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Molecular Identification of Postharvest Moldy Core Pathogens on Apple and Application of Biocontrol Products of Essential Oils (EOs) and *Trichoderma harzianum*

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Abstract: This study aimed to identify prevalent pathogens of a caused moldy core of postharvest apple fruits and the efficiency of essential oils (EO) of clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus globulus*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*), and *Trichoderma harzianum* filtrate to inhibit pathogens growth *of Alternaria alternata*, *Botrytis cinerea*, and *Penicillium griseofulvum*. The examined pathogens are recognized dependent on morphological and also molecular identification. In vivo, clove EO and *T. harzianum* filtrate were strongly restricted decay area on fruits with 82.36% and 81.69%, respectively when applied as direct inhibition. Growth of all examined pathogens was entirely stopped on fruits treated with both clove and thyme oils at 10%. The results also illustrated that *T. harzianum* filtrate and EOs exhibited considerable growth inhibition of *B. cinerea* and ranged between 86.53% and 100%. The lowest inhibitory potential of EOs 47.95% and 75.9% were observed with *P. griseofulvum*. *T. harzianum* filtrate was the most effective biocontrol that inhibited fruit decay by 64.5% followed by 45.9%, 38.6%, 37.5%, and 35.9% when utilized EOs of thyme, sage, eucalyptus, and clove, respectively. The growth of both pathogens *A. alternata* and *B. cinerea* depressed with up to 90% using *T. harzianum* filtrate followed by EOs of eucalyptus and thyme. Whereas fruits inoculated with *P. griseofulvum* were not frustrated when applied to each EOs or *T. harzianum*. Their systemic induction was restricted between 3.16% and 23.82%.

Keywords: Apple decay, Clove, Eucalyptus, PCR, Sage, Thyme.

Introduction

Several pathogenic and opportunistic fungi attacked postharvest pome fruits of apple and pear resulting in unremarkable and reduction of fruits quality and quantity. Fungal pathogens such as *Alternaria alternata, Botrytis cinerea*, *Penicillium expansum,* and *P. griseofulvum* are responsible for the main economic losses (Eid, 2013; Singh *et al.,* 2017). Several *Alternaria* species such as *A. alternata* and *A. tenuissma* and *A. arborescens* may be accompanied by apple decay (Gao *et al.,* 2013; Mc Leod, 2014; Ntasiou *et al.,* 2015). Thus, high diversity and few features are not critical factors for identification. Recently, phylogenetic analysis is definitive for recognizing *Alternaria* species (Andrew *et al.,* 2009). The identification of *Botrytis* spp. and *Penicillium* spp. have been based on

colony morphology and description of conidiophores supplemented with molecular characteristics depending on the sequence of internal transcribed spacer (ITS) is used for the genetic identification and fungus phylogenetic relationships (White *et al.,* 1990; Notte *et al.,* 2021). Despite using modern storage facilities and applying many fungicides to control apple decay pathogens, these chemicals are more expensive, generate resistant races, and are harmful to human health and the environment (Choudhury *et al.,* 2018; Fayyadh & Yousif, 2019). Thus, substantial means of biocontrol agents and essential oils (EOs) are promising to control plant diseases that produce extensive secondary metabolites of phenolic, steroid, and terpenoid compounds (Sanzani *et al.,* 2010; Parveen *et al.,* 2016). Furthermore, they are biodegradable (Sales *et al.,* 2016; Okla *et al.,* 2019; Behiry *et al.*, 2020; Mohamed *et al.,* 2020).

Essential oils of thyme and eucalyptus evaluated the antifungal effects against *A. alternata* on potato (Hadizadeh *et al.,* 2009). Abo-El-Seoud *et al.* (2005) estimated antimicrobial activities of paper mint and eucalyptus essential oils against *B. cinerea* and *P. italicum* clove extract and essential oils were reported to be effective on *P. digitatum* and used as alternative control. However, the antimicrobial activities of examined oils have been referred to as phenolic or flavonoid compounds (Campos *et al.,* 2015; Povi *et al.,* 2015). Antagonistic fungi such as *Trichoderma harzianum* is a good biocontrol agent invested for pre-and post-harvest diseases management, due to producing toxins, antibiotics, and effective enzymes against plant pathogens (Goes *et al.,* 2002; Mahde *et al.,* 2019) in addition to many articles asserted that *T. harzianum* induced systemic resistance for the spacious ambit of plants pathogens (Shoresh *et al.,* 2010; Salih & Mansoor, 2019). The current work aimed to evaluate essential oils (EOs) of clove buds, eucalyptus leaves, and foliage of sage and thyme in addition to filtrate *T. harzianum* against the moldy core of apple caused by *A. alternata*, *B. cinerea,* and *P. griseofulvum* pathogens after their molecular identified, in addition to the capability of treatments for determination pathogens growth through direct inhibition and induction of systemic resistance (ISR).

