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Effect of Certain Natural Materials on Tomato Diseases Control

[1]Hayyan Ismaeil Al-Taweil, [2]Khalid Al Jamhan

[1]Isra University – Faculty of Pharmacy, PO Box 22 & 33 Isra University Mail office 11622
Tel : 4711581 - 4711830 – 4711710, Ex : 2736 / 2730, Fax : 4711505

[2]The Promising Research Center in Biological Control and Agricultural Information, Qassim University, P.O. Box 6622, Burydah 51452, Kingdom of Saudi Arabia

Abstract: **This study aimed to evaluate the antagonistic effect of essential oils against *Botrytis cinerea*, and *Alternaria solani*. Mixtures of essential oils with yeast *"Saccharomyces cerevisiae"* showed high inhibitor effect against the pathogenic growth.**

On the light of these results the usage of the proposed combination of oil and yeast could be suggested for application against decay of tomato diseases in green houses. Therefore, as these agents are active at low concentrations, they could be used in the formulation of natural preparations.

Keywords: **Essential oils, mint, rosemary, lavender, antifungal activity, *Botrytis cinerea*, *Alternaria solani*, *Saccharomyces cerevisiae*, tomato**.

I. INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is grown as a commercial crop in Saudi Arabia with approximately 14,699 ha planted annually and an estimated yield of 522,152 tonnes (Ministry of Agriculture, Central Administration of Economic Studies and Statistics, 2008).

The application of synthetic fungicides is not an option for organic farms according to the National Organic Program (NOP), which defines an organic food as “Food produced without using most conventional pesticides; fertilizers made with synthetic ingredients or sewage sludge; bioengineering; or ionizing radiation” (USDA, 2011). These regulations, the continually increasing interest in preserving the environment, and public concern about pesticides residues in food, have resulted in increasing interest in organic products for plant disease control. Among these, essential oils, plant extracts, biological agents, soil extracts, and bicarbonate salt are acceptable materials for disease and pest management under the standards of the National Organic Program (NOP).

Fresh-market tomatoes (*Lycopersicon esculentum* Mill.) are grown in most countries around the world. Many greenhouse tomato growers have observed symptoms of foliar diseases. Leaf mold, also known as *Cladosporium*, was the most prevalent problem but gray mold or botrytis was also evident. Both are fungal diseases that need high humidity or wet plant surfaces to get going and while these diseases often start on leaves both can attack the stems, blossoms and fruits.

Gray mold is caused by the fungus *Botrytis cinerea*. Compared to Cladosporium leaf mold, *Botrytis* gray mold is more ubiquitous, that is the spores are everywhere all the time, especially in greenhouses, Also, unlike Cladosporium leaf mold which is host specific to tomato, *Botrytis* causes disease in just about any plant species if conditions are right.

Tomatoes are grown throughout the world, and the *Alternaria solani* pathogen can infect crops in any of these locations where they are planted. Therefore, the early blight of tomatoes disease, which is caused by the *A. solani* pathogen, is very widespread throughout the world. The infection of a plant initiates with the transfer of the *A. solani* conidia onto its host, the tomato plant, from a previously infected source. The conidia then germinate, produce germ tubes and hyphae, and penetrate their host with these extensions invading the inner tissue of the plant or fruit. As the conidia reproduce, they invade the host even further and cause a wound in the tissue. From that starting point, the hyphae continue to re-infect new parts of the same plant, and/or newly formed conidia transfer onto a new host.

Biological control has been advanced as an alternative to synthetic fungicides and considerable success in laboratory and pilot scale tests has been realized utilizing antagonistic microorganisms to control crops diseases. Several antagonistic yeasts and bacteria have been isolated and shown to have a broad spectrum of activity against a number of pathogens on a variety of fruit recently; interest has been shown in combining microbial biocontrol agents with other chemical components to increase their activity against crops pathogens (Droby et al 1998). Essential oils are also considered a promising alternative with many having antifungal properties (Hammer et al 2003, Ahmet at al 2005). Application of essential oil is a very attractive method for controlling diseases.

Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey et al 2001). Essential oils have been used successfully in combination with a variety of treatments, such as antibacterial agents, mild heat and salt compounds (Karatzas et al 2000).

Application of essential oil is a very attractive method for controlling diseases. Production of essential oils by plants is believed to be predominantly a defense mechanism against pathogens and pests and indeed, essential oils have been shown to possess antimicrobial and antifungal properties (Ahmet et al 2005).

Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploitation for potential multi-purpose functional use (Jobling 2000). Essential oils are made up of many different volatile compounds and the composition of the oil quite often varies between species (Mishra and Dubey 1994). It is difficult to associate the antifungal activity to single compounds or classes of compounds. It seems that the antifungal and antimicrobial effects are the result of many compounds acting synergistically (Bagamboula et al 2004). Thus, there would be negligible chance of development of resistant races of fungi after application of essential oils to fruit and vegetables. Consequently, essential oils are one of the most promising candidate groups of natural compounds for the development of safer antifungal agents.

Conventional and organic farming differ mainly in tillage methods, crop rotations, fertilizer applications, and pest control methods. The production of tomatoes is a very labor-intensive process, with each staked acre of tomatoes requiring approximately 350 hours of work (Konsler and Shoemaker, 1980). Despite the intensive work and good management practices, one of the greatest challenges to organic tomato growers in humid climates is disease management (Diver et al., 1999). The management of necrotrophic fungi in conventionally grown tomatoes is by the use of protectant fungicides like mancozeb and chlorothalonil or systemic fungicides in the strobilurin class (Zitter et al., 2005). In contrast, management of diseases in organically grown tomatoes is done by a combination of organic soil management practices, integrated pest management practices, natural remedies, and limited fungicide use (Diver et al., 1999). The use of tolerant or resistant cultivars is a management practice recommended to conventional farmers as well as organic farmers, but high levels of resistance to early blight in cultivated tomatoes is rare (Chaerani and Voorrips, 2006). Tomato varieties in the Mountain series, which are tolerant to early blight but not to sartorial leaf spot, are recommended to organic growers to manage the former (Diver et al., 1999).

In the majority of trials testing the efficacy of organic certified materials, copper-based products have provided the best results in terms of disease control but not in terms of yield. For example, in an evaluation of OMRI-certified materials such as CaCO3, SW-3 Seaweed, Humega and Champion WP (active ingredient (a.i.) copper hydroxide) to manage foliar disease in tomatoes in New York, some of them significantly reduced disease severity (Seaman et al., 2004, Wszelaki et al., 2003). In contrast, in a study in Iowa, copper fungicide not only controlled disease but also provided for yields 60% more than the other treatments (Joslin and Taber, 2003). Despite the fact that copper-based products have shown themselves to be very effective for controlling foliar disease in numerous trials, they also have failed in others trials (McGrath, 2007). Copper-based fungicides are routinely used for disease control in organic tomato production in the Eastern of United States; however, it can be toxic to earthworms and nitrogen-fixing soil microbes (blue-green algae) and can build up in the soil to phytotoxic levels to the plants (Diver et al., 1999).

The objective is to evaluate the effectiveness of the natural essential oils of mint, rosemary and lavender with yeast to inhibit pathogenic fungal growth in green houses.

II. MATERIALS AND METHODS

Pathogens and antagonists: One of each virulent pathogenic fungal isolates of *Botrytis cinerea*, and *Alternaria solani* are obtained from Plant Pathology Department of the Agriculture collage – Qassim University and the antagonistic yeast, *i.e. Saccharomyces cerevisiae* was obtained from local market (manufactured in France by S.I.Lesaffre)

Growth media: Potato dextrose agar (Difco Laboratories, Detroit, MI) and NYDB are used for growing fungal and yeast isolates tested in the present work. Fungal and yeast cultures will be maintained on PDA and NYD agar slant media at 5 °C as stock cultures until use. All isolates will be activated by growing at the optimum growth conditions at the beginning of the present experiments.

