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Studies on essential oils, Part 41. Chemical composition, antifungal, antioxidant and sprout suppressant activities of coriander (*Coriandrum sativum*) essential oil and its oleoresin

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ABSTRACT: Gas chromatography–mass spectrometry (GC–MS) analysis of the seed oil of coriander (*Coriandrum sativum*) showed the presence of 52 components, accounting for 98.45% of the total oil. The major components were linalool (75.30%), geranyl acetate (8.12%) and α-pinene (4.09%). Its oleoresin showed the presence of 28 components. Oleic acid (36.52%), linoleic acid (33.2%) and palmitic acid (11.05%) were the major components. The antifungal activities of coriander oil and its oleoresin against eight fungi were evaluated using the inverted petriplate and food poison techniques. Using the inverted petriplate method, the essential oil was found to be highly active against *Curvularia palliscens*, *Fusarium oxysporum*, *Fusarium moniliforme* and *Aspergillus terreus.* In the case of the oleoresin, more than 50% mycelial zone inhibition was obtained for the fungi *Fusarium oxysporum*, *Aspergillus niger* and *Aspergillus terreus*. Using the food poison technique, the essential oil showed 100% inhibition on the growth of *A. terreus*, *A. niger*, *F. graminearum* and *F. oxysporum*, whereas its oleoresin showed weaker fungitoxic activity, exhibiting 100% inhibition on the growth of *F. oxysporum* only. The oleoresin had a better antioxidant activity for sunflower oil than essential oil and synthetic antioxidants, BHA and BHT. The oil was also found to exert strong sprout suppressant activity (SSA) for potatoes. Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: *Coriandrum sativum*; essential oil; antioxidant activity; antifungal activity; sprout suppressant activity; oleoresin

Introduction

The growing interest in the substitution of 'traditional food preservatives', both antimicrobials and antioxidants, by natural ones has fostered research on vegetable sources and the screening of plant materials. It has been known since ancient times that spices and their essential oils have varying degrees of antimicrobial activity.[1–4] The major antimicrobial components of spices include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, carvacrol and thymol in oregano and thyme, and vanillin in vanilla beans. The antimicrobial activity of some essential oil components against food-borne pathogens, including mycotoxin-producing fungi, has also been tested.[5–7] More recently, plant extracts have also been developed and proposed for use in foods as natural antioxidants[8–10] and/or antimicrobials.[11–12] These findings prompted us to explore the coriander (*Coriandrum sativum*) essential oil and oleoresin, which could be exploited as natural food preservatives.

C. sativum (family Umbelliferae) is a native of the Mediterranean region and is extensively grown in India, Russia, Central Europe, Asia and Morocco. It is extensively cultivated in Deccan. The stem, leaves and fruits have a pleasant aromatic odour. The plant when young is used in preparing chutneys and sauces. The fruits are extensively employed for preparation of curry powder, pickling spices, sausages and seasonings. They are also used for flavouring pastry, cookies, buns and tobacco products. Coriander oil is a pale yellow liquid with a characteristic odour. Besides the essential oil, the seeds contain 19–21% of fatty oil having a dark, brownish-green colour and an odour similar to that of coriander seeds.[13] The oleoresin contains the volatile oil, non-volatile pungent principles, pigments, fats and other substances derived from seeds. The volatile oil isolated from coriander leaves has been found to be a potent source of fungicide.[14–16] Very little work has been reported on the chemistry[17,18] and activity[19–22] of the seed volatile oil of this plant, or on its oleoresin.[23–25] In continuation of our research programme on essential oils,[19,26–29] investigations of the chemical, antifungal, antioxidant and sprout-suppressant activities (SSA) of the seed essential oil and its oleoresin have been undertaken and the results are reported in this communication.

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Materials and Methods

Plant Material

Coriander seeds were purchased from the local market of Gorakhpur in India and voucher specimens were deposited at the Herbarium of the Science Faculty of the DDU Gorakhpur University, Gorakhpur, India.

Isolation of the Oil

Thoroughly washed and powdered coriander seeds (20 g) were hydrodistilled using a Clevenger-type apparatus for 6 h, in accordance with *European Pharmacopoeia* procedure.[30] Yellow oil (yield 2.2%) was obtained. It was dried over anhydrous sodium sulphate to remove traces of moisture and stored in a refrigerator in the dark at 4 °C.

