

EFFECT OF GROUND MATERIAL OF CERTAIN PLANT SPECIES AGAINST BOTRYTIS CINEREA

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Introduction

Botrytis cinerea causes the gray molds or gray mold of fruits and vegetables, both in the field and in storage. Almost all fresh fruits, vegetables, and bulbs are attacked by *B. cinerea* in storage (Agrios, 1997). In wine production, their most serious damage is qualitative, from the modified chemical composition of diseased berries. The fungus converts simple sugar (glucose and fructose) to glycerol and gluconic acid and produces enzymes that catalyze the oxidation of phenolic compounds. It also secretes polysaccharides such as β -glucan, which hinder the clarification of wine. Wine produced from rotten grape fruits has off-flavors and is fragile and sensitive to oxidation and bacterial contamination, making them unsuitable for aging (Jarvis, 1977, McClellan and Hewitt, 1973).

The use of synthetic chemicals to control plant diseases is restricted due to their possible carcinogenicity, acute toxicity, long degradation periods, environmental pollution and their effects on human beings (Lingk, 1991). Therefore alternative to synthetic pesticides are needed from microbial and plant sources. Effective phytocompounds are expected to be far more advantageous than synthetic pesticides, as they are easily decomposable, not environmental pollutants and possess no residual or phytotoxic properties (Tewari, 1990, Rao, 1990, Badei et al., 1996, Bishop and Thornton, 1997). The importance of spices and their derivatives (extracts, essential oils, decoctions, hydrosols) in crop protection is being increasingly recognized under the concept of Integrated Pest and Disease Management (IPDM). Under this concept, all possible modes of plant pests and disease control methods should be integrated to minimize the excessive use of synthetic pesticides (Beg and Ahmed, 2002).

During the last years there has been growing interest in testing natural compounds of different origins as defense for cultivated plants against phytopathogenic fungi (Thompson, 1989, Kishore et al., 1989, Tewari, 1995, Muller-Riebau et al., 1995, Wilson et al., 1997, Bowers and Locke,

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2000). In particular, essential oils were seen to exert good antifungal activities both in vitro and in vivo (Bhaskara Reddy et al., 1998, Caccioni et al., 1998, Tripathi et al., 2004). But antifungal activities of other derivatives have been less investigated against phytopathogenic fungi (Ozcan and Boyraz, 2000, Boyraz et al., 2003). In the present work we report in vitro and in vivo effect of ground material of certain aromatic plant species, carnation, garlic, lemon, cumin and ginger against *B. cinerea*.

Materials and Methods

Ground material of plant species

Six ground materials of certain plant species, *Syzygium aromaticum*, *Allium sativum*, *Carum carvi*, *Citrus limon*, *Myristica fragrans* and *Zingiber officinale* were chosen to study their antifungal activity against *B. cinerea* in vitro. They were used at three different concentrations (0.1, 0.5 and 1%). Ground material of carnation at 0.5 and 1% and garlic at 1% showing antifungal activity against *B. cinerea* in vitro were chosen for in vivo experiment. Sterile distilled water was used as control.

Pathogen inoculum

B. cinerea, isolated from apple fruits was cultivated on Botrytis Minimal Agar (BMA) (glucose 10 g/l, K₂HPO₄ 1.5 g/l., KH₂PO₄ 2 g/l, (NH₄)₂SO₄ 1.0 g/l, MgSO₄(7H₂O) 5.0 g/l, agar 20g/l) at room temperature in the dark. Fungal discs of 5 mm. in diameter were taken from 7 days old culture.

A spore suspension of the pathogen was prepared from 10-day-old culture dishes incubated at 25 °C by flooding with 10 ml of sterile distilled water. The spore concentration was determined with a hemacytometer and adjusted to 5×10⁵ spores per ml with sterile distilled water.

Assessment of inhibition of fungal growth

The effect of ground material of plant species, *Syzygium aromaticum*, *Allium sativum*, *Carum carvi*, *Citrus limon*, *Myristica fragrans* and *Zingiber officinale* were determined against *B. cinerea* growth using Botrytis Minimal Agar medium (BMA) (glucose 10 g/l, K₂HPO₄ 1.5 g/l., KH₂PO₄ 2 g/l, (NH₄)₂SO₄ 1.0 g/l, MgSO₄(7H₂O) 5.0 g/l, agar 20g/l). The 0.1, 0.5, 1.0% doses of ground material were added into bottle containing 100 ml media sterilized and cooled to 45 °C, separately. Then bottles were shaken thoroughly and the medium was poured into Petri dishes. Five millimeter discs of the test fungus taken from advancing edge of 7 day-old cultures were placed on the middle of the BMA medium containing the ground material, and then Petri dishes were incubated at 24 C for 5 days. Three replicates of each treatment were arranged according to a completely randomized design on incubatory shelves. Petri dishes containing BMA

medium free from ground material of the above mentioned plant species were served as control.