Preparation of fungal and yeast cells suspensions: Pathogenic fungal inocula are grown on PDA medium at 25 °C until an abundant heavy growth of conidia was evident. Conidia are harvested by scraping the surface of the colonies with a spatula, transferred to sterilized distilled water and filtered through nylon mesh. Meanwhile, antagonistic yeast bio-agents will be grown on NYDB medium and incubated in a rotary shaker at 200 rpm for 24 h at 28°C . The yeast cells are harvested by centrifugation at 6,000 rpm for 10 min, washed twice with 0.05 M phosphate buffer at pH 7.0, and resuspended in distilled water. The concentrations of yeast cells in the suspensions were adjusted to 3 $x10^8$ cells per milliliter.

Essential oils *i.e.* peppermint, rosemary and lavender oils at concentrations of 0, 0.25, 0.5 and 1% and yeast isolates (3 x 10^8 cell/mL) either as individual treatment or in combination were evaluated for their inhibitory effect against the linear growth of each pathogenic agents.

Essential oils: Commercial grade of essential oils, mint (*Mentha piperita* L.); Rosemary *(Rosmarinus officinalis* L) and Lavender (*Lavandula spica)* are obtained from the local market (Al- Ahlam for seeds oil production - Jeddah. The essential oils will be stored in dark glass bottles at 4 °C.

In vitro antifungal assay:

In vitro contact assay: PDA was autoclaved and cooled in a water bath to 40°C. The essential oils will be mixed with sterile PDA to obtain final concentrations 0, 0.25, 0.50, and 1.00 %

The PDA will be poured into 90 mm Petri plates, and then inoculated with 6 mm plugs from 7-days-old cultures of the pathogens. Three replicates were used per treatment. Plates will be incubated for 7 days at 34°C.

Fungal growth was recorded after 7 days. Growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control.

The inhibitory effect of essential oils at the same previous concentrations on colony formed by antagonistic yeast isolates will be assayed in NYPD broth using a modified method of (Piano et al1997.). Aliquots of 100 µL of the yeast cell suspension ($3x10^8$) were transferred to glass tubes containing 5 mL sterilized distilled water, then the tested emulsion essential oils were added individually to each tube to achieve the proposed concentration. All tubes were left for 6 h, then shaking well using magnetic stirrer for 5 min. One ml of each test tube was dispensed into Petri dish and about 20 mL of sterilized NYPD agar medium were poured into the inoculated plates and rotated gently to ensure equal distribution of the yeast inocula. Control check treatment was the yeast cell suspension free from essential oils. All plates were incubated for 72 h and then examined. Percent of yeast isolates formed colonies was calculated by comparing with their counts in check treatment. All treatments consisted of three replicates, and experiments were repeated three times (Hadizadeh at al 2009 and Abd- Allah et al 2009).

The interaction between yeast isolates and pathogenic fungi was evaluated as fungal growth inhibition *in vitro*. Dual culture technique after (Ferreira et al 1991) was followed. Yeast isolates (48-h-old) were streaked individually on one side of 9 cm Petri dishes containing PDA medium, while 5 mm disks of each individual fungal pathogen were placed on the opposite side of the yeast inoculated plates. Both tested microorganisms will be placed 2 cm from the plate edges. A set of only fungal inoculated plates will be used as control treatment. All plates were incubated at 34°C until full fungal growth occurred in check plates. Percentage of fungal growth reduction will be calculated in yeast treatments relative to the fungal growth in check treatment (Hadizadeh at al 2009 and Abd- Allah et al 2009).

