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# Protective and Curative Activity of QoI and DMI Fungicides Against *Botrytis cinerea* on Strawberry

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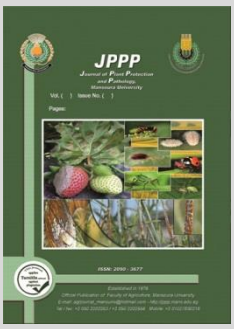


## ABSTRACT

Strawberry is considered to be an essential strategic crop due to its great economic value. Grey mold disease, induced by *Botrytis cinerea*, is a major constraint to strawberry production worldwide, causing considerable economic losses. Protective fungicides considered essential tool in management strategies. Recently, fungicides ineffectiveness observed widely in many countries due to the evolution of resistance in *B. cinerea* which poses an ongoing threat to strawberry production. Therefore, this investigation carried out to test the following fungicides Speedcide (difenoconazole DMIs), Tilt (propiconazole DMIs) and Tazer (azoxystrobin QoIs) against *B. cinerea* and to evaluate the fitness penalties of *B. cinerea* resistant isolates.the results indicated that among the collected isolates 13 (43.33%), 15 (50%) and 18 (60%) were resistant to difenoconazole, propiconazole and azoxystrobin. The assessment of fitness penalties of resistant isolates revealed that all the tested isolates were able to infect and develop lesions on strawberry leaves. Tilt (Propiconazole) had the strongest inhibition activity, as a protective and curative treatment with mean 91.89 and 71.45%, respectively. Finly, the application of Tilt fungicide inhibited spore germination on the leaf surface and proved its effectiveness against both resistant and sensitive isolates.

***Keywords*:** Strawberry, *Botrytis cinerea*, Fungicides, Fitness penalties.

## INTRODUCTION

Strawberry production is widespread in temperate locations around the world, with cultivation occurring in both open fields and protected greenhouses. Strawberry plants are vulnerable to infection by various phytopathogenic fungi, grey mold caused by *Botrytis cinerea* (*B. cinerea*) being one of the most serious diseases, not only for strawberries but also for a wide range of other crops. The disease leading to loses in crop production seriously as *B. cinerea* was discovered in the leftover plant components. High humidity levels promote the growth of *B. cinerea*, leading to infection of strawberry flowers, premature fruit drop, and reduced crop production (Adnan et al 2019).

To prevent the spread of infection, protective fungicides are essential in strawberry fields to effectively manage grey mold. The high genetic variability, polycyclic nature, and profuse sporulation of *B. cinerea* necessitate frequent fungicide applications to achieve effective control. The intense selective pressure drove the swift emergence of resistant fungal populations, rendering various fungicides ineffective in controlling the disease (Amiri *et al* 2013). Multiple studies have reported the development of resistance to various chemical classes of fungicides, including Quinone outside inhibitors (QoIs) and Demethylation inhibitors (DMIs) (Bardas *et al* 2010 and Fan *et al* 2017).

Egypt has registered multiple QoI fungicides, such as azoxystrobin, pyraclostrobin, kresoxim-methyl, and trifloxystrobin. To mitigate resistance, the Fungicide Resistance Action Committee (FRAC) in Europe offers recommendations for resistance management. The use of QoI fungicides is recommended in Egypt as a strategy to prevent or delay the development of resistant fungal populations. These fungicides exert their effect by inhibiting the energy production of plant pathogens, specifically by blocking electron transfer at the Quinone 'outside' site of the bc1 complex (complex III in the electron transport chain). The QoIs are effective against a range of plant diseases, including those caused by ascomycetes, basidiomycetes, and oomycetes, which are three major categories of plant pathogens.