Materials & Methods

Identification of pathogens

Morphological identification

Pathogens of *Alternaria alternata* (Fr.Keissl*.), Botrytis cinerea* (Pers**.**)*,* and *Penicillium griseofulvum* **(**Dierckx) were grown on Potato Dextrose Agar (PDA) at 25 ± 2°C and purified using single spore or hyphal tip techniques. Identification of the pure cultures was accomplished by cultural properties, morphological and microscopical characteristics according to Barnett & Hunter (1998) and Elad *et al.* (2007).

Molecular identification

DNA extraction, PCR amplification, and sequencing

Examined pathogens were grown in flasks 250ml containing 100ml potato dextrose broth at 25 ± 2˚C for seven days. The fungal mycelia were scribed and frozen at -20℃. DNA extraction was done according to commercial kits DNA preparation FATGK kit (BETA-BAYERN- Germany) protocol. The quality and quantity of extracted DNA were confirmed using Nanodrop 2000. Genomic DNA was played as a template for PCR amplification for its standard ITS region using ITS5/ITS4 universal primers (White *et al*., 1990). Polymerase chain reaction (PCR) was accomplished in a final

volume of 50µl containing 25µl (2x Taq) PCR Mix Master, 3µl of each forward primer and reverse (10 pm), 2µl of DNA genomic (50 ng. μl^{-1}) and 15µl of RNase Free water. Amplification was achieved in a GeneAmp PCR system PTC-200 thermocycler (Applied Biosystems) as follow: 95 ˚C for 3min., 35 cycles of 95 ˚C for 1min., 55 ˚C for 1min., 72 ˚C for 1min., and final amplification step of 72 ˚C for 10min.

Amplified PCR products were envisioned by 1% agarose gel electrophoresis stained with 3 µl of Pishgam- Fluorescent Gel Stain staining dye for DNA gel electrophoresis (Iran). The electrophoresis attained at 100 V. cm^{-1} gel, a voltage source (80V) for 40 min. Photography and illustration of bands were conducted using a trans-illuminator bear up with a digital camera. The sequencing was achieved at Microgen Company (South Korea). The result was investigated and aligned using BioEdit sequence alignment editor. The resemblance of sequences compared with homologous sequences deposited in GenBank and calculated using "Blast" tools depending on (NCBI) website.

Extraction essential oils (EOs)

Using steam distillation, purified EOs were extracted by (Adams, 2007) method without using any solvents. Every 100 gm of each grounded clove, eucalyptus, sage, and thyme was mixed with 400 ml water. The mixture was permitted at room temperature overnight for hydrolysis and hydro distilled at 100 °C. The liquid was separated using the Clevenger apparatus (Pyrex), and the water phase was discarded.

***In vivo*: effect of essential oils and *T. harzianum* filtrate on the pathogen's growth**

Direct inhibition of postharvest pathogens on apple fruit

Apple fruits were sterilized with 0.2 % Sodium hypochlorite, washed, dehydrated at room temperature, and pierced using a disinfected corkborer at the apical region (3mm depth, and wide, 3 wounds/fruit). 10 µl examined oils at (2.5%, 5%, 7.5%, and 10%), and *T. harzianum* filtrate was dropped for each wound. Conidial suspension 20 µ1 for each pathogen adjusted at (3 x 10^5 spores/ml) applied after 60 min. in the same wound before parceled in an incubator at 25 ±2 °C and 60 % humidity. Moldy rot was recorded after 7 days. The inoculated fruits with no essential oils are represented as control (Lopez-Reyes *et al.,* 2010).

Systemic resistance induction in fruits

Disinfected fruits wounded and the pathogen's spore suspension 20 µl of *A. alternata, B. cinerea,* and *P. griseofulvum* adjusted at 3 x 10^5 conidia/ml were applied in the second wound after 24 hrs., this wound is far away from the first one (treatments) by one-centimeter. Studied essential oils (2.5%, 5%, 7.5%, and 10%), and *T. harzianum* filtrate was dropped in each wound before incubating at 25 ±2°C and 60 % humidity. The necrosis was measured after a week.

Results & Discussion

Morphological and molecular identification

Phenotypic characterization:

***Alternaria alternata* (Fr. Keissl.):** Colonies on PDA were black or olive, with a diameter of 77-90 mm after 7 days, simple or branched conidiophores arising singly or in a small group. Conidia were ovoid or ellipsoidal 20-32 X 8.4 -11.4 µm. 3-5 transverse and several longitudinal septa Fig. (1) These observations were consistent with the descriptions of *A. alternata* (Ellis, 1970; Rotem, 1994; Simmons, 1995).

Fig. (1): *A. alternata* colony appearance on PDA after seven days with the morphology of conidia. Scale bar = 50µm.

***Botrytis cinerea* (Pers.):** Colonies on PDA were initially cottony white then changed to grayish-brown with a growth diameter 78- 90 mm after 7 days. Conidiophore branched with short sterigmata, aerial. Conidia were ovoid to ellipsoidal 9-12.5X 7.5 X 7.5-9.8µm. Sclerotia: Black elongate or spherical 3-6 mm (Fig. 2). This identification was similar to the results of Morgan (1971); Coley-Smith *et al.* (1980) and Notte *et al*. (2021).

