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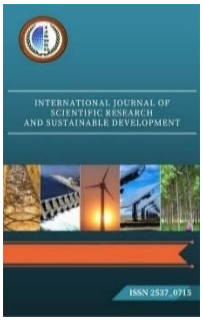


# Effect of Pre-Harvest Application of Salicylic Acid on Strawberry Growth and Resistance Against Gray Mold Disease

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## ABSTRACT

The present study aims to investigate the effect of salicylic acid treatment at various concentrations on strawberry (*Fragaria ananassa* var. Festival) growth, biochemical components, enzymes activity, and resistance to gray mold (*Botrytis cinerea*) during 2020-2021 growing season. Strawberry plants were planted in mid of September on raised beds in two open fields with two different types of soil (clay and sandy soil), in Qalyubia governorate–Egypt. Salicylic acid (SA), at 1, 2, 4 mM, and fenhexamid fungicide at Egyptian MOA recommended does, were applied at flowering stage. The results revealed that SA at all applied concentrations significantly improved the plant growth parameters and fruit yield compared to control or fenhexamid treatment. Maximum of plant height, root length, leaf and flower count/ plant, root and fruit fresh weight (g)/plant, and fruit volume ($cm^3$) were recorded by SA at the rate of 4 mM, which was followed by SA at 2mM. In addition, total chlorophyll, total phenol, and antioxidant enzymes activity increased significantly by SA 4- and 2-mM application rates. Treatment of SA *in vitro*, or under field's condition, reduced the diminished growth of gray mold more than Fenhexamid fungicide and control treatment. We can conclude that foliar application of salicylic acid significantly increased vegetative and fruit growth of strawberry and induced the treated plant defense against gray mold disease.

**Key words:** Strawberry, Salicylic acid, Fenhexamid, gray mold, growth parameter, plant pigments, phenols, antioxidant enzymes.

## Introduction

One of the most popular fruits in the world is the strawberry (*Fragaria ananassa* var. Festival), which is also a significant fruit grown on a large scale in Egypt. The minerals, amino acids, vitamins, and anthocyanin in strawberries are quite abundant (**Chauhan *et al.*, 2013**). Due to their high concentrations of phenolic compounds, including anthocyanin and vitamins C, E, and -carotene, which are chemicals linked to health benefits, these are also an important source of bioactive compounds. Ellagic acid, which has a wide range of biological activities, is present in



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quite high quality in it (**Meyers *et al*., 2003**). Because strawberries have a well-known perishable structure, their fruit is vulnerable to fungus assault. In addition, the fruit's high metabolic rate can rapidly reduce fruit quality after harvest.

The most dangerous strawberry disease is botrytis fruit rot (gray mold) and caused by the fungus *Botrytis cinerea* which is pervasive in nature. It may become dormant and stay on fruits as well as infect strawberry blooms, causing them to decay (**Raskin, 1992**). Significant yield losses are caused in Egypt by botrytis fruit rot, is the most severe and deadly disease of strawberries. When strawberry plants are not protected, frequently resulting in yield losses of up to 25% because it destroys strawberry blossoms', setting fruit, mature fruit, and leaves (**Sutton, 1990**). Gray mold is making a significant post-harvest loss factor during strawberry storage, transit, and shipment (**Sutton and Peng, 1993**). *Botrytis cinerea*, an airborne fungus, is a saprophyte, or unspecialized neurotropic fungus, that grows on rotting tissue as grayish masses of mycelium, conidiophores, and conidia (**Ciliberti *et al*., 2015**). According to **Kim *et al.* (2007)** and **Deising *et al.* (2008**), Botrytis has a very short incubation period and is quickly spread by wind, which makes it a major concern for all strawberry production during most of the year.

Several functions in plants, including heat production, disease resistance, seed germination, sex polarization, and ethylene synthesis, are regulated by salicylic acid (**Raskin, 1992**). Plants are vulnerable could be treated exogenously with salicylic acid in harmless amounts to increase their resistance to fungi. It also acts as a possible non-enzymatic antioxidant and plant growth regulator (**Zhang *et al*., 2011**). It has a high oral $LD_{50}$ (rat) of 891 mg/kg and has positive impacts on human health. Therefore, SA usage is safe for human health and is expected to enhance crop quality and stress resistance (**Peng and Jiang, 2006**).

Fenhexamid is a non-systemic molecule and acts by inhibiting spore germination, germ-tube elongation, and the mycelium growth of *Botrytis cinerea* inhibits germ-tube elongation and mycelia growth ( **Ha¨nßler and Pontzen, 1999**)

Due to that, the current study was planned to examine the effect of pre-harvest foliar application of salicylic acid on strawberry vegetative growth, fruit quality, and resistance to gray mold disease.

## Materials and Methods

### 1. Field preparation and planting

The current study was conducted at a private farm with two well-managed experimental fields of two types of soil: A (**clay soil**) and B (**sandy soil**) in Met-Kenana village, Toukh, Qalyubia governorate - Egypt, during 2020/2021 growing season. Tables 1 and 2 provide the physical and chemical characteristics of the experimental field soil types. Strawberries (*Fragaria ananassa* var. Festival) with one well-developed crown measuring 8–10 mm in diameter was transplanted as bare-rooted, cold-stored plants. The Strawberry and Non-Traditional Crops Improvement Center of Faculty of Agriculture - Ain Shams University is where the transplants were purchased. Strawberry transplants were planted at 10$^{th}$ and 14$^{th}$ September in clay and sandy soil fields, respectively. Raised beds that were 120 cm wide, and 15-20 cm high were used to grow the

transplants with a drip irrigation system, 30 cm apart. All cultural practices of cultivation (irrigation, fertilization, weeding, and pest management) were carried out in the two experimental fields in accordance with the recommendations of the Egyptian Ministry of Agriculture (EMOA).

