Antifungal Activity of Some Fungicide Alternatives on the Main Postharvest Pathogen of Tomato Fruits in Egypt

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ABSTRACT

Tomatoes crop and yield is suffered every year due to number of pathogenic diseases. Alternaria rot considered as the most common diseases of tomato fruits and causes heavy losses in quality of the fruits, thus rendering large quantity of tomato fruits unfit for consumption. Several essential oils and minerals were evaluated for their efficacy as potential fungicides against *Alternaria alternate*. Potato dextrose agar plates were inoculated with a disc of the 7 days old culture of tested fungus after a period of growth the growth inhibition was measured. Showed an ethanol extract of essential oils at concentrate 100 µl/l of clove oil has had the ability to inhibit fungal growth rate of 73.11% also jojoba oil gave inhibition of fungal growth rate of 32.22% while concentration gave 50 µl/l inhibitions by 40% for clove oil. On the other hand, out of four evaluated minerals, metal salts tested showed K_2SO_4 at 0.24% resulted *A. alternate* in maximum inhibition of growth (64.4%) followed by $MgSO_4$ by 52.2%. Chitosan, as antifungal agent, was found affected on the linear growth of *A. alternate* pathogen. The largest effect on *A. alternate* by 62.22% was observed at 6mg/ml. The obtained results in this study showed the possibility of usage K_2SO_4 and clove oils or chitosan to control fruit rot caused by *A. alternate*.

Keywords: Alternaria, Chitosan, fruit rot, tomato

1. INTRODUCTION:

Tomato is attacked by variety of pathogens; predominant being the fungal fruit rots. *Alternaria alternata* is a causal agent of black mold rot of tomato (*Lycopersicon esculentum*) fruit, a disease frequently causing substantial postharvest losses. There is a worldwide trend to explore new alternatives that control postharvest pathogenic diseases, giving priority to methods that reduce disease incidence and avoid negative

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and side effects on human health as a result of the excessive application of synthetic fungicides. [Johnson and Sangchote, 1994]. Recently, antimicrobial packaging has emerged as one of the most reliable and promising tool in the search for the next generation of 'active' packaging [Salleh, et al., 2007]. Identifying which essential oils or salt materials may have fungicidal effects against this organism is an important first step in developing a possible postharvest treatment.

2. OBJECTIVE:

To screen the antifungal activity of some fungicides alternatives such as essential oil, salts and chitosan on *Alternaria alternate* causing tomato fruits rot.

3. METHODS:

Alternaria alternata which was among several isolates isolated from tomato fruit rot, and proved to be the highest aggressive ones on tomato fruit. The antagonistic oil or salts were also used in the present work

The inhibitory effect of antagonistic materials, i. e, essential oils and chemical salts on the growth of *A. alternata* were evaluated using the culture technique [El-Mougy, et. al., 2004]. *In vitro* studies of tested microorganisms were performed on PDA medium in 9-cm-diameter Petri dishes. Procedures for growth inhibition measurements in all tests were done as the same followed technique. Tested chemicals were added to conical flasks containing sterilized PDA medium before its solidifying to obtain the proposed concentrations and rotated gently to ensure equal distribution of added chemicals. A separate PDA flask free of tested chemicals used as check treatment. The supplemented media were poured into sterilized Petri-dishes (9cm) approximately 20 ml per each. Mycelial disc (5mm) taken from the periphery of an actively growing PDA culture of tested fungus *A. alternata* was placed at the centre of the prepared Petri dishes, then incubated for seven days at 25±2°C. Three replicates were used for each treatment. The average linear growth diameter of colonies was measured and reduction in fungal growth was calculated in relative to check treatment. All experiments were repeated three times.

Different concentrations of Calcium carbonate, Potassium monohydrogen phosphate, Potassium sulphate, Magnesium sulphate (at concentrations of 0.25, 0.5, 1, 2 and 3g/l); Chitosan (at concentration of 1, 2, 3, 4, 5 and 6 g/L were tested. Certain weight or volumes of tested chemicals were added individually to conical flasks containing

sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri dishes (9-cm-diameter). Another set of conical flasks containing sterilized PDA medium free of tested chemicals was used as check control treatment. Petri dishes were individually inoculated at the centre with equal disks (5-mm) of tested fungus cultures. The average linear growth of each fungus was measured after 7 days of incubation at 25 ±2°C and reduction in fungal growth was calculated in relative to check treatment.