The efficacy of combined formula between essential oils and yeast isolates against the growth of pathogenic fungi are also evaluated. This test was carried out using Petri dishes containing PDA media supplemented with the above mentioned essential oils concentrations. Growth inhibition of pathogenic fungi affected by Yeast isolates in the presence of essential oils in the growth medium is evaluated following the dual culture technique (Hadizadeh at al 2009). All the procedures of PDA supplementation with essential oils concentrations, plates inoculation with the isolates of yeast and fungal inocula, plates incubation and growth reduction measurement were carried out as stated before (Hadizadeh at al 2009 and Abd-Allah et al 2009)

Statistical Analysis: Statistical analysis of the data obtained in the present study will be carried out in a completely randomized design layout with three replicates. Where a significant difference between means was verified based on ANOVA, the comparison of means of different treatments was performed using Tukey's test at $p = 0.05$.

III. RESULTS

Biological control is a relatively new approach and offers several advantages over conventional biological control (Wisniewski at al 1991). Several biological control agents have been developed in recent years, and a few have actually been registered for use on fruit crops. Yeasts such as *Pichia guilliermondii* (and *Cryptoccocus laurentii*, yeast that occurs naturally on apple leaves, buds, and fruit Roberts.1990) were the first to be applied for control of plants pathogens. The yeast, *Candida oleophilia* has been registered for control of postharvest decay on fruit crops.

The yeasts, *Cryptococcus infirmo-minutus* and *Candida sake* successfully control brown rot and blue mold on sweet cherry, and three diseases of apple (Spots et al 1998), respectively, and may be developed into commercial products. In the present study the efficacy of yeast isolates *S. cerevisiae* was evaluated against the growth tomato decay fungi *in vitro*. Result showed that (table 1) the yeast has inhibitory affect on the growth of the pathogenic fungi 38.7 and 41.9 % for *Botrytis* and *Alternaria* repetitively.

Compatibility with chemicals used during plant growth is also important. Indications are that biological control agents must be combined with other disease control strategies if they are to provide acceptable control. Several proposed non-fungicidal approaches, including the use of biological control with antagonistic microorganisms, heat treatment, induction of resistance, natural fungicides and plant extracts and essential oils have been extensively studied. Unfortunately, none of them, when used alone, can provide satisfactory levels of decay control when compared with synthetic fungicides (Doby et al 2003).

Furthermore, the individual inhibitory effect of tested essential oils against the linear growth of pathogenic fungi was shown in Tables 3 and 4mint oil at concentration of 1% showed superior inhibitor effect against the tested pathogenic fungi calculated as 55.5, 50.0 % reduction in the growth of *Alternaria solani*, and *Botrytis cinerea*, respectively.

As for lavender and rosemary oils they showed lesser inhibitor effect at the same concentration (1%) on the fungal growth. Percentages of the growth reduction were calculated as 38.8, 16.6, 28.6 and 14.4, 17.1, 15.3, in respective order. On the other hand, the synergistic or antagonistic effects of yeast and essential oils combination against the fungal growth were shown in Tables 3 and 4.

The data revealed that combination of yeast isolates and essential oils showed synergistic effect for inhibiting fungal growth, the less concentration of essential oils with yeast combination affect more than the highest concentration of essential oils as single treatment. *Alternaria* growth reduction in Mint Oil 1% as single treatment was 55.5% while the same effect obtained at the concentration of 0.25% with yeast combination, and same for other essential oils treatments.

Table 1: Reduction in fungal growth in response to antagonistic effect of yeast isolate

Yeast isolate	Fungal growth reduction			
	Botrytis cinerea		*Alternaria solani*	
	Linear growth mm	Reduction %	Linear growth mm	Reduction %
S. cerevisiae	55.2	38.7	52.3	41.9
Control	90		90	

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Table 2: Reduction of yeast growth in response to antagonistic of essential oils concentrations

Treatment	Conc. Of Essential oil %	*S. cerevisiae* growth reduction %	
		Colony numbers 10^6(cfu\ml)	Reduction %
Mint Oil	0.25	316a	9.7
	0.50	310a	11.4
	1.00	307a	12.2
Rosemary Oil	0.25	302a	13.7
	0.50	282b	19.4
	1.00	231c	34
Lavender Oil	0.25	300a	14.3
	0.50	289b	17.4
	1.00	278b	20.6
Control		350	

Data in each colum with the same letter are not significant difference (P=0.05) according to Tukey test

Table 3: Alternaria solani Growth reduction in response to essential oils in combination with yeast isolates *in vitro*.