Isolation of the Oleoresin

After extraction of the essential oil, the powdered seeds were dried in incubator at 40 °C. Oleoresin was obtained by extracting 20 g dried powdered seeds with 900 ml acetone for 6 h in a Soxhlet apparatus. The extract liquid was then concentrated to 20 ml at 60 °C. The remaining acetone was evaporated to dryness by placing the samples in a vacuum drier under reduced pressure and the oleoresin was stored in a freezer until use.

Chemical Investigations

Qualitative and quantitative analysis of essential oil and oleoresin were undertaken by gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS) techniques.

Gas Chromatography

Gas chromatograms of the oil and oleoresin were obtained using a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) and a silica column. The column was an HP-5 (30 m × 0.32 mm i.d.) whose injector and detector temperatures were maintained at 250 °C and 300 °C, respectively. The amount of the samples injected was 0.1 μl (in split mode, 80:1). The carrier gas was helium at a flow rate of 1.0 ml/min. The oven temperature was programmed as follows: 60 °C for 1 min, rising at 1 °C/min to 75 °C, held for 2 min, again rising at 0.5 °C/min to 81 °C, held for 2 min, and again rising at 3 °C/min to 180 °C and held for 7 min.

Gas Chromatography–Mass Spectrometry

The essential oil and oleoresin were subjected to GC–MS analysis using a Hewlett-Packard mass detector (Model 5973) and a HP-5MS column (30 m × 0.32 mm, film thickness 0.25 μm). The injector, GC–MS interface, ion source and selective mass detector temperatures were maintained at 270 °C, 280 °C, 230 °C and 150 °C, respectively. The oven temperature for the essential oil analysis was programmed as follows: 60 °C for 1 min, rising at 1.5 °C/min to 185 °C, then at 9 °C/min to 275 °C, then held for 2 min. The oven temperature for the oleoresin analysis was programmed as follows: 100 °C, rising at 5 °C/min to 280 °C, then held for 20 min.

Identification of Components

The oil and extract components were identified on the basis of comparison of their retention indices and mass spectra with published data[31,32] and computer matching with the WILEY 275 and National Institute of Standards and Technology (NIST 3.0) libraries provided with the computer controlling the GC–MS system. The results of the essential oil and extract analyses, given in Tables 1 and 2, respectively, were also confirmed by comparing the order of elution of the compounds with their relative retention indices in non-polar phase, as reported in the literature. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes, C_8–C_{16}.

Antifungal Investigations

The antifungal activity of the essential oil and oleoresin against the pathogenic fungal species *Aspergillus terreus* (AT), *Aspergillus niger* (AN), *Aspergillus flavus* (AF), *Trichothecium roseum* (TR), *Fusarium graminearum* (FG), *Fusarium oxysporum* (FO), *Fusarium moniliforme* (FM) and *Curvularia palliscens* (CP) were evaluated using the inverted petriplate and food poison techniques.[33,34] The fungal strains were collected from Microbial Type Culture Collection (MTCC), Chandigarh, India. The cultures of each of the fungi were maintained on Czapek (DOX) agar media, with the pH adjusted to 6.0–6.5, and the slants were stored at 5 °C. In the inverted petriplate method, the required doses (2, 4 and 6 μl) of undiluted sample were soaked on a small piece (diameter 12 mm) of Whatmann No. 1 filter paper and this was kept on the lid of the petriplate, which was in the inverted position, while in the food poison technique the required doses (2, 4 and 6 μl) of the undiluted sample were mixed with 20 ml culture medium. Each test was replicated three times. The toxicity was calculated in terms of percentage mycelial inhibition, using the following formula:

Table 1. Chemical composition of coriander essential oil

Compound	MS (%)	KI[a]
3-Methylbutanal	tr	—
α-Thujene	tr	0931
α-Pinene	4.09	0941
Camphene	0.05	0953
Verbenene	tr	0962
Sabinene	0.24	0975
β-Pinene	0.86	0980
6-Methyl-5-hepten-2-one	tr	0984
Myrcene	0.32	0993
α-Phellandrene	0.07	1007
δ-3-Carene	tr	1013
α-Terpinene	tr	1020
p-Cymene	0.45	1026
Limonene	0.63	1031
1,8-Cineole	0.43	1035
cis-β-Ocimene	tr	1040
trans-β-Ocimene	0.06	1050
γ-Terpinene	0.65	1064
1-Octanol	0.11	1072
cis-Linalool oxide	0.51	1076
Terpinolene	0.20	1088
trans-Linalool oxide	tr	1088
Linalool	75.30	1099
α-Campholene aldehyde	tr	1127
trans-Pinocarveol	0.08	1140
Camphor	0.14	1144
Citronellal	0.09	1156
Borneol	0.30	1167
Terpinen-4-ol	0.18	1147
α-Terpineol	0.42	1189
Verbenone	0.13	1205
Nerol	0.17	1230
Citronellol	0.10	1230
Cuminal	0.58	1246
Geraniol	0.81	1253
Undecanal	0.13	1308
Citronellyl acetate	0.29	1354
Neryl acetate	0.14	1366
α-Ylangene	tr	1377
Geranyl acetate	8.12	1382
β-Caryophyllene	0.39	1420
α-Humulene	0.09	1458
ar-Curcumene	0.11	1482
α-Zingiberene	0.14	1495
β-Bisabolene	0.09	1508
δ-Cadinene	tr	1524
β-Sesquiphellandrene	0.17	1526
Elemol	0.09	1550
Caryophyllene oxide	0.05	1583
ar-Turmerone	0.62	—
β-Turmerone	0.57	—
α-Turmerone	0.33	—
Total	98.45	

Percentages were obtained from electronic integration measurements using a selective mass detector.
[a] Retention indices were calculated using a homologous series of *n*-alkanes (C_8–C_{16}).
tr, trace < 0.05%.

$$\text{Mycelial zone inhibition (\%)} = \frac{dc - dt}{dc} \times 100$$

where *dc* and *dt* are average diameters of the mycelial colonies of the control and treated sets, respectively.

Table 2. Chemical composition of coriander oleoresin

Compound	MS (%)	KI[a]
Geranyl acetate	1.03	0712
Dodecanal	0.36	0763
β-Caryophyllene	tr	0795
Isovanillin	tr	0801
Undecanoic acid	tr	0902
ar-Curcumene	tr	0916
β-Bisabolene	tr	0997
β-Sesquiphellandrene	tr	1001
2(*E*)-Dodecenal	2.05	1096
β-Turmerone	0.91	1301
α-Turmerone	0.25	1376
5-Phenyl dodecane	tr	1424
4-Phenyl dodecane	tr	1433
Decyl-cyclopentane	0.12	1480
Myristic acid	2.04	1543
Pentadecanoic acid	0.03	1722
Palmitic acid	11.05	1981
Linoleic acid	33.21	2380
Oleic acid	**36.52**	2775
Octyl stearate	0.30	3214
Squalene	0.14	3284
Nonacosane	0.19	3379
α-Tocopherol	tr	3693
Ergost-5-en-3-ol	tr	3832
Stigmasterol	0.42	3885
γ-Sitosterol	0.53	3991
β-Amyrin	0.25	4045
Stigmast-4-en-3-one	tr	4271
Total	89.40	

Percentages were obtained from electronic integration measurements using a selective mass detector.
[a] The retention indices are calculated using a homologous series of *n*-alkanes (C_8–C_{16}).
tr, trace < 0.10%.

The results recorded by the inverted petriplate and food poison techniques are shown in Tables 3 and 4, respectively.

Antioxidant Activity

In order to assess the antioxidant activity[35–37] of coriander oil and oleoresin, refined sunflower oil without any antioxidant (Custom Laboratory, New Costom House, Mumbai) was used for the present investigation. The oil, having an initial peroxide value of 2.3 meq/kg, was selected for the present investigation due to its high degree of unsaturation and general use as an edible oil.

Peroxide Value (PV) Method

For measuring the peroxide value[38,39], the modified oven test[40] was used. The antioxidant activities of the essential oil and oleoresin were compared with synthetic antioxidants, such as BHA and BHT. The calculated quantities of essential oil and oleoresin (200 ppm) were added

Table 3. Antifungal investigations of coriander oil and its oleoresin by the inverted petriplate method