Effect of Some Essential Oils on Fungal Linear Growth, essential oils of Clove and Jojoba at 50 and 100 µl were used in the present work. Essential oils used in the study were prepared in Agric. Botany Dept. and extracting in petroleum Ether at room temperature for 48h. The inhibitory effect of the essential oils was evaluated against the linear growth of the tested *A. alternata* fungus *in vitro*. For each of the essential oil were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

4. Statistical Analysis

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between antagonistic inhibitor effect and linear growth of pathogenic fungi *in vitro*. A general linear model option of the analysis system SAS (1996) was used to perform the ANOVA. Duncan's multiple range test at $P \leq 0.05$ level was

5. RESULTS:

Results in Fig. (1) showed that tow tested essential oils have been found to have inhibitory effects against the mycelial growth of tested *A. alternata* isolates *in vitro*. Clove and jojoba oils had inhibitor effect on fungal mycelia. Fungal mycelial growth decreased significantly as the concentrations of essential oils were increased, to reach the fungal growth's minimum at the highest concentration used. It is possible that essential oils could be used in plant disease control as the main or as adjuvant antimicrobial compounds.

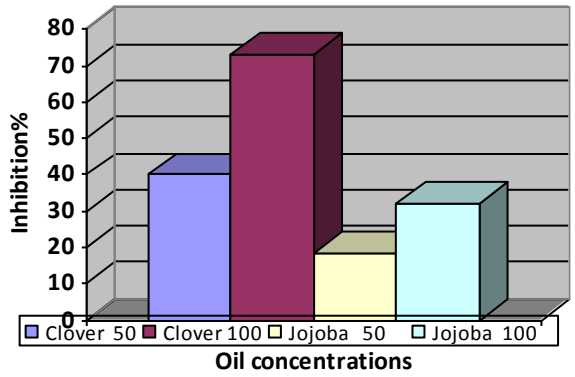


Fig. 1. Effect of essential oils on mycilial growth of *A. alternata*

Results in persented in Fig. (2) indicate that all evaluated plant resistance inducers significantly reduced the linear growth of tested isolate. The fungal mycelial growth reduced gradually by increasing of tested concentrations at the concentrations of 3% for Potassium phosphate and Magnesium phosphate. Also, it is observed that *A. alternata* showed more sensitivity against different concentrations of salts. In this regards, several types of treatment are reported to be partially effective in removing disease-causing organisms.

