

## EFFICACY OF SOME PLANT EXTRACTS AND FUNGICIDES TO CONTROL ALTERNARIA BLACK SPOTS CAUSED BY *ALTERNARIA ALTERNATA* ON POSTHARVEST MANGO FRUITS IN EGYPT

Ammar, Hager A.<sup>(1)</sup>; El-Helaly, Sahar H.<sup>(2)</sup> and Abdel-Rahman T. A.<sup>(3)</sup>

<sup>(1)</sup> Department of pesticides, Faculty of Agriculture, Menoufia University, Egypt.

<sup>(2)</sup> Department of Agricultural Botany, Faculty of Agriculture, Menoufia University, Egypt.

<sup>(3)</sup> The Central Agricultural Pesticide Lab, Nadi El-Said St., Giza, Egypt.

Received: Mar. 6, 2022

Accepted: Mar. 21, 2022

**ABSTRACT:** The aim of this study was to evaluate four the antifungal activity of aqueous plant extracts (*Laurus nobilis*, *Dianthus caryophyllus*, *Cinnamomum verum*, and *Thymus vulgaris*) as an alternatives to chemical fungicides (Imazalil and Thiabendazole) against *Alternaria alternata* which causing black spot of sugar mango fruits. Antifungal activities of plant extracts and chemical fungicides were tested both *in vitro* and *in vivo* conditions. The *in vitro* assay showed maximum inhibition of radial mycelial growth of *A. alternata* (100%) by *D. caryophyllus* extract, at concentrations of 2.5, 5, and 10% Similarly, the *in-vivo* assay showed a marked reduction in lesion diameter (2.5, 1.5, and 0.9 mm) and decreasing disease severity by 95.46, 97.04 and 98.22 %, respectively on the mango fruits treated with this extract. While the *in vitro* assay with Imazalil fungicide showed inhibition of radial mycelial growth of *A. alternate* by 82.13, 85.22, and 89.35%, respectively at 50, 100, and 200 ppm, respectively and reduced disease severity by 86.37, 88.90, and 93.43%, respectively. The residual activity of post-harvest treatment with imazalil (IMZ) and thiabendazole (TBZ) was studied at different dipping lengths and different cold storage times on mango fruit. IMZ and TBZ residue decreased with the number of cold storage days for all treatments and the residue increased with the duration of dipping for all treatments. Both fungicides residues were lower than MRL set by codex and EU Pesticides Database.

**Keywords:** Sugar mango, *A. alternate*, plant extracts, chemical fungicides, fungicide residue.

### INTRODUCTION

If post-harvest handling and storage conditions are not optimal fruit and vegetable postharvest losses can be extremely substantial, accounting for more than 25% of total fruit yields in industrial countries and more than 50% in developing ones. Many losses are caused by fungal pathogens because of the high amount of nutrients and water in fruit, low pH, and the fact that after harvest, fruits have lost the natural resistance that protected them when attached to the plant (Nunes, 2015).

The sugar mango (*Mangifera indica* L.) of the Anacardiaceae family is one of the most popular fruits growing in tropical locations (Al-Najada and Al-Suabey, 2014). In many subtropical countries, mango is an important part of the diet. Their appeal and flavour have improved the quality of life in places of the

world where living standards are low and nutritional deficiencies are severe (Kumlachew, 2014). Mango production, on the other hand, is plagued by a variety of diseases at every step of its development, from seedlings in the nursery to fruits in storage or transit (Prakash, 2004). Mango fruits are highly sensitive to pathogenic fungus during the period between harvest and consuming due to their high moisture and nutrient reserve. Mango fruits must be sold as soon as possible after harvesting due to their high susceptibility. *A. alternata* is one of the most dangerous fungal agents that causes black spots on preserved mango fruits and is one of the most serious fungal agents that causes mango post-harvest diseases (Haggag, 2010).

*A. alternata*, a common disease that causes significant postharvest losses in mango fruit, causing black spots on the fruit. Chemical pesticides can have a long-term endurance and

toxicity, entering the mango food chain and being exported to other nations (Sivakumar, *et al.*, 2011). Despite the fact that fungicides are quite effective in controlling some postharvest diseases, their widespread usage has resulted in the rise of fungicide-resistant species. Furthermore, the detrimental impact of fungicide residues on food and the environment is a major source of concern (El-Ghaouth *et al.*, 2003).

Various agrochemicals have been used for many years to control pre-and postharvest disease management in mango fruits (Deguine *et al.*, 2018). Mangoes have been protected from disease using agrochemicals like synthetic fungicides. Fungicide use prior to and after harvest has been found to be particularly effective in blocking *A. alternata* growth and development (Troncoso- Rojas and Tiznado-Hernandez, 2014). But a rising number of foreign marketplaces are no more approving fungicidal application for the importance of vegetables and fruits because synthetic fungicides are very harmful due to their maximum residue level (MRL) on consumer's health (Baibakova *et al.*, 2019). Imazalil (IMZ) and thiabendazole (TBZ) were systemic fungicides that used to treat many different types of fungal diseases in fruits, vegetables, and ornamentals (Tomlin, 1994). Both fungicides were commonly used in citrus packinghouse treatments to prevent postharvest deterioration (Eckert and Eaks, 1988).