## 2. Design and experiment

One month after Strawberry transplanting, Salicylic acid (2-Hydroxybenzoic acid, Molecular Weight equal 138.12g/mol., purity: 99%, Sigma-Aldrich),was dissolved in dimethyl sulphoxide (DMSO), then distilled water was added for preparing concentrations of 1, 2, and 4 milli Mole (mM). Fenhexamid SC 50% fungicide was applied according to Egyptian MOA's recommendation at the rate of 600$cm^3$/Fed to compare as a standard course of treatment. Salicylic acid treatments were performed at flowering stage, and repeated twice by 10 days intervals; meanwhile, Fenhexamid was treated once at the flowering stage. The treatments were laid out in Randomized Block design with four treatments which replicated thrice. All treatments were applied with a knapsack sprayer in the early morning. The control plants received identical doses of water and dimethyl sulphoxide at the appointed times. Since it was noted that rapid and efficient absorption by the lower leaf surface occurred, both the upper and lower leaf surfaces were sprayed until moist (**Hull *et al*., 1975**). The plot area was 6.0 $m^2$ included 50 plants.

## 3. Fruit quality and plant growth of strawberry

At the flowering stage, ten plants were randomly selected from each of the two inner rows of each experimental plot. The length of plant roots, leaves number and flowers per plant. Fresh fruit weight, and fruit volume were recorded after 90 days from transplantation.

## 4. Chemical determinations

After ten days from each treatment, some strawberry plants were randomly selected from each experiment plot, then the leaves were separated and divided into two parts: the first part was kept as fresh leaves (for pigments and enzyme activity determinations), and the second part was dried at 60$^o$C for two days in an electric oven, Fisher Isotemp., Senior model, then ground and stored in a deep freezer.

### 4.1. Pigment Contents

A test tube contains 5 ml of DMSO and 10 mg of strawberry fresh leaves tissue in fraction were added together. Without grinding, chlorophyll and carotenoids were extracted into DMSO solvent by incubation at 65°C overnight. For the determination of chlorophyll, absorbance was measured by spectrophotometer (Shanghai Lab-Spectrum Instrument Co., Ltd Model, Alpha-1102) at 644 and 662 nm for chlorophyll A and B, and 470 nm for carotenoids as specified by **Hiscox and Israelstam (1979**). The **Arnon equation (1949**) was used to compute total chlorophyll (Chl. a+b), chlorophyll a (Chl. a), and chlorophyll b (Chl. b), whereas **Cañal *et al.* (1985**) was used to calculate carotenoids.

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Arnon equation:

Chl. a = 12.7 x O.D 662 – 2.69 x O.D 644 mg/l
Chl. b = 22.9 x O.D 644 – 4.68 x O.D 662 mg/l
Chl. a+b = 20.2 x O.D 644 + 8.02 x O.D 662 mg/l

Cañal equation:

$$\text{Carotenoids} = \frac{A470 - 1.28\ (\text{Chl. a mg/l}) + 56.7\ (\text{Chl. b mg/l})\ \text{mg/l}}{256 \times 0.906}$$

### 4.2. Total Phenol contents

The Folin-Ciocalteau method was used to determine the total amount of phenolic compounds in the extracts of dried strawberry leaves for all treatments (1, 2, 4 mM of SA, Fenhexamid and control) according to **El-falleh *et al*. (2012**). The total phenolic compound in the extracts of dried strawberry leaves were determined and represented as mg/ml of extract equivalent Gallic acid.

### 4.3. Total proteins

Total proteins in the extracts of fresh strawberry leaves for all treatments (1, 2, 4 mM of SA, Fenhexamid and control) were determined by using **Bradford** method (**I976**).

### 4.4. Enzymes activity

According to **Ni *et al*.** (**2015**), the enzyme activity was tested in the extracts of fresh strawberry leaves for all treatments (1, 2, 4 mM of SA, Fenhexamid and control).

#### 4.3.1. Catalase activity

In accordance with **Aebi 1984,** a bio diagnostic kit (No., CA 2517), was used for catalase activity assessment in the extracts of fresh strawberry leaves for all treatments (1, 2, 4 mM of SA, Fenhexamid and control).

#### 4.3.2. Phenol oxidase activity

By the method of **Ishaaya** (**I971**), phenol oxidase activity was assessed in the extracts of fresh strawberry leaves for all treatments (1, 2, 4 mM of SA, Fenhexamid and control).

#### 4.4.3. Peroxidase activity

According to **Hammerschmidt *et al***. (**1982**), the peroxidase activity of the extracts of fresh strawberry leaves of all treatments (1, 2, 4 mM of SA, Fenhexamid and control) were determined. The change in absorbance/min/g of protein was used to express enzyme activity.

## 5. Evaluation of fungal diseases

### 5.1. A fungal pathogen's source.

Strawberry fruit samples that had naturally deteriorated and displayed a range of rot signs were gathered. According to **Hamouda *et al*.** (**2016**) infected strawberry fruit samples from each field were sent to the laboratory.

