

ORIGINAL PAPER

## Efficacy of Pre-harvest Applications of Various Bio and Agrochemical Products on Anthracnose Disease of Mango Fruits and Keeping Quality During Storage Conditions.

Hassan, M. S. S.<sup>1</sup> , Shehata, A. S. F.<sup>1\*</sup>  and Elmaghraby, I. M. K.<sup>2</sup> 

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### ABSTRACT

Mango anthracnose disease is an essential problem in Egypt and one of the more significant economic restrictions of mango production. The economic losses caused by anthracnose disease are mostly attributed to reduce fruit quality and marketability. Anthracnose disease caused by *Colletotrichum gloeosporioides* is one of the most widespread fungal diseases that affects mango worldwide. This study was to verify the effects of pre-harvest applications of various bio and agrochemical products, Fungisei 1% (*Bacillus subtilis* strain IAB/BSO3), Biocontrol T34 12% (*Trichoderma asperellum* strain T34), Trifmine 15% (Triflumizole), Divide 60% and calcium chloride ( $\text{CaCl}_2$ ) against natural and artificial infection by *C. gloeosporioides* on two mango cultivars cv. Naomi and cv. Keitt after storage at  $12\pm 1^\circ\text{C}$  and 85-90% RH for 50 days and their effect on fruit quality parameters. *C. gloeosporioides* was able to induce anthracnose disease on mango fruits. Keitt cultivar was more susceptible than cv. Naomi under artificial inoculation in pathogenicity tests of the two years of the investigation. All tested products reduced *C. gloeosporioides* linear growth on PDA medium compared to control. Divide 60%, Trifmine 15% and  $\text{CaCl}_2$  were the best effective treatments where each of them completely inhibited the growth of tested fungus. On the other hand, Biocontrol T34 12 % and Fungisei 1% recorded low efficacy in reducing the colony growth of the tested pathogen. All tested products were significantly better than the control in decrease the percentages of disease incidence and severity of anthracnose disease under natural and artificial infection with *C. gloeosporioides*. Calcium chloride, Trifmine 15% and Divide 60% their performance were the best compared with Fungisei 1% and Biocontrol T34 12 % for both natural and artificial infection during the two investigated seasons. Pre-harvest spraying any of  $\text{CaCl}_2$ , Trifmine 15% EC and Divide 60% totally controlled and inhibited anthracnose disease on mango fruits which had been treated with the previous products in the field and were subjected to the natural infection and cold storage at  $12\pm 1^\circ\text{C}$  and 85-90% RH for 50 days during 2023 and 2024. All tested products kept fruit quality parameters and had significant effect due to total soluble solids, titratable acidity, fruit firmness and vitamin C (Ascorbic acid content).

**Keywords:** Pre-harvest spraying, bio and agrochemical products, anthracnose, mango fruits.

\*Correspondence: Shehata, A.S.F.

E-mail: [arpp2022@arc.sci.eg](mailto:arpp2022@arc.sci.eg)

Mabrouk S. S. Hassan

<https://orcid.org/0000-0002-3477-339X>

Abou Ghanima, S. F. Shehata

<https://orcid.org/0000-0003-1698-7375>

1. Plant Pathology Research Institute,  
Agricultural Research Center, 12619, Giza,  
Egypt.

Ibrahim M. K. Elmaghraby

<https://orcid.org/0000-0002-2728-9751>

2. Central Lab. of Organic Agriculture,  
Agricultural Research Center, Giza 12619,  
Egypt.

### INTRODUCTION

A tropical fruit known for its sweet and fresh flavor is the mango (Zakawa *et al.*, 2020). Mango trees are grown all throughout the world, but particularly in

tropical nations. In 2019, 55.85 million metric tons of mangos were produced worldwide, a 37% growth over the previous ten years (FAO, 2021). Rich in proteins, vitamins A and C, carotene, malic and citric acids, and other elements, mango fruit has a high nutritional value. With an average yield of 10 tons per acre, Egypt's farmed acreage increased to 300,000 acres in 2022. With around 30,000 tons sent to markets in Europe, including France, Germany, the Netherlands, the UK, and Russia, as well as Gulf countries including the United Arab Emirates, Oman, Kuwait, and Saudi Arabia, in addition to Lebanon. Egypt is the tenth-largest exporter of mangos worldwide (Sharkawy *et al.*, 2023). The mango fruit promotes the development of its color, flavor, and texture by increasing its respiration rate and ethylene production after harvest. Fruit quality is decreased

during storage due to the faster growth of these characteristics, and fungus cause postharvest losses. Anthracnose and other postharvest diseases are brought on by these fungi. Mango fruit is attacked by phytopathogens belonging to the genus *Colletotrichum* (Xu *et al.*, 2017). *Colletotrichum gloeosporioides* has spread around the world and inflicted a significant economic damage. (Chung *et al.*, 2010). Mango fruit anthracnose has been found to be caused by *Colletotrichum* species. According to reports, it is one of the most aggressive pathogens that attack the various mango fruit varieties in Egypt (Ismail and El-Ganainy, 2022), Mexico (Tovar-Pedraza *et al.*, 2020), the Philippines (DelaCueva *et al.*, 2021), Australia (Giblinet *et al.*, 2018), China (Mo *et al.*, 2018) and Indonesia (Benatar *et al.*, 2021). *Colletotrichum* symptoms may be in a latent phase prior to harvest; dark patches and tissue putrefaction are signs of the disease that emerges after harvest (Tovar-Pedraza *et al.*, 2020). Although it can happen at any point in the fruits life cycle, anthracnose is most frequently found on mango fruits during storage and transportation. The infection on larger fruits may remain latent or inactive until the fruit ripens, at which point black, sunken necrotic lesions emerge on the fruit peel and rapidly enlarge (De Souza *et al.*, 2013). The young fruits are either aborted or mummified. During the flowering and fruit-setting seasons, anthracnose disease is most harmful under damp conditions. Mango infections can occur during flowering and the early stages of fruit production (Sarkar, 2016). The disease shortens the fruits' shelf life by infecting them while they are in the field, during transit and in cold storage (Qin *et al.*, 2019). The extent of disease resistance varies among cultivars. Two mango cultivars, "Keitt" and "Zill," were studied for their resistance to the anthracnose disease. When *C. gloeosporioides* infected commercially ripe or young fruit, the lesion sizes of "Keitt" fruit were less than those of "Zill" fruit (Gong *et al.*, 2013). "Keitt" had a lower disease index than "Zill" when non-

inoculated fruits were harvested at commercial maturity, suggesting that "Keitt" was more disease resistant than "Zill" (Gong *et al.*, 2013). Low temperatures and coatings are two methods that have been utilized to prevent deterioration, increase shelf life, and preserve mango fruit quality (Ravindra and Goswami, 2007). According to Tripathi and Dubey (2004), natural products are helpful and are being used as an alternative method of postharvest fruit deterioration and ripening delay. Most of the focus and effort in the fight against anthracnose has been on the use of fungicides. The application of fungicides reduces damage to fruit and inflorescence. Depending on the final destination of the exported fruit, different fungicides may be used. Early trials demonstrated the effectiveness of non-systemic fungicides such as zineb, maneb, or captan when treated weekly during flowering and subsequently monthly throughout fruit development (Sardrood and Goltapeh, 2018). In South Africa, it is advised to use copper oxychloride or copper oxychloride combined with zineb every 14 days in wet conditions and every 28 days in dry conditions as part of pre-harvest management to prevent anthracnose (Akem, 2006). Additionally, *C. gloeosporioides* has been reported to be resistant to systemic fungicides called benzimidazoles (Chung *et al.*, 2010). Despite the excellent effectiveness of chemical treatment with several fungicides against *C. gloeosporioides*, reports of the establishment of fungicide-resistant isolates of this disease have been made (Kongtragoul *et al.* 2011). Agronomic significance could be greatly increased by investigating different bio-control agents to manage different plant diseases. Mango pre- and post-harvest anthracnose may be managed using an environmentally favorable future method that involves the bio-formulation application of *Trichoderma harzianum*, *Bacillus subtilis*, and *Pichia anomala* (Sharma *et al.*, 2021). Chitinolytic activity was observed for *Bacillus* species and *Trichoderma* species