Accordingly, alternative solutions for reducing postharvest losses of fruits and vegetables caused by fungal diseases were being researched widely around the world. Plant extracts were currently being evaluated as a viable alternative to fungicides for post-harvest disease management because they were non-toxic, safe for humans and the environment, and exhibit antifungal action (Obagwu and Korsten, 2003). Therefore, the goal of this study was to compare the antifungal activity of natural plant extracts to chemical fungicides against *Alternaria* black spot in mango *in vivo* and *in vitro*. In addition, the residue levels of tested fungicides in mango fruits were determined.

## MATERIAL AND METHODS

### Isolation and identification of the fungal pathogen:

*A. alternata* which caused black spot disease of sugar mango was isolated from naturally infected sugar mango fruits. Infected tissues were surface sterilized for 1 minute in 1% sodium hypochlorite solution, then washed twice with sterilized water. To avoid bacterial contamination, sterilized pieces were put onto (PDA) medium containing streptomycin sulphate using a sterile scalpel in petri plates, the plates were incubated for 4–7 days at 25°C, and observations were recorded (Biligrani *et al.*, 1991). Individual hyphal-tips of developing fungi were transferred on new PDA plates and subsequently identified using morphological and microscopical characteristics outlined by (Barnett and Hunter, 1999). Identification was confirmed by staff of Botany Department, Faculty of Agriculture, Menoufia University, Egypt.

### Conidial suspension of *A. alternata*:

After, 14 days-old *A. alternata* culture plate was flooded with 15 ml of sterile water (SDW). The mycelium remains were collected and filtered through three layers of sterile muslin cloth after the colony's surface was softly scraped. A haemocytometer was used to adjust the spore suspension concentration ( $1 \times 10^4$  spores/ml) (Hosseini, *et al.*, 2020).

### Pathogenicity test:

Sugar mango fruits that showed no signs of disease were rinsed in water and surface-sterilized for 2 minutes with 1% sodium hypochlorite solution, then dipped three times in SDW. 10 µl of prepared conidial suspension was inoculated into each fruit. The inoculated mango fruits were then placed in a plastic tray and incubated at 25°C for 7 days. A tiny sample of the lesions was aseptically placed on PDA and incubated at 25°C for 7 days. The Koch postulates were confirmed and fulfilled by observing colony characteristics and microscope structure. Healthy control fruit was inoculated with SDW (10 µl). The tests were carried out in triplicate (Orak *et al.*, 2019).

***In vitro*: Valuation of some plant extracts and fungicides in controlling Alternaria black spot (*A. alternate*):**

**Preparation of plant extracts:**

All plant materials used in this study were collected and identified (common name, scientific name and used parts) (Table 1). Powders of four plant samples were purchased from a local market. About 100 gm of dry powder of each plant material was added to 1000 ml distilled water and mixed thoroughly then autoclaved with steam under pressure at 90 °C for 30 minutes (Metwally *et al.*, 2010). Each plant extract was tested at three concentrations: 2.5, 5, and 10%, with three replicates. The aqueous extracts were kept in dark glass bottles in the refrigerator for further studies. Plant extracts were prepared and evaluated for their bioactivity using the agar dilution method (Akaeze and Modupe, 2017).

**Antifungal activity of selected fungicides:**

This test aimed to evaluate the efficacy of different concentrations of two chemical fungicides Imazalil (Diabolo 10%SL) and Thiabendazole (Tecto 50%SC) against *A.alternata*. Three concentrations, *i.e.*, 50, 100 and 200 ppm (Hemeda, 2009), were tested individually to assess their effect on *A. alternate* growth inhibition in a petri dish plate. Inoculated plates were incubated for 5-7 days at 25°C and two diameters of every dish were measured daily until the full growth of the fungus was noticed in the control. The average diameter and growth reduction were calculated.

Three replicated plates for each treatment were maintained and the results were recorded when the control plate was full with the fungal growth. Fungitoxicity was measured as a percentage of mycelial growth inhibition against the tested fungus, which was calculated using the formula:

$$PI = \left[ \frac{(C - T)}{C} \right] \times 100$$

Where, PI: is the percent inhibition over control, C: is mycelial radial growth in control plate, T: is

mycelial radial growth in treatment (Shivapratap, *et al.*, 1996).

***In vivo*: Valuation of some plant extracts and fungicides in controlling Alternaria black spot (*A. alternate*):**

Fresh mango fruit, uniform in color and size, was washed and surface-sterilised with 1% sodium hypochlorite solution for 2 minute, then rinsed with SDW for three times and left to air-dry at 27°C. An *in vivo* assay method described by Rizwana, (2018) was used with some minor modifications. A sterile needle was used to wound the disinfected mangoes in two locations (injuries 2 mm wide and 2 mm deep). Each wound was inoculated with a conidial suspension and left for 30 minutes before being sprayed with 10 µl of each plant extract examined. The inoculated fruit was placed in sterile plates and wrapped in sterile plastic wrap after 1 hour. At 25°C, the trays were incubated for 7 days. The diameter of black lesions/spots was measured, and the method below was used to compute the % disease (lesion) inhibition. The conidial suspension was inoculated into control fruit without any treatments. The experiment was repeated three times, with three mangoes in each treatment.

$$D = (L_1 - L_2) / L_1 \times 100$$

Where, D: is the percentage disease inhibition, while L<sub>1</sub>: is the lesion diameters on control and L<sub>2</sub> is the lesion diameters on treated mangoes.