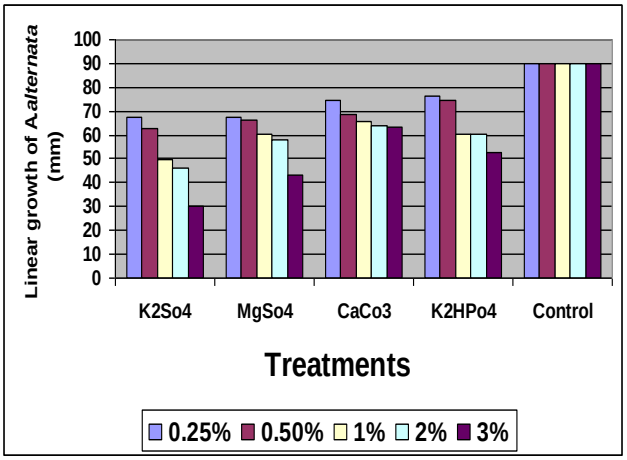


Fig. 2. Effect of inducers on mycilial growth of *A. alternata*

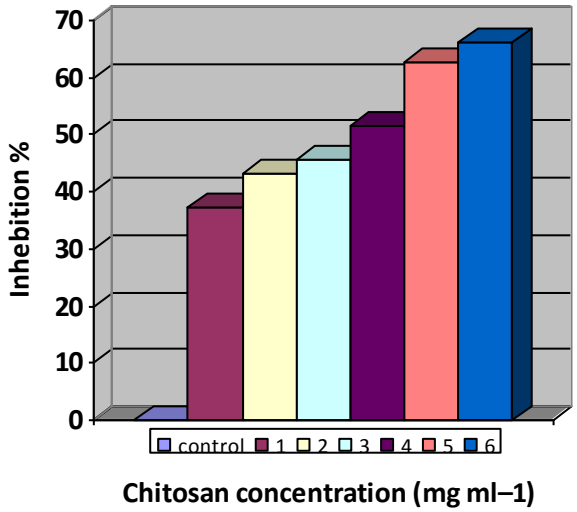
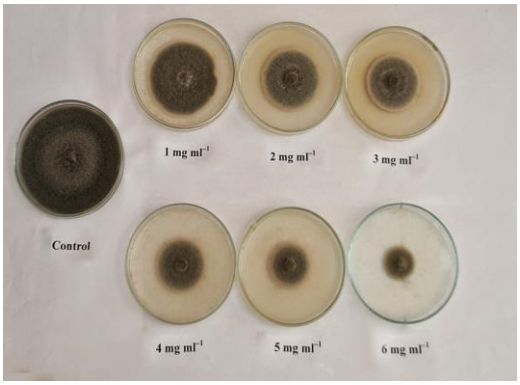
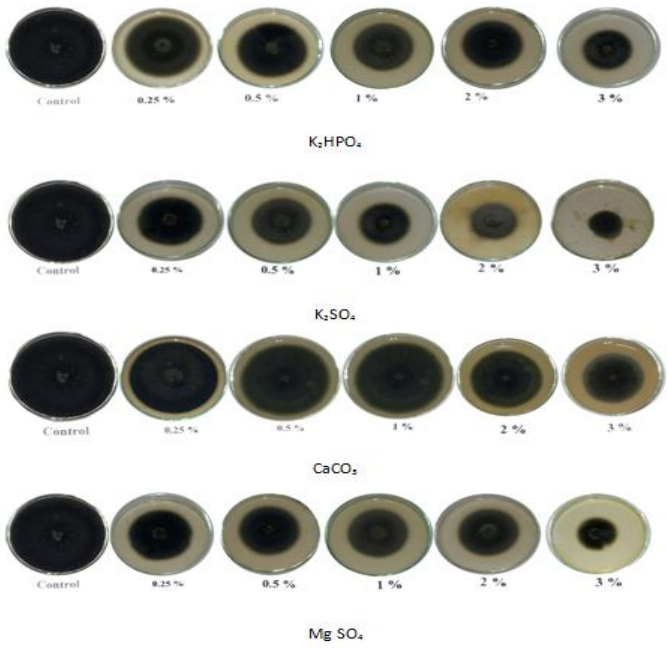


Fig. 3. Effect of chitosan on mycilial growth of *A. alternata*

Chitosan significantly inhibited mycelia growth of *A. alternata* ($P < 0.05$) (Fig. 3). chitosan at 6 mg/ml strongly inhibited mycelial growth of *A. alternata*.





The present study reported the different effects of essential oils, chitosan and some plant resistance inducers on developmental inhibition the mycelial growth of *A. alternate* the causal agent of tomato fruits rot. These results may lead to the conclusion that application of these control factors is applicable, safe and cost effective method for controlling such diseases under nursery and field conditions.

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نشاط مضاد الفطريات لبعض بدائل مبيدات على مسببات الأمراض الرئيسية ما بعد الحصاد للطماطم في مصر

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تهاجم ثمار الطماطم بالعديد من الامراض الفطرية التى تؤثر على جودتها وقيمتها لغذائية والتسويقية. عفن الثمار المتسبب عن فطر الالترناريا يتسبب فى قدر كبير من الخسائر وهو أكثر أعفان الثمار انتشاراً مما يجعل كمية كبيرة من ثمار الطماطم غير صالحة للاستهلاك. تم تقييم فاعلية بعض الزيوت النباتية لفعاليتها كمبيدات لفطر Alternaria alternate. تم تلقيح أطباق بترى محتوية على بيئة أجار دكستروز البطاطس بقرص من مزرعة فطر عمر سبعة ايام من الفطر المختبر بعد فترة من النمو (٧ ايام) تم قياس تثبيط النمو. أظهر مستخلص الإيثانول من الزيوت الأساسية عند لتركيز ١٠٠ ميكرولتر/ لتر من زيت القرنفل القدرة على تثبيط نمو الفطريات بمعدل ٧٣.١١٪ كما قدم زيت الجوجوبا تثبيط نمو الفطريات بمعدل ٣٢.٢٢٪ في حين أعطى زيت القرنفل عند التركيز ٥٠ ميكرولتر/ لتر تثبيط بنسبة ٤٠٪. من ناحية أخرى، من أصل أربعة املاح معدنية تم تقييمها، أظهر ملح K_2SO_4 عند تركيز ٠.٢٤٪ أعلى تثبيط لفطر الالترناريا (٦٤.٤٪)، يليه $MgSO_4$ بقدرة تثبيط ٥٢.٢٪. الشيتوزان، وعامل مضاد للفطريات، اظهر قدرة تثبيط للنمو الطولى لفطر الالترناريا. ولوحظ أكبر تأثير تثبيط ٦٢.٢٢٪ عند تركيز 6mg/ml. أظهرت النتائج التي تم الحصول عليها في هذه الدراسة إمكانية استخدام K_2SO_4 والقرنفل الزيوت أو الشيتوزان لمكافحة أعفان ثمار الطماطم لمتسببة عن فطر لفطر A. alternate.

الكلمات المفتاحية: الطماطم، الالترناريا، أعفان الثمار، الكيتوزان.