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# T34 Biocontrol ® (*Trichoderma asperellum* strain, T34), a Biocontrol Agent Reducing Strawberry Fruit Rots

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## ABSTRACT

Negative environmental impacts of chemical pesticides used for pest and disease management are an increasing issue. So, safe alternatives are being developed. One of the most effective methods is to use microbial biocontrol agents as antifungal biopesticides. The aim of this study was to explore the mode of action and evaluate the potentiality of the novel bio-fungicide T34 Biocontrol ® (*Trichoderma asperellum* strain T34) against strawberry fruit-rot-causing fungal pathogens. Different fungal plant pathogens were isolated from diseased fruits of strawberry showing rots symptoms, collected from a field (Royal Fruits Farm, fresh planting system) at Abo-Ghaleb Al-Mahatta, Abo-Ghaleb city region, Giza Gov., Egypt. Ten isolates were isolated and identified successfully. *Acremonium butyri*, *Alternaria tenuissima*, *Fusarium oxysporum*, *F. sporotrichioides*, *F. subglutinans*, *Mucor hiemalis*, *Rhizoctonia fragaria*, *R. solani* and *Trichothecium roseum* were associated with (as causal complex of) strawberry fruit rot diseases. The commercial formulation of T34 Biocontrol ® (*Trichoderma asperellum* strain, T34) was evaluated under lab conditions (*in vitro*) against these isolates throw two different techniques, growing on *Trichoderma asperellum*-mixed PDA medium plates and dual-culture. *Trichoderma asperellum* strain, T34 showed good antagonistic activities and significantly inhibited the fungal growth of all fungal pathogens tested. Also, the separation of chemical substances of partially purified culture filtrate of *T. asperellum* by GC-MS clarified that different chemical compounds with generally good antimicrobial, antibacterial, bactericidal, antifungal, antioxidant, anti-inflammatory, local anesthetic, antinociceptive, cicatrizing, antiseptic or anticancer properties can be produced by the tested biocontrol agent T34 and plays as antibiotics inhibiting the growth of plant pathogens. Separation of chemical substances of partially purified culture filtrate of *T. asperellum* by GCMS, showed that *T. asperellum* produces compounds (e.g., 9- Octadecenoic acid, Anthrone, Thymol, Glycerol, 1-Hexadecanol,2- methyl-, D-Mannitol, Palmitic Acid, Oleic Acid, Stearic acid, 1- Monopalmitin, Acetic acid, hydrazide, Citronellene, Carveol 1, Linalool, Stanozolol and Arachidonic acid) with antimicrobial activities according to available literature. *In vivo* assay of the biocontrol agent T34 Biocontrol ® under open field conditions against strawberry fruit rot diseases showed that the field application (spraying) of T34 significantly reduced the disease (fruit rots) incidence preharvest (on the field) and disease incidence and severity postharvest (during cooled storage) in both frequently tested winter seasons 2020-2021 and 2021-2022. T34 achieved highly efficient protective performance and led to a disease reduction percentage exceeding 90 % in some trials. Our results were nearly comparable with disease control achieved by comparative chemical fungicides of Fabolous 75%WP (Tebuconazole 12.5%+Chlorothalonil 62.5%(w/w)), Fabric 30%SC (Tebuconazole 15%+Kresoxim-Methyl 15%(w/v)), Fango 50%WG (Pyraclostrobin 50%(w/w)), Klop 50%WP (Difenoconazole 6%+Thiophanate methyl 44% (w/w)), Maven 18.7%WG (Pyraclostrobin 6.7%+Dimethomorph 12%(w/w)) and Mystic 20%WP (Pyrimethanil 20%(w/w)).

## INTRODUCTION

Strawberry (*Fragaria* x *ananassa* Duchesne) is an important soft fruit crop that is grown worldwide and considered one of the most important and widely distributed vegetable crops in Egypt (Awad and Al- Shennawy, 2015). Egypt is the fifth largest strawberry producer in the world, next to China, the USA, Mexico, and Turkey, respectively (FAO STAT, 2019). Egypt is also the largest exporter of frozen strawberries in the world (Food Export Council & International Trade Center (ITC), Daily News Egypt, September 4, 2022). Strawberries are beneficial to the human diet as a source of macro and micronutrients, vitamins, and health-promoting antioxidants (Basu *et al.,* 2014; Giampieri *et al.,* 2015 and Wang and Lin, 2000). Strawberry is affected by several pathogens including fungi, bacteria, viruses, and nematodes. The most economically impactful pathogens of strawberries are fungi, which can attack all parts of the plant leading to severe damage or death (Garrido *et al*., 2011). These pathogens affect fruit in the field, storage, transport, and market (Petrasch *et al.,* 2019).

Fruit rots are one of the most serious diseases affecting strawberries and the most important disease of strawberries worldwide. It often results in a large quantity of fruits rotting during harvest, storage, or transportation, causing serious economic losses (Hu *et al.,* 2019). Postharvest fungal diseases might be considered a minor problem for local markets with short periods between harvest and selling of vegetables and fruit, however, when the fruit is exported to foreign countries (Nallya *et al.,* 2012). The main causes of the decay of strawberry fruit during storage and shelf life are the development of rots caused by a range of fungi. Globally, tens of fungal species can cause rot diseases in strawberry fruits.

The traditional strategy to controlling postharvest strawberry decay relies on the application of synthetic chemical fungicides throughout the crop-growing cycle (Feliziani and Romanazzi, 2016). Undoubtedly, the main method of controlling strawberry fruit diseases throughout the world is through the frequent use of chemical fungicides. Over the past century, growers have relied intensively on the use of chemical fungicides to control strawberry diseases caused by fungi. Fungicide resistance coupled with current public concerns for both the environment and pesticide residues in food (Mehrotra *et al.,* 1997). Worldwide, the development of fungicide-resistant strains of fungal pathogens and increasing public concern about the level of fungicide residues on strawberry fruit, have led to the search for alternative control options, such as biological control (Card *et al.,* 2009).

Application of biopesticides is an eco-friendly approach to minimize the use of chemical pesticides and fertilizers in agriculture (Singh *et al.,* 2016). Biopesticides are developed from living microorganisms like viruses, nematodes, bacteria, and fungi (Gasic and Tanovic, 2013). Microbial biopesticides may be an alternative path in crop protection because of their safety for humans and non-target organisms, both in individual applications and within integrated pest management (IPM) programs (Gasic and Tanovic, 2013).

*Trichoderma* spp. is one of the most commonly used biocontrol agents against fungal plant pathogens (Segarra *et al.,* 2007). *Trichoderma* species have been investigated for over 80 years. They have been used recently as biocontrol agents and their isolates have become commercially available (Al-Obaidy and Al-Rijabo, 2010). *Trichoderma*-based bio-fungicides were also effective in integrated pest management (IPM) or integrated disease management (IDM) strategies on several crops. *Trichoderma* is commonly the most saprophytic fungal species found in the rhizosphere which holds the major share in the biopesticide industry (Keswani *et al.,* 2013 and Woo *et al.,* 2014). *Trichoderma* not only attacks other pathogenic fungi but also promotes the growth of host plants (Hermosa *et al.,* 2012 and Chen *et al.,* 2015). Its mode of action includes antibiosis, mycoparasitism and competition for nutrients and space (Keswani *et al.,* 2014). *Trichoderma asperellum*, a less-

studied fungus, is also a successful biological control agent for a wide range of fungal plant pathogens (Marcello *et al.*, 2010 and Wu *et al.*, 2017).

The aim of the present work was to evaluate the effectiveness of the promising novel bio-fungicide T34 Biocontrol ® (*Trichoderma asperellum* strain, T34), against strawberry fruit rot diseases under both lab, field conditions (preharvest) and during storage (postharvest), compared to locally registered and/or recommended synthetic chemical fungicides.

## MATERIALS AND METHODS

### 1.Tested Fungal Isolates:

#### 1.1. Source, Isolation, Identification, and Maintenance of Targeted Pant Pathogenic Fungal Isolates:

To isolate the causal of strawberry fruit rots, as usual, samples of rotten strawberry fruits showing slightly common fruit rot infection symptoms had been collected from different sites in the open (fresh planting system) strawberry field (Royal Fruits Farms) located in Abo-Ghaleb Al-Mahatta district, Giza Gov., Egypt. The samples were put into clean plastic boxes, then kept in a home refrigerator at 7°C (7 degree Celsius) for seven days to enhance the development of the growth of the fungal pathogens. Later the pathogens were isolated on PDA media, purified, and identified by Plant Quarantine Pathogens Lab (PQPL), Dept. of Fungi Survey & Taxonomy, Plant Pathology Research Institute, Agricultural Research Center (ARC), Egypt. The isolates were renewed on PDA media as necessary.