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### 5.2. Isolation and purification of the fungus

Strawberry fruits for each treatment (SA, fenhexamid, and control) were washed with distilled water, dried, and disinfected in a hypochlorite sodium solution (0.05%, w/v) for 2 min and rinsed in sterile water and allowed to dry. Sterile pieces were transferred to sterilized Potato Dextrose Agar (PDA) medium to be purified. On PDA solid medium, all fungal colonies were transferred and purified by using single-spore procedures. For subsequent research, purified fungi were stored at 25ºC on PDA slants (**sun *et al.*, 2010**).

### 5.3. Effect of SA and Fenhexamid on the linear growth *in vitro*

According to **Townsend and Heuberger (1943),** the warmed, sterilized PDA medium (19 ml) was mixed with one ml of three concentrations of salicylic acid (1, 2 and 4 mM) or Fenhexamid (recommended dose) individually before being poured into plates (9 cm in diameter). Following their solidification, the plates were infected with a disc (5 mm) cut from the peripheral active mycelia development of 5-day-old cultures of a fungal colony and placed in an incubator at 27 °C. Control plates were those that weren't treated. Three plates were employed. All plates' linear fungal growth averages were measured after one week. The mycelia growth inhibition percentage (MGI%), was calculated using the following formula:

**MGI% =DC-DT/DC x 100**

Where DC is the control's average mycelia growth's diameter.

DT stands for the treatment's average mycelia growth diameter.

### 5.4. Infection % of strawberry fruits during storage under lab condition

SA and Fenhexamid effects on infection rates on strawberry fruits were recorded in the 2020–2021 season at a commercial maturity stage of 3/4 of full fruit color. The identification was confirmed at the Disease Survey and Mycology Department, Plant Pathology Institute, Agriculture Research Center, Egypt. The fungal infection fruits from each treatment were transferred to storage for five days under lab conditions. Then the infection rate of strawberries was calculated according to **Abd-El-Moity, 1985)** as follows:

$$\text{Infection \%} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits}} \times 100$$

### 5.5. Disease incidence % of gray mold

Plant disease incidence can be defined as the number of plant units that are (visibly) diseased, usually relative to the total number assessed. The effect of SA and Fenhexamid on disease incidence % of strawberry fruits was evaluated according to **Romanazzi *et al*., 2000** as follows:

Disease incidence % = [Number of rotted fruits / Total number of tested fruits] × 100

**Analytical statistics**

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Using the COSTAT package application, the statistical analysis was carried out. An analysis of variance was performed on the data (ANOVA). Using Duncan's Multiple Range Test, the differences between data means were compared (**Waller and Duncan, 1969**). For all statistical calculations, P = 0.05 was used.

## Results and Discussion

### 1. Effects of SA and Fenhexamid on strawberry vegetative growth and fruit quality

#### 1.1. Strawberry vegetative growth

In Table 3, the impact of foliar SA treatments on the vegetative growth phenomenon of strawberries in fields with clay and sandy soil is depicted.

**In clay soil field,** application of SA showed no significant effect on leaves number per plant at the rate of 1- and 2-mM recording 7 and 8.3/plant, respectively. Meanwhile, treatments of SA (4 mM) and Fenhexamid significantly increased leaves number/plant being 9.7 and 9.5, respectively, compared to control.

Treatment of SA (1mM), and Fenhexamid, showed no significant effect on flower number / plant or the root length comparing with control, but it showed significant increase when treated with SA at the rate of 2 and 4mM recording 6.5 and 6.5 flowers / plant, or 21.3 and 23 cm root length, respectively.

Treatment of SA at the rate of 4 mM showed the highest significant increase fresh weight of root per plant compared to control followed by the rate of 2 and 1 mM of SA and Fenhexamid recorded 7.2, 5.3, 5.1 and 4.4g, respectively.

**In sandy soil field,** the application of SA at 4mM showed a significant increase in strawberry leaves number (8.3) compared to control (7). Conversely, there was a significant decrease in the leaves number when plants treated with SA (1mM) and Fenhexamid being 3.7 and 3.8, respectively.

The number of flowers per plant did not show significant differences between SA (2 and 4 mM), treatments and control. The plant root length showed no significant differences between SA treatment (2 mM), and control, meanwhile, whereas it recorded the maximum significant increase when applied at the rate of 4mM recording 21.5 cm. Application of Fenhexamid decreased the root length significantly (14cm) compared to control. Also, root fresh weight per plant showed no significant change between Fenhexamid and control. Conversely, SA treatments of 1 and 2 mM increased the root fresh weight significantly compared to control indicating 4.5 and 4.9g, respectively, and reaching its maximum increase when applied at rate of 4 mM (5.7g).

#### 1.2. Fruit quality

**In clay soil field** the highest significant fruit fresh weight was recorded by application of SA at 4mM comparing with the untreated being (30.7g), while there were no significant differences noticed when applied at 1and 2 mM. In contrast, Fenhexamid showed the lower significant fruit fresh weight (15.9g) compared to control. Also, the maximum significant increase in fruit volume was recorded by SA treatment (4mM) compared to control being 40cm$^3$, whereas the other treatments showed no significant change.