Efficacy of ground material of certain plant species used as a suspension against *B. cinerea* on tomato leaves

Detached tomato leaflet were collected from tomato plants grown in farm of Plant Protection Department, Debrecen University, Debrecen, Hungary. Surface sterilized detached tomato leaflets were rinsed first in a suspension of ground material of *Syzygium aromaticum* at concentrations of 0.5%, 1.0% and 2.0% and *Allium sativum* at 1.0% and 2.0%. Discs 6 mm. in diameter were used in inoculation. Artificial inoculation were performed by placing the inoculum discs (6 mm. in diameter) on the surface of sterilized detached tomato leaflets. The leaflets were surface sterilized by dipping in hypochloride solution for 15 minutes and then washed thoroughly in sterile water. Inoculated leaflets were kept in deep Petri- dishes at 25 °C. Control treatments were used in each trial and were rinsed only in tape water. Six replicates were used in each treatment.

Gray mold rot was estimated after 6 days as a percentage of the fruit area covered by gray lesions using a standard area diagram. The following numerical rates were suggested to facilitate visual determination and to give a satisfactory comparison:

- 0 = free from infection or nearly so
- 1 = trace - 25% leaf area covered
- 2 = 26 - 50% leaf area covered
- 3 = 51 - 75% leaf area covered
- 4 = 76 - 100% leaf area covered

The readings were converted to a disease index according to the following equation:

$$\text{Disease index} = \frac{\sum n \times r}{4N} \times 100$$

Where "n" is the number of leaves in each numerical rate "r" and "N" the total number of inoculated leaves multiplied by the maximum numerical rate "4".

n = number of leaves,

r = numerical rate,

4N = total number of inoculated leaves multiplied by 4

Efficacy of ground material of certain plant species used as a suspension against *B. cinerea* on apple fruits

A uniform 4-mm deep and 3-mm wide wound was made at the equator of apple fruits (Jonathan) cultivar using the tip of a sterile disinfecting needle. A 20 µl drop of a suspension of the ground material of *Syzygium aromaticum* at 0.5%, 1.0% and 2.0% and *Allium sativum* at 1.0% and 2.0% was pipetted onto each wound site. A 20 µl drop of sterile distilled water was used for control. One hour later, each wound was inoculated with a 20 µl of a suspension of 5×10^5 spores of *B. cinerea* per ml. Fruits were air dried, put into plastic trays and wrapped with high density polyethylene sleeves to keep high humidity (about 95%). Then fruits were kept at 25 °C. Lesion size in the fruits were measured after 6 days for fruits stored at 25 °C. Two diameters were measured at right angles across each lesion on the surface of infected fruit. Severity of decay was measured as the mean of the width and length of the lesion. Three replicates of 10 fruits per treatment with a complete randomization of trays in each test were used.

Efficacy of ground material of certain plant species used as a dust against *B. cinerea* on apple fruits

This experiment was carried out to study the effect of the ground material of *Syzygium aromaticum* and *Allium sativum* on fruit gray mould caused by *B. cinerea*. Inocula *B. cinerea* isolate were prepared by growing the isolate in Petri-dishes on Botrytis Minimal Agar (BMA) at room temperature in the dark for 5 days. Discs 6 mm. in diameter were used in inoculation. Surface sterilized apple fruits of 'Jonathan' variety were dusted first with ground material of *Syzygium aromaticum* at concentrations of 0.5%, 1.0% and 2.0% and *Allium sativum* at 1.0% and 2.0%. Concentrations were adjusted by adding Talc to the ground material (V/V). Then artificial inoculation in the laboratory were performed by placing the inoculum discs (5 mm. in diameter) on the surface of the apple fruits which was wounded before by using a tip of a sterile disinfecting needle. The fruits were surface sterilized by dipping in hypochloride solution for 15 minutes and then washed thoroughly in sterile water. Fruits were put into plastic trays and wrapped with high density polyethylene sleeves to keep high humidity (about 95%). Then, fruits were kept at 25 °C.