#### 1.2. Source of Fungal Pathogens:

Another group of diseased fruits of strawberries showing various types of rots symptoms were collected from the same field previously mentioned. Fruit samples transferred to lab, rinsed several times in sterilized distilled water (SDW) and surface sterilized by using 70% ethyl alcohol for two minutes and dried between sterilized filter papers. They were then cut into small pieces and placed in Petri dishes containing potato dextrose agar medium (PDA). The dishes were incubated for 7 days at 25°C ± 2°C. Developed mycelium was transferred, purified, identified, and kept on PDA plates as usual, and renewed on PDA Petri plates as necessary. All Petri plates used in laboratory experiments were plastic single-use plates and 9 cm in diameter.

### 2.Lab Experiments:

#### 2.1. Tested Pathogenic Fungi:

An identified isolates of strawberry fruit-rot-causing fungi were used for lab assays.

#### 2.2. Tested Fungicides:

Selected (locally-registered) fungicides were used for studying their effectiveness against strawberry fruit-rot-causing fungal pathogens compared to the bio-fungicide (Biological control agent) T34 Biocontrol ®, containing the living microorganism *Trichoderma asperellum* strain T34 as an active ingredient. Common names, active ingredients (a.i.), formulations and other important information (including information available in the updated FRAC (Fungicide Resistance Action Committee) Code List ©*2022, and information mentioned in Technical Recommendations for Agricultural Pest Control, 2022nd edition, by Agricultural Pesticides Committee (APC), Ministry of Agriculture and Land Reclamation (MALR), Arab Republic of Egypt (A.R.E)). The formulations of tested fungicides were explained in Table 1.

**Table 1.** key information of fungicides tested:

| Trade name, Conc. and Formulation | Active ingredient (s) (Common name (s)) | Group Name | Chemical or Biological group | WHO Toxicity Classification |
|---|---|---|---|---|
| **T34 12% WP ($1x10^9$ CFU/g** | *Trichoderma asperellum* strain T34 | microbial (*Trichoderma* spp.) | *Trichoderma* spp. and the fungicidal metabolites produced | U |
| **Fabolous 75% WP** | Tebuconazole 12.5% (w/w) + Chlorothalonil 62.5% (w/w) | DMI-fungicides (DeMethylation Inhibitors) SBI: Class I + chloronitriles (phthalonitriles) (unspecified mechanism) | Triazoles + chloronitriles (phthalonitriles) | Low III |
| **Fabric 30% SC** | Tebuconazole 15% (w/v) + Kresoxim-Methyl 15% (w/v) | DMI-fungicides (DeMethylation Inhibitors) SBI: Class I + QoI-fungicides (Quinone outside Inhibitors) | triazoles + oximino-acetates | U |
| **Fango 50% WG** | Pyraclostrobin 50% (w/w) | QoI-fungicides (Quinone outside Inhibitors) | methoxy-carbamates | U |
| **Klop 50% WP** | Difenoconazole 6% (w/w) + Thiophanate methyl 44% (w/w) | DMI-fungicides (DeMethylation Inhibitors) (SBI: Class I) + MBC- fungicides (Methyl Benzimidazole Carbamates) | triazoles + thiophanates | Low III |
| **Maven 18.7% WG** | Pyraclostrobin 6.7% (w/w) + Dimethomorph 12% (w/w) | QoI-fungicides (Quinone outside Inhibitors) + CAA-fungicides (Carboxylic Acid Amides) | methoxy-carbamates + cinnamic acid amides | U |
| **Mystic 20% WP** | Pyrimethanil 20% (w/w) | AP - fungicides (Anilino-Pyrimidines) | anilino-pyrimidines | U |

### 2.3. Effect of *Trichoderma asperellum* on Several Fungal Plant Pathogens:

#### 2.3.1. Ability of Fungal Plant Pathogens to Grow on T34-Inoculated PDA Plates:

PDA media was prepared and sterilized, as usual, T34 Biocontrol 12%WP Formulation was added to worm PDA flasks in two rates: 0.85 g/l (recommended dose) & 0.2125 g/L (1/4 of recommended dose), well mixed and poured into Petri plates. 5 mm discs of 7-10 days old cultures of fungal plant pathogenic isolates were placed onto the middle of Petri plates. No-added T34 PDA Petri plates were used as a control. Three plates were used as replicates for each treatment. All plates were incubated at 28°C till full growth of control. Fungal growth reduction percentages were calculated according to the following equation:

Fungal Growth Reduction% = ((C - T) / C) x 100

C: Median fungal growth diameter (cm) in control plates (pathogens control plates).

T: Median fungal growth diameter (cm) in treatment plates.

#### 2.3.2. Dual Culture Test on Petri Plates:

A dual culture technique was carried out according to Gao *et al.*, (2002) with some modifications. Petri dishes (9 cm in diameter), each containing 10ml, of PDA medium were inoculated with discs (5 mm in diameter) of any of the tested fungal plant pathogens, taken from the margin actively growing colony of 7 days old cultures. The discs were placed near (one cm) the edge of each Petri dish. 30 minutes later, the same plates were inoculated with equal discs of *T. asperellum* on the opposite side. Three plates were used as replicates for each treatment. PDA plates inoculated with the mycelial discs of each pathogen alone served as controls. All plates were incubated at 28°C either for two weeks or until the growth in the control treatment (pathogens control plates) reached the edge of the plates, whichever first. The results were recorded by measuring the radial linear growth of the pathogens growing

towards *Trichoderma*. The reduction percentage of fungal growth was calculated according to the following formula:

Fungal Growth Reduction% = ((C - T) / C) x 100

C: Median radial (linear) fungal growth (cm) in control plates (pathogens control plates).

T: Median radial (linear) fungal growth (cm) in treatment plates.

**2.4. Separation of Chemical Compounds (Components) of Partially Purified Culture Filtrate of *T. asperellum* by GC-MS:**

This experiment was carried out using the ready commercialized formulation of *T. asperellum*, T34 Biocontrol (WP 12% w/w, $1x10^9$ cfu/g) added -in the recommended dose (Field recommended concentration, 85 g of formulation / 100 L water = 0.85 g/L = $85x10^4$ cfu/ml)- to flasks (250 ml) containing 100 ml of warm sterilized potato dextrose broth (PDB) and then incubated at 27±2º C for one and two weeks, respectively. Then, the PDB (culture filtrate) content of volatile and non-volatile chemical compounds was determined by Chromatography Lab, Dept. of Food Poisons & Contaminants, Nutrition & Food Industries Institute, NRC, Egypt. After the two incubation periods, cultures were filtrated through filter paper (Watman No. 2). The supernatant was extracted three times with equal volumes of ethyl acetate using a separating funnel. The solvent was evaporated at 50º for 48 hrs. and the compounds of crude extract dissolved by ethyl acetate were separated by GC-MS.

**2.4.1. GC-MS Analysis for Volatile Compounds HS:**

**Gas Chromatography-Mass Spectrometry Analysis (GC-MS- HS):**

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. Headspace temperature program: oven temperature 80°C, needle temperature 120°C, transfer line temperature 140 °C and incubation time 20min. The GC was equipped with a DB-624 column (30 m x 320 µm internal diameter and 1.80 $\mu$m film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 3 ml/min at a splitless, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 7 °C /min to 250 °C and held for 5min. The injector and detector were held at 250 °C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 30-550. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

**2.4.2. Sample Derivatization:**

The sample was extracted, dried and resuspended in 50 µL of bis(trimethylsilyl) trifluoroacetamide (BSTFA)+trimethylchloro-silane (TMCS) 99:1 silylation reagent and 50 µL pyridine for derivatization sample functional groups to trimethylsilyl groups (abbreviated TMS) prior to GC analysis.