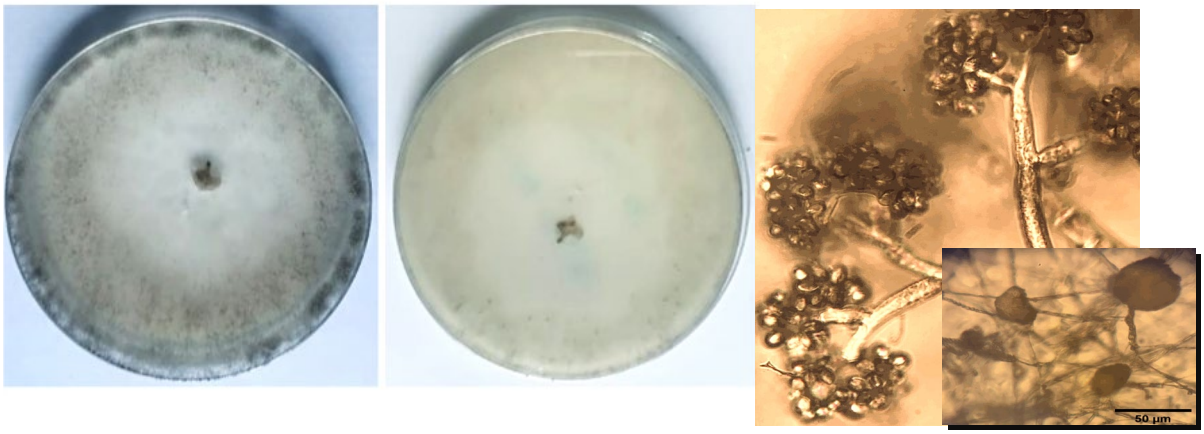

Fig. (2): *B. cinerea* colony appearance on PDA after seven days with the morphology of conidia. Scale bar = 33µ and Sclerotia 50 µ.

***Penicillium griseofulvum* (Dierckx)**: Colonies growth reached 20.4-25.7 mm after seven days. Colonies were gray-green to yellow-green, reverse view colored yellowish to orange-brown. Conidiophores were loose synnematous 400-460 µm, phialides more or less cylindrical with a very short neck. Conidia are subglobose 2-2.5 µm (Fig. 3). This morphological description corresponded to *P. griseofulvum* of Samson *et al.* (1981), and Banani *et al.* (2016).

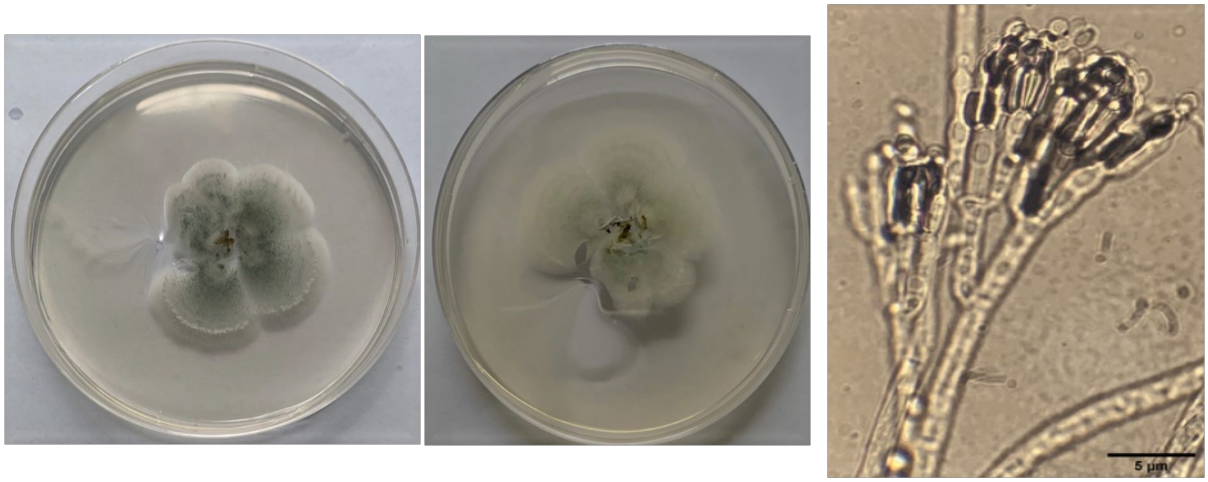

Fig. (3): *P. griseofulvum* colony appearance on PDA after seven days with the morphology of conidia. Scale bar = 5µ.

Molecular identification and phylogenetic tree of selected pathogens

PCR technique was conducted to approve the documentation of the pathogen by extension of the ITS region of rDNA. Nucleotides sequences of such regions were compared to those preserved at the GenBank sequence database. The selected isolates were *A. alternata* (accession number OK073893; amplicon size 600 bp) that showed 100% identity with the sequence of *A. alternata (a*ccession number MK774675 and MH716003). *B. cinerea* (accession number OK073895; amplicon size 600 bp) showed 100% identity with the sequence of *B. cinerea (a*ccession number MT573470 and MN844207). *P. griseofulvum* (accession number OK073894; amplicon size 600 bp) showed 100% identity with the sequence of *P. griseofulvum (a*ccession number MG975631 and MF034654). (Fig. 4).

