Potential of Certain Salts and Fungicides to Control Postharvest Gray Mould (*B. cinerea*) of Strawberry

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ABSTRACT

Effect of two fungicides (*i.e.* iprodione and cyprodinil+flydioxonil mixture) and two salts (sodium bicarbonate, SBC, and sodium benzoate, SBO) were evaluated separately or in mixtures in laboratory experiments as well as under field conditions against *Botrytis cinerea* instant of the fruit gray mould of strawberry. All tested salts and fungicides as well as their mixtures significantly decreased the radial growth of *B. cinerea in vitro*. Complete inhibition of radial growth was obtained with SBC at 2.0 and1.0 g/100ml. Also, high reductions were obtained with SBC at lower concentrations. The fungicide iprodione at 500 mg/L completely inhibited radial growth of *B. cinerea*, while complete inhibition was obtained with cyprodinil+flydioxonil at lower concentration (10 mg/L). Mixing the tested fungicides with salts increased their inhibition effect on *B. cinerea* growth at lower fungicide concentrations. iprodione at 0.1 mg/L mixed with SBO at 1g/100ml completely inhibited radial growth. Also, cyprodinil+flydioxonil at 0.1mg/L mixed with SBC at 0.2 g/100ml completely inhibited radial growth of *B. cinerea* while 0.001mg/L cyprodinil+flydioxonil fungicide with SBO at 2.0g/100 completely inhibited radial growth. On the other hand, the field experiments showed that all treatments with the tested salts and fungicides significantly decreased postharvest Botrytis gray mould on fruits harvested 1, 3, 7, 10 and 15 days after treatments and stored at 5°C for seven days. However, the treatment with 50% cyprodinil+flydioxonil + SBC 2% was the most effective and decreased mean percentage of infection to 4% compared to 23.86 % for the untreated control. This was followed by 50% iprodione +SBC 2%, 25% iprodione + SBC and 25% cyprodinil+flydioxonil + SBC 2%, and cyprodinil+flydioxonil, alone, and iprodione, alone, with percentages of infection being 7%, 8%, 8.5%, 8.5%, and 9%, respectively, while (SBC 2%, alone) treatment showed the least effect with 12.93 percentage of infection. Meanwhile, positive strong correlations (r = 0.981 to 0.943) were revealed between Infection (%) and harvest interval periods with two salts mixed with iprodione and cyprodinil + flydioxonil after stored at 5˚C. Also, the enzyme assay revealed that increases in the activity levels of pectolytic and cellulytic enzymes produced by the fungus were associated with high fruit rots incited by *B. cinerea*. Meanwhile, residues of all tested fungicides in treated strawberry fruits decreased with decreasing fungicide concentrations in the salt + fungicide mixtures. cyprodinil (25%) + SBO, and cyprodinil (25%) + SBC treatments exhibited the lowest residue values being 1.420, and 1.680 mg/kg fruit one hour after treatment while the other treatments showed higher residue values while the highest was recorded for iprodion at recommend dose being 29.81 mg/kg fruit. However, fungicide residues decreased with increasing time after preharvest treatments.

Key words: strawberry, gray mould, fungicides, sodium bicarbonate, sodium benzoate, *B. cinerea*.

INTRODUCTION

Strawberry (*Fragaria* X *ananassa* Duchesne) is an important and high-value crop worldwide. It is an excellent source of vitamin C, which contains an average 40–90 mg. Egypt is considered the third largest producer of strawberry in the world after the United States and Spain (Wu *et al*, 2012). In Egypt, strawberry is one of the most important vegetable crops for local consumption and exportation (Embaby *et al.*, 2016, and Barakat and Al-Masri, 2017). Unfortunately, strawberry is affected by several plant diseases that affect its yield and quality in both the pre- and post-harvest stages. Gray mold of strawberry caused by *B. cinerea* is one of the most important post-harvest diseases worldwide (Williamson *et al.*, 2007). However, *B. cinerea* can attack strawberry during all growth stages (flowering, fruiting and storage periods) causing destructive rots. *B. cinerea* is considered the main causal pathogen of strawberry postharvest decay (Sesan, 2003 and Droby and Lichter, 2004).

Traditionally, chemical fungicides have been used to maintain high quality of fruits and vegetables over extended periods of storage or transportation. However, the increased global chemo phobia and the reduced efficacy of chemicals due to pathogen resistant strains, have forced producers and researchers to search for more safe alternatives for controlling such postharvest diseases and to minimize the use of chemical fungicides to ensure good fruit quality (Sanzani, *et al*., 2009).