**2.5. Field Experiments:**

**2.5.1. The Biological Effectiveness of T34 (*Trichoderma asperellum* strain T34) Against Fruit Rots of Strawberry (cv. Fortuna) in An Open Field:**

T34 Biocontrol and presented synthetic fungicides (**Table 2**) were used to study their effect of reducing disease. Such experiment was conducted at a fresh planting system farm, strawberry cv. Fortuna) at Abo-Ghaleb Al-Mahatta, Abo-Ghaleb region, Giza Gov., Egypt.) in the middle (February) of 2020-2021 winter season.

The first field experiment was carried out in Royal Fruits Farm at Abo-Ghaleb Al-Mahatta, Abo-Ghaleb region, Giza Gov., Egypt.) in the middle (February) of 2020-2021 winter season, using three concentrations of T34 Biocontrol (*Trichoderma asperellum* strain T34) and the comparative (positive control) with synthetic fungicides of Fabric 30% SC, Fango 50% WG, Klop 50% WP, Maven 18.7% WG and Mystic 20% WP, respectively.

The on-field disease (Strawberry Fruit Rot) incidence (%) (DI%) for 200 moderately ripen fruits (4 Replicates, 50 fruits from each) was determined exactly (just) - one h. - before fungicides application (spraying). The fungicidal field spraying repeated two weeks later. A sample of 100 apparently healthy, not-wounded, not-infected strawberry fruits were collected from the whole tested (untreated) farm area (at zero time = 1 hour before first spraying) and packaged (stacked) gently into two clean plastic foam dishes (trays) (1 kg capacity) and tightly sealed by clean plastic bags, transferred to lab to be kept in refrigerator for 14 days. Strawberry fruits were examined, and disease (fruit rot) incidence and severity were recorded. The field fruit examination, sampling (collection) was repeated as explained in Table 3. A total number of 200 moderately ripen strawberry fruits (4 replicates, 50 fruits from each) was examined (randomly along two plant rows for each treatment) for fruit rot incidence, and that examination was repeated several times (Tables 2 and 3) after fungicides application.

The experiment was repeated for the next winter season (2021- 2022), (at the same farm, but on three strawberry cultivars: Festival, Fortuna, and Sensation) with modifications mentioned in Table 3. The results of this experiment presented as the determination of disease (strawberry fruit rots) incidence percentage on the field, which is estimated by the equation: (Number of rotten fruits examined / Total number of fruits examined) x100).

**Table 2.** First field trial in 2020-2021 winter season. (Application, examination and sampling schedule, Strawberry cv. Fortuna):

| No of treatment | Tested Fungicide (Trade name) | Active ingredients (Common names) | Rate of application | Times of application | Experimental units (Treated area) | Total volume sprayed | No. & times of field examination for Fruit Rot Incidence % | No. & times of samples taken |
|---|---|---|---|---|---|---|---|---|
| **1** | T34 12 WP ($1x10^9$ cfu/g, 12% (w/w) | *Trichoderma asperellum* strain T34 | Conc.1 = (0.5 of recommende dose) | Sat. Feb. 6, and repeated two wees later | Two rows (Row length x width = (95 x 0.95 m)) | 20 L / 2 rows (One full 20L knapsack sprayer) | Regularly, Every 4-6 days (Seven times with 5 days intervals) | Weekly (Five times with 7 days intervals) |
| **2** | | | Conc.2 = (Recommended dose (85g/100 L)) | | | | | |
| **3** | | | Conc.3 = (2 x Recommended dose) | | | | | |
| **4** | Fabric 30 SC | Tebuconazole 15% + Kresoxim-Methyl 15% | 40 ml/ 100L | | | | 200 fruits per experimental unit (100 fruit/row) | 25 fruits per single inspective sample (Only one sample) |
| **5** | Fango 50 WG | Pyraclostrobin 50% | 50 g/ 100L | | | | | |
| **6** | Klop 50 WP | Difenoconazole 6% + Thiophanate methyl 44% | 60 g/ 100L | | | | | |
| **7** | Maven 18.7 WG | Pyraclostrobin 6.7% + Dimethomorph 12% | 400 g/ 300L | | | | | |
| **8** | Mystic 20 WP | Pyrimethanil 20% | 300 g/ 100L | | | | | |
| **9** | Control | Neither fungicides nor water sprayed (Not treated) | | | | | | |

**Table 3.** Second field trial in 2021-2022 winter season. (Application, examination, and sampling schedule, three strawberry cultivars: Fortuna, Festival and Sensation):

| No. of Treatment | Tested Fungicide (Trade name) | Active ingredients (Common names) | Rate of application | Times of application | Experimental units (Treated area) | Total volume sprayed | No. & times of field examination for Fruit Rot Incidence % | No. & times of samples taken |
|---|---|---|---|---|---|---|---|---|
| **1** | T34 12 WP ($1x10^9$ CFU/g, 12% w/w) | *Trichoderma asperellum* strain T34 | (0.5 0f recommended dose) | Wed. Jan. 19, 2021 and repeated two other times with 2-3 weeks intervals | Two rows (Row length x width = (95 x 0.95 m)) | 20 L / 2 rows (One full 20L knapsack sprayer) | Regularly, Every week (Seven times with 7 days intervals) | Five times with 10 days intervals |
| **2** | | | Recommended dose (85g/100 L) | | | | | |
| **3** | | | 2 x Recommended dose | | | | | |
| **4** | Fango 50 WG | Pyraclostrobin 50% | 50 g/ 100L | | | | 200 fruits per experimental unit (100 fruit/row) | Four samples (replicates) per experimental unit (25 fruits per sample) |
| **5** | Fabolous 75 WP | Tebuconazole 12.5% + Chlorothalonil 62.5% | 60 g/ 100L | | | | | |
| **6** | Control | Neither fungicides nor water sprayed (Not treated) | | | | | | |

### 2.5.2. Determination of Disease Incidence (strawberry fruit rot) and Disease Severity Percentages After Two Weeks of Shelf-Life (cooled storage):

Regularly, a single inspective sample of 25 healthy strawberry fruits (for the first field experiment, winter 2021) was collected from each treated and control row (experimental unit) and packaged (stacked) gently into two clean plastic foam dishes (1 kg capacity) and tightly sealed in clean plastic bags, transported from the field, and stored into lab refrigerator for 14 days at 7 °C. For the repeated experiment in the second winter season 2021-2022) four samples of 25 fruit each were collected from each experimental unit. The same storage conditions and examination procedures were carried out.

After cool storage in a refrigerator for 14 days shelf life, fruits were examined for both rot incidence and rot severity. The fruits were given a 0 to 3 rating scale in which 0 = no visible infection (fruit rot) symptoms, 1 = up to one-third of fruit surface diseased, 2 = more than one-third to two-thirds of fruit surface diseased, 3 = more than two-thirds diseased. The percentage of disease (strawberry fruit rot) Incidence was determined according to the following formula:

Disease (strawberry fruit rot) Incidence % =

(Number of rotten fruits examined / total number of fruits examined) x 100

The percentage of fruit rot severity (Disease Severity Index, DS%) was estimated by the equation mentioned by El-ghanam *et al.* 2015.

$$\text{Disease severity DS\%} = ((\Sigma(NFC \times CR)) \div (TNF \times MSC)) \times 100$$

Where: CR= Class rate, NFC= No of fruits in each class rate, TNF=Total No of fruits in each treatment, MSC=Maximum severity class rate.

The percent of disease incidence reduction was estimated by the equation:

The percent of disease incidence reduction (DIR%) =

((Disease incidence % in control – Disease incidence % in treatment)/ Disease incidence % in control) x 100

The percent of disease severity reduction was also estimated by the equation:

DSR%= C-T / C x 100

Where:

DSR%= The percent of disease severity reduction.

C, T= Disease severity % in the control and treatment, respectively.

### 2.6. Data Analysis:

For analysis of variance (ANOVA), multiple comparisons between means were done using a least significant difference (LSD) test ($P \leqslant 0.05$). Statistical analysis was performed with SAS, version: 2005.

## RESULTS

### 1. Different Fungal Isolates, Associated with Strawberry Fruit Rots:

Ten fungal isolates (nine different isolates) associated with strawberry fruit rot diseases were successfully isolated on PDA plates, cleaned (sub-cultured) and identified. The isolates codes, genus and species were explained in Table 4. *Acremonium butyri*, *Alternaria tenuissima*, *Fusarium oxysporum*, *F. sporotrichioides*, *F. subglutinans*, *Mucor hiemalis*, *Rhizoctonia fragaria*, *Rhizoctonia solani* and *Trichothecium roseum* seems to be possible causal (causal complex) of strawberry fruit rot diseases.