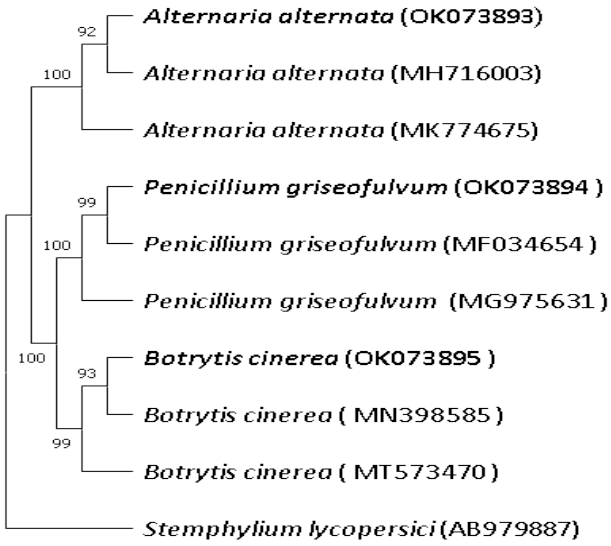

Fig. (4): Phylogenetic tree of isolated *A. alternata*, *B. cinerea*, and *P. griseofulvum* based on Neighbor-joining analysis ITS-rDNA sequences of the isolates *A. alternata*, *B. cinerea*, and *P. griseofulvum* .*Stemphylium lycopersici* used as out-group.

***In vivo*: effect of essential oils and *T. harzianum* filtrate on the pathogen's growth**

Direct inhibition of postharvest pathogens on apple fruit

The essential oils and *T. harzianum* filtrate have exposed encouraging results since the pathogen's growth was entirely stopped on apple fruits when treated with both clove and thyme oils at 10%. Previously, EOs of oregano at 1% and thyme at 10% prevented apple molds development (Lopez-Reyes *et al.,* 2010; Vieira *et al.,* 2018; Tzortzakis, 2019; Peralta- Ruiz *et al.,* 2021). The clove EO has the most noticeable antifungal activity against *P. italicum* and inhibited its growth at 24 µl.ml^{-1} after 15 days (Yahyazadeh *et al.,* 2008; Anjum & Akhtar, 2012).

Development of *B. cinerea* was inhibited entirely when using sage oil at 10% and eucalyptus EO at 5%, 7.5%, and 10%. The effective mean of examined EOs indicated that clove oil and *T. harzianum* filtrate considerably impaired decay progress with 82.36% and 81.69%, respectively. Researchers reported the efficiency of *Trichoderma* spp. in delaying spores' germination and pathogens growth (Lorito *et al.,* 1993; 1994; Schirmbock *et al.,* 1994). Moreover, they destroyed pectolytic and enzymes that are necessary for phytopathogenic fungi (Harman *et al.,* 2004). Generally, with increasing oil concentrations mycelial growth inhibition increased, and fruit decay caused by *B. cinerea* repressed 85.88%, followed by 72.21% and 58.78 % for *A. alternata* and *P. griseofulvum*, respectively. These findings supported by Kishore *et al.* (2007) and Xing *et al.* (2011) (Table 1 and Fig. 5).

Table (1): Direct inhibition of essential oils and *T. harzianum* on decay area of apple fruits.

Essential oils	%conc.	% Decay area inhibition ± SE			
		A. alternata	*B. cinerea*	*P.griseofulvum*	Mean
Clove	2.5	37.24 ± 4.4 r	80.7 ± 2.5 fg	48.33 ± 0.9 qr	82.36 a
	5	80.02 ± 2.9 fgh	91.81 ± 2.6 a-d	61.94 ± 1.8 lmn	
	7.5	94.95 ± 5.2 ab	100 ± 0.0 a	93.33 ± 6.6 abc	
	10	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	
Eucalyptus	0	0.0 ± 0.0 t	0.0 ± 0.0 t	0.0 ± 0.0 t	75.83 b
	2.5	69.41 ± 1.5 i-l	73.16 ± 2.7 g-j	44.48 ± 1.7 r	
	5	54.12 ± 4.3 opq	100 ± 0.0 a	57.75 ± 1.7 nop	
	7.5	86.79 ± 3.2 def	100 ± 0.0 a	57.06 ± 1.6 nop	
	10	100 ± 0.0 a	100 ± 0.0 a	67.25 ± 2.3 nop	
Sage	2.5	63.46 ± 0.3 lmn	72.49 ± 3.2 hij	44.62 ± 2.8 r	74.53 b
	5	71.12 ± 0.7 ijk	83.89 ± 3.0 ef	53.71 ± 2.8 opq	
	7.5	85.61 ± 3.2 def	97.62 ± 2.4 a	59.73 ± 1.9 mno	
	10	98.1 ± 1.9 a	100 ± 0.0 a	63.98 ± 2.6 k-n	
Thyme	2.5	33.37 ± 2.1 r	71.32 ± 1.1 ijk	33.52 ± 1.7 r	76.66 b
	5	89.31± 2.2 b-e	76.22 ± 1.1 ghi	51.04 ± 1.7 pqr	
	7.5	93.18 ± 3.6 abc	98.58 ±1.4 a	73.39 ± 3.1 g-i	
	10	100 ± 0.0 a	100 ± 0.0 a	100 ± 0.0 a	
T. harzianum		97.12 ± 2.7a	100 ± 0.0 a	47.95 ± 5.9 qr	81.69 a
Mean		75.21 b	85.88 a	58.78 c	