In Egypt, registered sterol demethylation inhibitors (DMIs) encompass a range of chemical classes, including piperazines, pyridines, pyrimidines, imidazoles, and triazoles, each comprising multiple active ingredients. The triazole group, for instance, includes difenoconazole, propiconazole, and several other active ingredients. Recently, the development of resistant populations has rendered many fungicides ineffective in controlling the disease (Lin et al 2009). Alternating fungicide usage is a recommended strategy in Egypt to prevent or delay the establishment of resistant fungal populations. DMIs, which are systemic fungicides, target the cell membrane by inhibiting C14 demethylation during sterol synthesis, ultimately disrupting fungal growth. Inhibitors of sterol biosynthesis have proven to be effective in controlling a wide range of fungal pathogens, including ascomycetes, basidiomycetes, and oomycetes, which are three major genera of plant pathogens, making DMIs a crucial component of control programs targeting significant plant diseases. Sterols and their derivatives play a crucial role in promoting and maintaining fungal growth and development by serving as essential membrane constituents and regulating metabolic processes. Research conducted by numerous scientists has revealed that several phytopathogenic fungi, including *Fusarium graminearum*, *Lasiodiplodia theobromae*, and *Botrytis*

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*cinerea*, have developed resistance to the fungicide difenoconazole. (Rekanović et al., 2010, Li et al., 2020 and Zhang et al., 2020).

Referring to fungicide resistance development, this pathogen is classified as a high-risk pathogen due to its short life cycle and prolific reproduction (Brent and Hollomon 1998). The development of fungicide resistance in *B. cinerea* often reduces the efficacy of control programs, resulting in unsuccessful disease management (Leroux 2007). In Egypt, many fungicides have been used extensively to control manage gray mold disease in strawberry over the last decade. Many authors in Egypt and around the world reported the emergence of resistance in *B. cinerea* to benzimidazole and QoI fungicides. Elsayed et al. (2023) reported that among 311 *B. cinerea* isolates collected from four governorates in Egypt (Beheira, Ismailia, Qalyubie and Dakahlia) 62.7% were resistant to difenoconazole. Moreover, al-Zahraa et al. (2022) stated that among 331 *B. cinerea* isolates collected from 4 governorates in Egypt 83.98% were resistant to pyraclostrobin. The sensitivity of 184 *B. cinerea* field isolates to QoI fungicide azoxystrobin was tested and the results showed that among the isolates collected, only seven showed resistance to azoxystrobin (Jiang et al. 2009). Maia *et al*., (2021) found that among 150 isolates of *B. cinerea*. The percentage of difenoconazole resistant isolates was 33.3%.

The current study conducted to detect the resistance among *B. cinerea isolates* collected and evaluate the effectiveness of commercial fungicides Speedcide (Difenoconazole), Tazer (Azoxystrobin) and Tilt (Propiconazole) against *B. cinerea* resistant isolates in vivo (Protective and Curative**)**

## MATERIALS AND METHODS

**Fungal isolates**

In this study, 30 isolates of *B. cinerea* were collected 2019. Strawberry fruits with characteristic grey mold symptoms were collected from commercial fields, selecting one fruit from each of different plants. Isolates of *B. cinerea* were cultured on potato dextrose agar medium (1000 ml of potato juice originated from 200 g of potato, 20 g of agar and 20 g of dextrose) at 20°C in darkness. The isolation and purification of the fungal strains was done using a single hyphal tip method. The isolates slants were preserved in 15-mL plastic tubes containing PDA medium at 4°C until use.

**Fungicides**

The commercial fungicides used in the study were

**Table 1. Shows Commercial Fungicides used in the study. (Name, active ingredient percentage and sources of samples are given in table):-**

| Fungicide or mixtures | Active-ingredient | Manufacturer |
|---|---|---|
| Speedcide | 25% EC (25% Difenoconazole a.i) | Channel corporation |
| Tazer | 25% SC (25% Azoxystrobin a.i) | Nufarm S.A.S corporation |
| Tilt | 25% EC (25% Propiconazole a.i.) | Syngenta corporation |

**Evaluate the effectiveness of commercial fungicides Speedcide (Difenoconazole), Tazer (Azoxystrobin) and Tilt (Propiconazole) against *B. cinerea***

To determine the sensitivity of *B. cinerea* isolates to propiconazole, difenoconazole, and azoxystrobin, 5 mm mycelial plugs were obtained from three-day-old colonies, and then transferred upside-down to media containing fungicides. After 3 days of incubation at 22°C, the diameter of the colonies was measured. Isolates were classified as resistant or susceptible to each fungicide based on their ability to grow on medium modified with discriminatory concentrations (10, 1, 10 µg/ ml for difenoconazole, propiconazole and azoxystrobin, respectively.