Control treatments used in each trial were dusted only by Talc. Three replicates of 10 fruits per treatment with a complete randomization of trays in each test were used.

Severity of gray mold rot of infected apple fruits was estimated after 6 days as a mean of the width and length of the gray mould lesion as previously mentioned.

Results

In vitro activity of ground material of certain plant species against *B. cinerea*

In regard to effects on fungal activity in the use of the ground material of Syzygium aromaticum, Allium sativum, Carum carvi, Citrus limon, Myristica fragrans and Zingiber officinale at 0.1%, 0.5% and 1% a great difference in the reduction percentage of the growth rate of B. Cinerea was observed (Table 1 and Fig. 1). No linear growth was found when ground material of Syzygium aromaticum at concentrations of 0.5% and 1% and Allium sativum at 1% were used. While a low effect on reduction of linear growth rate was observed when Myristica fragrans, Carum carvi and Zingiber officinale were used. On the other hand, no effect has been recorded on the linear growth rate by using the ground material Citrus limon at all the three different concentrations.

Table 1. Effect of ground material of certain plant species on mycelial growth of *Botrytis cinerea*

Ground matetial of Plant species	Concentrat ion	Reduction (%) of mycelial growth
Syzygium aromaticum	0.1	30
	0.5	100
	1.0	100
Allium sativum	0.1	7.6
	0.5	23
	1.0	100
Carum carvi	0.1	0.0
	0.5	9.0
	1.0	18.0
Citrus limon	0.1	0.0
	0.5	0.0
	1.0	0.0
Myristica fragrans	0.1	35
	0.5	28.5
	1.0	28.5
Zingiber officinale	0.1	21.4
	0.5	14.5
	1.0	14.5