Fungus	Mycelial zone inhibition (%) at different doses of oil*					
	Coriander essential oil			Coriander oleoresin		
	2 µl	6 µl	10 µl	2 µl	6 µl	10 µl
Aspergillus terreus (AT)	18.7	36.5	75.0#	25.0	50.0	62.5#
Aspergillus niger (AN)	12.5	18.8	37.5	18.8	18.8	50.0#
Aspergillus flavus (AF)	12.5	25.0	31.3	12.5	18.8	37.5
Trichothecium roseum (TR)	6.3	12.5	18.5	6.3	25.0	31.3
Fusarium graminearum (FG)	6.3	12.5	18.5	12.5	25.0	25.0
Fusarium oxysporum (FO)	25.0	31.3	70.0#	6.3	25.0	50.0#
Fusarium moniliforme (FM)	12.5	37.5	75.0#	12.3	18.8	25.0
Curvularia palliscens (CP)	18.8	50.0#	87.5#	6.3	12.5	18.8

* Average of three replicates.
# Statistically significant ($p < 0.05$) when single way analysis of variance was used for locating significant differences within different doses.

Table 4. Antifungal investigations of coriander oil and its oleoresin by the food poison technique

Fungus	Mycelial zone inhibition (%) at different doses of oil*					
	Coriander essential oil			Coriander oleoresin		
	2 µl	6 µl	10 µl	2 µl	6 µl	10 µl
Aspergillus terreus (AT)	25.0	75.0#	**100**#	12.5	18.8	31.3
Aspergillus niger (AN)	50.0	**100**#	**100**#	6.3	25.0	31.3
Aspergillus flavus (AF)	18.8	31.3	75.0#	6.3	25.0	50.0#
Trichothecium roseum (TR)	25.0	50.0	70.0#	6.3	12.5	18.8
Fusarium graminearum (FG)	75.0	**100**#	**100**#	25.0	31.3	75.0#
Fusarium oxysporum (FO)	31.3	75.0#	**100**#	18.8	50.0	**100**#
Fusarium moniliforme (FM)	18.8	50.0	75.0#	12.5	31.3	75.0#
Curvularia palliscens (CP)	31.3	50.0	87.5#	31.3	50.0	75.5#

* Average of three replicates.
# Statistically significant ($p < 0.05$) when single way analysis of variance was used for locating significant differences within different doses.

to 30 g sunflower oil in an open-mouthed beaker. The mixtures were thoroughly homogenized and placed into a thermostatted oven at 80 °C.

The peroxide values of all samples were determined[38,39] after 7, 14, 21 and 28 days. For this purpose, a known weight of sample (1 g) was dissolved in a mixture of CH_3COOH:$CHCl_3$ (3:2 v/v) and then a saturated solution of potassium iodide (1 ml) was added. The liberated I_2 was titrated against sodium thiosulphate (0.1 N), using starch as an indicator. A blank titration was also run parallel to the treated samples and the peroxide value (meq/kg) was calculated using the formula:

$$\text{Peroxide value (meq/kg)} = 1000SN/W$$

where S = volume (ml) of sodium thiosulphate used (blank corrected), N = normality of sodium thiosulphate used (viz. 0.01 N) and W = weight of sample (g).

Thiobarbituric Acid Value (TBA)

The test was performed according to the methods previously reported,[41,42] with small changes. Samples of sunflower oil with additives (essential oil, extract, BHA and BHT) similar to those prepared for the peroxide value method were used; 10 g sample was mixed with 0.67% aq. thiobarbituric acid (20 ml) and benzene (25 ml) solution. The mixture was shaken continuously for 2 h using a mechanical shaker. After 2 h, the supernatant layer was placed in a boiling waterbath for 1 h. After cooling, the absorbance of the supernatant layer was measured at 540 nm, using a Hitachi-U-2000 spectrophotometer. The TBA value (meq/g) was calculated using the following formula:

$$\text{TBA value} = \frac{3.5 \times \text{OD}}{0.15 \times \text{W}}$$

Table 5. Sprout inhibition activity (%) of coriander essential oil and its oleoresin on potatoes

Tests	Sprout inhibition activity (%) after weeks*									
	1	2	3	4	5	6	7	8	9	10
Essential oil	100	100	100	100	100	100	100	100	100	100
Oleoresin	80	80	60	60	40	20	20	20	20	20
Control	0	0	0	0	0	0	0	0	0	0

* Average of three experiments.

where OD = absorbance of supernatant solution and W = amount of sample (g).