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**In sandy soil field,** the fresh weight of fruit showed no significant changes when treated with 1mM of SA, Fenhexamid, compared to control (19.9, 19.2, and 18.2g, respectively), but it increased significantly and recorded 30.9g when treated with 4mM of SA compared to control. The volume of each fruit showed no significant differences when treated with 1mM of SA, Fenhexamid, comparing with the untreatment, meanwhile, the fruit volume showed significant increase when treated with 4mM of SA being 40 $cm^3$ compared to control.

These results are in match with **Karlidag *et al.* (2009**), who indicated that salicylic acid dramatically increased strawberry plant growth overall in terms of root length and fresh weight, and it also triggered flowering and fruit setting with higher fruit weight, volume and yield (**Fariduddin *et al*., 2003**, **Martin-Mex *et al*., 2005 and Yildirim *et al*., 2008**), According to **Lolaei *et al*. (2012)**, salicylic acid improved fruit output and quality while delaying strawberry ripening. According to **AOAC (2005); Mady (2009**) and **Jamali *et al.* (2011**), a salicylic acid treatment at 2.0 mM raised the fresh weights of the roots and shoots, the production of fruit, and the quality of the fruit which three times significantly enhanced the fresh and dry weights of the roots and the rate of vegetative growth when compared to the untreated ones.

**2. The impact of Fenhexamid and salicylic acid on the pigments of strawberry leaves at two different fields**

Table 4 showed the impact of foliar SA application on strawberry leaves pigments at two different fields (clay and sandy soils) at 10, 20, and 30 days after applications.

The data indicated that SA at the rate of 4mM increased all leaves pigments, Chl. A, B, and total chlorophyll, significantly compared to control and the other SA application rates (2 and 1 mM), recorded total chlorophyll value of 3.74 and 3.92 mg/g fw after 10 d., 3.2 and 3.65 mg/g fw after 20 d. and 5.95 and 5.36 mg/g fw after 30 d., from application in clay and sandy soil trial, respectively. Meanwhile, the other 2 applied SA rates (2 and 1 mM), did not show significant effect on the leaves pigments comparing with the untreatment. Conversely, Fenhexamid application showed significant decrease effect on the leaves pigment (Chl. A, B, and total chlorophyll), compared to control after 10, and 20 days after application being 3.18, and 2.36 mg/g fw, and 2.44 and 2.56 mg/g fw of total chlorophyll in the two-trial soil types: clay and sandy soil, respectively. Concerning to Carotenoids leaves content, all treatments did not reveal clear effect on carotenoids content at 10 and 20 days after application compared to control. After 30 days, SA treatment at the rate of 4 mM showed the highest significant increase effect on carotenoids, while Fenhexamid showed the lowest decrease effect being (0.28 and 0.25 mg/g fw), and (0.13 and 0.12 mg/g fw), in clay and sandy soil trial, respectively.

The above-mentioned results are in match with **Arfan *et al.*, 2007** who indicated that SA increased photosynthetic activity and it is essential to plant growth, ion absorption, and nutrient transport inside the plant. It has been implicated in a crucial function in plant water interactions, photosynthesis, and growth. Also, **Vicente and Plasencia, 2011** showed that SA is a photosynthesis regulator because of its ability to change the structure of the leaf and chloroplasts. Exogenous administration of SA has an impact on a variety of varied plant activities, including



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Academy of Scientific Research & Technology

stomata closure, ion uptake and transport, membrane permeability, photosynthetic and growth rates (**Khan and Kenhadji, 2003 and Gunes *et al.*, 2005**).

Fenhexamid causes a decline in Pn and in photosynthetic pigment concentrations 7 days after application and decrease in chlorophyll contents and carotenoids after fungicide treatments appeared to be dose-dependent (**Saladin *et al.*, 2003**).

### 3. The impact of SA and Fenhexamid on the total phenol in strawberry leaves

Data in Table 5 showed the impact of foliar SA treatments and Fenhexamid on the total phenol of strawberry leaves at two different fields, clay, and sandy soils.

The data indicated that SA treatment had significant increase effect on total phenol content in strawberry leaves at all application rates, in both of clay and sandy soil trial fields meanwhile Fenhexamid treatment showed the lowest significant decrease effect compared to control. The highest phenol content was recorded by SA treatment applied at the rate of 4 mM, being 34.89, 44.23 and 41.96 mg/ml in clay soil trial field, and 29.86, 46.27, and 38.03 mg/ml in sandy soil field after 10, 20 and 30 days from application, respectively.

These results are in agree with **Gad *et al.*, 2007 and El-Korany and Mohamed, 2008,** who indicated that salicylic acid resulted in appreciable increases in the total phenol when was sprayed with 4 mM.

### 4. The impact of SA and Fenhexamid on the activity of enzymes

#### 4.1. Catalase activity

Data in Table 6 showed that of SA at all application rates revealed significant increase effect on catalase activity of strawberry leaves at the two tested soil types while application of Fenhexamid showed no significant difference comparing with control. The highest increase effect is recorded by SA at the rate of 4 mM, being 67.1, 94.7 and 160.0 u/mg protein, in clay soil, and 72.4, 107.7, and 180.0 u/mg protein, in sandy soil, after 10, 20, 30 days from application, respectively.

#### 4.2. Phenol oxidase activity

Table 6 illustrated that foliar application of SA at 4 and 2 mM significantly increased phenol oxidase activity compared to the untreatment, meanwhile the treatment of SA at 2mM and Fenhexamid did not indicate significant effect at the two tested soil types after 10, 20, and 30 days comparing with control. The highest effect on phenol oxidase activity is recorded by SA treatment at the rate of 4 mM at 30 days after application being 505.0- and 645.0-units x 1000/min/mg protein in clay and sandy soil, respectively.