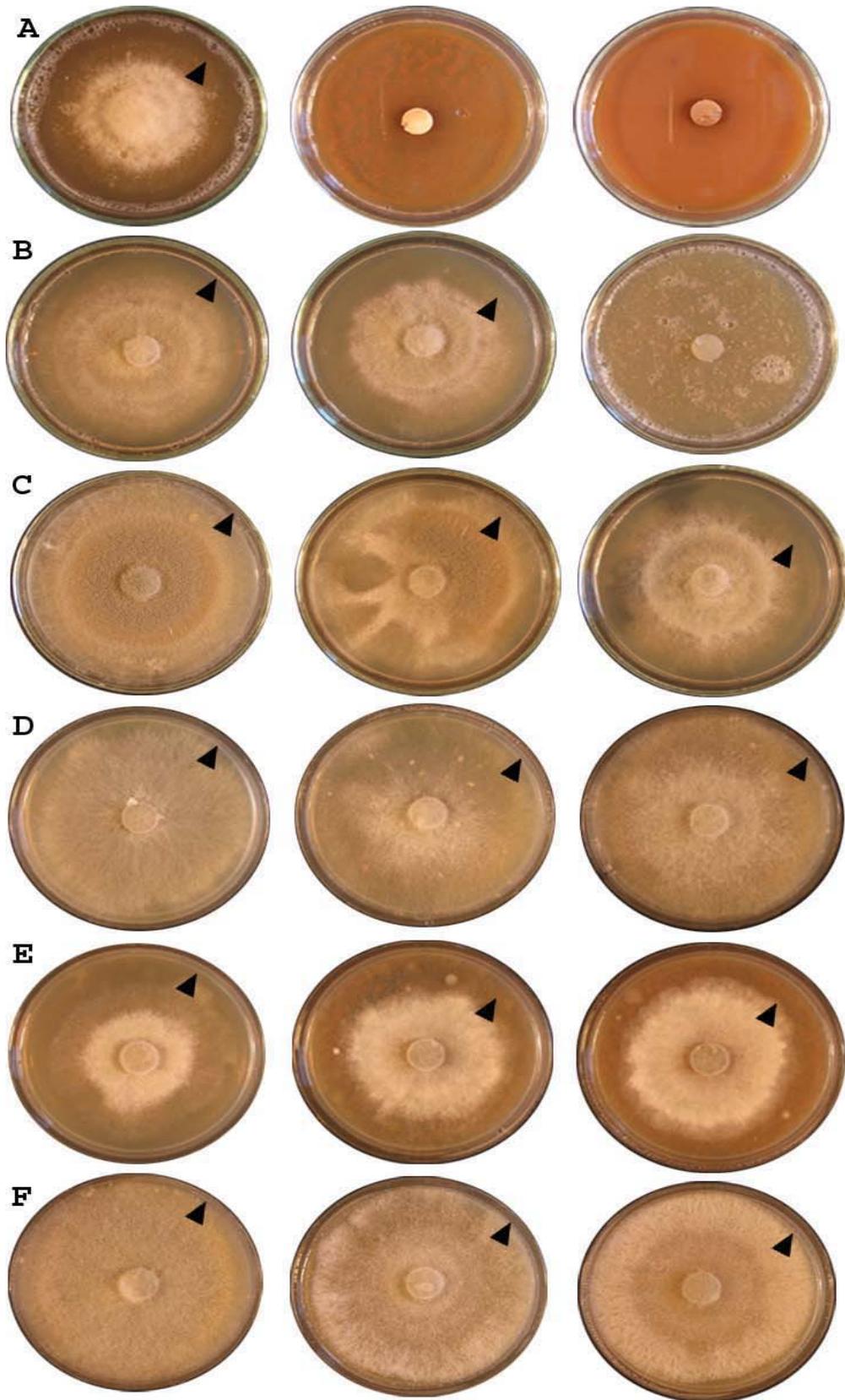


Fig. 1. Linear growth of *Botrytis cinerea* on BMA medium containing 0.1%, 0.5% and 1.0% of ground material of different plant species (A=*Syzygium aromaticum*, B=*Allium sativum*, C=*Carum carvi*, D=*Citrus limon*, E=*Myristica fragrans* and F=*Zingiber officinale*). The black arrows represent the growth margin of *Botrytis cinerea*

In vivo activity of ground material of certain plant species used as a suspension against *B. cinerea* on tomato leaves

Concerning the effect of treating tomato leaflets with a suspension of ground material of *Syzygium aromaticum* at 0.5 and 1.0% and *Allium sativum* at 1% on gray mold development, it is clear from data presented in Table 2 and demonstrated in Fig. 2 that the highest effect was observed when *Syzygium aromaticum* was applied at 1.0% (disease index = 10%) while the lowest was found when *Allium sativum* was applied at 1% (disease index = 50%).

Table 2. Disease index of gray mould caused by *B. cinerea* on tomato leaves

Treatments	Disease index
Control	75
<i>Syzygium aromaticum</i> at 0.5 %	30
<i>Syzygium aromaticum</i> at 1.0 %	10
<i>Allium sativum</i> 1.0 %	50



Fig. 2. Disease index of gray mould on tomato leaves treated with different concentrations of ground material (applied as suspension) of *Syzygium aromaticum* and *Allium sativum* (A=Control, B=*Syzygium aromaticum* at 0.5%, C=*Syzygium aromaticum* at 1.0%, D= *Allium sativum* 1.0)

In vivo activity of ground material of certain plant species used as a suspension against *B. cinerea* on apple fruits

Apple fruits treated by a suspension of the ground material of *Syzygium aromaticum* at 0.5, 1.0 and 2% and *Allium sativum* at 1.0 and 2% were infected by *B. cinerea* at 25 °C and no differences were observed as to disease severity expressed by lesion diameter of gray mould. No inhibition effect of the ground material of both plant species at all different concentration was found (Table 3 and Fig. 3).

Table 3. Diameter of fruit lesions produced by *Botrytis cinerea*, the cause of gray mould disease of apple

Treatments	Disease severity [diameter of lesion (cm)]
Control	6.0
<i>Syzygium aromaticum</i> at 0.5 %	5.5
<i>Syzygium aromaticum</i> at 1.0 %	5.5
<i>Syzygium aromaticum</i> at 2.0 %	5.0
<i>Allium sativum</i> 1.0 %	6.0
<i>Allium sativum</i> 2.0 %	5.5