**Post-harvest treatment with tested fungicides:**

**Plant material:**

Mature mangoes were obtained from a horticulture farm, faculty of agriculture, Menoufia university. Fruits were transported to the laboratory immediately after harvest. They were sorted for uniform size and free from defects. With commercially available Diabolo % SL (Certis company, Holland) and Tecto 50% SC (Syngenta Agro, Switzerland), Imazalil and Thiabendazole aqueous solutions with distilled water were prepared, respectively. **Treatments:** The Mangos fruits were divided into four groups for the following treatments:

- Group (1): Dipped in a 1000 mg/L of IMZ aqueous solution at 15°C for 5 sec.
- Group (2): Dipped in a 1000 mg/L of IMZ aqueous solution at 15°C for 10 sec.
- Group (3): Dipped in a 1000 mg/L of TBZ aqueous solution at 15°C for 5 sec.
- Group (4): Dipped in a 1000 mg/L of TBZ aqueous solution at 15°C for 10 sec.

After treatment, all fruits were air-dried at 15°C and stored for 14 days at 5°C and 85-90 RH.

**Analysis of selected fungicides:**

The IMZ and TBZ residues on the fruits were determined on randomly picked fruit triplicates after treatment at 0, 3, 7, and 14 days of cold storage. The fruits were chopped into pieces with a knife, homogenized for 1 minute, and kept at -20°C until analysis. The procedure QuEChERS was used. In brief, mango samples were extracted using the method outlined and modified by (Lehotay *et al.*, 2010), 10 g of the homogenized sample was weighed into a 50 ml centrifuge tube. Ten milliliters of 1.0% acidified acetonitrile with acetic acid were added; the screw cap was closed and vigorously shaken for 1 min using a vortex mixer at maximum speed. After that, 4 g of anhydrous MgSO<sub>4</sub>, 1 g of NaCl, 1 g sodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate were added, and the extract was shaken rapidly on a

vortex for 2 minutes before centrifuging at 5,000 rpm for 10 minutes. An aliquot of 3 ml was transferred from the supernatant to a new clean 5-ml centrifuge tube and cleaned by dispersive solid-phase extraction with 75 mg of PSA and 500 mg of magnesium sulfate. After that, centrifugation at 6,000 rpm for 5 minutes was performed. The supernatant was aliquoted (2 ml) and filtered using a 0.2-µm PTFE filter (Millipore, USA) before being analyzed with an Agilent 1100 HPLC-DAD.

**Method validation:**

The proposed analytical method (HPLC-DAD) was validated in accordance with SANCO document 10684/2009. For HPLC-DAD, linearity was assessed by creating matrix matched calibration curves in the range of 0.1–20 µg/L. Samples spiked with the studied pesticides at three different levels (1, 0.01, and 0.001 mg/kg) were used to determine method sensitivity and recovery. After the fortified samples were extracted as described previously, the average recovery percentages for fortified samples were calculated. The pesticide concentration that causes a peak signal-to-noise ratio of 3:1 and 10:1, respectively, was used to determine the limits of detection (LOD) and quantitation (LOQ). Table (2) summarized the prior procedures.

**Table (1): Plant materials used in preparing aqueous extracts for bioassay against *Alternaria black spot (A. alternata)*:**

Plant extracts			
Common name	Scientific name	Family	Used part
1-Bay leaf	<i>Laurus nobilis</i>	Lauraceae	Leaves
2-Carnation	<i>Dianthus caryophyllus</i>	Caryophyllaceae	Flower buds
3-Cinnamon	<i>Cinnamomum verum</i>	Lauraceae	Bark
4- Thyme	<i>Thymus vulgaris</i>	Lamiaceae (Labiatae)	Leaves

**Table (2). HPLC conditions and Percent recovery from fortified mango samples and the minimum detection limits (mg/ kg) for numerous pesticides**

Pesticides	Mobile Phase (v/v)	Flow Rate (ml/min)	Detectors Wave length (nm)	Recovery %	LOD*	LOQ**	r2
<b>Imazalil</b>	methanol-acetonitrile (70:30)	1	197	93.55	0.001	0.003	0.996
<b>Thiabendazole</b>	methanol-acetonitrile (70:30)	1	210	94.33	0.01	0.03	0.995

\*LOD: Limit of Detection \*\*LOQ: Limit of Quantification

Reference standards of all tested insecticides were of >98% purity and obtained from Central Agricultural Pesticides Laboratory (Egypt). Pesticide stock solutions were made in acetonitrile and kept at 18°C. All HPLC grade organic solvents, methanol, and acetonitrile were purchased from Sigma (Sigma GmbH, Germany). Primary secondary amine (PSA, 40 µm Bondesil) sorbent was purchased from Supelco (Supelco, Bellefonte, USA). Analytical reagent grade sodium acetate and anhydrous magnesium sulphate were procured from Merck Ltd. These were activated by heating at 150 °C overnight and kept in desiccators. The HPLC analysis was performed using an Agilent 1100 system, which included a degasser, binary pump, autosampler, column oven, and UV-DAD detector. The chromatographic separation was performed with the Zorbax EclipsePlus C18 (3.5 µm, 3.6 mm x 150 mm) chromatographic column. Each pesticide's mobile phase, flow rate, and detection wavelength are listed in the table (2). Data analysis was performed using Chemstation software.

### Statistical analysis:

The data was analyzed using one-way analysis of variance (ANOVA) following by LSD test for mean separation. Statistically significance was defined as P value <0.05 (CoStat-statistic software, CoHort software).