**Table 4.** Different fungal isolates, associated with strawberry fruit rots:

| Isolate No. | Isolate code | Identification |
|---|---|---|
| **1** | SFR4 | *Acremonium butyri* |
| **2** | SFR19 | *Alternaria tenuissima* |
| **3** | SFR37 | *Fusarium oxysporum* |
| **4** | SFR38 | *Fusarium oxysporum* |
| **5** | SFR8 | *Fusarium sporotrichioides* |
| **6** | SFR2 | *Fusarium subglutinans* |
| **7** | SFR17 | *Mucor hiemalis* |
| **8** | SFR18 | *Rhizoctonia fragaria* |
| **9** | SFR16 | *Rhizoctonia solani* |
| **10** | SFR36 | *Trichothecium roseum* |

### 2. Ability of Fungal Plant Pathogens to Grow on T34-inoculated PDA Petri Plates:

The results in Table 5. showed the fungal growth reduction (FGR) percentages of the tested fungal isolates grown on Petri plates containing PDA media mixed with T34 Biocontrol ® (*Trichoderma asperellum* strain, T34) in two different doses (rates). Adding T34 Biocontrol to the media in the recommended dose and a quarter of recommended dose both caused significantly high fungal growth reduction percentages in all tested isolates, exceeding 94% in some isolates. These highly reduction percentages in fungal growth caused by T34 Biocontrol could be comparable to and/or competitive with any other conventional fungicide.

**Table 5.** Ability of fungal plant pathogens to grow on T34-inoculated PDA Plates:

| Isolate code No. | Identification (Host plant) | Fungal Growth diameter (cm) or state (Control) | Fungal Growth diameter (cm) or state (Grown on T34-mixed PDA Petri plates in ¼ rec. dose) | Fungal Growth diameter (cm) or state (Grown on T34-mixed PDA Petri plates in rec. dose) | Fungal Growth Reduction% (Grown on T34-mixed PDA Petri plates in ¼ rec. dose) | Fungal Growth Reduction% (Grown on T34-mixed PDA Petri plates in rec. dose) | F. Value | LSD at 0.05 level |
|---|---|---|---|---|---|---|---|---|
| **SFR2** | *Fusarium subglutinans* | 8.43 ± 0.05 a | 0.70 ± 0.00 b | 0.6 ± 0.00 c | 91.70 | 92.88 | 54529.0 | 0.0835 |
| **SFR4** | *Acremonium butyri* | 7.03 ± 0.05 a | 1.27 ± 0.05 b | 1.1 ± 0.00 c | 81.94 | 84.35 | 15409.5 | 0.1181 |
| **SFR8** | *Fusarium sporotrichioides* | 8.40 ± 0.00 a | 0.80 ± 0.00 b | 0.7 ± 0.00 c | 90.48 | 91.67 | Infty | Zero |
| **SFR16** | *Rhizoctonia solani* | 8.40 ± 0.00 a | 0.70 ± 0.00 b | 0.7 ± 0.00 b | 91.67 | 91.67 | Infty | Zero |
| **SFR17** | *Mucor hiemalis* | 8.50 ± 0.00 a | 0.60 ± 0.00 b | 0.6 ± 0.00 b | 92.94 | 92.94 | Infty | Zero |
| **SFR19** | *Alternaria tenuissima* | 8.57 ± 0.05 a | 0.60 ± 0.00 b | 0.5 ± 0.00 c | 93.00 | 94.17 | 57847.0 | 0.0835 |
| **SFR37** | *Fusarium oxysporum* | 8.43 ± 0.00 a | 0.60 ± 0.00 b | 0.5 ± 0.00 b | 92.88 | 94.07 | 30786.1 | 0.1123 |

**Values within a row followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

### 3. Dual Culture Test on Petri Plates:

The results presented in Table 6. show the antagonistic activity of *Trichoderma asperellum* strain, T34 against the tested fungal isolates. A dual culture technique was used to determine the radial growth inhibition percentages caused by the studied biocontrol agent T34 Biocontrol. The tested bio-fungicide T34 Biocontrol significantly reduced the radial fungal growth of all fungal isolates tested, causing fungal growth inhibition percentages from 69% up to 95 %. In addition to that the antagonistic fungi *Trichoderma asperellum* strain, T34 could overgrow and parasitize all tested fungal isolates. In some cases (four isolates of eight ones), inhibition zones were observed clearly along the margin between the growth of the two antagonistic fungi, *Trichoderma asperellum* and opposite fungi (plant pathogenic fungi). That phenomenon supported the thought that *Trichoderma* spp. can even secrete inhibiting substances or antibiotics against other unwanted (plant pathogens) microbes.

**Table 6.** Effect of T34 Biocontrol ® (*T. asperellum*) on fungal growth of tested fungal plant pathogens:

| Isolate Code: | Plant Pathogenic Fungus | Radial Fungal Growth (cm) (Control=Plant Pathogenic Fungus only) | Radial Fungal Growth (cm) (*T. asperellum* x P. P. fungus) | Radial Fungal Growth (cm) of Antagonistic bio-control agent alone (*T. asperellum*) | Fungal Growth Reduction percentage (%) | F. Value | L.S.D. | Observations Noticed | |
|---|---|---|---|---|---|---|---|---|---|
| **SFR2** | *Fusarium subglutinans* | 7.0 ± 0.00 a | 1.0 ± 0.00 b | 7.5 ± 0.00 | 85.71 | Infty | Zero | Inhibition Zone | Over Growth |
| **SFR4** | *Acremonium butyri* | 3.7 ± 0.05 a | 0.2 ± 0.08 b | | 94.60 | 2704.00 | 0.1851 | Inhibition Zone | Over Growth |
| **SFR8** | *Fusarium sporotrichioides* | 8.0 ± 0.05 a | 1.8 ± 0.16 b | | 77.50 | 2632.69 | 0.3337 | Inhibition Zone | Marginal over growth |
| **SFR16** | *Rhizoctonia solani* | 8.0 ± 0.00 a | 0.5 ± 0.22 b | | 93.75 | 2410.71 | 0.4241 | - | Over Growth |
| **SFR17** | *Mucor hiemalis* | 8.0 ± 0.00 a | 2.3 ± 0.25 b | | 71.25 | 1032.14 | 0.4897 | - | Over Growth |
| **SFR18** | *Rhizoctonia fragaria* | 5.1 ± 0.33 a | 1.6 ± 0.00 b | | 68.63 | 0220.73 | 0.6478 | - | Over Growth |
| **SFR19** | *Alternaria tenuissima* | 8.0 ± 0.00 a | 1.2 ± 0.13 b | | 85.00 | 6003.57 | 0.2449 | Inhibition Zone | Over Growth |
| **SFR37** | *Fusarium oxysporum* | 7.0 ± 0.05 a | 1.2 ± 0.05 b | | 82.86 | 14792.0 | 0.1309 | - | Over Growth |

**Values within a row followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

### 4. Separation of Chemical Compounds (components) of Partially Purified Culture Filtrate of *T. asperellum* by GC-MS:

Data in Tables (7 and 8) respectively, showed the volatile and non-volatile chemical substances with antimicrobial, anti-fungal, or antibacterial properties can be secreted into PDB liquid media on which T34 Biocontrol (*T. asperellum*) was grown for one week, as secondary metabolites. GC-MS analysis of liquid culture filtrate (PDB) of T34 Biocontrol (*T. asperellum*) of one-week incubation clarified that volatile substances like: 9-Octadecenoic acid (Z)- and Anthrone, and non-volatile substances like: Thymol, Glycerol, 1-Hexadecanol, 2-methyl-, D-Mannitol, Palmitic Acid, Oleic Acid, Stearic acid and 1-Monopalmitin can be secreted in the media, and these chemical substances generally have some good antimicrobial, antibacterial, bactericidal, antifungal, antioxidant, anti-inflammatory, local anesthetic, antinociceptive, cicatrizing, antiseptic and anticancer properties, according to the available review.