* Within each independent factor and interaction means followed by the same letter(s) aren't significantly different ($p \leq 0.05$).

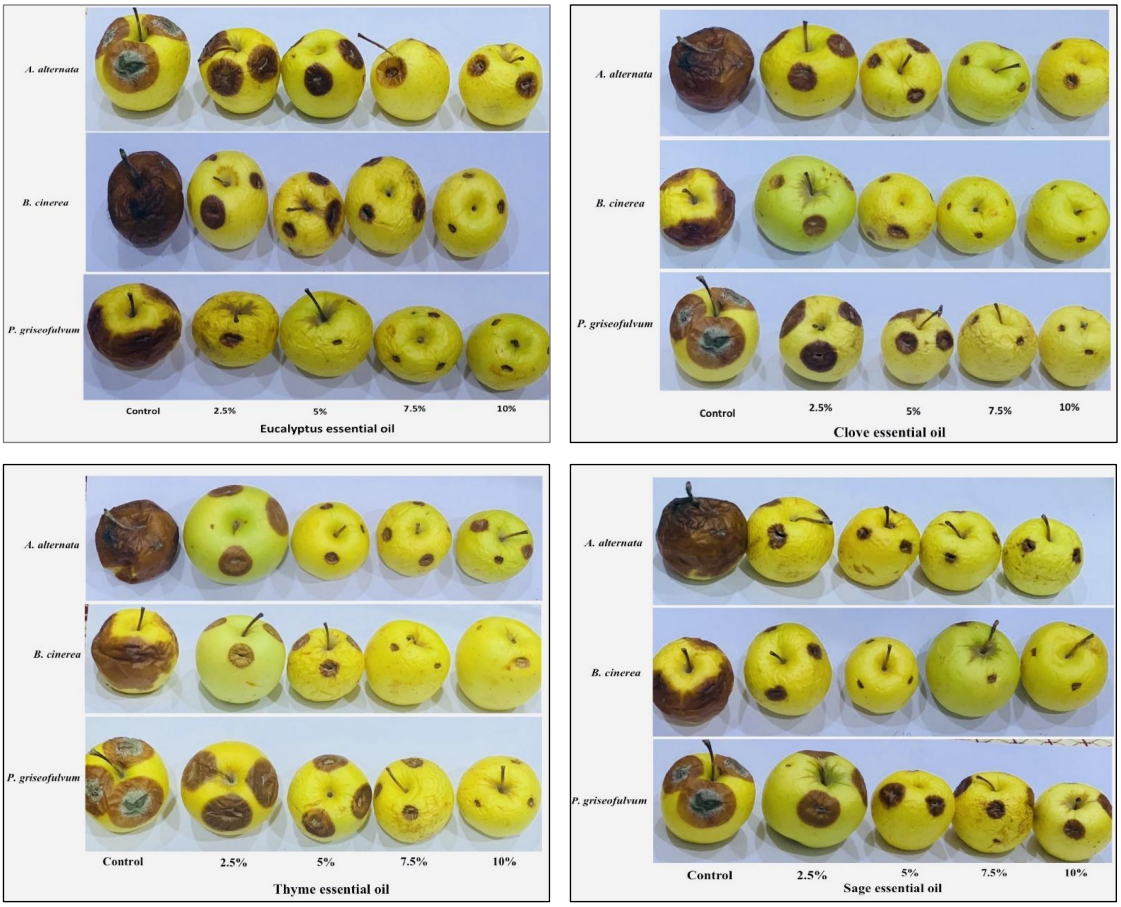

Fig. (5): Representative photographs of apple fruits infected with pathogens as a result of direct inhibition of inoculated pathogens using essential oils of clove, eucalyptus, sage, and thyme at different concentrations.

Application of EOs at different concentrations and *T. harzianum* filtrate were significantly inhibited apple decay compared to control treatment; clove and thyme oils at 10% showed complete inhibitory effect followed by eucalyptus and sage 89.08% and 87.36%, respectively (Fig. 6).