## RESULTS AND DISCUSSION

### Pathogenicity test:

After 7 days of incubation, the sugar mango fruit artificially inoculated with a conidial suspension of *A. alternata* revealed typical alternaria black spot symptoms. Sunken areas were noticed around the inoculation sites three days after inoculation. The sunken spots had developed into black lesions on the seventh day, the sunken areas had enlarged into black lesions, Control fruit (inoculated with distilled water) did not show any lesions. The fungus was re-isolated from the inoculated mango fruit's lesions, thus fulfilling to Koch postulates. In terms of colony, hyphae, and conidia morphology, the lesion was identical to that of the inoculated isolates

previously described. As a result, *A. alternata* was identified as the pathogen, and alternaria black spot was identified as the disease. These results are in agreement with the findings of (Diedhiou, *et al.*, 2007, Amin *et al.*, 2011, Mohsan *et al.*, 2011, Allam, 2017 and Qadri *et al.*, 2020).

### ***In vitro*: Valuation of tested plant extracts and fungicides against *A. alternata*:**

In the present study, all tested plant extracts concentrations were effective in inhibiting fungal growth of *A. alternata* in all experimental trials in petri dishes. The increase in concentration resulted significant increase in the percentage of mycelial inhibition. As showed in Table 3 and Fig. 1, Carnation extracts gave the maximum mycelial growth inhibition (100% at 2.5, 5 and 10 % concentration), followed by Cinnamon extract (65.42, 73.12 and 85.81) and Thyme extract (62.56, 71.97 and 84.15) respectively. The present results are also showed that treatment with Imazalil fungicide significantly reduced mycelial growth inhibition by 82.07, 85.22 and 90.50 at 50, 100 and 200 ppm, respectively. Also, thiabendazole fungicide reduced mycelial growth inhibition by (62.56, 71.97 and 82.075 %). Carnation extract has previously been proven to inhibit anthracnose produced by (*Colletotricum gloeosporioides*) Carla, (2012) and fruit rot caused by (*Lasiodiplodia theobromae*) Allam (2017) both of which are similar to our findings. Several polar chemicals, including alkaloids, flavonoids, tannins, phenols, and terpenoids, are extracted by aqueous extracts, and they turn the fungal cell membrane permeable, causing loss of cytoplasmic content, which could explain the variable antifungal activity found by Solomon-Wisdom *et al.*, (2014).

### ***In vivo*: Valuation of tested plant extracts and fungicides against *A. alternata*:**

The application of plant extracts in controlling black spot on mango fruits was determined by comparing the decay area

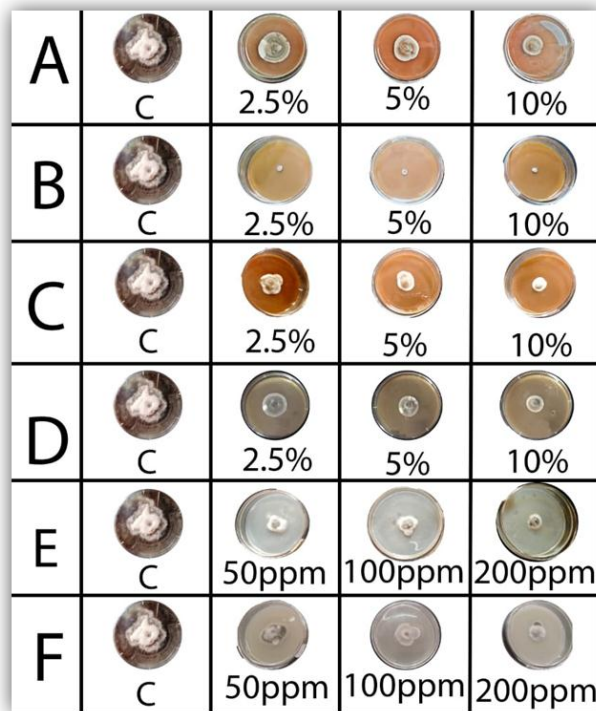
indicated by lesion diameter to the control. Consistent with our *in vivo* findings, aqueous Carnation extract at concentration of 2.5, 5 and 10 % reduced disease severity by 95.46, 97.04 and 98.22%, respectively (Fig. 2 and Table 4) relative to that in the control on mango fruits. Conversely, aqueous extracts of Cinnamon, Thyme and Bay leaf achieved significantly antifungal activity *in vivo* assay, with 85.90, 84.13 and 82.18% at 10 % concentration, respectively. A similar study found that aqueous extracts of *Annona muricata* and *D. caryophyllus*

reduced the size of black spots when compared to a control group (Humaira *et al.*, 2021). Plant extracts and essential oils employed as coatings on fruit surfaces, have demonstrated promising benefits in preventing postharvest fungal incidence as well as disease prevention (Qadri *et al.*, 2020). Plants are a rich source of bioactive substances, including secondary metabolites that operate as chemical signals and indicate activity against various fungal phytopathogens (Ramirez-Gomez *et al.*, 2019).