(Huang *et al.*, 2005). Fungal mycelia are lysed by *Bacillus* species (Podile and Prakash 1996). *Bacillus subtilis* showed strong antagonistic action against *C. gloeosporioides* in the dual plate assay. According to Ashwini and Srividya (2014), microscopic investigations demonstrated a definite hyphallysis and fungal cell wall degradation. One of the key elements influencing the growth and quality of horticultural crops is mineral nutrition. Calcium (Ca), one of the mineral nutrients, is thought to be a major factor in determining the quality and shelf life of fruits and is known to have a significant impact on plant cell processes. According to Liu *et al.* (2017), postharvest calcium application has been shown to benefit fruit in a variety of ways, such as lowering physiological disorders, maintaining membrane permeability, preventing softening, slowing ripening processes, and reducing fruit decay as well as increasing fruit acceptability. It has previously been mentioned that  $\text{CaCl}_2$  could be added to food to enhance the texture of the meat in goose meat preparations (Li *et al.*, 2017). This study aimed to control anthracnose on mango fruits and keeping quality during storage conditions at  $12 \pm 1$  °C and 85-90% RH for 50 days during 2023 and 2024 on both Naomi and Keitt cultivars by performing pre-harvest spray of five bio and agrochemical products *i.e.* Fungisei 1% SC 150ml/100L, Biocontrol T34 12 %WP 200g/100L, Calcium chloride ( $\text{CaCl}_2$ ) 100g/100L, Trifmine 15% EC 25ml/100L and Divide 60% WG 100g/100L water.

## MATERIALS AND METHODS

This study was conducted using two mango varieties, Naomi and Keitt that were grown in El-Qanater El-Khayria, Qaliobiya governorate, Egypt, during 2022, 2023, and 2024 seasons.

### Pathogen isolation:

In Qaliobiya governorate, mango fruits (cv. Naomi and cv. Keitt) exhibiting naturally occurring symptoms of anthracnose disease were collected from marketplaces, cold room storage and fields. After being chopped into small pieces, the diseased tissues were surface sterilized by

immersing them in 1% sodium hypochlorite for 0.5 min. After being cleaned using sterile distilled water, the tissues were cultured on PDA medium. After that, the Petri plates were incubated for a week at 22°C. The hyphal tip method (HTM) was used to purify the developing fungal colonies. According to Sutton (1980) the purified isolates were identified depending on their morphological and cultural characters and maintained on PDA media.

### Pathogenicity test:

Mango fruits cv. Naomi and cv. Keitt were collected from the Agricultural Research Center (ARC), El-Qanater El-Khayriya Horticultural Research Station (EHRS) during 2023 and 2024. There were no wounds or rotting on the mango fruits. Three replicates of nine fruits from each cultivar were used. After being cleaned and surface sterilized, the mango fruits were slightly scraped and inoculated with spore suspension of *Colletotrichum gloeosporioides* ( $10^6$  spores/ml) for one minute using 7 days old cultures (Hassan *et al.*, 2021). Additionally, the same numbers of unwounded fruits were inoculated by spraying spore suspension ( $10^6$  spores/ml) of *Colletotrichum gloeosporioides* (Hassan and Shehata, 2024). After 15 days of room temperature incubation at  $24 \pm 1$  °C, the inoculated and non-inoculated fruits were evaluated for disease symptoms using the disease incidence (%) and disease severity (%) scales according to Lakshmi *et al.* (2011) (Table 1). The causal fungus of anthracnose disease was re-isolated again for identity confirmation.

$$\text{Disease incidence (\%)} = \frac{n}{N} \times 100$$

Where, n= Number of infected mango fruits with anthracnose disease and N= the total number of all tested fruits.

$$\text{Disease severity (\%)} = \frac{\sum n \times v}{5N} \times 100$$

Where:

5 = Maximum disease severity grade,

n = Number of infected fruits in each category (grade),

N = Total number of the inspected fruits,

v = Numerical value of each category as follows:

**Table (1):** Disease severity scale:

Rating	Where:
0	No of infected fruit.
1	5% of fruit affected area.
2	Between 5 and 10% of fruit affected area.
3	Between 10 and 20% of fruit affected area.
4	Between 20 and 50% of fruit affected area.
5	More than 50% of fruit affected area.

***In vitro* experiment:**

*In vitro*, the inhibitory efficacy of five bio and agrochemical products (Table 2); Fungisei 1% SC (*Bacillus subtilis* strain (IAB/BSO3  $1 \times 10^8$  CFU/ml)), Biocontrol T34 12% WP (*Trichoderma asperellum* strain T34), calcium chloride ( $\text{CaCl}_2$ ), Trifmine 15% EC (Triflumizole) and Divide 60% WG (Metiram 55% +

Pyraclostrobin 5%) against *C. gloeosporioides* was assessed. Before solidifying, treatments were added each alone to conical flasks containing autoclaved PDA. They were then gently mixed and then poured into 90 mm plates. After solidification, equal disks 5 mm in diameter were cut from the periphery of the active mycelial growth of 7-day-old cultures of *C. gloeosporioides* to inoculate each plate separately at the center. At  $22 \pm 1$  °C, the plates were incubated. The control group consisted of untreated PDA plates that had been inoculated with fungus. For every treatment, six plates were used. The following formula was used to compute the average colony diameter (mm) and percentage inhibition once the control plates had completed full development (Hassan *et al.*, 2021).

$$\% \text{ Inhibition} = \frac{\text{Mean of colony diameter in control plates} - \text{Mean of colony diameter of test plates}}{\text{Mean of colony diameter in control plates}} \times 100$$

**Table (2):** Tested bio and agrochemical compounds.

Product	Active ingredients	Status	Conc./100 L water
Fungisei 1%	<i>Bacillus subtilis</i> strain (IAB/BSO3 $1 \times 10^8$ CFU/ml)	SC	150 ml
Biocontrol T34 12%	<i>Trichoderma asperellum</i> strain T34	WP	200 g
Calcium chloride	$\text{CaCl}_2$	WP	100 g
Trifmine 15%	Triflumizole	EC	25 ml
Divide 60%	Metiram 55% + Pyraclostrobin 5%	WG	100 g

**Field experiment:**

During 2023 and 2024, field experiments were conducted on 12-year-old mango trees, cv. Naomi and cv. Keitt, at the Agricultural Research Center, El-Qanater El-Khayriya Horticultural Research Station (EHRS). Three mango trees were intended for each treatment, with three trees per replicate. Five sprays of each investigated bio and agrochemical products were applied; the first was sprayed at the start of fruit set, and the interval between sprays was 20 days. Water was sprayed on control mango trees. Mango fruits were harvested at the physiological maturity stage and split into two groups. Eighteen fruits were selected at random from each cultivar treatment. As mentioned in the pathogenicity test, the first group of mango fruits which were treated with Fungisei 1%,

Biocontrol T34 12%, Calcium chloride ( $\text{CaCl}_2$ ), Trifmine 15%, and Divide 60% were surface sterilized and artificially inoculated with the prepared spore suspension of *Colletotrichum gloeosporioides* ( $10^6$  spores/ml). Without any artificial inoculation, the second group of mango fruits which had been treated with the identical bio and agrochemical products that had been tested in the field were subjected to a natural infection. For 50 days, mango fruits were kept in the EHRS Central Lab at  $12 \pm 1$  °C and 85-90% relative humidity (RH) in cardboard boxes. The disease incidence and severity of anthracnose, fruit firmness (lb/inch<sup>2</sup>), total soluble solids (TSS), titratable acidity (TA) and vitamin C (Ascorbic acid as mg/100 g fresh weight) were assessed at the first and the end of the cold storage.

**Disease assessment:****Disease incidence and disease severity (%):**

Anthrachnose disease incidence (%) and disease severity (%) were determined as mentioned before according to **Lakshmi et al. (2011)**.

**Physical characteristics:****Fruit firmness (lb/inch<sup>2</sup>):**

Using a hand pressure tester as lb/inch<sup>2</sup> fruit firmness was calculated according to **Hassan et al. (2023)**.

**Chemical Properties:****Total soluble solids (TSS):**

Using Digital refractometer PR32 the percentage of TSS was calculated in mango fruit juice as recorded by **Abdel-Rahman et al. (2021)** and **Hassan et al. (2023)**.