In this aspect, Jhalegar *et al.* (2014) confirmed that essential oils can be recommended as a safe method for extending its storage life while maintaining fruit quality through reducing fruit respiration, ethylene production, and metabolic activity. Therefore, the concentration of CO_2 might speed up fruit's ripening during storage which led to decay symptoms (Calvo & Sozzi, 2004; Peralta- Ruiz *et al.,* 2021). Also, EOs at 7.5% and 10% suppressed the decayed area. Fungistatic and fungicidal effects of oils like eucalyptus were observed at lower and higher doses, respectively (Shahi *et al*., 2003). Generally, the biological activity of examined oils was proportional to increasing their concentrations.

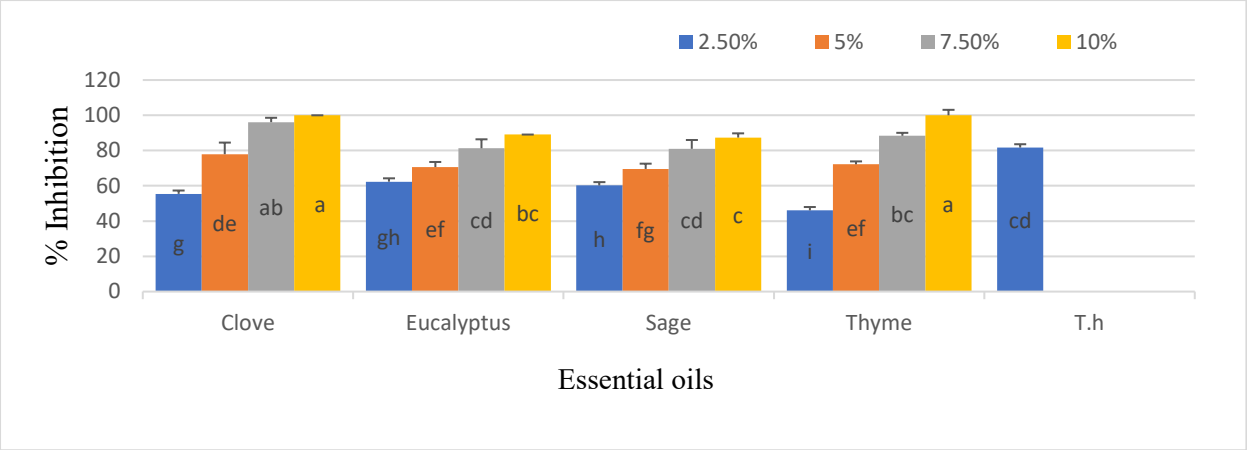

Fig. (6): Direct effect of essential oils concentration and *T. harzianum* on decay area of apple fruits.

Results of fig. (7) exhibited the interaction of direct inoculation with EOs and *T. harzianum* filtrate X pathogens. *T. harzianum* proved remarkable and entire inhibition of *B. cinerea* and 97.12% for *A.alternata*. Both clove and eucalyptus coincided with *T. harzianum* effectiveness in decay depression up to 93%. Eucalyptus EO increased the metabolic activity of the fruit itself noticed as a browning disorder (Xylia, *et al.,* 2021). In contrast, the lowest inhibitory potential of EOs was observed with *P. griseofulvum* and ranged between 47% and 75%

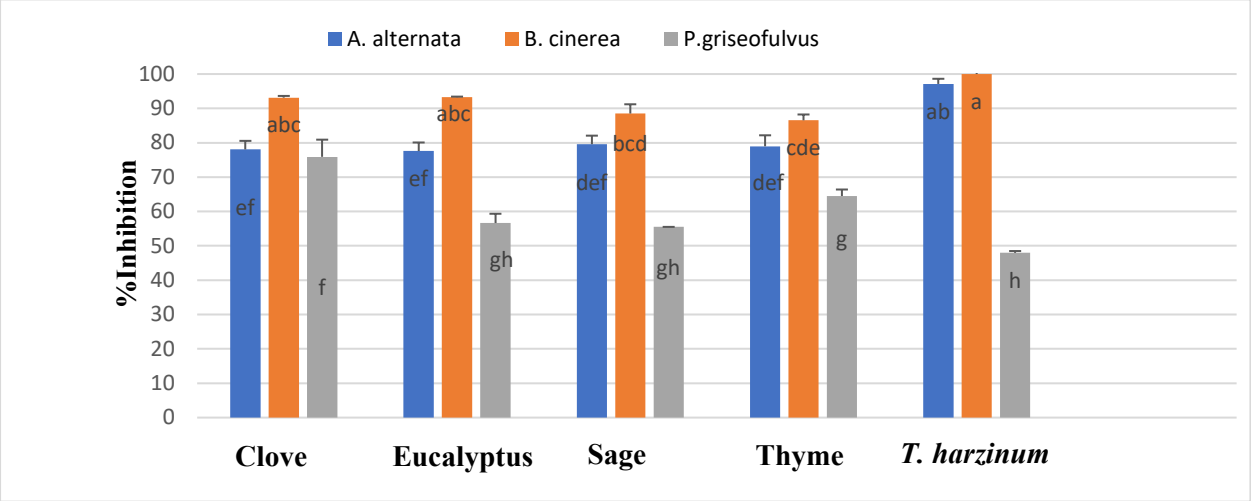

Fig. (7): Interaction of direct effect of essential oils and *T. harzianum* X pathogens on decay area of apple fruits.