**Fitness Penalties in the Evolution of Fungicide Resistance**

The pathogenicity test of the selected isolates (one sensitive isolates and five resistant isolates per fungicide) was performed using intact primary leaves of strawberry plants by measuring the gray mold lesions. Leaves washed with sterile distilled water, ethanol 70% and sterile distilled water respectively. Mycelial plugs (5 mm in diameter) of each isolate were transferred to leaves. The lesion area on each leave was measured after 7 days. Five replicate leaves represented each isolate, and the experiment was performed twice.

For counting spores production number, the isolates were grown on PDA media for 15 days at 25°C. After that, spores were collected from plates by gently rubbing the agar surface after adding 0.5ml of Tween-80 solution (0.02% v/v) to the sporulated cultures in each plate. The spore suspension was filtered through cheese cloth to remove mycelial fragments, which combined and centrifuged at 10 ml for 5 min and the number of spores was recorded in ml by using Haemocytometer slide.

**Assessment of Fungicides' Protective and Curative Efficacy against *B. cinerea***

A study was conducted to assess the activity of commercial fungicides (Speedcide 25% EC, Tazer 25% SC, and Tilt 25% EC) against *B. cinerea* using detached strawberry leaves of uniform size and shape. Randomly selected resistant isolates (n=5) to difenoconazole, propiconazole, and azoxystrobin were used for the experiment. Leaf surfaces were sterilized by washing with distilled water, 70% ethanol, and then re-washing with distilled water, followed by air-drying. The fungicides Speedcide, Tazer, and Tilt were applied at recommended rates of 50 ml/100L, 50 ml/100L, and 15 ml/100L, respectively. A hand-held sprayer was used for application. Each fungicide was tested against a set of 6 isolates, including five resistant isolates and one sensitive isolate. To assess the protective activity of the fungicides, five leaves per isolate were treated with the recommended dose, allowed to air-dry, and then inoculated with mycelial plugs. For curative treatments, leaves were inoculated 24 hours before fungicide application. Control leaves remained untreated. The test was performed in triplicate.

Following treatment, leaves were incubated at 25°C and 80% relative humidity for 72 hours. Control efficacy (CE) was determined using the formula: CE = [1 - (lesion area of treated leaves / lesion area of untreated control leaves)] × 100.

Statistical analysis

The results are presented as the mean ± standard error (SE). The statistical analysis was conducted using SAS software (SAS Institute Inc., Version 9.1.3. SAS Cary, NC, USA). The data underwent a one-way analysis of variance (ANOVA), followed by Tukey's test to determine significance at $P < 0.05$.

## RESULTS AND DISCUSSION

**Evaluate the effectiveness of the tested fungicides**

Among the collected isolates (n=30), 13 (43.33%), 15 (50%) and 18 (60%) isolates were resistant to difenoconazole,

propiconazole and azoxystrobin, respectively which may be returned to significant selection pressure (Table 2).

**Table 2. Resistance frequencies to fungicides among Botrytis cinerea isolates collected during 2019**

| Fungicides | Number of isolates | Number of | | % of resistance |
|---|---|---|---|---|
| | | Sensitive isolates | Resistant isolates | |
| Difenoconazole | 30 | 17 | 13 | 43.33% |
| Propiconazole | 30 | 15 | 15 | 50% |
| Azoxystrobin | 30 | 12 | 18 | 60% |

In the same trend with our results, Leroch *et al.*, (2013) stated that strains of *B. cinerea* showed resistance to the fungicide QoI azoxystrobin at very high frequencies among the 173 isolates collected during the years 2008 and 2011 in the strawberry growing areas of Germany at a rate of 83.3% in the north, 76.3% in the center and an average of 84.0% in the south.

Juliana *et al.*, (2018) collected *B. cinerea* isolates and evaluated the effective concentration of azoxystrobin in addition to assessing the resistance frequencies. The results showed that resistance to azoxystrobin was observed in 87.5% of collected isolates from conventional fields and 31.4% of the isolates collected from organic fields. Also, Maia *et al.*, (2021) found that among 150 of *B. cinerea* isolates 33.3% were resistant to difenoconazole. The sensitivity test was done by using a discriminatory concentration of 10 µg/ml. Zhang *et al.*, (2021) collected strains of *B. cinerea* from 5 places in China from North, Central, and South China during 2011. The frequency resistance rate to difenoconazole has reached 11.7%.