**Titrate acidity (TA) %:**

TA was calculated by titrating the mango juice against 0.1 N NaOH using phenolphthalein indicators and expressed as percentage of citric acid according to **Abdel-Rahman et al. (2021)**.

**Vitamin C (Ascorbic acid):**

Vitamin C content (Ascorbic acid as mg/100g fresh weight) was evaluated according to **Shao et al. (2013)** using the method of 2, 6-dichlorophenol indophenol.

**RESULTS****Pathogen isolation:**

Using its cultural and morphological characteristics, the isolated fungus from mango fruits (cv. Naomi and cv. Keitt) showing natural infection with anthracnose disease symptoms and were collected from fields, cold room storage and markets in Qaliobiya governorate during 2022 was purified and identified as *Colletotrichum gloeosporioides* Penz (Fig. 1).

**Pathogenicity test:**

*Colletotrichum gloeosporioides* was able to cause anthracnose disease on mango fruits, according to the pathogenicity test (Fig. 2). Mango fruits cv. Naomi and cv. Keitt were subjected to a pathogenicity test, which was verified. According to data in Table (3), the infection by *C. gloeosporioides* on cv. Keitt caused the highest disease severity (anthracnose) percentage on the cultivars examined during 2023 and 2024. According to the pathogenicity test conducted throughout the two-years of the investigation, cv. Keitt was more susceptible to artificial inoculation than cv. Naomi. Little amounts of the disease severity (%) on the unwounded fruits after inoculation by spore suspension using *Colletotrichum gloeosporioides* after the incubation period (Fig. 3).

**Table (3):** Pathogenicity test as anthracnose disease incidence (%) and disease severity of *Colletotrichum gloeosporioides* during the 2023 and 2024.

Cultivars	Disease incidence (%)		Disease severity (%)	
	2023	2024	2023	2024
Naomi	100a	100a	37.77b	26.66b
Keitt	100a	100a	71.11a	48.88a

No significant differences between the treatments with the same letter/s in the same column at  $P \leq 0.05$

**In vitro experiment:**

All tested bio and agrochemical products decreased *Colletotrichum gloeosporioides* linear growth on PDA medium compared to control, according to data in Table (4). The most successful treatments were Divide 60%, Trifmine 15%

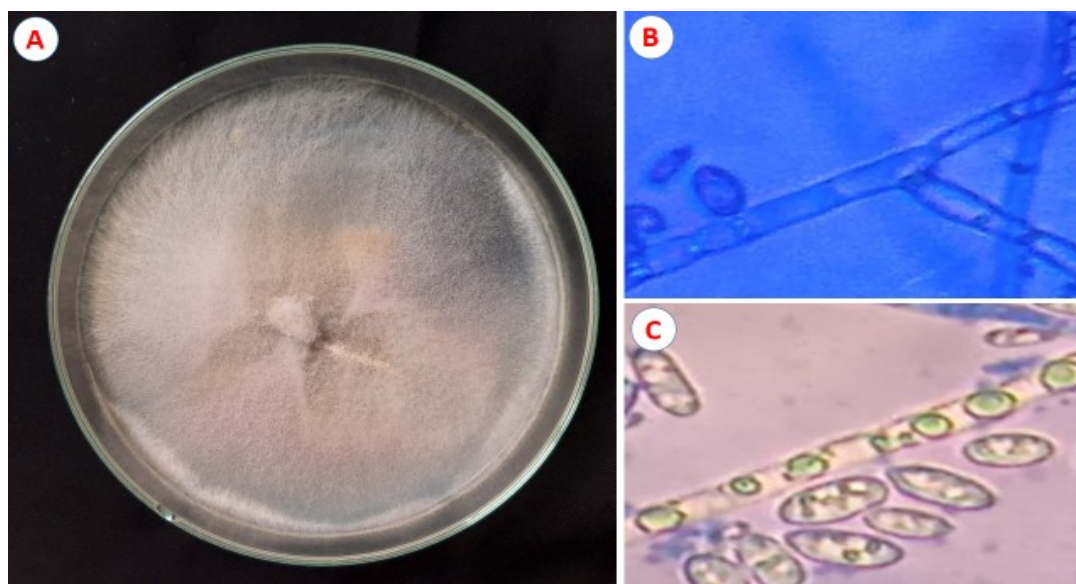
and Calcium chloride ( $\text{CaCl}_2$ ) where all tested materials inhibited the development of the tested fungus. However, the effectiveness of Biocontrol T34 (12%) and Fungisei 1% in lowering the studied pathogens colony growth was only 77.77 % and 72.77%, respectively.



**Table (4):** Effect of the tested bio and agrochemical products on the linear growth (mm) of *Colletotrichum gloeosporioides* *in vitro*.

Tested chemical	Con./L	<i>Colletotrichum gloeosporioides</i> linear growth	
		Mm	%Eff.
<b>Fungisei 1%</b>	1.5 ml	25.00 b	72.77
<b>Biocontrol T34 12 %</b>	2 g	20.00c	77.77
<b>CaCl<sub>2</sub></b>	1 g	0.00d	100
<b>Trifmine 15%</b>	0.25ml	0.00d	100
<b>Divide 60%</b>	1 g	0.00d	100
<b>Control</b>	..	90.00a	00

No significant differences between the treatments with the same letter/s in the same column at  $P \leq 0.05$   
 mm = Colony growth, millimeters. % Eff. = efficacy to control (untreated). Con. /L= concentration/ litre media

**Fig. (1):** *Colletotrichum gloeosporioides* isolated from mango fruits 7 days after incubation at  $22 \pm 1$  °C on PDA medium (A); (B) Conidiophore and (C) conidiospores.**Fig. (2):** Symptoms of infection by *Colletotrichum gloeosporioides* on wounded artificially inoculated mango fruits. (A) cv. Naomi; (C) cv. Keitt and control (B & D).



**Fig. (3):** Little amounts of the disease severity (%) on the unwounded fruits after inoculation by spore suspension using *Colletotrichum gloeosporioides* after the incubation period. (A) cv. Naomi; (B) cv. Keitt and control (C & D).

#### Field experiment:

##### Disease incidence and disease severity (%):

**Effect of pre-harvest spraying of the tested bio and agrochemical products on anthracnose disease incidence (%) and severity (%) under natural infection and artificially inoculated mango fruits by *C. gloeosporioides* after storage at 12±1°C and 85-90% RH for 50 days:**

After 50 days storage period at 12±1 °C and 85-90% RH, the effects of bio and agrochemical products, Fungisei 1% (*Bacillus subtilis* strain (IAB/BSO3 1×10<sup>8</sup> CFU/ml)), Biocontrol T34 12% (*Trichoderma asperellum* strain (T34)), Calcium chloride (CaCl<sub>2</sub>), Trifmine 15% EC (Triflumizole), and Divide 60% (Metriam 55% + Pyraclostrobin 5%) are

shown on two mango cultivars in Table (5 and 6). In general, the highest percentage of disease incidence and severity was seen in the control treatment (Fig. 4 and 5). During the two seasons under investigation, calcium chloride (CaCl<sub>2</sub>), Trifmine 15% EC, and Divide 60% performed the best when compared to Fungisei 1% and Biocontrol T34 12% for both natural and artificial infections. On naturally infected mangos, CaCl<sub>2</sub>, Trifmine 15% EC, and Divide 60% completely inhibited and controlled anthracnose disease during 50 days of cold storage at 12±1 °C and 85-90% RH in 2023 and 2024 (Table 5). On the other hand in the artificial infections, CaCl<sub>2</sub> recorded the lowest disease incidence and severity % in both seasons on both tested cultivars.

**Table (5):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars on anthracnose disease incidence (%) and disease severity (%) caused by *Colletotrichum gloeosporioides* under natural infection after storage at 12±1 °C and 85-90% RH for 50 days during 2023 and 2024 seasons.