#### 4.3. Peroxidase activity

In clay soil field, Table 6 illustrates that all SA treatments revealed a substantial rise in peroxidase at 4, 2, 1 mM application rates compared to control after 10 days from application recorded 564.7, 523.0, and 501.0 ΔO.Dx1000/ min/mg protein, respectively. Meanwhile there was no significant effect at 20 and 30 days from application except the rate of 4 mM which shows the

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highest significant rises comparing with control being 650.0 and 846.7 ΔO.Dx1000/min/mg protein, respectively. In contrast, Fenhexamid showed no significant effect.

In sandy soil, the presented data (Table 6), illustrated that all SA treatments and Fenhexamid showed significant effect on peroxidase activity after 20 and 30 days from treatment. The highest rise effect was recorded by SA (4mM), being 11053 and 1211.7 ΔO.Dx1000/min/mg protein, after 20 and 30 days,

The results of strawberry enzymes activity agree with **Khan *et al*., 2012; and Shafiee, *et al*., 2010** who found that SA has been demonstrated to be a significant signal molecule that can activate a specific enzyme that catalysis biosynthetic reaction. Exogenous SA has been applied to plants in recent years at non-toxic concentrations to control a variety of processes, including biotic and abiotic stressors. Also, **Hayat *et al.* (2010**) indicated that SA an endogenous plant hormone is produce antioxidant enzymes.

## 5. Impact of SA and Fenhexamid on fungus

### 5.1. Effectiveness on mycelia growth inhibition *in vitro.*

Data indicated in figure 1 revealed that SA treatments showed a promising inhibition effect on *B. cinerea* MGI %. Fenhexamid fungicide and SA showed positive linear inhibition effect to the mycelia growth by increasing application rate. Salicylic acid treatment showed close inhibition effect to Fenhexamid at the lower tested rate of 200, 400, and 600 ppm being (51.3 and 55.4%), (71.5 and 80.7%) or (87.1 and 89.5%), respectively. At the higher application rate (800 ppm), each of SA and Fenhexamid showed the same inhibition effect recoding 94.4%.

### 5.2. Salicylic Acid and Fenhexamid effects on strawberry fruit gray mold when being stored under laboratory conditions.

Results in Table 7 demonstrated the infection % of strawberry fruits collected from clay and sandy soil trial, during storage under laboratory conditions. Gray mold infection % of SA at the different application rates and Fenhexamid were markedly lower than control. According to the data, SA at the rate of 1 mM, and Fenhexamid treatment (at the recommended dose) showed equivalent effect and reduced the infection % by 21.4, and 19.4% or 21.4 and 18.6% of clay and sandy soil trial, respectively. Application of SA at 4 mM showed high positive effect and reduced the infection % more than Fenhexamid fungicide being 10.9 and 19.5% or 10.9 and 18.7% for clay and sandy soil trial, respectively.

### 5.3. Effectives of SA and Fenhexamid on disease incidence%

Data in Table 8 indicated the effect of SA and Fenhexamid on the disease incidence % of strawberry gray mold under field conditions.

The data showed that the incidence of strawberry fruit gray mold in SA and Fenhexamid treatments was markedly lower than the untreatment. Treatment of SA at 1 mM showed a slight difference effect comparing to Fenhexamid fungicide, being 27.3 and 24.6 % or 26.7 and 23.4 % of clay and sandy soil trial, respectively. At the higher application rates of SA (2 and 4 mM), were

markedly reduced the infection % compared to Fenhexamid treatment, recorded (15.2 and 4.3%) or (24.6 and 24.7%) in clay soil, or (15.0 and 4.2%) or (23.6 and 23.6%) in sandy soil, respectively.

These results are in match with **Ezzat*et al.*, 2017** who indicated that **t**he application of salicylic acid to strawberry seedlings resulted in much less gray mold on the seedlings and improved seedling development as compared to the control. Also, phenolic compounds will protect seedlings by enhancing their disease resistance, promoting seedling growth, increasing their tolerance to environmental challenges, and improving plant growth and productivity. Also, it could be due that SA treatment efficiently lower fruit ethylene production, inhibit fungal rot, and maintains overall fruit quality (**El-Morsy *et al.*, 2022)**. Also, SA postharvest treatment of fruit inhibits the growth and spread of rot fungi in strawberry fruits (**Doares *et al*. (1995); Huang *et al*. (2000); Amborabe *et al*., (2002); Lu and Chen (2005); Babalar *et al.* (2007) and Vicente and Plasencia, 2011).**