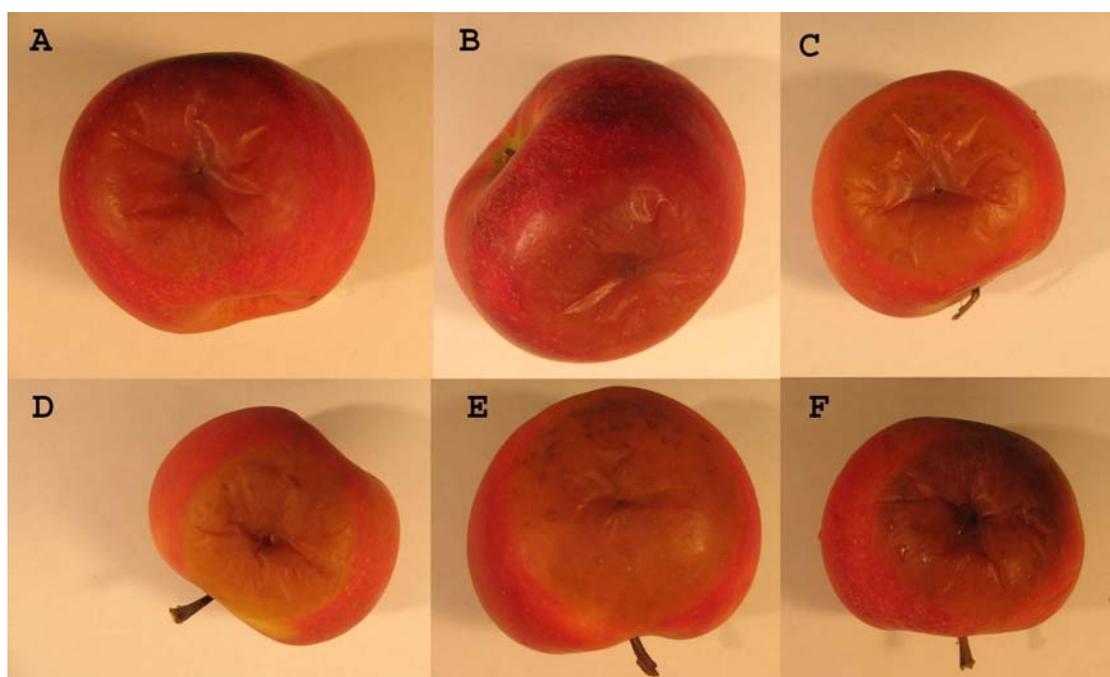


Fig. 3. Diameter of gray mould lesions in apple fruits treated with different concentrations of ground material (applied as suspension) of *Syzygium aromaticum* and *Allium sativum* (A=Control, B=*Syzygium aromaticum* at 0.5%, C=*Syzygium aromaticum* at 1.0%, D= *Syzygium aromaticum* at 2.0%, E= *Allium sativum* 1.0 %, F= *Allium sativum* 2.0 %

In vivo activity of ground material of certain plant species applied as a dust against *B. cinerea* on apple fruits

Concerning the effect of application *Syzygium aromaticum* and *Allium sativum* on gray mold development on apple fruits., it is clear from data presented in Table 4 and demonstrated in Fig. 4 that the highest effect was observed when *Syzygium aromaticum* and *Allium sativum* were applied at 2% (diameter of lesion = 0.0% and 0.5 respectively). Good results were found when both *Syzygium aromaticum* and *Allium sativum* were applied 1% (diameter of lesion = 0.5 and 1.0 respectively). The lowest effect was found when *Syzygium aromaticum* was applied at 0.5% (diameter of lesion =2.0).

Table 4. Diameter of fruit lesions produced by *Botrytis cinerea*, the cause of gray mould disease of apple

Treatments	Disease severity [diameter of lesion (cm)]
Control	6.0
<i>Syzygium aromaticum</i> at 0.5 %	2.0
<i>Syzygium aromaticum</i> at 1.0 %	0.5
<i>Syzygium aromaticum</i> at 2.0 %	0.0
<i>Allium sativum</i> 1.0 %	1.0
<i>Allium sativum</i> 2.0 %	0.5