Treatments	Conc. /100L	2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		DI%	DS%	DI%	DS%	DI%	DS%	DI%	DS%
Fungisei 1%	150 ml	33.33b	13.33b	22.22b	8.88b	44.44b	8.88b	22.22b	8.88b
Biocontrol T34 12 %	200 g	33.33b	13.33b	22.22b	8.88b	44.44b	8.88b	22.22b	8.88b
CaCl <sub>2</sub>	100 g	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c
Trifmine 15%	25 ml	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c
Divide 60%	100 g	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c	00.00c
Control	Water	44.44a	17.77a	55.55a	15.55a	66.66a	26.66a	77.77a	33.33a

No significant differences between the treatments with the same letter/s in the same column at P≤0.05

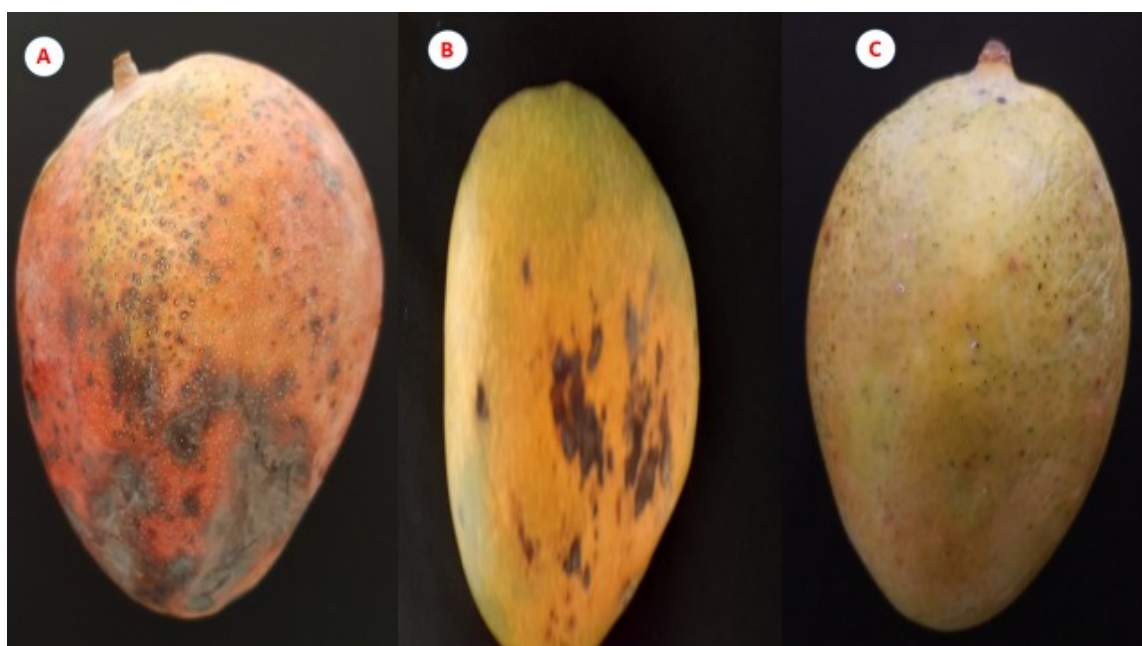
DI%=Disease incidence, DS%= disease severity.

**Table (6):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars on anthracnose disease incidence (%) and severity (%) caused by *Colletotrichum gloeosporioides* under artificial inoculation after storage at 12±1 °C and 85-90% RH for 50 days during 2023 and 2024 seasons.

Treatments	Conc. /100L	2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		DI%	DS%	DI%	DS%	DI%	DS%	DI%	DS%
Fungisei 1%	150 ml	66.66c	17.77c	77.77b	26.66b	77.77b	31.11b	77.77b	26.66b
Biocontrol T34 12 %	200 g	88.88b	28.44b	77.77b	26.66b	77.77b	31.11b	77.77b	26.66b
CaCl <sub>2</sub>	100 g	44.44e	11.11e	55.55d	15.55e	33.33d	20.00c	55.55d	15.55e
Trifmine 15%	25 ml	55.55d	13.33d	66.66c	22.22d	66.66c	20.00c	66.66c	22.22d
Divide 60%	100 g	55.55d	13.33d	77.77b	24.44c	66.66c	20.00c	77.77b	24.44c
Control	Water	100.00a	40.00a	100.00a	60.00a	100.00a	33.33a	100.00a	31.33a

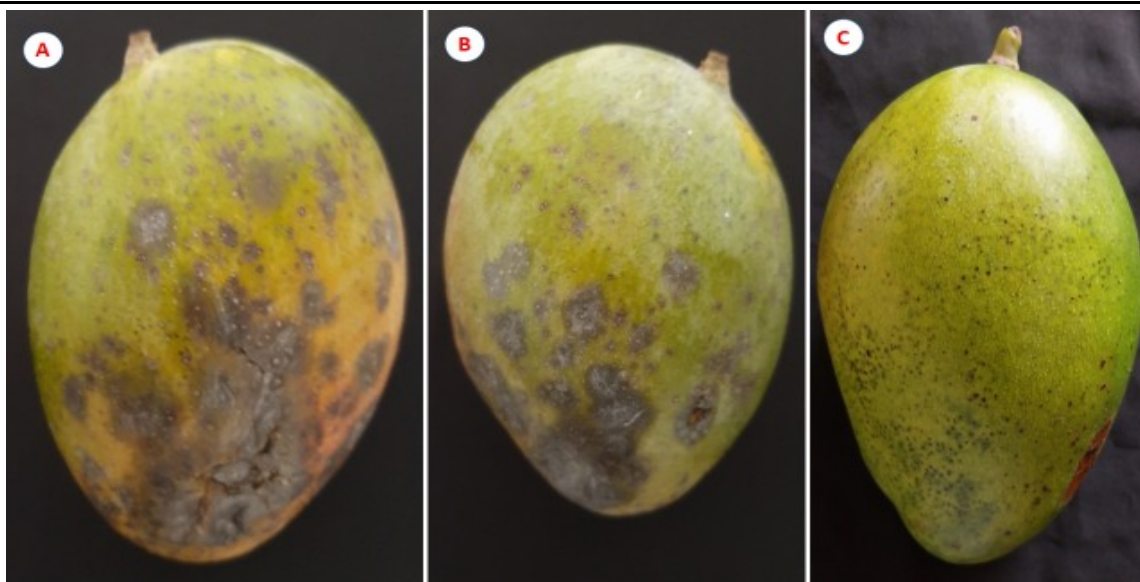
No significant differences between the treatments with the same letter/s in the same column at P≤0.05

DI%=Disease incidence, DS%= Disease severity.



**Fig. (4):** Symptoms of *Colletotrichum gloeosporioides* infection on artificially inoculated mango fruits cv. Naomi after storage at 12±1 °C and 85-90% RH for 50 days; (A) control; (B) treated fruit with bio products and (C) treated fruit with agrochemical products.





**Fig. (5):** Symptoms of *Colletotrichum gloeosporioides* infection on artificially inoculated mango fruits cv. Keitt after storage at  $12\pm 1$  °C and 85-90% RH for 50 days; (A) control; (B) treated fruit with bio products and (C) treated fruit with agrochemical products.

#### Fruit firmness (lb/inch<sup>2</sup>):

**Effect of pre-harvest spraying of the tested bio and agrochemical products on fruit firmness (lb/inch<sup>2</sup>) under natural infection and artificially inoculated mango fruits by *C. gloeosporioides* after cold storage at  $12\pm 1$  °C and 85-90% RH for 50 days:**

After 50 days of cold storage at  $12\pm 1$  °C and 85-90% RH, the effect of the tested products on the fruit firmness of two mango cultivars against both natural and artificial infection with anthracnose (*C. gloeosporioides*) is shown in Tables (7 and 8). According to the data, fruit firmness was decreased as storage time increased. After 50 days of cold storage at  $12\pm 1$  °C and 85-90% relative humidity, the firmness of the

mango fruit began to decline more noticeably than before. In comparison to the control treatment, all of the evaluated bio and agrochemical products increased the firmness values of mango fruit at harvest and after cold storage in 2023 and 2024. During the two seasons of the study, pre-harvest spraying with Divide 60%, Trifmine 15% EC, and calcium chloride ( $\text{CaCl}_2$ ) generally resulted in higher mango fruit firmness values than Fungisei 1% and Biocontrol T34 12% in both tested cultivars. Generally, after 50 days of cold storage at  $12\pm 1$  °C and 85-90% RH, the treated mango fruits with artificial anthracnose infection had lower firmness values compared to the naturally infected mango fruits.