Induction of systemic resistance in fruits

To verify the effectiveness of EOs or *T. harzianum* filtrate in inducing apple resistance, the wounded fruits were treated with oils before the pathogen's inoculation in the second wound after 24h. *B. cinerea* reduced decay development by more than 68% compared to 30.74% and 16.74% for *A. alternata* and *P. griseofulvum,* respectively. *T. harzianum* filtrate was the most capable bioproduct which induced resistance with 64.5% decay inhibition compared to 45.9%, 38.6%, 37.5%, and 35.9% of applied EOs of thyme, sage, eucalyptus, and clove, respectively. Several publications have confirmed the efficiency of *T. harzianum* inhibitory potential against the

development of infectious fungi (Lorito *et al.,* 1994; Schirmbock *et al.,* 1994; Made *et al.*, 219). *B. cinerea* and *A. alternata* showed more susceptibility for *T. harzianum* filtrate and decay areas confined with 97.9% and 92.5%, respectively. The highest concentration of examined EOs 10% exhibited remarkable resistance and considerable decay inhibition with up to 90% (Table 2). This study observed that thyme EO or *T. harzianum* possess the ability to induce defenses against *B. cinerea* more than A. *alternata* and *P. griseofulvum*. However, the effectiveness of these bioproducts was proportioned to increasing their concentrations.

Table (2): Induction of systemic resistance of essential oils and *T. harzianum* filtrate against apple decay.

Essential oils	%Conc.	% Decay area inhibition ± SE			
		A. alternata	*B. cinerea*	*P.griseofulvum*	Mean
Clove	2.5	6.89 ±1.6 qrs	29.07 ± 2.1 hij	2.95 ± 0.7 rs	35.94 c
	5	12.04 ± 1.5 n-q	40.75 ± 2.2 g	8.47 ± 2.4 pqr	
	7.5	17.24 ± 2.9 l-o	78.98 ± 2.3 c	11.03 ± 3.1 opq	
	10	60.29 ± 2.2 e	90.81 ± 1.1 ab	72.81 ± 3.4 cd	
Eucalyptus	2.5	5.74 ± 1.4 qrs	62.01± 2.5 e	5.88 ± 1.8 qrs	37.59 c
	5	10.85 ± 0.7 opq	74.49 ± 1.6 cd	15.12 ± 1.9 m-p	
	7.5	18.78 ± 2.6 lmn	88.65 ± 1.1 b	20.64 ± 1.2 klm	
	10	27.29 ± 2.1 ijk	94.38 ± 2.9 ab	26.98 ± 1.0 jk	
Sage	2.5	26.03 ± 3.9 jk	34.11 ± 0.6 ghi	5.08 ± 2.8 qrs	38.61 c
	5	27.55 ± 4.3 ijk	59.06 ± 0.9 e	15.42 ± 3.1 m-p	
	7.5	38.69 ±1.5 g	71.5 ± 0.9 d	18.28 ± 2.6 lmn	
	10	50.44 ± 3.1 f	93.74 ± 3.2 ab	23.41 ± 2.2 jkl	
Thyme	2.5	26.46 ± 1.2 jk	58.21± 1.1 e	6.05 ± 2.7 qrs	45.99 b
	5	35.37 ± 3.0 gh	73.28 ± 2.2 cd	18.72 ± 3.5 lmn	
	7.5	41.04 ± 1.3 g	92.34 ± 2.1 ab	21.16 ± 2.2 klm	
	10	56.14 ± 1.4 ef	97.14 ± 2.9 a	25.97 ± 1.5 jk	
T. harzianum		92.51 ± 4.1 ab	97.97 ± 2.0 a	3.16rs ± 0.9 rs	64.55 a
Mean		30.74 b	68.69 a	16.74 c	0 d

* Within each independent factor and interaction means followed by the same letter(s) aren't significantly different ($p \leq 0.05$).

The data of fig. (8) clarified the superiority of *T. harzianum* filtrate in the inhibition of both *A. alternata* and *B. cinerea* with average of 90%, followed by EOs of eucalyptus and

thyme. On the contrary, *P. griseofulvum* was not stopped when applied to each of EOs or *T. harzianum* and their systemic induction ranged between 3.16% and 23.82%, respectively. Corresponding results of *Trichoderma* spp. was confirmed by Terry & Joyce (2004), Walters *et al.* (2005) and Harman (2006).

