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THE EFFECTS OF THYME OIL AND CHLORINE DIOXIDE ON THE GERMINATION AND PRODUCTION OF CONIDIA AND MYCELIAL GROWTH OF *MONILINIA FRUCTICOLA* (G. WINTER) HONEY

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ABSTRACT
This study aims to research the *in vitro* effectiveness of thyme oil and chlorine dioxide (ClO_2) on mycelial growth, conidia production and conidia germination of *Monilinia fructicola* (G. Wint.) Honey which causes brown rot in plums.While a 300 ppm dose of chlorine dioxide inhibits mycelial growth by 77.2%, thyme oil at 30 ppm inhibits by 100%. The MIC value of chlorine dioxide is above 300 ppm. The ED_{50} values of thyme oil and chlorine dioxide have been determined as <3 and 15.3 ppm, respectively. While the spore germination of the pathogen is suppressed at high rates by 100 and 300 ppm chlorine dioxide (70 and 90%, respectively), the same doses of thyme oil suppress by 47 and 75%. Parallel to the effectiveness on germination the ED_{50} values of chlorine dioxide and thyme oil are 18 and 112.2 ppm. The test chemicals affected the fruit yield of the fungus at varying rates. Doses of 30, 100 and 300 ppm of chlorine dioxide affected the spore production at rates of 23.75, 37.5 and 43.75%. Doses of 3 and 10 ppm thyme oil affected the spore production by 77.7 and 96.5% while doses 30 µl/ml and above inhibited fructification by 100%. The research results prove that thyme oil, especially, and chlorine dioxide inhibit mycelial growth, spore germination and fructification of *M. fructicola* by a significant amount, indicating that, after *in vivo* studies, both chemical compounds may be used against monilia disease for plums after harvest.

Keywords: antifungal activity, thyme oil, chlorine dioxide, *Monilinia fructicola*, postharvest diseases.

INTRODUCTION

Monilinia fructicola [(G. Wint.) Honey] takes first place among diseases causing economic loss of fruit beginning before harvest and continuing during storage after harvest. *Monilinia fructicola*, which causes brown rot, generally begins with inoculum produced by apothecia forming on overwintered mummified fruit which cause blossom blight in spring. In appropriate environmental conditions blossom blight may progress to twig blight and branch canker, forming the second inoculum source and causing latent infection in mature stone fruits before and after harvest and in unripened green fruit (BOEHM ET AL., 2001).

Apricot, cherry, morello cherry, plum, almond and peach are among the hosts for this disease. The first signs on fruit are brown stains during the ripening period. In the advanced period of the disease it develops within the fruit flesh and causes drying and shriveling of the fruit over time. Infection discovered near harvest or during harvest may cause growth of rotting after harvest. Rotting after harvest is an important factor limiting the storage life of the fruit (ESTI ET AL., 2002).

Due to problems with chemical residues after harvest it is not possible to use synthetic fungicides such as carbendazim, captan, cyprodinil, dodine, iprodion, thiram and tebuconazole to treat monilia disease in the period before harvest. As a result in recent years to control the disease after harvest applications of biocontrol agents, food additives, plant activators, saline solutions, heat and hot water, ultraviolet light, disinfectants, plant extracts and essential oils have been used

(PUSEY ET AL., 1988, MARGOSAN ET AL., 1997, HONG ET AL., 1998, EKINCI ET AL., 2006, AKBUDAK AND KARABULUT, 2002, MARI ET AL., 1999). WILSON ET AL. (1986) identified that benzaldehyde, benzyl acetate, benzyl alcohol, δ-decalactone, γ-caprolactone, γ-decalactone, γ-octalactone, methyl salicylate and γ-valerolactone, naturally found in fruit, inhibited the spore germination of *Monilinia fructicola* and *Botrytis cinerea* by a large amount. They found that ethyl benzoate had a fungicidal effect on *M. fructicola* and a fungistatic effect on *B. cinerea* while methyl salicylate and benzaldehyde had fungicidal effects on both fungi. HONG AND MICHAILIDES (1998) in a study on brown rot in plums after harvest found 3 isolates of *Trichoderma spp.* (New, Ta291 and 23-E-6) inhibited *M. fructicola* by a significant amount (67-100%) while the BI-54 isolate of *Rhodotorula sp* had an inhibition rate of 54%.

The aim of this study is to research the *in vitro* effect of chlorine dioxide and thyme oil on the mycelial growth, spore density and spore germination of an isolate of *M. fructicola* from an infected plum.

MATERIAL AND METHOD

Material

The fungal material for the study was isolated from *M. fructicola* from an infected plum. Some properties of the test chemicals used during the study are given in *Table 1*.