**Table 7.** Determination of volatile chemical compounds of partially purified 7 days old (one-week-old) liquid culture filtrate (PDB) of T34 Biocontrol (*T. asperellum*) by GC-mass:

| Peak | RT (min)[a] | Name: (Possible compound) | Formula | Area | Area Sum % | Biological Activity | Ref. |
|---|---|---|---|---|---|---|---|
| **26** | 25.164 | 9-Octadecenoic acid (Z)- | $C_{18}H_{34}O_2$ | 261945.36 | 0.95 | Antibacterial | **Pu *et al.*, (2010)** |
| **28** | 28.369 | Anthrone | $C_{14}H_{10}O$ | 1501597.5 | 5.47 | Antibacterial & antioxidant | **Oumera *et al.*, (2014) & Asamenew *et al.*, (2011)** |

**Table 8.** Determination of other (non-volatile) chemical compounds of partially 7-day-incubated (one-week-old) liquid culture filtrate of T34 Biocontrol (*T. asperellum*) by GC-MS:

| Peak | RT (min)[a] | Name: (Possible compound) | Formula | Area | Area Sum % | Biological Activity | Ref. |
|---|---|---|---|---|---|---|---|
| **1** | 7.134 | Thymol, TMS derivative | $C_{13}H_{22}OSi$ | 4944538.8 | 0.49 | antioxidant, anti-inflammatory, local anesthetic, antinociceptive, cicatrizing, antiseptic, and especially antibacterial and antifungal | **Marchese *et al.*, (2016)** |
| **3** | 14.951 | Glycerol, 3TMS derivative | $C_{12}H_{32}O_3Si_3$ | 14755627 | 1.46 | Antibacterial | **Singh, (2014)** |
| **4** | 21.287 | 1-Hexadecanol, 2-methyl- | $C_{17}H_{36}O$ | 6419080.6 | 0.63 | Antimicrobial (antibacterial) | **Hamed & Hussein, (2020)** |
| **8** | 24.065 | D-Mannitol, 6TMS derivative | $C_{24}H_{62}O_6Si_6$ | 12438516 | 1.23 | Week antimicrobial activity. Better antibacterial than antifungal activity. | **Inaba *et al.*, (2020). Zhang *et al.*, (2018)** |
| **10** | 24.854 | Palmitic Acid, TMS derivative | $C_{19}H_{40}O_2Si$ | 159962278 | 15.78 | Bactericidal properties | **Casillas-Vargas *et al.*, (2021)** |
| **11** | 26.425 | Oleic Acid, (Z)-, TMS derivative | $C_{21}H_{42}O_2Si$ | 43990584 | 4.34 | Antibacterial | **Zheng *et al.*, (2005)** |
| **12** | 26.592 | Stearic acid, TMS derivative | $C_{21}H_{44}O_2Si$ | 49339792 | 4.87 | Antibacterial | **Casillas-Vargas *et al.*, (2021)** |
| **14** | 29.438 | 1-Monopalmitin, 2TMS derivative | $C_{25}H_{54}O_4Si_2$ | 3490598.9 | 0.34 | Antimicrobial & anticancer activities | **Elsayed *et al*, (2020)** |

Also, volatile, and non-volatile chemical substances -with antimicrobial properties - can detected in PDB liquid media (culture filtrate) on which T34 Biocontrol (*T. asperellum*) grown for two weeks Tables (9 and 10). GC-MS analysis of partially purified 14-dayold (two weeks) culture filtrate of *T. asperellum* showed that different volatile chemical compounds like: Acetic acid, hydrazide, Citronellene, Carveol 1, Linalool and Stanozolol, and non-volatile compounds like: Thymol, Glycerol, Palmitic Acid, Oleic Acid, Stearic acid, Arachidonic acid and 1-Monopalmitin can be secreted (produced) by *T. asperellum*.

**Table 9.** Determination of volatile chemical compounds of partially purified 14 days old liquid PDB culture filtrate of *T. asperellum* by GC-mass:

| Peak | RT (min)[a] | Name: (Possible compound) | Formula | Area | Area Sum % | Biological Activity | Ref. |
|---|---|---|---|---|---|---|---|
| **1** | 2.135 | Acetic acid, hydrazide | $C_2H_6N_2O$ | 563613.78 | 11.14 | Antimicrobial | **Popiołek and Biernasiuk, (2016)** |
| **11** | 6.616 | Citronellene | $C_{10}H_{18}$ | 64883.54 | 1.28 | Possible antibacterial | **Lopez-Romero *et al.*, (2015)** |
| **12** | 8.515 | Carveol 1 | $C_{10}H_{16}O$ | 38721.92 | 0.77 | Antibacterial | **Lopez-Romero *et al.*, (2015)** |
| **14** | 13.738 | Linalool | $C_{10}H_{18}O$ | 68572.61 | 1.36 | Antibacterial | **Liu *et al.*, (2020)** |
| **15** | 25.876 | Stanozolol | $C_{21}H_{32}N_2O$ | 191333.34 | 3.78 | Antibacterial | **Dogan *et al.*, (2017)** |

**Table 10.** Determination of other (non-volatile) chemical compounds of partially 14-day-incubated liquid culture filtrate of T34 Biocontrol (*T. asperellum*) by GC-MS:

| Peak | RT (min)[a] | Name: (Possible compound) | Formula | Area | Area Sum % | Biological Activity | Ref. |
|---|---|---|---|---|---|---|---|
| **1** | 7.112 | Thymol, TMS derivative | $C_{13}H_{22}OSi$ | 3115206.1 | 1 | Antimicrobial (antibacterial & antifungal) | **Marchese *et al.*, (2016)** |
| **3** | 14.943 | Glycerol, 3TMS derivative | $C_{12}H_{32}O_3Si_3$ | 14368079 | 4.6 | Antibacterial | **Singh, (2014)** |
| **8** | 24.065 | D-Mannitol, 6TMS derivative | $C_{24}H_{62}O_6Si_6$ | 13167288 | 4.21 | Weak antimicrobial activity | **Inaba *et al.*, (2020) Zhang *et al.*, (2018)** |
| **10** | 24.839 | Palmitic Acid, TMS derivative | $C_{19}H_{40}O_2Si$ | 60284832 | 19.29 | Bactericidal properties | **Casillas-Vargas *et al.*, (2021)** |
| **11** | 26.417 | Oleic Acid, (Z)-, TMS derivative | $C_{21}H_{42}O_2Si$ | 17693920 | 5.66 | Antibacterial | **Zheng *et al.*, (2005)** |
| **12** | 26.584 | Stearic acid, TMS derivative | $C_{21}H_{44}O_2Si$ | 22130997 | 7.08 | Antibacterial | **Casillas-Vargas *et al.*, (2021)** |
| **14** | 27.192 | Arachidonic acid, TMS derivative | $C_{23}H_{40}O_2Si$ | 1598920.5 | 0.51 | Antibacterial | **Beavers *et al.* (2019)** |
| **16** | 29.438 | 1-Monopalmitin, 2TMS derivative | $C_{25}H_{54}O_4Si_2$ | 6787672.7 | 2.17 | Antimicrobial & anticancer activities | **Elsayed *et al.* (2020)** |

## 5. Field Experiments:

### 5.1. The Biological Effectiveness of T34 (*Trichoderma asperellum* strain T34) Against Fruit Rots of Strawberry (cv. Fortuna) in An Open Field (preharvest) and Postharvest (during storage) (2020-2021 winter season):

Results are obtained in **Table 11.** showing that all tested fungicides significantly reduced the percentage of strawberry fruit rot incidence on the field and gave an acceptable general disease incidence percentage from 55.25% up to 82.86% for the tested chemical or biological fungicides. T34 Biocontrol (*Trichoderma asperellum* strain T34) was the best, followed by Mystic 20 WP & Fabric 30 SC, respectively. Table 12, clarified the numerically disease (fruit rot) incidence % and severity % after 14 days of refrigeration (cooled storage) (2020-2021 winter season). All fungicides tested reduced both disease (fruit rot) incidence % and severity %, numerically. For disease incidence %: Fabric 30 SC was the best, followed by Klopp 50 WP, Fango 50 WG and T34 (in the highest concentration tested), respectively. For disease severity %: Klopp 50 WP was the best, followed by T34 (in the highest concentration tested), Fango 50 WG and Fabric 30 SC, respectively.