In this context, there is a considerable interest in the use of bicarbonates for controlling various fungal diseases in plants (Smilanick *et al*., 1999 and Pankaj and singh, 2017), as these salts are inexpensive, easy, safe in application, and non-hazardous for humans and the environment. Sodium salts were shown to inhibit fungal pathogens of fruits, field crops, vegetables and ornamentals (Oliver *et al*., 1999 and Melanie *et al*., 2002). Sodium benzoate is a preservative which is bacteriostatic and fungistatic under acidic conditions. Also, sodium bicarbonate (NaHCO3), commonly known as baking soda, shown antimicrobial activity and has been used to control rots caused by *Phytophthora infestans* (Mills *et al*., 2004), *Penicillium digitatum* (Smilanick *et al.*, 1999), *Penicillium expansum* (Wan *et al*., 2003) and *B. cinerea* in table grape, tomato, apple, and cherry (Droby *et al*., 2003, Ippolito *et al*., 2005, Nigro *et al*., 2006, and Fayza *et al*., 2017).

Consequently, the objectives of the present study was to evaluate the potential of the antimicrobial effect of sodium biocarbonate and sodium benzoate salts singly or in mixtures with low doses of fungicides (*i.e.* iprodione and cyprodinil+fludioxonil mixture) for the inhibition of mycelial growth of *B.cinerea in vitro* and under field conditions to control postharvest gray mould of strawberry during storage. In addition, effects of such treatments were evaluated for pectinase (PC) and celluloses (CX) produced in fruits by fungal pathogen, as well as the influence of these treatments on the residue levels of these fungicides in treated strawberry fruits.

MATERIALS AND METHODS

1- Fungal culture.

Fruit samples of strawberry showing gray mould symptoms were collected during 2015-2016 winter season from different fields (fresh planting) in El Tahreir region. Diseased fruits were rinsed several times in sterilized water; surface disinfested using 70% ethyl alcohol for two minutes and dried between sterilized filter papers, cut into small pieces, each containing diseased tissues with adjacent healthy ones. Sample pieces were, then, cultured on PDA medium and incubated for 4 days at 24 ˚C. The growing fungi were purified by using single spore isolation technique and were identified according to Gilman (1957) and Barnett and Hunter (1972) and transferred on PDA slants.

1.1- The *in vitro* effect of certain salts, fungicides on mycelial growth of *B. cinerea*.

Sodium bicarbonate and sodium benzoate (Sigma) were mixed with sterilized and non-solidified PDA media at the concentrations 2.0, 1.0, 0.5, 0.2, 0.05 and 0.02%, while the fungicides at concentrations of 500, 100, 10, 1, 0.1, 0.01 and 0.001 a.i. mg/L for iprodione and mixture of cyprodinil+flydioxonil Table (1) were prepared in distilled water and incorporated into the molten PDA before pouring the plates, 20 ml of PDA/plate (Smilanick *et al*., 2008). Petri plates were then, inoculated in the center with 0.7cm diameter mycelium disks of 5-day- old culture of *B. cinerea* and incubated at 20˚C. Diameters of the developed colonies were measured in cm daily until the fungal colonies in controls reached the edge of the plates (Sabebaro *et al.*, 2014, and EL-Mougy *et al*., 2015).

1.2- The *in vitro* effect of salt and fungicide mixtures to inhibit the growth of *B. cinerea*.

Sodium bicarbonate at the concentrations of 0.20, 0.10 and 0.05% and sodium benzoate at the concentrations of 2.0, 1.0 and 0.5%, while the fungicides at concentrations of 10, 1.0 and 0.1 a.i mg/L for iprodione and 0.1, 0.01 and .001 a.i. mg/L for the cyprodinil+flydioxonil mixture (Smilanick *et al*., 2008), were tested in combination for their inhibitory effect on the mycelial growth of *B. cinerea*.

2. Filed experiments:

Field experiments were conducted at El-Tahreer regions during 2016-2017 winter season. Healthy strawberry transplants cv. Festival were used. Salts and fungicides, (iprodion, Rovral, 50%WP, and cyprodinil+flydioxonil mixture, Switch, 62.5% WG) were prepared in water and sprayed (at recommended dosage, half and fourth recommended dosage mixing with salts at 2%) two times a week before harvest (preharvest) in 15thJanuary at the beginning of the flowering stage, when 10 - 20% of the flowers had opened and at 50 - 75% bloom (Blacharski et al., 2001 and Haydu, 2003), four replicates were prepared for each treatment each contained 50 plants. Check treatment was sprayed with water. Plants were managed as usual for strawberry cultivation but no fungicides were applied except the tested treatments.

Fruits from the different treatments, as well as controls were collected 1, 3, 7, 10 and 15 days after the second spray application at random from sampling, and placed in sterilized plastic container contained ice packages. Fruits were incubated at 5˚C for 7 days and observed daily for Botrytis fruit rot development (Hongyin et al., 2007).