**Fitness penalties of resistant isolates**

The data presented in (Table.3) showed that lesion diameter rates of all the azoxystrobin resistant isolates were significantly decreased after 48 and 96 hours comparing with the sensitive isolate. Moreover, spores production for all the tested resistant isolates significantly increased in comparing with the sensitive isolate. The high ability of azoxystrobin resistant isolates to produce spores will complicate the control process in case of wide spread.

**Table 3. lesion growth rates and number of spores production In vivo to azoxystrobin:-**

| Isolates | phenotype | lesion growth *In vivo* | | | Spore production ($\times 10^7$ ml$^{-1}$) | |
|---|---|---|---|---|---|---|
| | | Post-48 hrs. | Post-72 hrs. | Post-96 hrs. | N. of Spores | |
| | | Mean±SD | Mean±SD | Mean±SD | Mean±SD | |
| 208 | R | 2.620$^{d}$±0.027 | 13.91$^{c}$±0.136 | 21.86$^{e}$±0.298 | 265.0$^{ab}$ | 22.65 |
| BN80 | R | 4.141$^{c}$±0.126 | 12.58$^{d}$±0.373 | 23.63$^{d}$±0.374 | 260.7$^{ab}$ | 17.47 |
| MB185 | R | 3.654$^{d}$±0.020 | 12.58$^{d}$±0.373 | 19.63$^{f}$±0.374 | 235.0$^{b}$ | 2.00 |
| MB315 | R | 4.779$^{b}$±0.019 | 16.26$^{b}$±0.042 | 28.24$^{c}$±0.233 | 285.0$^{a}$ | 11.14 |
| S244 | R | 4.151$^{c}$±0.061 | 22.15$^{a}$±0.099 | 29.83$^{b}$±0.353 | 240.0$^{ab}$ | 6.24 |
| S226 | S | 5.654$^{a}$±0.020 | 22.28$^{a}$±0.445 | 40.13$^{a}$±0.187 | 165.0$^{c}$ | 25.51 |
| LSD 0.05 | | 0.1642 | 0.7966 | 0.8547 | 45.291 | |

**a-f=Means with the same letter in each column are not significantly different at P ≤0.05 using Tukey test.**

The data presented in (Table.4) demonstrated that after 48 hours, lesion diameter rates of all difenoconazole resistant isolates significantly reduced in comparing with the sensitive isolate (BN212). While after 96 hours, it was clear that all difenoconazole resistant isolates significantly reduced comparing with the sensitive isolate. The present results indicated that spores production for all the tested resistant isolates significantly decreased in comparing with the sensitive isolate.

**Table 4. lesion growth rates and number of spores production In vivo to difenoconazole:-**

| Isolates | phenotype | lesion growth *In vivo* | | | Spore production ($\times 10^7$ ml$^{-1}$) |
|---|---|---|---|---|---|
| | | Post-48 hrs. | Post-72 hrs. | Post-96 hrs. | N. of Spores |
| | | Mean±SD | Mean±SD | Mean±SD | Mean±SD |
| MB185 | R | 2.429$^{d}$±0.290 | 9.48$^{f}$±0.250 | 22.71$^{e}$±0.284 | 139.0$^{d}$±6.56 |
| MB19 | R | 3.753$^{c}$±0.106 | 17.87$^{c}$±0.154 | 43.74$^{c}$±0.260 | 214.0$^{bc}$±20.52 |
| S225 | R | 2.620$^{d}$±0.027 | 13.91$^{e}$±0.136 | 34.44$^{d}$±0.449 | 185.0$^{bc}$±26.51 |
| S244 | R | 4.464$^{b}$±0.391 | 18.58$^{b}$±0.403 | 48.71$^{b}$±0.365 | 218.0$^{bc}$±19.70 |
| S44 | R | 2.895$^{d}$±0.045 | 14.93$^{d}$±0.081 | 34.66$^{d}$±0.372 | 252.0$^{b}$±21.63 |
| BN212 | S | 5.552$^{a}$±0.254 | 20.11$^{a}$±0.200 | 51.78$^{a}$±0.264 | 311.00$^{a}$±19.97 |
| LSD 0.05 | | 0.629 | 0.6275 | 0.6275 | 55.102 |