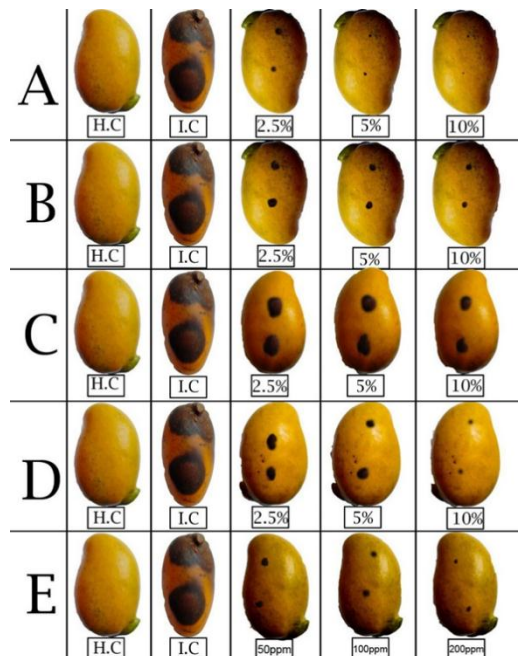
**Table (3): Effect of different concentrations of tested aqueous plant extracts and agriculture fungicides on the mycelium growth of *A. alternata*:**

Treatment	Conc. (%)	Linear growth (mm)	*Growth reduction (%)
<b>Plant Extracts</b>			
Bay leaf	2.5	32.44 <sup>b</sup>	62.61 <sup>i</sup>
	5	24.70 <sup>d</sup>	71.53 <sup>g</sup>
	10	15.67 <sup>f</sup>	81.94 <sup>e</sup>
Carnation	2.5	00.00 <sup>j</sup>	100.00 <sup>a</sup>
	5	00.00 <sup>j</sup>	100.00 <sup>a</sup>
	10	00.00 <sup>j</sup>	100.00 <sup>a</sup>
Cinnamon	2.5	30.00 <sup>c</sup>	65.42 <sup>h</sup>
	5	23.32 <sup>e</sup>	73.12 <sup>f</sup>
	10	12.31 <sup>h</sup>	85.81 <sup>c</sup>
Thyme	2.5	32.51 <sup>b</sup>	62.56 <sup>i</sup>
	5	24.32 <sup>d</sup>	71.97 <sup>g</sup>
	10	13.75 <sup>g</sup>	84.15 <sup>d</sup>
<b>Fungicides</b>			
Thiabendazole (Tecto® 50 SC)	50	32.48 <sup>b</sup>	62.56 <sup>i</sup>
	100	24.32 <sup>d</sup>	71.97 <sup>g</sup>
	200	15.55 <sup>f</sup>	82.07 <sup>e</sup>
Imazalil (Diabolo® 10 SL)	50	15.55 <sup>f</sup>	82.07 <sup>e</sup>
	100	12.82 <sup>h</sup>	85.22 <sup>c</sup>
	200	8.24 <sup>i</sup>	90.50 <sup>b</sup>
Control	-	86.77 <sup>a</sup>	00.00 <sup>j</sup>
L. S. D. 0.05	-	0.8294	0.7037

Means within the similar column followed by the same letter(s) are not significantly different ( $P \geq 0.05$ , LSD test). \*Growth reduction (%): is calculated relative to the control [control – treated / control x 100].



**Fig (1):** *In vitro* assay. Inhibitory effects of different concentrations of aqueous extracts of tested plants and fungicides on the growth of *A. alternata* grown on PDA medium, **A:** Bay leaf extract **B:** Carnation extract **C:** Cinnamon extract **D:** Thyme extract. **E:** Imazalil fungicide **F:** Thiabendazole fungicide.



**Fig (2):** *In vivo* assay. Inhibitory effects of different concentrations of aqueous extracts of tested plants and fungicides on the Disease severity of *A. alternata* on mango fruits: **H.C:** Healthy control , **I.C.:** Infected control without any treatment, **A:** Carnation extract, **B:** Cinnamon extract, **C:** Bay leaf extract, **D:** Thyme extract, **E:** Imazalil fungicide.

**Table (4): Effect of different concentrations of tested aqueous plant extracts and agriculture fungicides on the lesion diameter of *A. alternata* on mango fruits:**

Treatment	Conc. (%)	Lesion diameters (mm)	Disease severity reduction (%)
<b>Plant Extracts</b>			
Bay leaf	2.5	9.77 <sup>b</sup>	80.72 <sup>i</sup>
	5	8.13 <sup>bc</sup>	83.96 <sup>h</sup>
	10	6.50 <sup>cde</sup>	86.52 <sup>g</sup>
Carnation	2.5	2.50 <sup>hij</sup>	95.46 <sup>c</sup>
	5	1.50 <sup>ij</sup>	97.04 <sup>ab</sup>
	10	0.9 <sup>j</sup>	98.22 <sup>a</sup>
Cinnamon	2.5	6.91 <sup>cd</sup>	86.37 <sup>g</sup>
	5	4.3 <sup>fg</sup>	91.45 <sup>e</sup>
	10	2.50 <sup>hij</sup>	95.46 <sup>c</sup>
Thyme	2.5	6.50 <sup>cde</sup>	87.17 <sup>g</sup>
	5	4.90 <sup>ef</sup>	90.33 <sup>e</sup>
	10	3.25 <sup>gh</sup>	93.60 <sup>d</sup>
<b>Fungicides</b>			
Thiabendazole (Tecto® 50% SC)	50	7.85 <sup>c</sup>	84.51 <sup>h</sup>
	100	5.63 <sup>def</sup>	88.9 <sup>f</sup>
	200	4.33 <sup>fg</sup>	91.45 <sup>e</sup>
Imazalil (Diabolo® 10% SL)	50	4.33 <sup>fg</sup>	91.45 <sup>e</sup>
	100	3.11 <sup>ghi</sup>	93.86 <sup>d</sup>
	200	1.98 <sup>hij</sup>	96.15 <sup>bc</sup>
Control	-	50.7 <sup>a</sup>	00.00 <sup>j</sup>
L. S. D. 0.05	-	1.6457	1.3929

Means within the same column followed by the same letter(s) are not significantly different ( $P \geq 0.05$ , LSD test).