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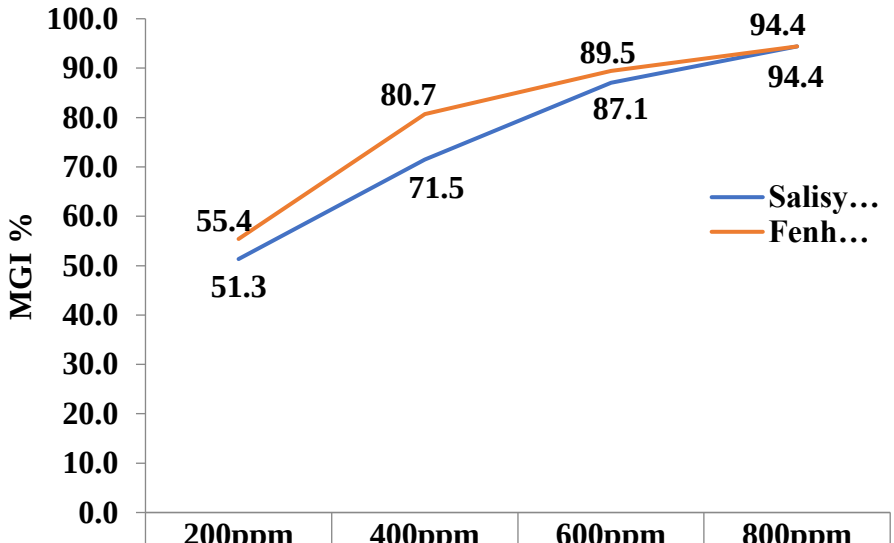


| | 200ppm | 400ppm | 600ppm | 800ppm |
|---|---|---|---|---|
| Salisylic Acid | 51.3 | 71.5 | 87.1 | 94.4 |
| Fenhexamid | 55.4 | 80.7 | 89.5 | 94.4 |

**Fig. 1: Efficacy of Salicylic acid and fenhexamid on mycelia growth inhibition of strawberry fruits rot caused by *Botrytis Cinerea in vitro***

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**Table 1: Chemical and physical properties of the expremintal field location A**

| Physical properties | | | | | SP% | EC | pH |
|---|---|---|---|---|---|---|---|
| Clay% | Silt% | Sand% | Total% | Texture | | DS/m | |
| 6 | 8 | 86 | 100 | Clay | 50 | 0.18 | 8.3 |

| Chemical properties | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soluble cations (meq/L) | | | | Soluble anions (meq/L) | | | | Mineral elements (ppm) | | | | | | |
| $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{-2}$ | $HCO_3^{-1}$ | $Cl^{-1}$ | $So_4^{-2}$ | N | P | K | Cu | Fe | Mn | Zn |
| 0.8 | 0.4 | 0.4 | 0.2 | - | 0.2 | 1 | 0.6 | 30 | 42 | 128 | 1.3 | 25.68 | - | 0.58 |

**Table 2: Chemical and physical properties of the expremintal field location B**

| Physical properties | | | | | SP% | EC | pH |
|---|---|---|---|---|---|---|---|
| Clay% | Silt% | Sand% | Total% | Texture | | DS/m | |
| 10.88 | 10 | 79.12 | 100 | Sand | 50 | 0.5 | 8.2 |

| Chemical properties | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soluble cations (meq/L) | | | | Soluble anions (meq/L) | | | | Mineral elements (ppm) | | | | | | |
| $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $CO_3^{-2}$ | $HCO_3^{-1}$ | $Cl^{-1}$ | $So_4^{-2}$ | N | P | K | Cu | Fe | Mn | Zn |
| 3.2 | 0.6 | 1 | 0.2 | - | 1 | 2.5 | 1.5 | 40 | 39 | 288 | 1.58 | 16.5 | - | 0.02 |

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**Table (3) Effect of salicylic acid applications on Strawberry vegetative growth phenomenon at two different soil types of fields at 10, 20 and 30 days after treatment.**

| Treatment | Appl. Rate | Clay soil field | | | | | | | | | | | | Sandy soil field | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Leaves No./ Plant | | Flower No./ Plant | | Root L/ Plant (Cm) | | Root FW/ Plant (g) | | Fruit FW g/ fruit | | Fruit Vol. ($cm^3$)/ fruit | | Leaves No./ Plant | | Flower No./ Plant | | Root L/ Plant (Cm) | | Root FW/ Plant (g) | | Fruit FW g/ fruit | | Fruit Vol. ($cm^3$)/ fruit | |
| Salicylic Acid | 4 mM | 9.7 | a | 6.5 | a | 23.0 | a | 7.2 | a | 30.7 | A | 40.0 | a | 8.3 | a | 6.2 | a | 21.5 | a | 5.7 | a | 30.9 | a | 40.0 | a |
| | 2 mM | 8.3 | ab | 6.5 | a | 21.3 | a | 5.3 | b | 22.2 | B | 30.0 | b | 6.5 | b | 5.7 | a | 19.0 | b | 4.9 | ab | 25.1 | b | 30.0 | b |
| | 1 mM | 7.0 | bc | 4.0 | b | 17.0 | b | 5.1 | b | 19.5 | C | 20.0 | c | 6.3 | b | 3.7 | c | 16.3 | c | 4.5 | b | 19.9 | c | 20.3 | c |
| Un-Treat. | --- | 6.3 | c | 3.3 | b | 16.7 | b | 2.7 | c | 18.5 | C | 20.3 | c | 7.0 | b | 5.0 | ab | 18.7 | b | 2.7 | c | 18.2 | c | 21.0 | c |
| Fenhexamid SC 50% | 600 CC/ Fed. | 9.5 | a | 4.5 | b | 14.3 | b | 4.4 | b | 15.9 | D | 19.7 | c | 6.8 | b | 3.8 | bc | 14.0 | d | 2.8 | c | 19.2 | c | 20.0 | c |
| LSD (0.05) | | 1.467 | | 1.834 | | 4.088 | | 0.830 | | 1.229 | | 1.558 | | 1.151 | | 1.243 | | 1.979 | | 0.810 | | 1.759 | | 2.048 | |