**Table 11.** Determination of disease (strawberry fruit rot) incidence % on the field (2020-2021 winter season, cv. Fortuna):

| Times of examination | | Disease Incidence % | | | | | | General Disease Incidence % | General Disease Incidence Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| **Dates of examination** | **Zero time Sat. 6-2-2021** | **5 days later** | **10 days later** | **15 days later** | **20 days later** | **25 days later** | **30 days later** | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | | 48.5 a | 24.0 a | 14.0 a | 17.5 a | 26.5 a | 16.5 a | 24.5 | |
| **T34 (Conc. 1)** | | 23.5 bc | 15.5 ab | 05.0 b | 12.5 ab | 07.0 b | 06.5 b | 11.7 | 55.25 |
| **T34 (Conc. 2)** | | 15.0 bcd | 08.0 bc | 01.5 b | 08.5 bc | 07.0 b | 02.5 b | 07.1 | 71.02 |
| **T34 (Conc. 3)** | | 7.5 d | 04.5 c | 01.5 b | 03.5 c | 05.5 b | 02.5 b | 04.2 | 82.86 |
| **Fabric 30 SC** | | 9.5 cd | 05.0 c | 04.5 b | 05.5bc | 06.0 b | 03.0 b | 05.6 | 77.14 |
| **Fango 50 WG** | 10.5 | 16.0 bcd | 02.0 c | 03.0 b | 05.5 bc | 08.5 b | 02.5 b | 06.3 | 74.29 |
| **Klop 50 WP** | | 7.5 d | 09.0 bc | 03.0 b | 04.5bc | 07.5 b | 04.5 b | 06.0 | 75.51 |
| **Maven 18.7 WG** | | 28.5 b | 08.0 bc | 04.0 b | 10.0 abc | 09.0 b | 06.0 b | 10.9 | 55.51 |
| **Mystic 20 WP** | | 10.0 cd | 06.0 bc | 01.5 b | 04.5 bc | 04.0 b | 05.0 b | 05.2 | 78.78 |
| **F. Value** | | **19.78** | **11.32** | **05.70** | **06.05** | **08.88** | **08.74** | | |
| **L.S.D.** | | **7.1742** | **4.7694** | **3.8852** | **4.4863** | **5.3788** | **3.5615** | | |

**Values within a column followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

**Table 12.** Determination of numerically disease (fruit rot) incidence % and Severity % after 14-days refrigeration (cooled storage) (2020-2021 winter season, cv. Fortuna):

| Times of examination | Disease Incidence % and (Severity %) | | | | | General Disease Incidence % | General Disease Severity % | General Disease Incidence Reduction % | General Disease Severity Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | | |
| Dates of examination | Zero time Sat. 6-2-2021 | Week later | Two weeks later | Three weeks later | Four weeks later | | | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | 85.38 (45.32) | 100 (84.0) | 100 (89.3) | 100 (84.0) | 100 (64.0) | 100 | 80.33 | | |
| **T34 (Conc. 1)** | | 96 (76.0) | 92 (72.0) | 100 (70.7) | 64 (24.0) | 88 | 60.68 | 12 | 24.46 |
| **T34 (Conc. 2)** | | 92 (72.0) | 92 (72.0) | 96 (68.0) | 60 (24.0) | 85 | 59.00 | 15 | 26.55 |
| **T34 (Conc. 3)** | | 92 (60.0) | 84 (54.7) | 96 (53.3) | 60 (24.0) | 83 | 48.00 | 17 | 40.25 |
| **Fabric 30 SC** | | 92 (66.7) | 96 (78.7) | 84 (50.7) | 40 (16.0) | 78 | 53.03 | 22 | 34.00 |
| **Fango 50 WG** | | 88 (54.7) | 100 (74.7) | 92 (64.0) | 48 (17.3) | 82 | 52.68 | 18 | 34.42 |
| **Klopp 50 WP** | | 76 (40.0) | 100 (74.7) | 84 (45.3) | 64 (28.0) | 81 | 47.00 | 19 | 41.49 |
| **Maven 18.7 WG** | | 88 (65.3) | 100 (72.0) | 100 (78.7) | 84 (45.3) | 93 | 65.33 | 07 | 18.67 |
| **Mystic 20 WP** | | 92 (54.7) | 100 (69.3) | 96 (78.7) | 80 (41.33) | 92 | 61.01 | 08 | 24.05 |

## 5.2. The Biological Effectiveness of T34 (*Trichoderma asperellum* strain T34) Against Fruit Rots of Strawberry (cultivars of Fortuna, Festival and Sensation) in An Open Field (preharvest) and Postharvest (during storage) (2021-2022 winter season):

Tables 13, 14 and 15. are showing the Disease (strawberry fruit rot) incidence % on the field (2021-2022 winter season, for cultivars: Fortuna, Festival and Sensation, respectively). In general, all tested fungicides led to a significant reduction in fruit rot incidence percentages. For the first cultivar of Fortuna, Fabolous 75WP was the best, followed by T34 Biocontrol -in the heist concentration used (Conc.3=double of recommended rate)- and Fango 50WG, respectively. For the cultivar of Festival, T34 (Conc.3) was the best, followed by Fabolous 75WP, T34(Conc.2) and Fango 50WG, respectively. But for the third cultivar of Sensation, T34 (Conc.3) was the best, followed by Fango 50WG and Fabolous 75WP, respectively.

**Table 13.** Determination of disease (strawberry fruit rot) incidence % on the field (2021-2022 winter season, cv. Fortuna):

| Times of examination | Disease Incidence % | | | | | | | General Disease Incidence % | General Disease Incidence Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| Dates of examination | Zero time 19-1-2022 | Week later | 2 weeks later | 3 weeks later | 4 weeks later | 5 weeks later | 6 weeks later | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | 6.5 | 8.0 a | 12.0 a | 15.5 a | 7.0 a | 19.5 a | 25.0 a | 14.5 | |
| **T34 (Conc. 1)** | | 2.5 b | 8.0 ab | 5 b | 2.0 b | 13.0 a | 5.0 ab | 5.92 | 59.17 |
| **T34 (Conc. 2)** | | 1.5 b | 7.0 ab | 4.5 b | 2.0 b | 13.0 a | 4.0 b | 5.33 | 63.24 |
| **T34 (Conc. 3)** | | 1.5 b | 4.5 b | 4.5 b | 1.0 b | 11.5 ab | 2.0 b | 4.17 | 71.24 |
| **Fango 50 WG** | | 1.5 b | 4.5 b | 6.0 b | 1.5 b | 13.0 a | 2.5 b | 4.83 | 66.69 |
| **Fabolous75WP** | | 1.0 b | 4.5 b | 3.0 b | 3.0 ab | 4.0 b | 1.5 b | 2.83 | 80.48 |
| **F. Value** | | **7.95** | **4.80** | **9.28** | **5.46** | **6.27** | **5.88** | | |
| **L.S.D.** | | **2.1187** | **3.0542** | **3.3604** | **2.1021** | **4.4474** | **3.7919** | | |

**Values within a column followed by the same letter(s) are not significantly different, according to the LSD test at P = 0.05.

**Table 14.** Determination of disease (strawberry fruit rot) incidence % on the field (2021-2022 winter season, cv. Festival):

| Times of examination | Disease Incidence % | | | | | | | General Disease Incidence % | General Disease Incidence Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | |
| **Dates of examination** | **Zero time 19-1-2022** | **Week later** | **2 weeks later** | **3 weeks later** | **4 weeks later** | **5 weeks later** | **6 weeks later** | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | 1.0 | 7.5 a | 2.5 a | 8.5 a | 3.0 a | 8.5 a | 8.0 a | 6.33 | |
| **T34 (Conc. 1)** | | 1.0 b | 2.0 a | 1.5 b | 1.5 a | 4.5 b | 3.0 b | 2.25 | 64.46 |
| **T34 (Conc. 2)** | | 0.5 b | 1.0 a | 1.0 b | 1.5 a | 1.0 bc | 2.0 b | 1.17 | 81.52 |
| **T34 (Conc. 3)** | | 0.5 b | 0.5 a | 1.0 b | 1.0 a | 0.5 c | 0.0 b | 0.58 | 90.84 |
| **Fango 50 WG** | | 0.5 b | 1.5 a | 2.0 b | 2.0 a | 1.0 bc | 0.5 b | 1.25 | 80.25 |
| **Fabolous75WP** | | 0.5 b | 1.5 a | 1.5 b | 0.5 a | 2.0 bc | 0.5 b | 1.08 | 82.94 |
| **F. Value** | | **11.26** | **00.64** | **12.55** | **01.09** | **14.02** | **18.99** | | |
| **L.S.D.** | | **1.8913** | **1.9819** | **1.8539** | **1.8539** | **1.8539** | **1.5442** | | |