Our results revealed that treatment with Imazalil fungicide decreased black lesions diameter (3.11mm) comparative to that in the control (50.7 mm), constituting disease inhibition of 93.86% at 100 ppm concentrate. Also, thiabendazole treatment at a concentration of 100

ppm decreased the lesions diameter (5.63mm) comparative to that in the control (50.7 mm), constituting disease inhibition of 88.90 %. According to (Mohsan *et al.*, 2011) postharvest applications of various fungicidal treatments (imazalil, benomyl, mancozeb, and



thiabendazole) considerably reduced the severity of black spots disease on mango fruits. Despite the fact that fungicides are particularly successful in controlling several postharvest diseases, their extensive use has led to the emergence of fungicide-resistant strains, as previously mentioned. Furthermore, the harmful effects of fungicide residues on food and the environment have aroused serious concerns (El- Ghaouth *et al.*, 2003).

### Analysis of fungicide residues in mango fruits:

Imazalil residue decreased with the number of cold storage days for all treatments and the residue increased with duration of dipping for all treatments although (Table 4). The residue level of Imazalil in mango fruit after treatment with 1000 mg/L at 15°C for 5 and 10 sec was increased from 0.0063 mg/kg to 0.0074, respectively. Similarly, dipping with 1000 mg/L Thiabendazole at 15 °C for 5 and 10 sec increased the residues from 1.5 mg/kg to 1.71 mg/kg, respectively. Data in Table (5) shown that % of fungicides loss increased with storage duration 0, 3, 7, 14 days from 0% in zero day to 21.27% in 21days for IMZ fungicide, and from 0% in zero day to 40.12% in 21 days for TBZ fungicide. The fungicides residues decreased

gradually with duration of storage for both fungicides but average loss in TBZ more than IMZ. Both fungicides residues were lower than MRL set by codex and EU Pesticides Database. Cabras *et al.*, (1999) found that IMZ residues shown a great persistence in citrus fruit during storage when applied separately, and >83% of active ingredient was present after 9 weeks of storage. IMZ residues increased with dip length, doubling when dip time increased from 0.5 to 3 min. In contrast, TBZ residues did not change with the different dip times. Similar results were obtained by Brown and Dezman (1990) and Smilanick *et al.*, (1997) by increasing the treatment duration with IMZ.

### Conclusion:

Plant extracts in the present work efficiently reduced the mycelial growth of black spot fungi, *A. alternata*. Moreover, the extracts, significantly reduced the disease incidence in an *in vivo* assay. The levels of tested fungicides in mango fruits were lower than MRL set by Codex and EU pesticide databases. Thus, our results in this research can be applied to the preparation of natural plant products that may be applied in crops targeting to reduce or replace the application of conventional chemical fungicides.

**Table (5). Dissipation rate of fungicides residues, mean (mg/kg) and % loss detected in mango fruits**

Time	Imazalil				Thiabendazole			
	5 s	% loss	10 s	% loss	5 s	% loss	10 s	% loss
Zero	0.0063	0	0.0074	0	1.50	0	1.71	0
3 days	0.0058	8.57	0.0062	16.96	1.26	16.1	1.51	11.69
7 days	0.005	14.37	0.0055	26.42	0.99	34.32	1.26	26.31
14 days	0.0046	21.27	0.0053	29.12	0.90	40.12	1.00	41.52
MRL(ppm)*	0.05				5			

\* set by Codex and EU Pesticides Database Ciscato et al., (2015)