Table 1. Chemicals used during the experiment

Trade Name	Active Ingredient	Percentage Active Ingredient	Formulation	Company
Thymol (Thyme oil)	2-isopropyl-5-methylphenol, IPMP	≥99.5%	Liquid	Sigma-Aldrich
Chlorine dioxide	ClO_2	10%	WP	AgrOx

Method

Effect of test chemical on mycelial growth

To determine the effectiveness of the test chemicals, chlorine dioxide and thyme oil, on the isolates doses of 3, 10, 30, 100 and 300 ppm were used. Only 1 ml sterile pure water and 1% diluted acetone were added to control petris. During the study chlorine dioxide was diluted with sterile pure water, while thyme oil was diluted with 1% acetone. After sterilization of the test chemicals the PDA broth was cooled to 45 °C and the concentrations listed above were spread across the surface until it was covered. The medium treated with control and chemicals were later inoculated with 4 mm disks, made with the aid of a cork-borer, of 7 day *Monilinia fructicola* grown in PDA broth. After inoculation the petri dishes were left to incubate at 23±2 °C. Colony growth was measured at 24 hour periods over 5 days.

Experiments were completely randomized and repeated 3 times. The effectiveness of the test chemicals on mycelial growth was identified by using the Abbott formula to compare the radius of the colonies on the petri dish with colonies on the control petri dishes. The ED_{50} value was found by using a semilogarithmic graph.

Effect of the test chemicals on spore germination

To determine the effect of the test chemicals on the spore germination of the pathogen, 1 ml 10^5 spore/ml spore suspensions prepared from *M. fructicola* isolates were spread on PDA in petris with the test chemicals at the same concentrations as used to test the effect on mycelial growth. After inoculation the petris were incubated in the dark at 23±2 °C and spore germination was examined under a microscope at 21 and 48 hours, recorded as germinated, non-germinated and rudimentary spores. Forty-eight hours after the spore suspension was administered to stop germination the blotting paper on the lid of every petri was treated with 0.5 ml 9% formaldehyde. Spores with clear germ tubes ½ their length were accepted as germinated, while swollen germ tubes or broken-tipped ones were accepted as rudimentary; no germination or germ tube less than ½ the length were accepted as non-germinated. ED_{50} values were found by using a semilogarithmic graph.

Effect of the test chemicals on sporulation

The effectiveness of the test chemicals on sporulation was determined by measuring the sporulation yield of the fungus culture in petris used to determine effectiveness against mycelial growth. To identify sporulation 10 ml pure water and 50 µl Tween 20 were added to the petris and using a glass rod the spores were placed in the water. The solution including spore and mycelium pieces was filtered through a single layer of cheesecloth and sterile pure water was added to reach a volume of 20 ml. Evaluation was made with the aid of hemacytometer as spore density per ml.

RESULTS

Thyme oil and chlorine dioxide showed effectiveness against mycelial growth of *Monilinia fructicola* at different levels. The effectiveness of the chemicals on the mycelial growth of the fungus is shown in *Figure 1*.

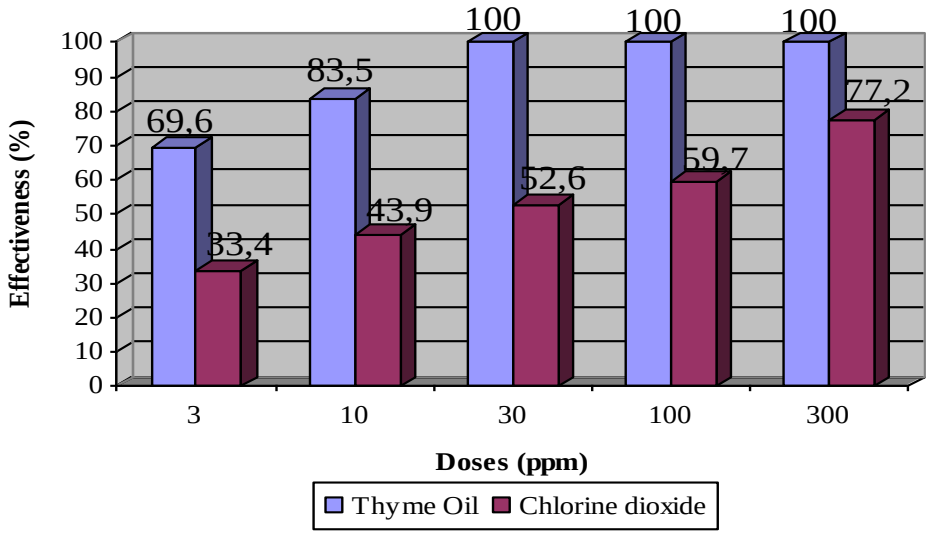

Figure 1. Effectiveness of the chemicals on the mycelia development of *Monilinia fructicola*