**a-f=Means with the same letter in each column are not significantly different at P ≤0.05 using Tukey test.**

The data in Table 5 showed that all resistant isolates were clever enough to infect strawberry leaves that weren't treated with fungicides. After 48 hours, lesion diameter rates of all propiconazole-resistant isolates significantly increased in comparing with the sensitive isolate. The increase in lesion diameter rates continued after 72 hours and 96 hours in comparing with the sensitive isolate. Moreover, spores production for all the tested resistant isolates significantly increased in comparing with the sensitive isolate.

The results obtained in the present study were in harmony with Zhang *et al*., (2021) where physique penalties were tested on tomato fruits using 6 resistant isolates of *B. cinerea* and two sensitive isolates to confirm the degree of resistance to difenoconazole on separate fruits. By measuring the affected area on the surface of tomato fruits, the results showed that all isolates were capable of infestation on tomatoes that were not treated with fungicides. Also, Kim and Xiao (2011) in Washington State studied the throwing and pathogenic fitness of 22 isolates of the fungus *B. cinerea* showing different phenotypic patterns of sensitivity to pyraclostrobin. The results indicated a clear variation in fungal growth and spore production between pyraclostrobin-sensitive isolates as well as pyraclostrobin-resistant isolates.

**Table.5 lesion growth rates and number of spores production In vivo to Propiconazole:-**

| Isolates | phenotype | lesion growth *In* vivo | | | Spore production ($\times 10^7$ ml$^{-1}$) |
|---|---|---|---|---|---|
| | | Post-48 hrs. | Post-72 hrs. | Post-96 hrs. | N. of Spores |
| | | Mean±SD | Mean±SD | Mean±SD | Mean±SD |
| 180 | R | 4.46$^{b}$±0.285 | 19.88$^{b}$±0.79 | 49.93$^{b}$±0.231 | 270.0$^{ab}$±24.52 |
| H3(7) | R | 5.84$^{a}$±0.183 | 22.78$^{a}$±0.29 | 54.87$^{a}$±0.243 | 312.0$^{a}$±15.10 |
| MB185 | R | 3.46$^{c}$±0.298 | 16.87$^{c}$±0.25 | 41.88$^{d}$±0.198 | 294.3$^{ab}$±17.56 |
| S225 | R | 3.70$^{c}$±0.202 | 14.93$^{d}$±0.13 | 38.63$^{e}$±0.371 | 246.0$^{bc}$±19.52 |
| S44 | R | 5.00$^{a}$±0.154 | 18.72$^{b}$±0.36 | 46.32$^{c}$±1.245 | 216.0$^{c}$±21.66 |
| MB15 | S | 1.91$^{d}$±0.203 | 7.92$^{e}$±0.48 | 25.80$^{f}$±0.327 | 104.0$^{d}$±17.52 |
| LSD 0.05 | | 0.6224 | 1.1959 | 1.5625 | 53.63 |

**a-f=Means with the same letter in each column are not significantly different at P ≤0.05 using Tukey test.**

**Protective and Curative Activity of Commercial Fungicides against *B. cinerea***

The application of fungicides against *B. cinerea* as pre-infection (protective treatments) yields better control results compared to post-infection (curative treatments).

The data presented in (Table 6) described the protective and curative efficiency of Speedcide commercial fungicide against *B. cinerea* isolates. Generally, the fungicide gives high efficiency against the sensitive isolate when it is used as a protective treatment in comparing with curative treatment. Relatively low efficiency was observed against the resistant isolates when it was used as a protective treatment such as (MB185, MB19, S225 and S244). While, the efficiency was lower in the case of curative treatment as significant variations were observed between the sensitive and resistant isolates. The spores number produced by the sensitive and resistant isolates were counted after using the fungicides as protective and curative treatment. It was clear that the sensitive isolate lost the ability to produce spores in the protective treatment in comparing with the resistant isolates (MB185, MB19, S225 and S244). While, in the case of curative treatment there were significant variations between the spores count produced by the sensitive and the resistant isolates. Spores produced by all resistant isolates were reduced in in comparing with the sensitive isolate.