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**Table (4) Effect of salicylic acid applications on Strawberry leaves pigments (mg/g fresh weight) at two different soil types of fields at 10, 20 and 30 after treatment.**

| Treatments | Appl. Rate | 10 Days | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Clay soil field | | | | | | | | | Sandy soil field | | | | | | | | |
| | | Carotene | | Chl. A | | Chl. B | | Total Chl. | | A/B ratio | Carotene | | Chl. A | | Chl. B | | Total Chl. | | A/B ratio |
| Salicylic Acid | 4 mM | 0.27 | a | 2.59 | a | 1.15 | a | 3.74 | A | 2.25 | 0.28 | a | 2.60 | a | 1.32 | a | 3.92 | a | 1.97 |
| | 2 mM | 0.23 | b | 2.35 | ab | 1.05 | b | 3.39 | B | 2.24 | 0.19 | b | 2.18 | b | 1.03 | b | 3.21 | b | 2.19 |
| | 1 mM | 0.23 | b | 1.91 | c | 1.04 | b | 2.95 | C | 1.84 | 0.18 | b | 1.91 | c | 0.94 | bc | 2.85 | c | 2.04 |
| Un-Treat | --- | 0.27 | a | 2.18 | b | 1.14 | a | 3.32 | B | 1.91 | 0.27 | a | 2.40 | ab | 1.01 | b | 3.41 | b | 2.38 |
| Fenhexamid SC 50% | 600 CC/ Fed. | 0.23 | b | 2.16 | b | 1.02 | b | 3.18 | Bc | 1.79 | 0.18 | b | 1.60 | d | 0.78 | c | 2.38 | d | 2.06 |
| LSD | | 0.0205 | | 0.2451 | | 0.0477 | | 0.2600 | | | 0.0290 | | 0.2544 | | 0.1902 | | 0.3483 | | |
| Treatment | Appl. Rate | 20 Days | | | | | | | | | | | | | | | | | |
| | | Carotene | | Chl. A | | Chl. B | | Total Chl. | | A/B ratio | Carotene | | Chl. A | | Chl. B | | Total Chl. | | A/B ratio |
| Salicylic Acid | 4 mM | 0.17 | a | 2.65 | a | 0.64 | a | 3.29 | A | 4.12 | 0.19 | a | 2.99 | a | 0.66 | a | 3.65 | a | 4.53 |
| | 2 mM | 0.15 | ab | 2.40 | ab | 0.54 | b | 2.94 | Bc | 4.47 | 0.17 | a | 2.44 | b | 0.63 | ab | 3.07 | bc | 3.91 |
| | 1 mM | 0.13 | b | 2.29 | b | 0.53 | b | 2.82 | C | 4.32 | 0.13 | b | 2.25 | b | 0.57 | bc | 2.81 | cd | 4.03 |
| Un-Treat. | --- | 0.16 | ab | 2.62 | a | 0.52 | b | 3.14 | Ab | 5.03 | 0.18 | a | 2.59 | b | 0.62 | abc | 3.22 | b | 4.15 |
| Fenhexamid SC 50% | 600 CC/ Fed. | 0.14 | ab | 2.00 | c | 0.44 | c | 2.44 | D | 4.55 | 0.12 | b | 2.02 | c | 0.54 | c | 2.56 | d | 3.72 |
| LSD | | 0.0282 | | 0.2467 | | 0.0469 | | 0.2504 | | | 0.0300 | | 0.3310 | | 0.0778 | | 0.3278 | | |
| Treatment | Appl. Rate | 30 Days | | | | | | | | | | | | | | | | | |
| | | Carotene | | Chl. A | | Chl. B | | Total Chl. | | A/B ratio | Carotene | | Chl. A | | Chl. B | | Total Chl. | | A/B ratio |
| Salicylic Acid | 4 mM | 0.28 | a | 4.73 | a | 1.23 | a | 5.95 | a | 3.86 | 0.25 | a | 4.33 | a | 1.04 | a | 5.36 | a | 4.18 |
| | 2 mM | 0.23 | b | 3.93 | b | 0.75 | c | 4.69 | b | 5.22 | 0.22 | b | 4.22 | a | 0.97 | ab | 5.19 | a | 4.35 |
| | 1 mM | 0.23 | b | 2.85 | c | 0.92 | b | 3.77 | c | 3.11 | 0.21 | b | 2.78 | c | 0.94 | ab | 3.72 | c | 2.96 |
| Un-Treat. | --- | 0.22 | b | 4.02 | b | 0.96 | b | 4.98 | b | 4.19 | 0.22 | b | 3.45 | b | 1.01 | a | 4.45 | b | 3.42 |
| Fenhexamid SC 50% | 600 CC/ Fed. | 0.13 | c | 3.68 | b | 0.93 | b | 4.60 | b | 3.99 | 0.12 | c | 3.36 | b | 0.88 | b | 4.24 | b | 3.87 |
| LSD (0.05) | | 0.0194 | | 0.3465 | | 0.1082 | | 0.4311 | | | 0.1999 | | 0.2424 | | 0.1084 | | 0.2470 | | |