**Values within a column followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

**Table 15.** Determination of disease (strawberry fruit rot) incidence % on the field (2021-2022 winter season, cv. Sensation):

| Times of examination | Disease Incidence % | | | | | | | General Disease Incidence % | General Disease Incidence Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | | |
| **Dates of examination** | **Zero time 19-1-2022** | **Week later** | **2 weeks later** | **3 weeks later** | **4 weeks later** | **5 weeks later** | **6 weeks later** | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | 13.5 | 14.0 a | 13.0 a | 3.5 a | 12.5 a | 5.0 a | 12.0 a | 10.0 | |
| **T34 (Conc. 1)** | | 6.0 b | 4.0 b | 3.0 a | 2.5 b | 1.5 ab | 1.5 b | 3.10 | 69.0 |
| **T34 (Conc. 2)** | | 6.0 b | 3.0 b | 1.5 a | 2.0 b | 1.0 b | 1.0 b | 2.42 | 75.8 |
| **T34 (Conc. 3)** | | 2.0 b | 0.5 b | 1.5 a | 2.0 b | 0.5 b | 0.5 b | 1.17 | 88.3 |
| **Fango 50 WG** | | 5.0 b | 1.5 b | 1.0 a | 0.5 b | 0.0 b | 0.5 b | 1.42 | 85.8 |
| **Fabolous75WP** | | 2.5 b | 4.0 b | 0.5 a | 1.5 b | 0.5 b | 0.5 b | 1.58 | 84.2 |
| **F. Value** | | **15.43** | **20.54** | **1.89** | **11.55** | **04.22** | **16.73** | | |
| **L.S.D.** | | **2.4702** | **2.2158** | **1.9098** | **2.9491** | **1.9995** | **2.4985** | | |

**Values within a column followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

Data obtained in Tables 16, 17 and 18, showed disease (fruit rot) incidence % and severity % after 14-days refrigeration (cooled storage) (2021-2022 winter season, for cultivars of Fortuna, Festival and Sensation, respectively). Generally, all tested fungicides reduced the disease incidence and severity numerically, and significantly in some cases. For cv. Fortuna, in both disease incidence % and disease severity: Fabolous 75 WP was the best (general disease severity reduction of 51.82%), followed by T34 (recommended rate) and Fango 50 WG, respectively. For cv. Festival, for both disease incidence % and disease severity Fabolous 75 WP was the best (general disease severity reduction of 59.58%), followed by T34 and Fango 50 WG, respectively (The same previous result for cv. Fortuna). While the results for cv. Sensation, for disease incidence %, Fabolous 75 WP was the best, followed by Fango 50 WG and T34, respectively. For disease severity: Fango 50 WG was the best (general disease severity reduction of 49.49%), followed by Fabolous 75WP and T34, respectively.

**Table 16.** Determination of disease (fruit rot) incidence % and severity % after 14-days refrigeration (cooled storage) (2021-2022 winter season, cv. Fortuna):

| Times of examination | Disease Incidence % & (Severity %) | | | | | General Disease Incidence % | General Disease Severity % | General Disease Incidence Reduction % | General Disease Severity Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | | |
| **Dates of examination** | **Zero time 19-1-2022** | **10 days later** | **20 days later** | **30 days later** | **40 days later** | | | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | 77.19 (24.0) | 92.00 (70.22) a | 100.0 (67.56) a | 97.33 (67.56) a | 100 (52.00) a | 97.33 | 64.34 | | |
| **T34 (Recommended dose)** | | 74.67 (42.67) a | 72.00 (30.67) ab | 65.33 (28.00) b | 86.67 (37.78) a | 74.67 | 34.78 | 23.28 | 45.94 |
| **Fango 50 WG** | | 78.67 (50.22) a | 56.00 (24.89) b | 80.00 (35.56) ab | 88.00 (33.33) a | 75.67 | 36.00 | 22.25 | 44.02 |
| **Fabolous 75 WP** | | 69.33 (39.11) a | 89.33 (51.11) a | 65.33 (23.56) b | 28.00 (10.22) b | 63.00 | 31.00 | 35.27 | 51.82 |
| **F. Value** | | **03.70** | **09.59** | **06.20** | **21.55** | | | | |
| **L.S.D.** | | **5.6986** | **7.0806** | **6.9179** | **7.8077** | | | | |

**Values within a column followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

**Table 17.** Determination of disease (fruit rot) incidence % and severity % after 14-days refrigeration (cooled storage) (2021-2022 winter season, cv. Festival):

| Times of examination | Disease Incidence %& (Severity %) | | | | | General Disease Incidence % | General Disease Severity % | General Disease Incidence Reduction % | General Disease Severity Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | | |
| **Dates of examination** | **Zero time 19-1-2022** | **10 days later** | **20 days later** | **30 days later** | **40 days later** | | | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | 60.00 (31.56) | 54.67 (29.78) a | 89.33 (49.78) a | 94.67 (48.00) a | 100.0 (60.44) a | 84.67 | 47.00 | | |
| **T34 (Recommended dose)** | | 37.33 (16.44) a | 29.33 (13.33) b | 65.33 (23.56) bc | 85.33 (38.67) ab | 54.33 | 23.00 | 35.83 | 51.06 |
| **Fango 50 WG** | | 38.67 (18.67) a | 25.33 (9.33) b | 66.67 (24.00) b | 98.67 (53.33) a | 57.34 | 26.33 | 32.29 | 43.98 |
| **Fabolous 75 WP** | | 40.00 (20.89) a | 32.00 (12.44) b | 41.33 (13.78) c | 80.00 (28.89) b | 48.33 | 19.00 | 42.92 | 59.58 |
| **F. Value** | | **01.75** | **13.81** | **15.98** | **08.45** | | | | |
| **L.S.D.** | | **6.9179** | **9.2444** | **6.1783** | **3.8487** | | | | |

**Values within a column followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

**Table 18.** Determination of disease (fruit rot) incidence % and severity % after 14-days refrigeration (cooled storage) (2021-2022 winter season, cv. Sensation):

| Times of examination | Disease Incidence %& (Severity %) | | | | | General Disease Incidence % | General Disease Severity % | General Disease Incidence Reduction % | General Disease Severity Reduction % |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | | | | |
| Dates of examination | Zero time 19-1-2022 | 10 days later | 20 days later | 30 days later | 40 days later | | | | |
| **Fungicides tested** | | | | | | | | | |
| **Control** | 62.67 (27.11) | 72.00 (35.11) a | 100.0 (79.56) a | 92.00 (33.78) a | 100.0 (65.33) a | 91.00 | 53.45 | | |
| **T34 (Recommended dose)** | | 50.67 (22.22) a | 85.33 (37.78) ab | 53.33 (18.67) b | 90.67 (38.67) ab | 70.00 | 29.34 | 23.08 | 45.11 |
| **Fango 50 WG** | | 57.33 (24.89) a | 76.00 (28.89) b | 68.00 (22.67) ab | 76.00 (31.56) bc | 69.33 | 27.00 | 23.81 | 49.49 |
| **Fabolous 75 WP** | | 50.67 (23.56) a | 73.33 (35.56) b | 64.00 (23.56) b | 66.67 (25.78) c | 63.67 | 27.12 | 30.03 | 49.26 |
| **F. Value** | | **02.17** | **05.53** | **07.06** | **11.30** | | | | |
| **L.S.D.** | | **7.7344** | **5.7977** | **6.9589** | **5.0068** | | | | |

**Values within a column followed by the same letter(s) are not significantly different, according to the LSD test at $P = 0.05$.