Determination of Antioxidant Activity in the Linoleic Acid System

Antioxidant activity was investigated using the method proposed by other authors,[43,44] with minor changes. Samples (4 mg) in 99.5% ethanol were mixed with 2.5% linoleic acid in 99.5% ethanol (4.1 ml), 0.05 M phosphate buffer (pH 7.0, 8 ml) and distilled water (3.9 ml) and kept in screw-cap containers in the dark at 40 °C. Then 0.1 ml of this solution was added to 9.7 ml 75% ethanol and 0.1 ml 30% ammonium thiocyanate. After 3 min, 0.1 ml 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The absorbance of red colour was measured at 500 nm in the spectrophotometer every 2 days. The control and standard samples (BHA and BHT) were subjected to the same procedure except for the control, where there was no addition of sample, and for the standard 4 mg sample were replaced with 4 mg BHA or BHT. The incubation time was plotted vs. absorbance (Figure 3).

Sprout-suppressant Activity (SSA)

Potatoes were purchased from a local market. Essential oil (0.1 ml) and a corresponding amount of oleoresin, dissolved in acetone, were soaked in cotton swab and wrapped in sterilized muslin cloth. This was placed at the bottom of $12 \times 10\ cm^2$ plastic vials and a group of five potatoes were taken in each vial. All the vials were pored for aeration. A control was run in parallel, using a blank sterilized cotton swab. The potatoes were examined weekly for sprout formation at room temperature and the average values for SSA from three sets of experiments are summarized in Table 5.

Statistical Analysis

Quantitative data for the major components of the oil and the acetone extract were statistically examined by analysis of variance (ANOVA) and the significant differences among several groups of data were examined using Ducan's multiple range test.

Results and Discussion

Analysis of coriander seed essential oil by GC and GC–MS techniques (Table 1) showed the presence of more than 52 components. The major components were linalool (75.30%), geranyl acetate (8.12%) and α-pinene (4.09%). The oleoresin showed the presence of 28 components (Table 2). Oleic acid (36.5%), which is a monounsaturated fatty acid[45], was the major component, followed by linoleic acid (33.21%) and palmitic acid (11.05%). Recently, Bardoni *et al.*[16] reported 20 components in the essential oil, accounting for 96.6–99.7% of the total oil composition. The main constituents were linalool, γ-terpinene, camphor, α-pinene, geraniol and geranyl acetate. This variation in chemical composition is mainly due to genetic, environmental, developmental and other differences.

Using the inverted petriplate method (Table 3), the essential oil was found to be highly active against CP, FM, FO and AT, as more than 70% zone inhibition was obtained. In comparison, the oleoresin was found to be less effective against the tested fungi . The oleoresin exhibited about 50% mycelial zone inhibition on the growth of AN, AT and FO. Using the food poison technique (Table 4), the essential oil showed a potent inhibitory effect on the growth of all the fungi tested, completely inhibiting the growth of AT, AN, FG and FO, whereas the oleoresin showed 100% inhibition of FO and more than 50% inhibition of AF, FG, FM and CP, but was found to be less effective against the other fungi tested.

The changes of PV (Figure 1) in sunflower oil of all investigated samples were determined to define the results of the changes of primary oxidation products more precisely. It must be noted that PV is widely used to measure primary lipid oxidation, indicating the amount of peroxides formed in the fats and oils during oxidation. Sunflower oil oxidation was measured at timed periods during 28 days of storage. During this time the PV of the blank sample increased to 248 meq/kg, whereas the PV of oleoresin, essential oil, BHA and BHT were 132.9,