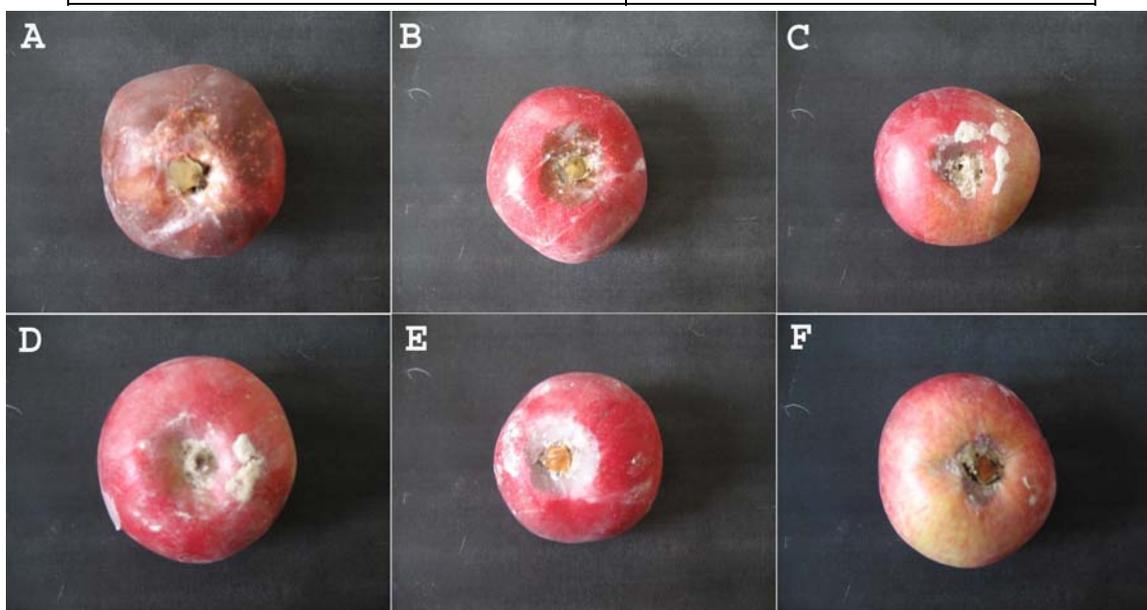


Fig. 4. Diameter of gray mould lesions in apple fruits treated with different concentrations of ground material (applied as dust) of *Syzygium aromaticum* and *Allium sativum* (A=Control B=*Syzygium aromaticum* at 0.5%, C=*Syzygium aromaticum* at 1.0%, D= *Syzygium aromaticum* at 2.0%, E= *Allium sativum* 1.0 %, F= *Allium sativum* 2.0 %

Discussion

Studies on the inhibitory effects of herbs, spices and their derivatives such as essential oils, extracts and ground materials have been conducted on the antimicrobial properties (Farag et al., 1989, Dormen and Deans, 2000, Ozcan and Boyraz, 2000, Bowers and Locke, 2000, Erkmen and Ozcan, 2003, Sagdic and Ozcan, 2003, Ozkan et al., 2003, Sagdic et al., 2003). Additionally, it is known that the composition of ground materials and their antimicrobial effects depend on plant species and their regional conditions. Some researcher reported that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity (Deans and Svoboda, 1990).

The fungitoxic abilities of spices are also a particularly interesting field for applications within food, stored products. The active fraction of ground materials is probably responsible for the antimicrobial activity.

Partial studies are recommended on the use of selected spices and their derivatives during production of foods. Combinations of ground material and essential oils may provide an efficacious mixture for the inactivation of pathogenic and spoilage microorganisms in plant and foods. So, knowledge of how to protect some stored food products from pathogens and saprophytic fungi can probably be gained from different concentrations of spice derivatives including ground material.

It is recommended from the obtained results of application the ground materials of *Syzygium aromaticum* and *Allium sativum* in two different forms of use (suspension or dust) to apply the ground material of both previous plant species in the form of dust application since this form is much more effective in controlling gray mould caused by *B. cinerea* on apple fruits.

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Results of testing six ground material of *Syzygium aromaticum*, *Allium sativum*, *Carum carvi*, *Citrus limon*, *Myristica fragrans* and *Zingiber officinale* *in vitro* at different concentrations for their antifungal activity against *Botrytis cinerea* revealed a different inhibitory effect on mycelial growth. *Syzygium aromaticum* and *Allium sativum* at relatively high concentrations completely prevented mycelial growth. On the other hand no inhibitory effect on mycelial growth was recorded when *Carum carvi* and *Citrus limon* were used.

Application of the ground material of *Syzygium aromaticum* and *Allium sativum* in the form of suspension at all different concentration *in vivo* had no effect on the development of gray mould of apple fruits. On the other hand, application of the ground material of *Syzygium aromaticum* and *Allium sativum* in the form of dust *in vivo* and its effect on the development of gray mould of apple fruits resulted in a reduction in gray mould severity on apple fruits. The effect is increased by increasing the concentration, since the development of gray mould is prevented at 2.0%. As to application of the ground material *Syzygium aromaticum* and *Allium sativum* in the form of suspension on tomato leaflets, high effect was observed of the ground material of *Syzygium aromaticum* at 1% on the development of gray mould on tomato leaflets while low effect was found when *Allium sativum* was applied at 1%.

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