In the case of Tilt commercial fungicide the protective and curative efficiency against *B. cinerea* isolates were tested. Generally, the fungicide gives high efficiency against the sensitive isolate when it is used as a protective treatment in comparing with curative treatment. Relatively low efficiency was observed against the resistant isolates when it was used as protective treatment such as (S244, MB185 and S225). While, the efficiency was lower in the case of curative treatment as significant variations observed between the sensitive and resistant isolates. Declared that the sensitive isolate lost the ability to produce spores in the protective and curative treatments in comparing with the most of resistant isolates (MB185, S225 and S244). It was interesting to find that the resistant isolates (MB19 and S44) lost the ability to produce spores in the protective treatment.

**Table 6. Protective and curative activity of Speedcide, Tilt and Tazer against B. cinerea:-**

| Treatment | Isolates | Sensitivity | Protective activity | | Curative activity | | P value[4] |
|---|---|---|---|---|---|---|---|
| | | | Mean± SD | No. of spores (*10$^4$ ml$^{-1}$)[5] | Control efficacy[3] | No. of spores (*10$^6$ ml$^{-1}$)[6] | |
| Speedcide 25% EC | MB185 | R | 70.210$^{b}$±4.119 | 113$^{c}$ | 60.467$^{ab}$±2.191 | 289$^{d}$ | 0.022 |
| | MB19 | R | 67.927$^{b}$±5.152 | 63$^{d}$ | 38.380$^{cd}$±9.360 | 78$^{e}$ | 0.009 |
| | S225 | R | 65.34$^{b}$±11.222 | 332$^{a}$ | 33.900$^{d}$±4.607 | 514$^{b}$ | 0.0109 |
| | S244 | R | 70.837$^{b}$±3.875 | 205$^{b}$ | 48.633$^{bc}$±12.110 | 355$^{c}$ | 0.039 |
| | S44 | R | 92.937$^{a}$±0.106 | 0$^{e}$ | 61.757$^{a}$±4.558 | 51$^{e}$ | 0.0001 |
| | 208 | S | 97.900$^{a}$±0.026 | 0$^{e}$ | 66.530$^{a}$±2.243 | 781$^{a}$ | 0.0001 |
| | LSD 0.05 | | 9.8641 | 21.062 | 12.28 | 35.958 | |
| Tilt 25% EC | MB185 | R | 75.897$^{c}$±1.522 | 63$^{b}$ | 63.737$^{c}$±1.594 | 173$^{b}$ | 0.001 |
| | MB19 | R | 97.963$^{a}$±0.107 | 0$^{d}$ | 78.973$^{b}$±0.980 | 96$^{c}$ | 0.0001 |
| | S225 | R | 87.507$^{b}$±3.014 | 49$^{c}$ | 68.727$^{c}$±1.334 | 159$^{b}$ | 0.001 |
| | S244 | R | 56.330$^{d}$±5.052 | 125$^{a}$ | 30.640$^{e}$±8.226 | 380$^{a}$ | 0.0100 |
| | S44 | R | 97.937$^{a}$±0.106 | 0$^{d}$ | 54.607$^{d}$±2.282 | 84$^{c}$ | 0.0001 |
| | MB358 | S | 99.147$^{a}$±0.012 | 0$^{d}$ | 91.213$^{a}$±0.949 | 0$^{d}$ | 0.0001 |
| | LSD 0.05 | | 4.414 | 11.228 | 6.458 | 18.493 | |
| Tazer 25% SC | MB185 | R | 98.15$^{a}$±0.06 | 0$^{c}$ | 54.52$^{b}$±7.17 | 323$^{b}$ | 0.0001 |
| | MB19 | R | 79.40$^{b}$±0.79 | 125$^{b}$ | 66.466$^{ab}$±6.2273 | 223$^{d}$ | 0.0234 |
| | MB315 | R | 50.55$^{c}$±4.39 | 177$^{a}$ | 40.13$^{c}$±4.44 | 206$^{d}$ | 0.045 |
| | S244 | R | 76.49$^{b}$±8.61 | 196$^{a}$ | 64.25$^{b}$±1.08 | 294$^{c}$ | 0.071 |
| | S273 | R | 98.03$^{a}$±0.19 | 0$^{c}$ | 57.37$^{b}$±12.97 | 330$^{b}$ | 0.006 |
| | MB7 | S | 99.28$^{a}$±0.01 | 0$^{c}$ | 78.92$^{a}$±4.29 | 429$^{a}$ | 0.001 |
| | LSD 0.05 | | 7.045 | 20.323 | 12.53 | 23.354 | |