## DISCUSSION

T34 Biocontrol ® (*Trichoderma asperellum* strain, T34) inhibited the growth of fungal plant pathogens tested when added to PDA media plates. Results obtained by Pastrana *et al.* (2016) found that dual plate confrontation experiments demonstrated the antagonistic effects of *T. asperellum* by inhibiting radial growth of *M. phaseolina* and *F. solani* by more than 36%, and the previous results are similar to our results but the studied biocontrol T34 could achieve superiorly fungal inhibition percentages *in vitro* exceeded 90%. *T. asperellum* parasitized all tested fungal plant pathogens. Similar results mentioned by Hermosa *et al.* (2012) confirmed that *Trichoderma* (teleomorph Hypocrea) is a fungal genus found in many ecosystems. *Trichoderma* spp. can reduce the severity of plant diseases by inhibiting plant pathogens in the soil through their highly potent antagonistic and mycoparasitic activity. Cardoza *et al.* (2005) also clearly reported the *in vitro* antagonism in dual cultures of *Trichoderma harzianum* and *Botrytis cinerea*.

*T. asperellum* also produced a group of secondary metabolites (specific chemical substances) in the media on which it is grown, a lot of these substances have good antimicrobial properties. Khan *et al*. (2020) reported that the use of biocontrol agents and their secondary metabolites (SMs) is one of the potential approaches used today. *Trichoderma* spp. is a well-known biocontrol agent used globally.Many Trichoderma species are the most prominent producers of SMs with antimicrobial activity against phytopathogenic fungi. Vinale *et al.* (2014) referred that many secondary metabolites (like such produced here by *T. asperellum* strain: t34) may also have antibiotic properties, which enable the producing microbe to inhibit and/or kill other microorganisms i.e. competing for a nutritional niche. That phenomenon supported the thought that *Trichoderma* spp. can even secrete substances or antibiotics inhibiting other unwanted microbes (plant pathogens). Our results of both *in vitro* (lab) and *in vivo* (on the field) are in complete harmony with the results obtained by Rashid *et al.* (2022) who studied some biological control agents; *Bacillus subtilis*, *Bacillus megatherium*, *Trichoderma album* and *Trichoderma asperellum* (T34), *Trichoderma viride*, compared to Switch (synthetic fungicide) and tap water as control treatment. All biological control agents showed the

highest linear growth inhibition of fruit rots pathogens under laboratory conditions. The tested treatments gave the best effects as they decreased the disease incidence (D.I.) of fruit rots in the field. The fruits were harvested at a commercial maturity 3/4 color stage and stored at 0 °C and 95-98 % RH for 20 days. The results obtained by Rashid *et al.* (2022) indicated that pre-harvest spraying of strawberry fruits with *Trichoderma asperellum* (T34) was the most effective treatment for delaying fruit deterioration through reducing color changing, decay maintaining good appearance, firmness, acidity, TSS% and weight loss.

**Conclusions**

*In vitro* testing of T34 Biocontrol (*Trichoderma asperellum* strain T34) efficiency against fungal plant pathogens causing strawberry fruit rots confirmed that it strongly inhibited the radial fungal growth of all tested isolates and secreted (produced) a lot of substances with good antimicrobial, antifungal, antibacterial or antiseptic properties. *In vivo* study evidenced that T34 Biocontrol (*Trichoderma asperellum* strain T34) field application (spraying) can significantly reduce the incidence and severity of strawberry fruit rot diseases not only on the field (preharvest) but also during transportation or cooled storage (postharvest) and may be superior in protecting strawberry fruits than chemical fungicides, with big safety margin, as a biocontrol agent. *Trichoderma*-based bio fungicides in general, and T34 Biocontrol ® (*Trichoderma asperellum* strain, T34) in particular, seem to be promising in controlling pre and postharvest diseases, especially fruit rots. T34 Biocontrol® and such biocontrol agents may play an important role in integrated pest and disease management, specifically in organic and exported crop production systems. So, these biocontrol agents are still needing more expanded studies under local Egyptian agricultural conditions.

**Declarations:**

**Ethical Approval**: Not applicable.

**Competing interests**: The authors declare no conflict of interest.

**Authors Contributions:**

I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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**Availability of Data and Materials:** All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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## ARABIC SUMMARY

**T34 بيو كونترول 12% WP (*Trichoderma asperellum* strain, T34) ® T34 Biocontrol، عامل مكافحة حيوية لأعفان ثمار الفراولة**

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تعتبر التأثيرات البيئية السلبية لاستخدام المبيدات الكيميائية التقليدية المستخدمة في مكافحة آفات وأمراض النبات مشكلة متنامية. لذا يتم البحث عن بدائل أكثر أماناً. ويعتبر استخدام الكائنات الحية الدقيقة كعوامل مكافحة حيوية لمسببات أمراض النبات أحد أفضل هذه البدائل. استهدفت هذه الدراسة استكشاف طريقة تأثير وتقييم فاعلية المبيد الحيوي الواعد بيوكونترول T34 12% مسحوق قابل للبلل (*Trichoderma asperellum* strain, T34 Biocontrol ®, T34, 12% WP) تجاه الفطريات المسببة لأعفان ثمار الفراولة تحت الظروف المصرية. تم أولاً عزل وتعريف عشرة عزلات لأنواع مختلفة من فطريات عُزِلت من ثمار مصابة جُمِعت من مزرعة رويال فروتس بمنطقة أبوغالب المحطة، مدينة أبوغالب، منشأة القناطر، محافظة الجيزة، مصر. تم عزل فطريات *Acremonium butyri*، *Alternaria tenuissima*، *Fusarium oxysporum*، *F. sporotrichioides*، *F. subglutinans*، *Mucor hiemalis*، *Rhizoctonia fragaria*، *R. solani* و *Trichothecium roseum* المسببة لأعفان ثمار الفراولة. تم تقييم فاعلية المستحضر التجاري للمبيد الحيوي T34 Biocontrol ® (*Trichoderma asperellum* strain, T34, 12% WP) تحت ظروف المعمل من خلال تقنيتين مختلفتين، الأولي تنمية الفطريات علي بيئة غذائية صلبة (أطباق بيئة بطاطس دكستروز أجار) مضاف إليها مستحضر المبيد الفطري الحيوي T34، والثانية إختبار تضاد حيوي بتقنية مزرعة مزدوجة علي أطباق آجار. أظهر عامل المكافحة الحيوي T34 نشاطاً تضادياً جيداً، مع خفض معنوي للنسبة المئوية لنمو جميع الفطريات المختبرة. كما تم باستخدام جهاز كروماتوجرافي غازي مزود بمطياف كتلة GC-MS، فصل وتعريف عدد من المركبات الحيوية ذات الخصائص المضادة للميكروبات، للفطريات أو للبكتيريا من بيئة غذائية سائلة (مستخلص بطاطس ودكستروز). من هذه المركبات 9-Octadecenoic acid (Z)-، Anthrone، Thymol، Glycerol، 1-Hexadecanol, 2-methyl-، D-Mannitol، Palmitic Acid، Oleic Acid، Stearic acid، 1-Monopalmitin، Acetic acid, hydrazide، Citronellene، Carveol 1، Linalool، Stanozolol و Arachidonic acid. أوضح التقييم الحيوي للمبيد الفطري الحيوي T34 تحت ظروف الحقل المفتوح أن الرش الحقلي للمبيد أدي لخفض نسبة إصابة ثمار الفراولة بأمراض أعفان الثمار في الحقل (قبل الحصاد) بدرجة معنوية، كما أدي أيضاً إلي خفض النسبة المئوية لحدوث الأعفان والنسبة المئوية لشدة حدوث الاعفان معنوياً أثناء التخزين البارد (بعد الحصاد) في كلٍ من موسِمَي الدراسة المتتاليين، شتاء 2020-2021 و شتاء 2021-2022. كما حقق كفاءة وقائية عالية بخفض نسبة الإصابة بأعفان الثمار تجاوزت 90% في بعض المعاملات. كفاءة المبيد الحيوي T34 كانت مقاربة لكفاءة المكافحة المتحققة بالمبيدات الكيميائية المخلقة (التقليدية) المقارنة (Fabolous 75%WP, Tebuconazole 12.5%+Chlorothalonil 62.5%(w/w)) (Fabric 30%SC, Tebuconazole 15%+Kresoxim-Methyl 15%(w/v))، (Fango 50%WG, Pyraclostrobin 50%(w/w)) (Klop 50%WP Thiophanate methyl 44%+Difenoconazole 6%(w/w)، (Maven 18.7%WG, Pyraclostrobin 6.7%+Dimethomorph 12% (w/w)) و (Mystic 20%WP, Pyrimethanil 20%(w/w)).