## REFERENCES

- Akaze, O.O. and Modupe A. (2017). Fusarium wilt disease of tomato: Screening for resistance and *in vitro* evaluation of botanicals for control; the Ngeia case. *Journal of Microbiology, Biotechnology and Food Science* 123: 353-362.
- Al-Najada, A.R. and Al-Suabeyl, M.S. (2014). Isolation and classification of fungi associated with spoilage of post-harvest mango (*Mangifera indica* L.) in Saudi Arabia. *Journal of Microbiol. Res*, 8: 685-688.
- Amin, M.; Aman Ullah, M.; Ahmed, A. Khan and Nazir, J. (2011). Potential of Fungicides and Plant Activator for Postharvest Disease Management in Mangoes. *Int. J. Agric. Biol.* Vol. 13 (5): 671-676.
- Baibakova, E.V.; Nefedjeva, E.E.; Suska-Malawska, M.; Wilk, M. and Zheltobriukhov, V.F. (2019). Modern fungicides: Mechanisms of action, fungal resistance and phytotoxic effects. *Annul. Res. Rev. Biol.* 32: 1-16.
- Barnett, H.L. and Hunter, B.B. (1999). *Illustrated Genera of imperfect fungi* (fourth ed.), APS Press, St. Paul, Minnesota, USA. 218pp.
- Bilgrami, K.S.; Jamaluddin, S. and Rizwi, M.A. (1991). *Fungi of India Part III. List and Rerences*. Today and Tomorrow's Printers and Publishers, New Delhi.
- Brown, G. E. and Dezman, D. J. (1990). Uptake of imazalil by citrus fruit after postharvest application and effect of residue distribution on sporulation of *Penicillium digitatum*. *Plant Dis.* 74: 927-930.
- Cabras, P.; Schirra, M.; Pirisi, F. M.; Garau, V. L. and Angioni, A. (1999). Factors affecting Imazil Imazalil and Thiabendazole Uptake and Persistence in citrus fruits following DIP treatments. *J. Agric. Food Chem.* 47: 3352-3354.
- Carla, A. Nunes (2012). Biological control of postharvest diseases of fruit. *Journal of Plant Pathology* 133:182-196.
- Chung, K.R. (2012). Stress Response and Pathogenicity of the Necrotrophic Fungal Pathogen *Alternaria alternata*. *Scientifica*, 2012, Article ID 635431, 17 pages.
- Ciscato, C. H. P.; Ozaw, K. S.; Barbosa, C. M. and Gebara, A. B. (2015). Evaluation of pesticide residues in mango by a multi-residue analysis and GC/MS Triple Quadrupole. *Journal of Agricultural Science and Technology B* 5: 530-53.
- Deguine, J.P.; Jacquot, M.; Marquier, M.; Gloance, C. and Vanhuffel, L. (2018). Agroecological protection of mango orchards in La Reunion. *Sustain Agric Rev* 28: 249-307.
- Diedhiou, P.M.; Mbaye, N.; Drame, A. and Samb, P.I. (2007). Alteration of postharvest diseases of mango *Mangifera indica* through production practices and climatic factors. *African J. Biotech.* 6(9): 1087-1094.
- Eckert, J. W. and Eaks, I. L. (1988). Postharvest disorders and diseases of citrus fruit. In *The Citrus Industry*; Reuther, W.; Calavan, E. C.; Carman, G. E.; Eds., University of California Press: Los Angeles, CA. Vol. 5, pp 179-260.
- El Ghaouth, A.; Wilson, C.L. and Wisniewski, M. (2003). Control of postharvest decay of apple fruit with *Candida saitoana* and induction of defense responses. *Phytopathology* 93, 344–348.
- El- Ghaouth, A.; Wilson, C.L. and Wisniewski, M. (2003). Control of postharvest decay of apple fruit with *Cadida saitoana* and induction of defense responses. *Phytopathology* 93: 344-348.
- Haggag, W. M. (2010). Mango diseases in Egypt. *Agric. Biol. J. North Am.* 1: 285-289.
- Hemeda, Amal, A. (2009). Control of bean root-rot diseases by using resistance chemical inducers. *Alexandria Journal of Agricultural Research.* 54(1) : 175-187.
- Humaira, R.; Najat A. Bokahri; Sarah, A. A.; Amal, S. Al Showiman; Rawan, M. A. and Horiah, A. Aldehaish (2012). Postharvest disease management of *Alternaria* spots on tomato fruit by plant extracts. *Saudi Journal of Biological Sciences* 28 :2236–2244.
- Hosseini, S.; Amini, J.; Saba, M.K.; Karimi, K. and Pertot, I. (2020). Preharvest and