**a-b= Means with the same letter in each column are not significantly different at p≤0.05.**

While, for Tazer commercial fungicide activity against *B. cinerea* isolates generally, the fungicide gives high efficiency against the sensitive isolate when it was used as a protective treatment in comparing with curative treatment. Relatively low efficiency observed against the resistant isolates when it was used as protective treatment such as (MB19, MB315 and S244). While, the efficiency was lower in case of curative treatment as significant variations observed between the sensitive and resistant isolates. The spores number produced by the sensitive and resistant isolates were counted after using the fungicides as protective and curative treatment. It was clear that the sensitive isolate lost the ability to produce spores in the protective treatment in comparing with the resistant isolates (MB19, MB315 and S244). While, in case of curative treatment there were significant variations between the spores count produced by the sensitive and the resistant isolates. Spores produced by all resistant isolates reduced in in comparing with the sensitive isolate.

Tilt (propiconazole) was 91.89% effective against *B. cinerea* in protective applications and they were better in activity comparing to Speedcide (difenoconazole) was 80.96% and Tazer (azoxystrobin) was 84.50%. While, curative applications to Tilt (propiconazole) was 71.45% effective against *B. cinerea* and they were better in activity comparing to Speedcide (difenoconazole) was 55.55% and Tazer (azoxystrobin) was 59.04% (Table.7).

**Table 7. Mean percentage effectiveness of commercial fungicides (Speedcide, Tazer and Tilt) as protective and curative activity against B. cinerea**

| Fungicides | Resistant isolates | | P value | Sensitive isolates | | P value |
|---|---|---|---|---|---|---|
| | protective | curative | | protective | curative | |
| | Mean±SD | Mean±SD | | Mean±SD | Mean±SD | |
| Speedcide | 80.96$^{a}$±14.65 | 55.55$^{b}$±12.52 | 0.0001 | 99.300$^{a}$±1.212 | 66.947$^{b}$±10.201 | 0.0055 |
| Tazer | 84.50$^{a}$±20.00 | 59.04$^{b}$±14.46 | 0.0004 | 99.760$^{a}$±0.416 | 78.180$^{ab}$±4.872 | 0.0016 |
| Tilt | 91.89$^{a}$±9.51 | 71.45$^{a}$±13.14 | 0.0001 | 99.717$^{a}$±0.491 | 91.720$^{a}$±3.361 | 0.0151 |
| P value | 0.1502 | 0.0058 | | 0.7457 | 0.0124 | |
| LSD | 11.298 | 9.8721 | | 1.5831 | 13.604 | |

**a-b= Means with the same letter in each column are not significantly different at p≤0.05.**

The application of Tilt (propiconazole) resulted in outstanding protection against *B. cinerea* spores on strawberry leaves, as the fungicide inhibited spore germination on the leaf surface and proved its effectiveness against both resistant and sensitive isolates (Table 8).

**Table 8. Number of spores production after using the fungicides protective and curative activity against B. cinerea**

| Fungicides | Protective(*$10^4$ ml$^{-1}$) | Curative(*$10^6$ ml$^{-1}$) | P value | Protective(*$10^4$ ml$^{-1}$) | Curative(*$10^6$ ml$^{-1}$) | P value |
|---|---|---|---|---|---|---|
| | Mean±SD | Mean±SD | | Mean±SD | Mean±SD | |
| Speedcide | 1.43$^{a}$±1.21 | 257.33$^{ab}$±180.88 | 0.0001 | 0.00±0.00 | 781.00$^{a}$±7.55 | 0.0001 |
| Tazer | 1.00$^{ab}$±0.88 | 275.20$^{a}$±54.30 | 0.0001 | 0.00±0.00 | 429.00$^{b}$±11.27 | 0.0001 |
| Tilt | 0.47$^{b}$±0.48 | 178.47$^{b}$±110.64 | 0.0001 | 0.00±0.00 | 0.00$^{c}$±0.00 | 1.000 |
| P value | 0.0227 | 0.0952 | | 1.000 | 0.0001 | |
| LSD | 0.6681 | 93.122 | | --------- | 15.647 | |