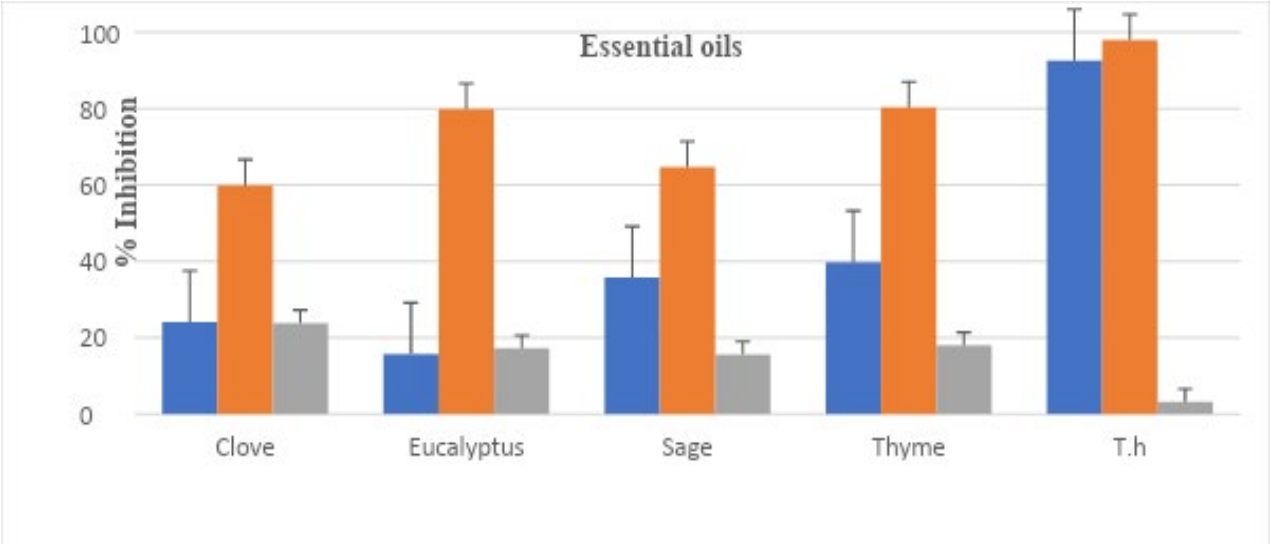

Fig. (8): Interaction of indirect effect of essential oils and Th. X pathogens on inhibition decay area of apple.

Conclusion

The natural products, such essential oils of clove, eucalyptus, sage, and thyme and bioagent of *T. harzianum* that applied in the current work gave promising evidence for providing an alternative control of apple decay instead of using hazardous chemical fungicides. Theses natural products reduced postharvest infections caused by the major causes of diseases *A. alternata, B. cinerea,* and *P. griseofulvum*; antifungal activity of the oils confirmed effective and obvious inhibition of the fungi mycelial growth, then prevented the rotting of fruits during storage. These friendly bioproducts particularly *T. harzianum* filtrate and clove EO also induced the systemic resistance in the treated fruits to prevent a moldy core attack.

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Contributions of authors

D.K.K.: Sample collection, Laboratory methodology, and writing the manuscript.
W.H.A.: Suggest a title of the research, graphs, and statistical analysis.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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التشخيص الجزيئي لممرضات تعفن التفاح ما بعد الجني ومقاومتها حيويا باستخدام الزيوت العطرية والفطر *Trichoderma harzianum*

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المستخلص: استهدفت الدراسة تشخيص ممرضات تعفن ثمار التفاح ما بعد الجني و اختبار كفاءة زيوت القرنفل *Syzygium aromaticum* و اليوكالبتوس *Eucalyptus globulus* و الزعتر *Salvia officinalis* والميرمية *Salvia officinalis* وراشح *T. harzianum* في تثبيط نمو الممرضات *Alternaria alternata,* و *Botrytis cinerea* و *Penicillium griseofulvum*. شخصت الممرضات مورفولوجيا وجزيئيا، ثبطت زيت القرنفل و *T. harzianum* مساحة الثمار المتعفنة بنسب عالية بلغت 82.63% و81.69% على التوالي عند استخدامه بطريقة التثبيط المباشر، وتوقفت نمو الممرضات كليا على الثمار المعاملة بزيوت القرنفل والزعتر بتركيز 10%. أظهرت الدراسة أيضا ان راشح *T. harzianum* والزيوت المختبرة ثبطت نمو *B. cinerea* معنويا وبنسب عالية تراوحت بين 86.53% و100% وبلغت اقل قدرة تثبيطيه للزيوت 47.95%و75.9% عند استخدامها ضد *P. griseofulvum*تبين ان *T. harzianum* هو الأكثر فعالية في تثبيط تعفن الثمار وبنسبة 64.5% يتبعه زيوت الزعتر، الميرمية، اليوكالبتوس والقرنفل وبنسب بلغت 45.9%، 38.6%، 37.5% و35.9% على التوالي. تثبطت تمو الممرضين *Alternaria alternata*, و*Botrytis cinerea* بنسب اعلى من 90% عند استخدام*T. harzianum* تليها زيوت اليوكالبتوس والزعتر بينما الثمار الملقحة بالممرض*P. griseofulvum* لم تتثبط نموها عند استخدام أيا من الزيوت او*T. harzianum* ، واستحثت مقاومة جهازية بنسب منخفضة تراوحت بين 3.16% و23.82%.

الكلمات المفتاحية: PCR تعفن التفاح، القرنفل، اليوكالبتوس، الميرمية، الزعتر.