**Table (7):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars fruit firmness (lb/inch<sup>2</sup>) under natural infection of mango fruits with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at  $12\pm 1$  °C and 85-90% RH for 50 days.

Treatments	Conc. /100L	Fruit firmness (lb/inch <sup>2</sup> )							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	12.00a	4.99e	12.60b	3.50c	12.00b	4.00c	12.00b	4.50b
Biocontrol T34 12 %	200 g	12.00a	5.50d	12.60b	4.00b	12.00b	4.00c	12.00b	4.50b
$\text{CaCl}_2$	100 g	12.33b	6.00a	12.66a	5.00a	12.50a	6.00a	12.50a	5.00a
Trifmine 15%	25 ml	12.33b	5.90b	12.66a	5.00a	12.50a	5.50b	12.50a	5.00a
Divide 60%	100 g	12.33b	5.70c	12.66a	5.00a	12.50a	5.50b	12.50a	5.00a
Control	Water	11.30c	3.55f	11.70c	3.00d	11.11c	3.30d	11.50c	3.00c

No significant differences between the treatments with the same letter/s in the same column at  $P \leq 0.05$

**Table (8):** Effect of pre-harvest spraying of the tested products on two mango cultivars fruit firmness (lb/inch<sup>2</sup>) under artificial infection of mango fruits with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at 12±1 °C and 85-90% RH for 50 days.

Treatments	Conc. /100L	Fruit firmness (lb/inch <sup>2</sup> )							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	12.00b	4.00b	12.60b	3.50c	12.00b	4.00b	12.00b	4.00b
Biocontrol T34 12 %	200 g	12.00b	4.00b	12.60b	4.50b	12.00b	4.00b	12.00b	3.50c
CaCl <sub>2</sub>	100 g	12.33a	5.00a	12.66a	5.00a	12.50a	5.00a	12.50a	4.00b
Trifmine 15%	25 ml	12.33a	5.00a	12.66a	5.00a	12.50a	5.50a	12.50a	4.00b
Divide 60%	100 g	12.33a	5.00a	12.60b	5.00a	12.50a	5.00a	12.50a	5.00a
Control	Water	11.30c	3.50c	11.70c	3.50c	11.11c	3.00c	11.50c	3.00d

No significant differences between the treatments with the same letter/s in the same column at P≤0.05

### Total soluble solids (TSS):

**Effect of pre-harvest spraying of the tested bio and agrochemical products on total soluble solids (TSS) under natural infection and artificially inoculated mango fruits by *C. gloeosporioides* after cold storage at 12±1 °C and 85-90% RH for 50 days:**

Data concerning total soluble solids (TSS) content in mango fruits cv. Naomi and cv. Keitt due to pre-harvest spraying of some bio and agrochemical products Fungisei 1%, Biocontrol T34 12 %, calcium chloride (CaCl<sub>2</sub>), Trifmine 15% EC and Divide 60% under natural and artificial infection by anthracnose (*C. gloeosporioides*) after cold storage at 12±1 °C and 85-90% RH for 50 days are reported in Tables (9 and 10). Data showed that, TSS

values were increased significantly with the progress of storage period and reached the highest level at the end of storage period compared with fruits at harvest. Partly, during 2023 and 2024, all tested products decreased TSS values at harvesting and after cold storage for 50 days compared with control treatment. In most cases, pre-harvest spraying of Fungisei 1% and Biocontrol T34 12 % decreased TSS values compared with Divide 60%, Trifmine 15% EC and CaCl<sub>2</sub> and control treatment in both tested cultivars during the two seasons of investigation. TSS values were increased under artificial infection than natural infection with *C. gloeosporioides* after cold storage at 12±1 °C and 85-90% RH for 50 days.

**Table (9):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars total soluble solids (TSS) under natural infection with anthracnose (*Colletotrichum gloeosporioides*) after 50 days of cold storage at 12±1 °C and 85-90% RH.

Treatments	Conc. /100L	Total soluble solids (TSS)							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	11.30c	17.50e	11.50e	17.20c	11.60d	17.00d	11.30c	16.00c
Biocontrol T34 12 %	200 g	11.30c	17.66d	11.55d	17.20c	11.60d	17.00d	11.30c	16.00c
CaCl <sub>2</sub>	100 g	12.00b	18.33c	11.90c	18.00b	12.00c	18.00c	11.55b	17.50b
Trifmine 15%	25 ml	12.00b	18.55b	11.95b	18.00b	12.00c	19.00b	11.55b	17.50b
Divide 60%	100 g	12.00b	18.55b	11.95b	18.00b	12.30b	19.00b	11.55b	17.50b
Control	Water	12.50a	19.00a	12.11a	18.66a	12.80a	19.22a	12.00a	18.00a

No significant differences between the treatments with the same letter/s in the same column at P≤0.05

**Table (10):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars total soluble solids (TSS) under artificial infection with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at 12±1 °C and 85-90% RH for 50 days.

Treatments	Conc. /100L	Total soluble solids (TSS)							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	11.30c	17.90c	11.50e	18.50f	11.60d	18.70d	11.30c	19.00c
Biocontrol T34 12 %	200 g	11.30c	17.90c	11.55d	18.80e	11.60d	18.70d	11.30c	19.00c
CaCl <sub>2</sub>	100 g	12.00b	19.50b	11.90c	19.50c	12.00c	19.90b	11.55b	19.50b
Trifmine 15%	25 ml	12.00b	19.50b	11.95b	19.00d	12.00c	19.50c	11.55b	19.50b
Divide 60%	100 g	12.00b	19.50b	11.95b	19.80b	12.30b	19.50c	11.55b	19.50b
Control	Water	12.50a	21.00a	12.11a	21.60a	12.80a	21.25a	12.00a	22.00a

No significant differences between the treatments with the same letter/s in the same column at P≤0.05

#### Titrateable acidity (TA) %:

**Effect of pre-harvest spraying of the tested bio and agrochemical products on titrateable acidity (TA) under natural infection and artificially inoculated mango fruits by *C. gloeosporioides* after cold storage at 12±1 °C and 85-90% RH for 50 days:**

Concerning the response of titrateable acidity (TA) content on mango fruits cv. Naomi and cv. Keitt to pre-harvest spraying of some bio and agrochemical products; Fungisei 1%, Biocontrol T34 12 %, calcium chloride (CaCl<sub>2</sub>), Trifmine 15% EC and Divide 60% under natural and artificial infection with anthracnose (*C. gloeosporioides*) after cold storage at 12±1 °C and 85-90% RH for 50 days, results are reported in Tables (11 and 12). Data showed

that, during 2023 and 2024, all products caused a significant decrease in titrateable acidity (TA) after cold storage for 50 days compared with 0 day. All tested products recorded increasing in TA compared with control in the two tested cultivars during the two years of the investigation. In most cases, pre-harvest spraying of Divide 60% gave the highest TA values followed by Trifmine 15% EC and CaCl<sub>2</sub> compared with control. Pre-harvest spraying of the products increased TA% values in cv. Keitt compared with cv. Naomi. TA values were decreased in mango fruits under artificial infection with anthracnose (*C. gloeosporioides*) compared to naturally infected one in both tested cultivars during the two seasons of investigation.

**Table (11):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars titrateable acidity (TA) under natural infection with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at 12 ±1 °C and 85-90% RH for 50 days.

Treatments	Conc. /100L	Titrateable acidity (TA) %							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	0.95d	0.45d	0.98c	0.48d	0.90d	0.45e	0.99b	0.45c
Biocontrol T34 12 %	200 g	0.97c	0.49c	1.90b	0.55c	0.99c	0.55d	1.99a	0.45c
CaCl <sub>2</sub>	100 g	0.99b	0.69b	1.90b	0.75b	0.99c	0.60c	1.99a	0.80b
Trifmine 15%	25 ml	0.99b	0.69b	1.90b	0.75b	1.03b	0.68b	1.99a	0.80b
Divide 60%	100 g	1.03a	0.72a	1.96a	0.77a	1.11a	0.74a	1.99a	0.88a
Control	Water	0.69e	0.29e	0.77d	0.33e	0.69e	0.45f	0.77c	0.36d

No significant differences between the treatments with the same letter/s in the same column at P≤0.05

**Table (12):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars titratable acidity (TA) under artificial infection with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at 12±1 °C and 85-90% RH for 50 days.