**a-b= Means with the same letter in each column are not significantly different at p≤0.05.**

In the same trend of our results, Nosehy et al. (2019) tested the protective and curative efficacy of several fungicides against squash powdery mildew. The study revealed that the combination of azoxystrobin and difenoconazole had the highest control efficiency, with 83.3% in protective treatments, outperforming azoxystrobin alone (70.7%) and difenoconazole alone (62.3%). In curative treatments, the azoxystrobin and difenoconazole mixture showed 69.3% efficiency, superior to azoxystrobin alone (57.6%) and difenoconazole alone (44.2%). The findings demonstrated that azoxystrobin, both as a standalone and in combination with difenoconazole, was more effective than difenoconazole alone in both protective and curative applications. The protective method was notably more effective than the curative approach, and the combined use of azoxystrobin and difenoconazole surpassed all other treatments tested. In addition, Anesiadis et al. (2015) evaluated azoxystrobin's protective, curative, and eradicant activities against *Cercospora beticola* and *Erysiphe betae* on sugar beet under controlled conditions. Difenoconazole was used as the standard fungicide. Azoxystrobin at 16 µg/ml achieved 89-94% control of *C. beticola* and 95-97% control of *E. betae*. Curative treatments with azoxystrobin at 8 or 16 µg/ml provided over 90% control of Cercospora leaf spot when applied within 24 hours post-inoculation. Difenoconazole (8 µg/ml) showed excellent protective and curative efficacy against both pathogens.

## CONCLUSION

Understanding fitness penalties is vital for predicting effectiveness and optimizing disease control strategies. Studies indicate initial findings on fitness penalties. Given *Botrytis cinerea* potential for resistance to multiple fungicides, it is essential to rotate fungicides like difenoconazole, propiconazole, and azoxystrobin.

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## النشاط الوقائي والعلاجي لمبيدات الفطريات QoI و DMI ضد البوترايتس سيناريا على الفراولة

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### الملخص

تعتبر الفراولة محصولاً استراتيجياً أساسياً نظراً لقيمتها الاقتصادية الكبيرة. يعد مرض العفن الرمادي، الناجم عن فطر *Botrytis cinerea*، عائقًا رئيسيًا أمام إنتاج الفراولة في جميع أنحاء العالم، مما يسبب خسائر اقتصادية كبيرة. تعتبر مبيدات الفطريات الوقائية أداة أساسية في استراتيجيات الإدارة. في الآونة الأخيرة، لوحظ عدم فعالية مبيدات الفطريات على نطاق واسع في العديد من البلدان بسبب تطور المقاومة في *B. cinerea* الذي يشكل تهديدا مستمرا لإنتاج الفراولة. لذلك، تم إجراء هذا البحث لاختبار مبيدات الفطريات التالية Speedcide (difenoconazole DMIs)، (Tilt (propiconazole DMIs وQoIs (azoxystrobin Tazer) ضد *B. cinerea* ولتقييم عقوبات اللياقة البدنية للعزلات المقاومة لـ *B. cinerea*. أشارت النتائج إلى أن من بين العزلات المجمعة 13 (43.33٪)، 15 (50٪) و 18 (60٪) كانت مقاومة للفطر. ديفينوكونازول، بروبيكونازول وأزوكسيستروبين. أظهر تقييم عقوبات اللياقة البدنية للعزلات المقاومة أن جميع العزلات المختبرة كانت قادرة على إصابة وتطور الآفات على أوراق الفراولة. كان لمبيد Tilt (بروبيكونازول) أقوى فعالية تثبيطية كعلاج وقائي وعلاجي بمتوسط 91.89 و 71.45% على التوالي. وأخيرا، أدى استخدام المبيد الفطري Tilt إلى تثبيط إنبات الجراثيم على سطح الورقة وأثبت فعاليته ضد العزلات المقاومة والحساسة.