- postharvest application of garlic and rosemary essential oils for controlling anthracnose and quality assessment of strawberry fruit during cold storage. *Microbiol.* 11, 1855.
- Kumlachew, A. (2014). Dynamics and Management of major postharvest fungal diseases of mango fruits. *Journal of Biology, Agriculture and Healthcare*, 4:13-21.
- Lehotay, S.J.; Son, K.A.; Fu, W.; Mastovska, K.; Hoh, E. and Leepipatpiboon, N. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruit and vegetables, *J. Chromato. A.*, 1217: 2548-2560.
- Melo, T. A.; Serra, I. M.; Sousa, A.A. and Pascholati, S. F. (2018). Effect of *Ascophyllum nodosum* seaweed extract on post-harvest "Tommy Atkins" mangoes. *Rev Brasil Frutic* 40: 621-630.
- Metwally, H.M.A.; Omar, M.A. and Badaiwy, M. (2002). *Microsporum gallinae* growth response to some plant extracts. Faculty of Applied Science, Umm Al-Qura University, Makkah, Kingdom of Saudi Arabia. 1-8.
- Mohsan, M., Hassan, M. I. and Liaquat, A. (2011). Chemotherapeutic management of Alternaria black spot (*Alternaria alternata*) in mango fruits. *J. Agric. Res.* 49(4): 499-506.
- Allam, M. W. (2017). Exploitation of natural products as an alternative strategy to control stem end rot disease of mango fruit in Pakistan. *Pakistan Journal of Agricultural Science.* 54(2):503-511.
- Nunes, C. A. (2015). Biological control of postharvest diseases of fruit. *Journal of plant pathology* 133: 181-196. nited Nations. Database. Available at <http://www.fao.org/faostat/en/#data/QC/>.
- Obagwu, J. and Korsten, L. (2003). Control of citrus green and blue molds with garlic extract. *Eurp. J. Plant Pathol.* 109 : 221-225.
- Orak, H.H.; Bahrisefit, I.S. and Sabudak, T. (2019). Antioxidant activity of extracts of Soursop (*Annona muricata* L.) leaves, fruit pulps, peels and seeds. *Pol. J. Food Nutr. Sci.* 69: 359-366.
- Prakash, O.M. (2004). Diseases and Disorders of mango and their management. *Diseases of Fruits and vegetable* 1: 511-619.
- Qadri, R.; Khan, I.; Yang, Y.; Ejaz, S.; Akram, M.T. and Khan, M.A. (2020). Conventional and modern technologies for the management of post-harvest diseases. In: Ul Haq, I., Ijaz, S. (Eds.) *Plant Disease Management Strategies for Sustainable Agriculture Through Traditional and Modern Approaches. Sustainability in Plant and Crop Protection*, vol 13. Springer, Cham.
- Ramírez-Gómez, X.S.; Jiménez-García, S.N. Vicente Beltrán Campos, ma. Lourdes and García Campos, (2019). Plant Metabolites in Plant Defense Against Pathogens in Plant Diseases-Current Threats and Management Trends pp 1-19.
- Rizwana, H. (2018). Postharvest control of anthracnose lesions and its causative agent, *Colletotrichum musae* by some oils. *Cell. Mol. Biol. Noisy-Le- Grand* 64: 52-58.
- Shivapratap, H.R.; Philip, T. and Sharma, D.D. (1996). The species concept in Fusarium. *Indian Journal of Seri*, 35 (2) :107 -110.
- Sivakumar, D.; Jiang, Y. and Yahia, E.M. (2011). Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. *Food Res. Ins* 44: 1254-1263.
- Solomon-Wisdom, G. O.; Ugoh, S.C. and Mohamed, B. (2014). Phytochemical screening and antimicrobial activities of *Annona muricata* leaf extract. *Am. J. Bio. Chem. Pharma Sci.* 2: 1-7.
- Smilanick, J. L.; Michael, I. F.; Mansour, M. F.; Mackey, B. E.; Margosan, D.A.; Flores, D. and Weist, C. F. (1997). Improved control of green mold of citrus fruit with imazalil with warm water compared with its use in wax. *Plant Dis.* 81: 1299-1304.
- Tomlin, C.D. (1994). *The Pesticide Manual*, 10th ed.; BCPC: Farnham, U.K. pp 580-581.
- Troncoso-Rojas, R. and Tiznado-Hernandez, M.E. (2014). *Alternaria alternata* (black rot, black spot). In: Bautista-Banos, S. (Ed). *Postharvest Decay of fruits and vegetables: Control Strategies*, Elsevier, Atlanta, GA, USA, pp. 147-188.

## فاعلية بعض المستخلصات النباتية ومبيدات الآفات في مقاومة التبغ الألترناري الأسود (*Alternaria alternata*) على ثمار المانجو بعد الحصاد في مصر

هاجر علي عمار<sup>(١)</sup> - سحر حسن عبد اللطيف الهلالي<sup>(٢)</sup> - طارق عبد العليم عبد الرحمن<sup>(٣)</sup>

<sup>(١)</sup> قسم مبيدات الآفات - كلية الزراعة - جامعه المنوفية

<sup>(٢)</sup> قسم النبات الزراعي (أمراض النبات) - كلية الزراعة - جامعة المنوفية

<sup>(٣)</sup> المعمل المركزي للمبيدات - شارع نادي الصيد - الجيزة - مصر

### الملخص العربي:

كان الهدف من هذه الدراسة هو تقييم أربعة مستخلصات نباتية مائية وهي مستخلص (ورق اللوري -القرنفل- القرفة- الزعتر) كبديل للمبيدات الفطريات الكيميائية (Imazilil و Thiabendazole) ضد *Alternaria alternata* العامل المسبب للبقع السوداء في ثمار المانجو السكري. تم اختبار النشاط المضاد للفطر باستخدام المستخلصات النباتية ومبيدات الفطريات الكيميائية في كل من الظروف المعملية والحيوية. أوضحت الاختبارات المعملية أن أقصى تثبيط لنمو الفطر (١٠٠٪) عند استخدام مستخلص القرنفل علي ثلاث تركيزات (٢,٥ - ٥ - ١٠٪) وخفض قطر البقع المتكونة علي الثمار المعاملة بهذا المستخلص (٢,٥ - ١,٥ - ٩,٠ مم) وخفض شدة المرض بنسبة (٩٥,٤٦ - ٩٧,٩٧ - ٩٨,٩٨ ٪). أظهر الفحص الحيوي باستخدام مبيد الفطريات Imazalil تثبيط النمو لفطر الألترناريا بنسبة (١٣, ٨٢ - ٢٢, ٨٥ - ٨٩,٣٥ ٪) وتقليل شدة المرض بنسبة (٨٦,٣٧ - ٨٨,٩٠ - ٩٣,٤٣ ٪) عند استخدام تركيزات ٥٠ - ١٠٠ - ٢٠٠ جزئ في المليون. تم دراسة متبقيات المبيدات في الثمار المعاملة بعد الحصاد باستخدام مبيد (Imazilil و Thiabendazole) بعد النقع علي فترات مختلفة وظروف تخزين علي البارد مختلفة لثمار المانجو. انخفضت متبقيات IMZ و TBZ مع عدد أيام التخزين البارد لجميع المعالجات وزادت متبقيات المبيد مع مدة الغمس لجميع المعالجات. بالرغم من ذلك، كانت كل من بقايا مبيدات الفطريات أقل من الحد الأقصى للبقايا التي حددتها مخطوطة الاتحاد الأوروبي وقاعدة بيانات مبيدات الآفات في الاتحاد الأوروبي.