Treatments	Conc. /100L	Titratable acidity (TA) %							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	0.95b	0.25d	0.98c	0.45c	0.90d	0.35c	0.99b	0.25c
Biocontrol T34 12 %	200 g	0.94c	0.29c	1.96a	0.45c	0.99c	0.35c	1.99a	0.25c
CaCl <sub>2</sub>	100 g	0.94c	0.32b	1.96a	0.65b	0.99c	0.36b	1.99a	0.45b
Trifmine 15%	25 ml	0.94c	0.32b	1.96a	0.65b	1.03b	0.34d	1.99a	0.45b
Divide 60%	100 g	1.03a	0.38a	1.93b	0.77a	1.11a	0.38a	1.99a	0.47a
Control	Water	0.69d	0.20e	0.77d	0.33d	0.69e	0.35e	0.77c	0.30d

No significant differences between the treatments with the same letter/s in the same column at P≤0.05

### Vitamin C (Ascorbic acid):

**Effect of pre-harvest spraying of the tested bio and agrochemical products on fruit vitamin C (Ascorbic acid) content under natural infection and artificially inoculated mango fruits by *C. gloeosporioides* after cold storage at 12±1 °C and 85-90% RH for 50 days:**

Data in Tables (13 and 14) and Fig. (6) during 2023 and 2024, show a significant decrease in vitamin C (Ascorbic acid) mg/100g after cold storage for 50 days compared with 0 day. All tested bio and agrochemical products caused an increase in vitamin C (Ascorbic acid) compared with control in the two tested mango cultivars

during the two years of the investigation. In most cases, pre-harvest spraying of Fungisei 1% and Biocontrol T34 12 % gave the highest vitamin C values followed by calcium chloride (CaCl<sub>2</sub>) compared with control. Pre-harvest spraying of bio and agrochemical products increased vitamin C (Ascorbic acid) values in cv. Keitt compared with cv. Naomi. Under artificial infection with anthracnose (*C. gloeosporioides*), after cold storage at 12±1 °C and 85-90% RH for 50 days, vitamin C values were decreased compared with natural infected mango fruits in both tested cultivars during the two seasons of investigation.

**Table (13):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars vitamin C content (Ascorbic acid) (mg/100g) under natural infection with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at 12±1 °C and 85-90% RH for 50 days.

Treatments	Conc. /100L	Vitamin C (Ascorbic acid) mg/100g							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	49.33c	38.50a	70.55b	53.50a	49.50a	36.50b	72.55a	60.50a
Biocontrol T34 12 %	200 g	49.58a	38.50a	71.50a	53.50a	49.50a	35.50c	72.55a	60.50a
CaCl <sub>2</sub>	100 g	49.50b	38.50a	68.50c	53.50a	49.50a	37.50a	70.50b	60.50a
Trifmine 15%	25 ml	46.23e	34.20b	49.25e	50.20b	48.00c	33.20d	55.60c	55.20b
Divide 60%	100 g	46.28d	34.20b	49.27d	44.20c	48.20b	33.20d	55.20d	45.20c
Control	Water	45.53f	28.50c	47.56f	39.50d	45.57d	30.50e	47.57e	39.50d

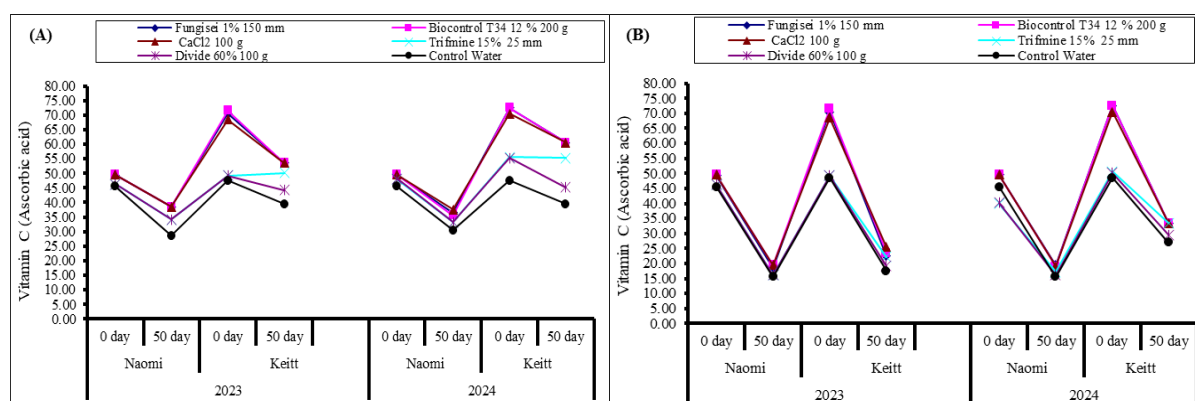
No significant differences between the treatments with the same letter/s in the same column at P≤0.05



**Table (14):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars vitamin C content (Ascorbic acid) under artificial infection with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at 12±1 °C and 85-90% RH for 50 days.

Treatments	Conc. /100L	Vitamin C (Ascorbic acid) mg/100g							
		2023				2024			
		Naomi		Keitt		Naomi		Keitt	
		0 day	50 day	0 day	50 day	0 day	50 day	0 day	50 day
Fungisei 1%	150 ml	49.33c	18.50b	70.55b	22.50c	49.50a	19.50a	72.55a	33.50a
Biocontrol T34 12 %	200 g	49.58a	19.50a	71.50a	23.50b	49.50a	19.00b	72.55a	33.50a
CaCl <sub>2</sub>	100 g	49.50b	19.50a	68.50c	25.50a	49.50a	19.00b	70.50b	33.50a
Trifmine 15%	25 ml	46.23e	16.20c	49.25e	22.20d	40.00d	17.20c	50.60c	33.50a
Divide 60%	100 g	46.28d	16.20c	49.27d	19.20e	40.20c	16.20d	50.20d	29.50b
Control	Water	45.53f	15.50d	48.56f	17.50f	45.57b	15.50e	48.57e	27.00c

No significant differences between the treatments with the same letter/s in the same column at P≤0.05



**Fig. (6):** Effect of pre-harvest spraying of the tested bio and agrochemical products on two mango cultivars vitamin c (Ascorbic acid) mg/100g under natural infection (A) and artificial infection (B) with anthracnose (*Colletotrichum gloeosporioides*) after cold storage at 12±1°C and 85-90% RH for 50 days.

## DISCUSSION

A tropical and seasonal fruit, mangos are typically accessible in Egypt during the summer months, from June through September serving as the primary harvest gathering time. With its superb exotic flavor, the mango (*Mangifera indica* L., Family Anacardiaceae) is regarded as the fruit's queen. About 60 million tons of mango fruits were produced worldwide (FAO, 2022). Mangos are the second most popular fruit crop in Egypt, after citrus. In the reclaimed land, the area planted with mango trees has grown very quickly. Keitt and Naomi, two previously introduced mango cultivars with exceptional fruit quality, were successfully cultivated in Egypt under various regional conditions (El-Agamy *et al.*, 2018). Many diseases can affect mangos, some of which are quite

destructive and reduce the crop's yield and productivity. The most destructive of the mango diseases is anthracnose, which is caused by *Colletotrichum gloeosporioides*. As a post-harvest disease of harvested fruits, it mostly targets leaves, blossoms, young fruits, and twigs. Leaf spots, blossom blight, fruit spots, and eventually fruit rot are caused by black, irregularly shaped, and somewhat sunken spots that develop on leaves and fruits and progressively become larger. There is a significant prevalence of disease in moist or extremely humid environments, which are common in most mango-growing regions (Berardini *et al.* 2005). Mango availability, trade, and export are hampered by their high perish ability and vulnerability to postharvest infections. More than 25-30% of mangos are lost