Treatment	Conc. Of Essential oil %	Fungal growth reduction %	
		Alternaria alternata	
		Linear Growth Mm	Reduction %
Mint Oil	0.25	80a	11.11
	0.50	66 b	26.7
	1.00	40 f	55.6
Mint Oil + Yeast	0.25	40 f	55.6
	0.50	35 c	61.11
	1.00	19 d	78.9
Rosemary Oil	0.25	85 a	5.6
	0.50	55 e	38.9
	1.00	42 cf	53.33
Rosemary Oil + Yeast	0.25	40 f	55.6
	0.50	33 c	63.3
	1.00	21 d	76.7
Lavender Oil	0.25	84a	6.7
	0.50	70b	22.2
	1.00	53e	41.1
Lavender Oil + Yeast	0.25	48 e	46.7
	0.50	36c	60
	1.00	26d	71.1
Control		90	

Data in each colum with the same letter are not significant difference (P=0.05) according to Tukey test

Table 4: B.cinerea Growth reduction in response to essential oils in combination with yeast isolates *in vitro*.

Treatment	Conc. Of Essential oil % Reduction %	Fungal growth reduction %	
		B.cinerea	
		Linear Growth Mm	Reduction %
Mint Oil	0.25	86a	4.4
	0.50	69 c	23.3
	1.00	43 f	52.2
Mint Oil + Yeast	0.25	42 f	53.3
	0.50	41 f	54.4
	1.00	30 e	66.7

Rosemary Oil	0.25	87a	3.3
	0.50	78 b	13.3
	1.00	53 d	41.1
Rosemary Oil + Yeast	0.25	44 f	51.1
	0.50	43 f	52.2
	1.00	35 cf	61.1
Lavender Oil	0.25	87a	3.3
	0.50	71 c	21.1
	1.00	50 g	44.4
Lavender Oil + Yeast	0.25	48 fg	46.7
	0.50	35 cf	61.1
	1.00	29 e	67.8
Control		90	

Data in each colum with the same letter are not significant difference (P=0.05) according to Tukey test

IV. DISCUSSION

Since no alternative to chemical control alone is as consistently effective as fungicides in reducing postharvest decay, promising alternatives of biological control with beneficial yeasts and plant essential oils treatments were tested to develop a strategy to provide satisfactory control of greenhouse decay on tomatoes. In order to enhance biocontrol activity of antagonists against fungal pathogens, certain strategies, such as adding calcium salts, carbohydrates, amino acids and other nitrogen compounds to biocontrol treatments, were suggested (Janisiewicz et al 1998).

Essential oils are made up of many different volatile compounds and the composition of the oil quite often varies between species (Mishra and Dubey 1994). The antifungal and antimicrobial effects are the result of many compounds acting synergistically (Bagamboula et al 2004). Thus, there would be negligible chance of development of resistant races of fungi after application of essential oils to fruit and vegetables. Consequently, essential oils are one of the most promising candidate groups of natural compounds for the development of safer antifungal agents.

To achieve a suitable efficacy and avoid antagonistic effect that could be happened in the essential oil-yeast formula, melon and rose oils were neglected to be tested as combined factor with yeast isolates referring to their inhibitor effect on the viability of yeast isolates (Table, 2).

The results in the present work indicate that the using of combination between yeast and essential oil enhanced the efficacy of decay incidence of tomato in green houses better than each individual component.

The obtained results in the present study revealed that the potential of using essential oils supplemented with *S. cerevisiae* and peppermint oil to control artificially-inoculated tomato fruits resulted in significant reduction in incidence and decay development infected plants. On the light of these results the usage of the proposed combination could be suggested for application against decay of tomato greenhouses (Abd-Alla et al 2009).

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