Fungistatic / Fungicidal action (100% inhibition) of the tested oils against five fungi causing cotton seedling damping – off disease.

Oils	Concent. (ppm)	*M. phasolina*	*S. rolfsii*	*E. solani*	*R. solani*	*Pythium sp.*
Thyme	300	S	S	S	S	S
	500	C	C	S	C	S
	1000	C	C	C	C	S
Cumin	500	S	S	S	S	S
	1000	S	C	S	S	S
	2000	S	C	S	C	S
Mint	500	S	S	S	S	S
	1000	S	C	S	S	S
	2000	S	C	S	C	S

S : Fungistatic action C : Fungicidal action

Effect of essential oils and rizolex T50 as seed treatment on 5 fungi causing cotton seedling damping – off

The role of essential oils and rizoles–T50 in minimizing the percentage of pre- and post emergence cotton damping- off disease were presented in Table (5). All treatments decreased the percentage of disease incidence caused by the tested fungi as compared with the control, untreated infested soil except with *F. solani*. Seed treatment with rizolex- T50 caused higher reduction in the percentage of damping–off than the most tested essential oils. Only cumin essential oil caused

higher reduction against *F. solani* than rizolix–T50. Generally, the tested essential oils revealed similar reduction effect in disease incidence, but lower than rizolex-T50. This finding is in agreement with **Deans, 1991** who reported that there are a number of problems must be addressed prior the general use of essential oils as crop protection agents. In the contrary, **Singh *et al* 1983** found that dementhaloised oil was effective against *Helminthosporium oryzae* than commercial fungicides. Also **El-Shazley, 2000** who, reported that thyme and blue gum showed the best results in minimizing the pre-and post emergence damping off as compared with another essential oils and fungicides.

Table 5. Effect of seed treatment with certain essential oils and the fungicide rizolex-T50 on fungi causing cotton seedling damping – off disease.

Fungi	Treatment	Disease incidence (%)			Survived (%)
		Pre-emergence	Post - emergence	Total	
M. phaseolina	Thyme oil	33.30	15.23	48.53	51.47
	Cumin oil	45.80	5.56	51.36	48.64
	Mint oil	41.60	7.60	49.20	50.80
	Rizolex- T50	16.60	10.00	26.60	73.40
	Control	45.80	15.30	61.10	38.90
S. rolfsii	Thyme oil	44.40	15.73	60.13	39.87
	Cumin oil	58.30	5.00	63.30	36.70
	Mint oil	54.10	15.30	69.40	30.60
	Rizolex- T50	54.10	11.70	65.80	34.20
	Control	62.50	22.20	84.70	15.30
F.solani	Thyme oil	47.16	12.50	59.66	40.34
	Cumin oil	41.63	10.53	52.16	47.84
	Mint oil	54.10	8.80	62.90	37.10
	Rizolex- T50	45.80	15.30	61.10	38.90
	Control	58.30	00.00	58.30	41.71
R. solani	Thyme oil	50.00	14.16	64.16	35.84
	Cumin oil	45.00	10.00	55.00	45.00
	Mint oil	60.00	16.60	76.60	23.40
	Rizolex- T50	35.00	00.00	35.00	65.00
	Control	50.00	25.00	75.00	25.00
Pythium sp.	Thyme oil	50.00	8.30	58.30	41.70
	Cumin oil	52.00	5.80	57.80	42.20
	Mint oil	54.10	4.55	58.65	41.44
	Rizolex- T50	25.00	5.50	30.50	69.50
	Control	41.65	21.40	63.05	36.95

L.S.D for rizolex – T50 against *R. solani* at 5% = 1.16

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التأثير المضاد الفطري لبعض الزيوت الطيارة تجاه الفطريات المسببة لمرض سقوط بادرات القطن

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تم تقييم النشاط المضاد الفطري لثمانية زيوت نباتية طيارة هي الشبت ، العتر ، الكسبرة، الريحان ، الزعتر ، البردقوش ، النعناع الفلفلي والكمون تجاه الفطريات الممرضة *M.phaseolina, S. rolfsii, F. solani , R. solani , Pythium sp.* المسببة لعفن بذور وموت بادرات القطن.

وقد أظهرت كل الزيوت المختبرة نشاطا مضادا تجاه الفطريات المختبرة، ولكن بدرجات متباينة توقفت علي طبيعة الزيت وجنس الفطر. سجلت زيوت الزعتر والنعناع والكمون أعلي نشاطا مضادا للفطريات حيث سببت زيوت الكمون والنعناع تثبيطا كليا للنمو الميسليومي لبعض الفطريات ، بينما سبب زيت الزعتر تثبيطا كليا لجميع الفطريات المختبرة وأيضا فإن أبخرة الزيوت المختبرة قد احدثت تأثيرا متشابها بعد فترات مختلفة من التحضين. تسببت أبخرة زيوت الزعتر والكمون والنعناع في إيقاف كامل للنمو الميسليومي لجميع الفطريات ، ماعدا زيت النعناع تجاه فطر *F. solani* والذي تسبب فقط في تثبيط مقداره ٧٥,٥٢%. هذا وقد استمر النشاط المضاد لأبخرة هذه الزيوت ثابتا حتي ١٠ أيام ، وذلك بالرغم من أن تأثير بعض الزيوت الأخري تناقص تدريجيا بمرور الوقت . وقد أظهرت قيم التركيز الفعال (EC_{50}) لزيوت الزعتر و النعناع والكمون عند مقارنتها بالمبيد الفطري ريزولكس - ت٥٠ فعالية أكثر للمبيد عن الثلاث زيوت السابقة. ومن ناحية اخري فإن هذه الزيوت قد أظهرت تأثيرا مانعا للنمو (Fungistatic) بكل التركيزات المختبرة تجاه بعض الفطريات ، بينما كانت طبيعة التأثير مانعة للنمو فقط بالتركيزات المنخفضة ، وإبادية بالتركيزات العالية تجاه فطريات أخري وذلك طبقا لطريقة الاختبار المتبعة ، وقد أظهر تقييم الفعالية للزيوت الثلاثة في مكافحة مرض عفن بذور وسقوط بادرات القطن مقارنة بمبيد الريزولكس - ت٥٠ تحت ظروف الصوبة أن للزيوت الثلاثة تأثيرا متشابها في اختزال حدوث المرض ، ولكن بدرجة أقل من المبيد.

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