during handling and storage, according to a nationwide survey (**Prasad *et al.*, 2019**). According to **Medlicott *et al.* (1990)**, mature green mango fruits should be kept between 10 and 12°C. In order to preserve fruit quality and prevent chilling injury, **Pesis *et al.* (1997)** kept mango fruits (cvs. Alphonso and Keitt) at various cold storage temperatures. They discovered that at 10°C, chilling injury symptoms manifested as pitting and red spots on the peel, while at lower temperatures, black spots emerged. When Pairi mango fruits were kept at 25°C, **El-Khoreiby *et al.* (1998)** found that the proportion of rotten fruits was 13% in the first week and 37% in the second. In the present study, *C. gloeosporioides* was able to induce anthracnose disease on mango fruits. Keitt cultivar was more susceptible than cv. Naomi under artificial inoculation in pathogenicity tests of the two years of the investigation. All tested bio and agrochemical products reduced *C. gloeosporioides* linear growth on PDA medium compared with control. Divide 60%, Trifmine 15% and calcium chloride (CaCl<sub>2</sub>) were the best effective treatments where they completely inhibited the growth of tested fungus. On the other hand, Biocontrol T34 12 % and Fungisei 1% recorded low efficacy in reducing the colony growth of the tested pathogen. All tested products were significantly better than the control in decrease the percentages of disease incidence and disease severity of anthracnose disease under natural and artificial infection with *C. gloeosporioides*. In general, the performance of CaCl<sub>2</sub>, Trifmine 15% and Divide 60% were the best compared with Fungisei 1% and Biocontrol T34 12 % for both natural and artificial infections during the two investigated season. Pre-harvest spraying any of CaCl<sub>2</sub>, Trifmine 15% EC and Divide 60% totally controlled and inhibited anthracnose disease on the second group of mango fruits which had been treated with the previous products in the field and were subjected to a natural infection after cold storage at 12±1 °C and 85-90% RH for 50 days during 2023 and

2024 growing seasons. These results are consistent with a prior study that found that *C. gloeosporioides* caused 20-30% of mango fruits in Hyderabad to decay. Mango anthracnose disease reduced Bangladesh's mango yield by 25-30%, according to **Hossain and Ahmed (1994)**. In Thailand, **Sardsud *et al.* (2003)** found that mango anthracnose disease caused 62.8% of mangos loss during harvest and 63.2% in the markets. China's mango harvest can be reduced by 30-60% every year due to anthracnose (**Li *et al.*, 2019**). Despite this, producers and sellers continue to offer low-quality fruits on the local market, with nations that export mangos, such as Ghana, suffering the most from the revenue gap between export and local markets (**Kankam *et al.*, 2022**). Due to senescence and physiological changes, mango fruits are susceptible to post-harvest infections while being stored (**Prusky and Keen, 1993**). **Khaliq *et al.* (2016)** examined the physiological and biochemical characteristics of mango (*Mangifera indica* L. cv. Choke Anan) fruit in relation to the effects of 10% Arabic gum (AG) and 1% chitosan (CH) edible coatings. During the storage period, they found no discernible differences between fruits treated with CH 1% and fruits treated with AG 10% + CH 1%. At the conclusion of the storage period, the control samples had the highest decay percentage. Coating has been shown to be effective in inhibiting the growth of fungi in a variety of horticultural products (**Tripathi and Dubey, 2004**). A number of methods have been tested to prevent the development of rot and postpone the ripening of mango fruit by employing natural bio preservatives rather than fungicides. According to **Elsabee and Abdou (2013)**, chitosan helps prevent postharvest fruit diseases and possesses potent antibacterial and antifungal qualities. The most promising fungicide in fields, according to **Sharma and Verma (2007)**, was Saaf (carbendazim 12% + mancozeb 63%) @ 0.2%, which can be advised for the management of mango anthracnose. According to **Gud and Raut**

(2008), the most effective fungicides for completely inhibiting the mycelial growth of mango anthracnose were propiconazole 0.1%, thiophanate methyl (0.2%), and Emisan 0.2%. **The same authors (2008)** mentioned that many *Trichoderma* species are effective at preventing the growth of the mycelia of *C. gloeosporioides*, which causes mango anthracnose. All of the isolated fungi linear growth was totally reduced by 0.5ml/L Difenconazole 25% (Score 25% EC), 0.5g/L Boscalid 25.2% + Pyraclostrobin 12.8% (Bellis 38%WG), and 1ml/L Thiabendazole (Tekto 50% SC), **(Hassan et al., 2024)**. In the two seasons of the study, using both tested cultivars, cv. Montakhab El-Qanater and cv. Ewais, Score 25% EC was the most effective at reducing mango fruit drop. Bellis 38% WG and Tekto 50% SC came next. Azoxystrobin 20% (25 ml/100 L water) + Tebuconazole 30% (Dovex 50% SC) and 40 ml/100 L water Difenconazole 15% + Propiconazole 15% (Craft 30% EC) provided the lowest efficacy. Pre-harvest spraying of loquat trees with the best concentrations of Score 25% EC (Difenconazole) 1 ml/litre, Scar Nat 70% WP (Thiophanate-methyl) 1 g/litre, Pyrmadol 40% SC (Pyrimethanil) 1 ml/litre, and Decent 32.5 EC (Azoxystrobin 20% + Difenconazole 12.5%) 1 ml/litre helped control fruit rot, leaf spot, and inflorescence blight diseases **(Hassan and Shehata 2024)**. The three main pathogens that cause avocado fruit rot in Egypt are *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, and *Penicillium expansum*.

In Egypt, the characteristic symptoms of fruit rot were caused by artificial inoculation of these fungi. Under market conditions, avocado fruit rots may also be managed with pre-harvest treatments that contain the highest concentration of Imazalil and copper oxide nanoparticles. The research also demonstrated the viability of employing essential oils and alternative therapies as Serenade ASO (*Bacillus subtilis* QST 713) as management strategies for the prevention of avocado fruit rot disease both before and after

harvest **(Hassan et al., 2021)**. According to **Begum et al. (2008)**, the *Trichoderma* isolates coiled around *C. truncatum* hyphae, further limiting its ability to develop and disseminate. *Trichoderma* species produce a variety of antibiotics that suppress fungal development and sporulation, including trichotoxin, harzianolide, trichodermin, and trichodermol **(Yonas and Amare 2008)**.

Many *Trichoderma* species are known to produce siderophores and indole acetic acid, as well as a many of extracellular enzymes that aid in the breakdown of pathogenic fungi cell walls, such as chitinase, pectinase, amylase, and protease **(Sallam et al., 2021)**. According to **Yoshida et al. (2001)**, *B. subtilis* has the potential to produce antimicrobial peptide substances, such as subtilin, bacilysin and mycobacillisyn, which can alter hyphal morphology in *C. acutatum* and *C. gloeosporioides* hyphal cells, causing hyphal swelling, distortion, and cytoplasm aggregation. Anthracnose disease was successfully controlled on mango trees by spraying *Streptomyces aureofaciens* bacterial filtrate **(Haggag et al., 2011)**. **Korsten et al. (1991)** recorded that *Bacillus subtilis* ( $10^7$  cells/ml) has been used both before and after harvest to reduce avocado post-harvest infections. Additionally, *B. subtilis* decreased the severity of stem-end rot, the Dothiorella / Colletotrichum fruit rot complex, and anthracnose on Fuerte avocado fruit. Additionally, the fruit dipping treatment with *B. subtilis* was just as effective as or even more successful as a prochloraz. Mango anthracnose was managed by fruit dip and challenge inoculation techniques with promising bio-agents. Up to two days of incubation on the Alphonso variety, fruits treated with the bio-agents *Trichoderma harzianum*, *T. asperellum*, *B. subtilis*, *P. fluorescens*, and the yeasts *Meyerozyma caribbica* and *Torulaspora delbrueckii* showed no symptoms; nevertheless, following that, the fruits symptom expression was progressively increased. Bacterial bio-agents *P. fluorescens* and *B. subtilis* had the lowest

disease incidence among the bio-agents examined, at 37.70% and 33.81%, respectively. In contrast, the disease index percentages for fruits treated with *Trichoderma asperellum* and *T. harzianum* were 43.75 and 46.33%, respectively. On the tenth day of incubation, the disease index for the control (untreated fruits) recorded 85.50%, while the disease index for the yeast, *M. caribbica*, and *T. delbrueckii*, recorded 40.00 and 41.82 percent, respectively. Similar outcomes were obtained using the challenge inoculation strategy on the Alphonso variety (Sudha *et al.*, 2021).