Thyme oil at doses of 30 ppm and above completely inhibited (100%) the mycelial growth while 3 and 10 mm doses caused a significant rate of inhibition (69.6 and

83.5%, respectively) (*Figure 1*). Different doses of chlorine dioxide affected mycelial growth at different rates. The lowest effectiveness was 3 ppm (33.4%) while the highest was with 300 ppm (77.2%). The minimum inhibition concentration (MIC) and ED_{50} values for the radial mycelial growth of *M. fructicola* are given in *Table 2*.

Table 2. MIC and ED_{50} of the test chemicals on the mycelial growth of *Monilinia fructicola*

Test chemical	MIC(ppm)	ED_{50}
Thyme oil	30	<3
Chlorine dioxide	>300	15.3

While thyme oil at 30 ppm completely inhibited the radial mycelial growth, the MIC value for chlorine dioxide was above 300 ppm. The ED_{50} value for thyme oil was less than 3 ppm, while it was 15.3 ppm for chlorine dioxide.
The effectiveness of the test chemicals on the germination of *M. fructicola* conidia are given in *Table 3*.

Table 3. Effectiveness of the chemicals on conidia germination of *Monilinia fructicola* (%)

Dose (ppm)	Chlorine dioxide	Thyme oil
3	0	0
10	44	4
30	55	5
100	70	47
300	90	75

The lowest concentrations of the chemicals did not inhibit germination of conidia (*Table 3*). While 30 ppm dose of thyme oil prevented 5% of conidia germination, chlorine dioxide at the same dose inhibited 55%. Chlorine dioxide and thyme oil at 300 µg/ml concentrations inhibited conidia germination by significant amounts (90 and 75%, respectively). Parallel to the effectiveness against germination, the ED_{50} values were 18 ppm for chlorine dioxide and 112.2 ppm for thyme oil.

Table 4. Effect of chemicals on conidia yield of *Monilinia fructicola* (spore count/ml)

Dose (ppm)	Chlorine dioxide	Thyme oil
3	$4.2x10^5$	$3.5x10^5$
10	$2.55x10^5$	$5.5x10^4$
30	$3.05x10^5$	0
100	$2.5x10^5$	0
300	$2.25x10^5$	0
Control	$4x10^5$	$1.57x10^6$

The spore density per ml of *M. fructicola* in sterile pure water was found to be $4x10^5$, while for acetone (1%) this value was $1.57x10^6$. At 3 and 10 ppm doses of

thyme oil the fructification yield of the fungus was significantly reduced while at 30, 100 and 300 ppm fructification yield was completely inhibited. A 3 ppm dose of chlorine dioxide did not affect conidia yield of the fungus but at 10, 30, 100 and 300 ppm doses the fructification reduced by 50% compared to control with a similarity between dose and spore production (*Table 4*).

CONCLUSIONS

This study investigated the *in vitro* effect of chlorine dioxide and thyme oil on the mycelial growth, conidia germination and fructification of *M. fructicola*. Thyme oil was found to be more effective against mycelial growth and fructification than chlorine dioxide. However chlorine dioxide prevented spore germination at a higher rate than thyme oil.

Only at 300 ppm doses did chlorine dioxide prevent the mycelial growth of the fungus at high rates, while thyme oil at doses ≥30 ppm totally inhibited growth. Increasing doses of chlorine dioxide prevented conidia germination more than thyme oil. At 300 ppm dose chlorine dioxide inhibited conidia germination by 90% while the same dose of thyme oil inhibited 75%. A study by LAZAR-BAKER ET AL. (2011) found doses of thyme oil ≥250 ppm completely inhibited mycelial growth of *M. fructicola* while 1000 ppm doses prevented 98% of spore germination. FATHI ET AL. (2012) reported that concentrations of thyme oil ≥400 ppm completely prevented the mycelial growth of *M. fructicola*. MARI ET AL. (1999) identified that a 100 ppm dose of chlorine dioxide completely inhibited the conidia germination of *M. laxa*. The highest effectiveness against fructification of *M. fructicola* was shown by thyme oil with doses ≥30 ppm completely inhibiting fructification. Chlorine dioxide at 300 ppm dose reduced conidia yield by 36.2%.

In conclusion, the test chemicals, especially thyme oil, were determined to have a large effect on the mycelial growth, conidia germination and fructification of *M. fructicola in vitro*. After *in vivo* studies, both chemicals may provide effect results in controlling brown rot in plums after harvest.

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