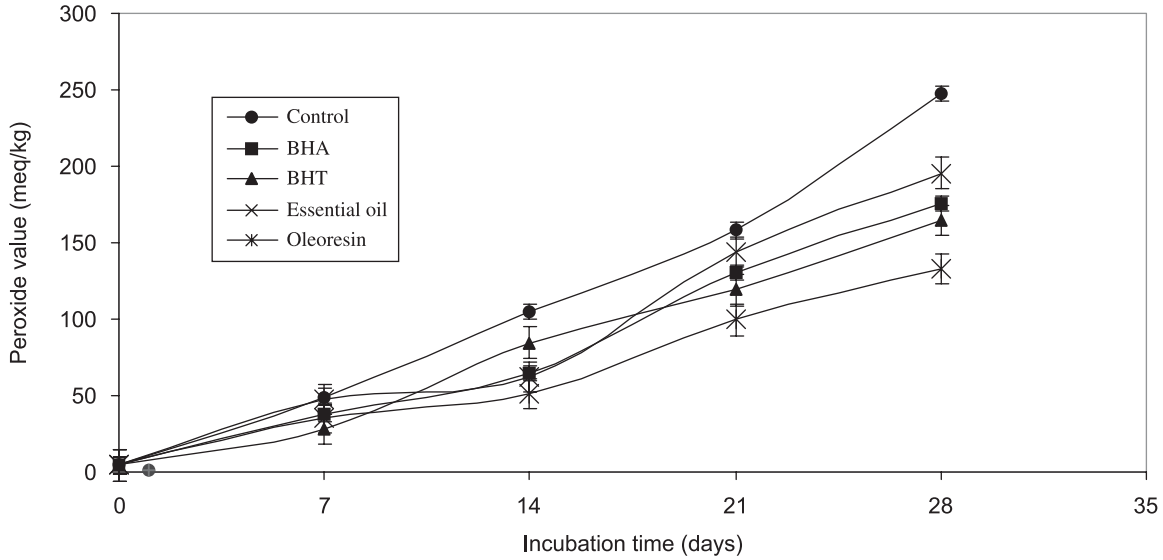

Figure 1. Inhibitory effect of coriander essential oil and its oleoresin on the peroxidation of sunflower oil, using the PV method at 80 °C. Each value is expressed as ± standard deviation. The data were found to be significant ($p < 0.05$)

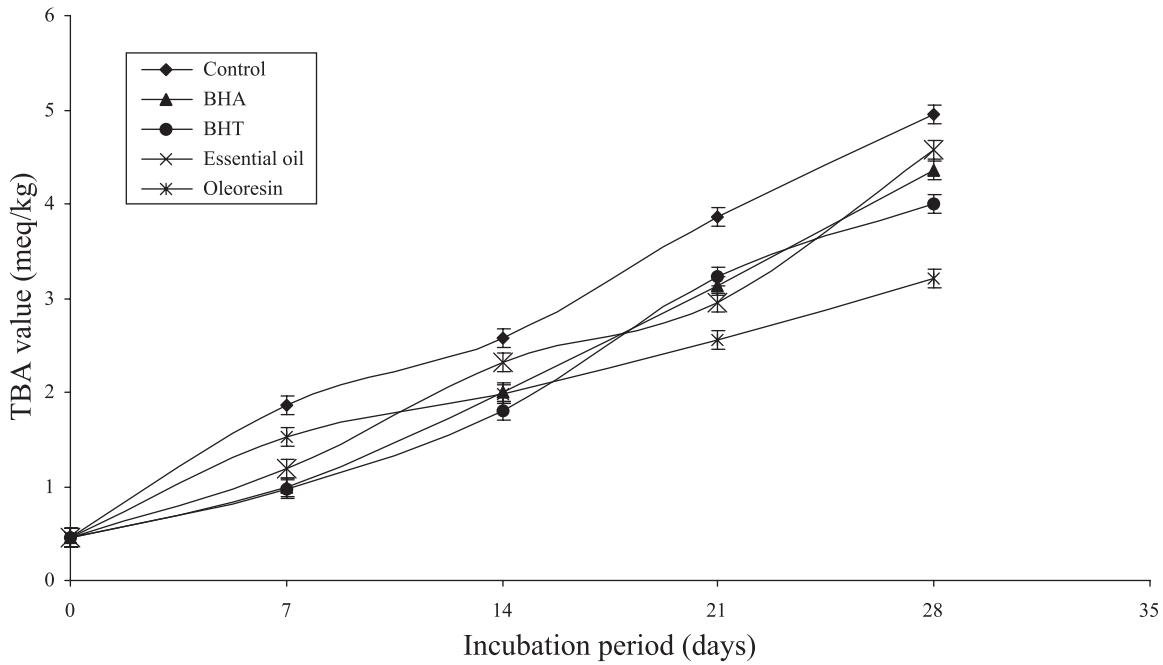

Figure 2. Inhibitory effect of coriander essential oil and its oleoresin on peroxidation of sunflower oil, using TBA at 80 °C. Each value is expressed as ± standard deviation. The data were found to be significant ($p < 0.05$)

195.6, 175.6 and 165.0 meq/kg, respectively. In terms of retarding the formation of primary oxidation products, the relative effectiveness of the treatments at a concentration of 200 ppm was determined to be oleoresin > BHT > BHA > essential oil.