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**Table (5) Effect of salicylic acid applications on total phenol contents (mg/ml) of Strawberry leaves at two different soil types of fields at 10, 20 and 30 days after treatment.**

| Treatment | Appl. Rate | Clay soil field | | | | | | Sandy soil field | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 d | | 20 d | | 30 d | | 10 d | | 20 d | | 30 d | |
| Salicylic Acid | 4 mM | 34.89 | a | 44.23 | a | 41.96 | a | 29.86 | a | 46.27 | a | 38.03 | a |
| | 2 mM | 33.72 | b | 36.69 | b | 40.16 | b | 28.73 | b | 44.47 | b | 36.03 | b |
| | 1 mM | 24.83 | c | 31.42 | c | 34.71 | c | 26.59 | c | 39.90 | c | 33.10 | c |
| Un-Treat. | --- | 23.42 | d | 22.11 | d | 28.96 | d | 22.44 | d | 31.96 | d | 32.56 | c |
| Fenhexamid SC 50% | 600 CC/ Fed. | 21.84 | e | 21.80 | e | 23.83 | e | 18.87 | e | 25.13 | e | 25.76 | d |
| LSD (0.05) | | 0.855 | | 0.815 | | 0.626 | | 0.412 | | 0.603 | | 1.096 | |

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**Table (6) Effect of salicylic acid and fenhexamid on Strawberry leaves enzymes at two different soil types of fields at 10, 20 and 30 days after treatment.**

| | Treatments | Clay soil field | | | | | | Sandy soil field | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 d | | 20 d | | 30 d | | 10 d | | 20 d | | 30 d | |
| Catalase (unit/mg protein) | SA-4mM | 67.1 | a | 94.7 | a | 160.0 | a | 72.4 | a | 107.7 | a | 180.0 | a |
| | SA-2mM | 60.8 | b | 86.0 | b | 125.0 | b | 62.2 | b | 92.0 | b | 142.3 | b |
| | SA-1mM | 53.0 | c | 72.0 | c | 117.7 | bc | 56.7 | c | 82.0 | c | 132.0 | bc |
| | Fenhexamid | 53.2 | c | 68.0 | cd | 113.7 | c | 53.1 | d | 74.0 | d | 122.7 | c |
| | control | 51.8 | c | 64.0 | d | 117.0 | bc | 52.9 | d | 69.0 | e | 116.7 | c |
| | LSD | 2.2537 | | 4.6973 | | 9.718 | | 2.3407 | | 3.5773 | | 16.93 | |
| Phenol oxidase (Units x1000/min/mg protein) | SA-4mM | 386.0 | a | 437.0 | a | 505.0 | a | 404.0 | a | 445.0 | a | 645.0 | a |
| | SA-2mM | 369.3 | b | 431.7 | a | 483.3 | b | 388.0 | b | 432.7 | b | 550.0 | b |
| | SA-1mM | 364.7 | bc | 422.0 | b | 462.0 | d | 372.0 | c | 428.0 | b | 506.7 | c |
| | Fenhexamid | 364.3 | bc | 418.0 | bc | 468.0 | C | 368.0 | cd | 421.0 | c | 511.7 | c |
| | control | 360.7 | c | 412.7 | c | 461.0 | d | 366.0 | d | 405.3 | d | 505.3 | c |
| | LSD | 6.7256 | | 5.9602 | | 5.6563 | | 5.5181 | | 6.2317 | | 11.6774 | |
| Peroxidase (ΔO. Dx1000/min/mg protein) | SA-4mM | 564.7 | a | 650.0 | a | 846.7 | a | 1035.0 | a | 1105.3 | a | 1211.7 | a |
| | SA-2mM | 523.0 | b | 620.0 | b | 725.0 | b | 907.3 | b | 1002.0 | b | 1180.0 | b |
| | SA-1mM | 501.0 | c | 594.7 | c | 720.0 | b | 710.0 | c | 801.0 | c | 975.0 | c |
| | Fenhexamid | 485.0 | d | 591.0 | c | 722.3 | b | 663.3 | cd | 760.0 | d | 915.7 | d |
| | control | 481.7 | d | 584.7 | c | 715.0 | b | 620.0 | d | 706.0 | e | 850.0 | e |
| | LSD (0.05) | 7.4863 | | 12.49 | | 94.5833 | | 51.1319 | | 11.6015 | | 15.4299 | |

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**Table (7): Efficacy of pre-harvest application of salicylic acid and fenhexamid on gray mold infection % of strawberry fruits during storage under laboratory conditions.**

| | Clay soil | | | | Sandy soil | | |
|---|---|---|---|---|---|---|---|
| Concentrations | 1mM | 2mM | 4mM | | 1mM | 2mM | 4mM |
| Salicylic Acid | 21.4 | 13.2 | 10.9 | | 21.4 | 13.3 | 10.9 |
| Fenhexamid (Recom. dose) | 19.4 | 19.5 | 19.5 | | 18.6 | 18.5 | 18.7 |
| Control | 50.2 | 50.2 | 50.2 | | 50.2 | 50.2 | 50.2 |

**Table (8): Efficacy of salicylic acid and fungicide treatments on gray mold infection % under field conditions.**

| | Clay soil | | | | Sandy soil | | |
|---|---|---|---|---|---|---|---|
| Concentrations | 1mM | 2mM | 4mM | | 1mM | 2mM | 4mM |
| Salicylic Acid | 27.3 | 15.2 | 4.3 | | 26.7 | 15.0 | 4.2 |
| Fenhexamid (Recom. dose) | 24.6 | 24.6 | 24.7 | | 23.4 | 23.6 | 23.6 |
| control | 48.6 | 48.6 | 48.6 | | 48.6 | 48.6 | 48.6 |