In the present study, all tested bio and agrochemical products kept fruit quality parameters and had significant effect due to total soluble solids TSS, TA and fruit firmness. All tested bio and agrochemical products increased mango fruit firmness values at harvesting and after cold storage at  $12 \pm 1$  °C and 85-90% RH for 50 days compared with control treatment. In most cases, pre-harvest spraying of Divide 60%, Trifmine 15% EC and  $\text{CaCl}_2$  increased mango fruit firmness values compared with Fungisei 1% and Biocontrol T34 12 % in both tested cultivars during the two seasons of investigation. Mango fruit firmness values were decreased under artificial infection with anthracnose (*C. gloeosporioides*) than natural infected mangos after cold storage at  $12 \pm 1$  °C and 85-90% RH for 50 days. TSS values were increased significantly with the progress of storage and reached the highest level at the end of storage period compared with fruits at harvest. Partly, during 2023 and 2024, all tested bio and agrochemical products decreased TSS values at harvesting and after cold storage at  $12 \pm 1$  °C and 85-90% RH for 50 days compared with control treatment.

In most cases, pre-harvest spraying of Fungisei 1% and Biocontrol T34 12 % decreased TSS values compared with Divide 60%, Trifmine 15% EC and calcium chloride ( $\text{CaCl}_2$ ) and control treatment in both tested cultivars during the two seasons of investigation. TSS values were increased due to artificial

infection than under natural infection with *C. gloeosporioides* after cold storage at  $12 \pm 1$  °C and 85-90% RH for 50 days. All bio and agrochemical products caused a significant decrease in TA after cold storage for 50 days compared with 0 day. All tested bio and agrochemical products caused increase in TA compared with control in the two tested cultivars during the two years of the investigation. In most cases, pre-harvest spraying of Divide 60% gave the highest TA values followed by Trifmine 15% EC and calcium chloride ( $\text{CaCl}_2$ ) compared with control. Pre-harvest spraying of bio and agrochemical products increased TA% values in cv. Keitt compared with cv. Naomi. Under artificial infection with anthracnose (*C. gloeosporioides*) after cold storage at  $12 \pm 1$  °C and 85-90% RH for 50 days TA values were decreased compared to natural infected mango fruits in both tested cultivars during the two seasons of investigation. Significant decrease in vitamin C (Ascorbic acid) after cold storage for 50 days compared with 0 day. Pre-harvest spraying of Fungisei 1% and Biocontrol T34 12 % gave the highest vitamin C values followed by  $\text{CaCl}_2$  compared to control. Pre-harvest spraying of bio and agrochemical products increased vitamin C (Ascorbic acid) values in cv. Keitt compared with cv. Naomi. Vitamin C values under artificially infected mango fruits with anthracnose (*C. gloeosporioides*), after cold storage at  $12 \pm 1$  °C and 85-90% RH for 50 days, were decreased compared to natural infection in both tested cultivars during the two seasons of investigation. These results are consistent with Ali *et al.* (2004), who found that ripening of mango fruits causes a decrease in firmness over the progress of the storage period. This is because ripening and cold storage increase the activity of enzymes that hydrolyze cell walls, including pectatylases, polygalacturonase, pectinesterase, and pectin methylesterase. The hydrolysis of carbs to sugars due to moisture loss and a decrease in acidity may be the source of the larger increase in mango fruit content in TSS during the



progress of the storage period (**Golding et al., 2005**). Starch hydrolyzes into simple sugars throughout the ripening process; in ripe fruits, glucose, fructose, and sucrose predominate. The enzymes amylase, invertase, and sucrose synthase all boosted their activity and hydrolyzed the starch to produce sucrose (**Kumar et al., 1994**). According to a study by **Mohamed et al. (2017)** on the impact of chitosan coating on the storability of mango cv. "Zibda" fruit, the amount of mango fruit in titratable acidity (TA) dropped progressively and considerably over the course of the trial's two seasons. All pre-harvest coating applications considerably decreased the pace at which the titratable acidity (TA) of the fruit changed during cold storage. Fast senescence of mango fruits is linked to a rapid decrease in their TA content during storage (**Abbasi et al., 2009**). The conversion of citric acid into sugars and subsequent use of these sugars in different fruit metabolic processes may be the cause of the decrease in acidity over the storage period (**Rathore et al., 2007**).  $\text{CaCl}_2$  has been shown to be a safe and efficient way to manage postharvest illness and prevent fruit decay, despite the fact that the use of some chemicals may have unintended lingering effects on human safety (**Shen and Yang 2017**).  $\text{CaCl}_2$  and salicylic acid (SA) are signal response molecules that activate downstream reactions, such as the manufacture of antimicrobial compounds and cell wall reinforcement mechanisms, which may explain their impact on fruit disease prevention (**Kumar 2014**). The fungal pathogen *C. gloeosporioides* was stopped from growing by the applications of  $\text{CaCl}_2$ , SA, and *M. pulcherrima* yeast. They also decreased the decay index, stopped the fruit from softening, stopped changes in the TSS and vitamin C contents, and increased the activity of crucial defense enzymes. Additionally, it was discovered that the combination of SA, *M. pulcherrima* yeast, and  $\text{CaCl}_2$  was more effective than either treatment alone. Accordingly, we propose that SA,  $\text{CaCl}_2$ , and *M. pulcherrima* yeast may be used as a

safe and efficient postharvest method to prevent anthracnose while also preserving and increasing mango postharvest life (**Shao et al., 2019**). **Karemera et al. (2013)** reported that mango fruits cv. Mallika exhibited a significant delay, with significantly higher fruit length, breadth, thickness, volume, weight, and pulp weight. Additionally, trees sprayed with 1.50%  $\text{CaCl}_2$  30 days before harvest showed also significantly higher TSS of fruits, a significantly higher percentage of total sugars and the lowest percentage of titratable acidity. Higher calcium levels in fruit can cause lower respiration and ethylene production rates, which may be the reason of the  $\text{CaCl}_2$ -induced ripening delay (**Hewajulige et al., 2003**).  $\text{CaCl}_2$  sprays may prolong the shelf life of fruits because, when fruits are picked at the right time, calcium plays a variety of roles, such as increasing fruit firmness compared to control **Gore (2005)**. The fruit quality improvement that resulted from giving trees  $\text{CaCl}_2$  may have been caused by its effects on improving the formation and transformation of carbohydrates and carbohydrate enzymes; other possible explanations include the reduction of abscission and the crucial role that calcium plays in maintaining the middle lamella cells (**Kumar et al., 2006**). The conversion of fruit organic compounds to TSS during enzymatic activities under the presence of  $\text{CaCl}_2$  may be the cause of the increase in TSS during storage periods (**Wahdan et al., 2011**).

## CONCLUSION

In this study, *Colletotrichum gloeosporioides* was able to induce anthracnose disease on mango fruits. Keitt cultivar was more susceptible than cv. Naomi under artificial inoculation in pathogenicity tests. All tested bio and agrochemical products reduced *C. gloeosporioides* linear growth on PDA medium compared with control. Divide 60%, Trifmine 15% and Calcium chloride ( $\text{CaCl}_2$ ) were the best effective treatments where each completely inhibited the growth of the tested fungus. On the other

hand, Biocontrol T34 12 % and Fungisei 1% recorded low efficacy in reducing the colony growth of the tested pathogen. All tested bio and agrochemical products were significantly better than the control in decrease the percentages of disease incidence and disease severity of anthracnose disease under natural and artificial infection with *C. gloeosporioides*. The performance of Calcium chloride, Trifmine 15% and Divide 60% were the best compared with Fungisei 1% and Biocontrol T34 12 % for both natural and artificial infections during the two investigated seasons. Pre-harvest spraying using  $\text{CaCl}_2$ , Trifmine 15% and Divide 60% totally controlled and inhibited anthracnose disease on naturally infected mango during cold storage at  $12 \pm 1$  °C and 85-90% RH for 50 days during 2023 and 2024. All tested products kept fruit quality parameters and had significant effect due to total soluble solids, titratable acidity, fruit firmness and vitamin C values.

#### Author's contribution

Majority contribution for the whole article belongs to the author(s). The authors read and approved the final manuscript.

#### Competing interests

The author declares that he has no competing interests.

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