During the oxidation processes, peroxides are gradually decomposed to lower molecular weight compounds. One such compound is malonaldehyde, which is measured by the TBA method. Lipid oxidation was assessed on the basis of malonaldehyde, which is used as an index of lipid peroxidation. The malonaldehyde formation of all the additives increases with storage time. Figure 2 demonstrates the effect of oil and oleoresin on secondary oxidation products. The essential oil showed much lower inhibition on the lipid peroxidation than BHA and BHT. The inhibition effect of the oleoresin on oxidation was

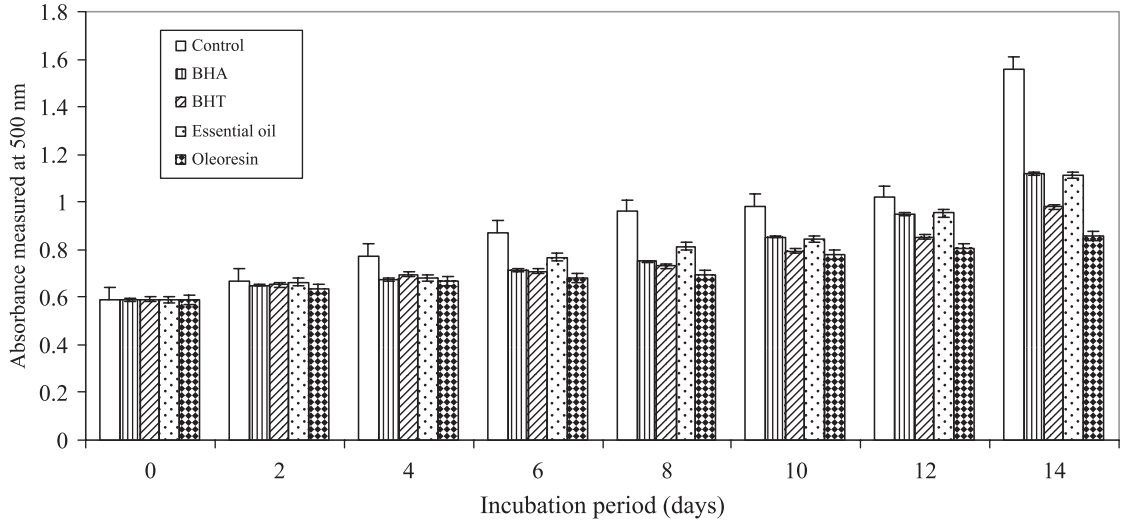

Figure 3. Inhibitory effect of coriander essential oil and its oleoresin on linoleic acid peroxidation at 40 °C. Each value is expressed as ± standard deviation. The data were found to be significant ($p < 0.05$)

best among all the additives and there was a significant difference between the blank and antioxidants ($p < 0.05$). These results are also well correlated with the results of the PV method.

The effect of essential oil and oleoresin on the formation of primary and secondary oxidation products was assessed by measuring the changes in the absorption values of linoleic acid emulsion at 500 nm over 12 days. The absorbance of the linoleic acid emulsion of the blank or with additives increased rapidly and there was a significant difference between the blank and antioxidants ($p < 0.05$). However, in general, the results obtained by spectrophotometric absorptions at 500 nm (Figure 3) were closely related with PVs. Recently, Melo *et al.*[46] reported that aqueous and etheric coriander extracts, mainly containing phenolics and carotenoids, exhibit considerable antioxidant activity. In 2004, Ramdan *et al.*[21] investigated the oxidative stability of crude and stripped coriander seed oils. These researchers reported that the oxidative stabilities of the crude oils were stronger than those of the stripped oils.

Potato tubers are kept in cold stores to preserve their quality and food value. Therefore, sprout suppression during storage is extremely important, especially for the potato-processing industry. We therefore studied the SSA activity of coriander oil and its oleoresin and found that the oil has strong SSA but the oleoresin does not (Table 5). Singh *et al.*[26] reported that coriander essential oil, due to the presence of linalool as a major constituent, exerts strong SSA. This may be due to the vapour action of the oil. The oleoresin does not vapourize and hence it can not show any activity.

Summarizing these results, it can be concluded that the volatile oil of *Coriandrum sativum* contain linalool as the main component, possesses potent antifungal activity against all species tested at 10 µl and can be used as natural sprout suppressant, while the oleoresin, rich in oleic acid and linoleic acid, may be used as an alternative source of natural antioxidant.

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