Table 1: Chemical name, common name, recommended dose and active ingredient of the investigated fungicide were as follows:

Fungicide	Chemical name	Trade name	Dose/ 100L	Active ingredient (ai)
Switch 62.5%	4-cyclopropyl-6-methyl-N-phenyl-2-pyrimidinamine + 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile	Switch	75g	cyprodynil+ flydioxyonil
Rovral 50%	3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboxamide	Rovral	90 g	iprodione

Percentage of infection with Botrytis gray mold on strawberry fruits was calculated at the end of storage as follow:

$$\text{Infection (\%)} = \frac{\text{Number of rotted fruits}}{\text{Total number of tested fruits}} \times 100$$

2.1- Assessment of cellulose and pectinase activities:

Activities of pectinase (PC) and cellulase (CX) enzymes produced by B. cinerea in stored strawberry fruits were determined under laboratory conditions according to methods described by Talboys and Busch (1970) in fruit tissue extract prepared by Luke et al. (1981) The reduction in viscosity of the reaction mixtures containing 2 ml of crude enzyme (tissue extract), 5ml, 1.5% citrus pectin solution in 0.1 M phosphate buffer at PH 5 adjusted by 0.3 M Na OH or HCl. The reaction mixtures were incubated at 28˚C and the loss in viscosity of the mixture was measured after 30 minutes against blank containing boiled inactivated extracts. PC and CX activity assay methods were based on the loss in viscosity by the Fenske-Ostwald viscometer, according to (Bell *et al*., 1955).

$$\text{Loss in viscosity (\%)} = \frac{T0 - T1}{T0 - Tw} \times 100$$

T0 = time of flow blank (zero time)

T1 =the time of flow at a given time interval

Tw = the time of flow to distilled water

2.2- Determination of fungicide residues:

Strawberry fruits were obtained from the field experiment at the different intervals (0, 3, 7, 10, 15 days) after last spray. Three replicates, 350 g from each treatment as well as the control, were put in polyethylene bags. The three replicates were mixed to form one representative sample and homogenized. Two sub-samples each 50 g were taken, one for extraction instantly and the second was kept in a deep freezer at -20 ˚C as a stock.

Multi residue method: AOAC (1995) was followed with some modification where the sample and solvent volume were only half of those in the AOAC method and a rotary evaporator and blowing down with air. After drying, pesticide residues were reconstituted in n-hexane/acetone for gas chromatography determination according to (Abada et al., 2005).

3. Statistical analysis:

All data were statistically analyzed and means were compared using LSD test at 5% level of probability according at Gomez and Gomez (1984). All statistical analyses were performed using analysis of variance technique by means on Costat (1985) computer software package.

RESULTS AND DISCUSSION

1. Laboratory Experiments

1.1. The *in vitro* effect of certain salts and fungicides on growth of B. *cinerea.*

Results illustrated in Figure 1 (A and B) showed that both of the tested salts (SBC & SBO) significantly decreased the radial growth of *B. cinerea*. However, complete inhibition of radial growth was obtained with SBC at 2.0 and 1.0 g/100 ml. Also, high reductions were obtained with SBC at lower concentrations of 0.50, 0.20 and 0.10 g/ 100 ml, where mean radial diameter decreased to 1.50, 2.77 and 3.55 cm, respectively, compared to 9.0 cm for the untreated control. Meanwhile, the illustrated data showed that use of SBO at 2.0, 1.0 and 0.5 g/100 ml decreased mean radial diameter to 1.65, 3.00 and 4.58 cm, respectively.

On the other hand, data illustrated in Figures 1 (C and D) showed that the two tested fungicides *i.e*., iprodione and mixture cyprodinil+flydioxonil significantly decreased radial growth of *B. cinerea* at the different tested concentrations. iprodione fungicide at 500 mg/L completely inhibited radial growth of *B. cinerea*, while concentrations of 100 and 10 mg/L decreased radial growth (diameter) to 1.58 and 2.07 cm, respectively, compared to 9.0 cm for the untreated control.

However, complete inhibition was obtained with cyprodinil+flydioxonil low concentrations of 100 and 10 mg/L. Also, high reductions of radial growth were obtained being 1.85, 2.67, 4.00 and 4.67 cm at lower concentrations of 1.0, 0.10, 0.01 and 0.001 mg/L, respectively. These findings are in harmony with those of Palmer et al. (1997) and Nigro et al. (2006) who reported that sodium bicarbonates inhibited radial growth of *B. cinerea.* Also, Zaker (2014) showed that potassium and sodium bicarbonate were effective in reducing growth and spore germination of *B. cinerea*. Also, Helalia and Sameer (2014) indicated similar results with SBC which caused inhibition rates ranged between 7.44 and 87.00 % on radial growth of *P. digitatum* when SBC applied at 250- 3000 µg/ml. Meanwhile, El-Gamal et al. (2014) found that sodium benzoate, at concs., 0.25, 0.5, 1.0 and 1.5%, significantly decreased the radial growth of *Alternaria alternata* and *Rhizopus stolonifer*. Also, they indicated that 1.0% for sodium benzoate showed high radial growth reduction by 63.3% and 61.1%, respectively, compared to the untreated control. Such results are in harmony with those reported by (Menzel *et al*., 2016 and Barakat and Al-Masri, 2017).

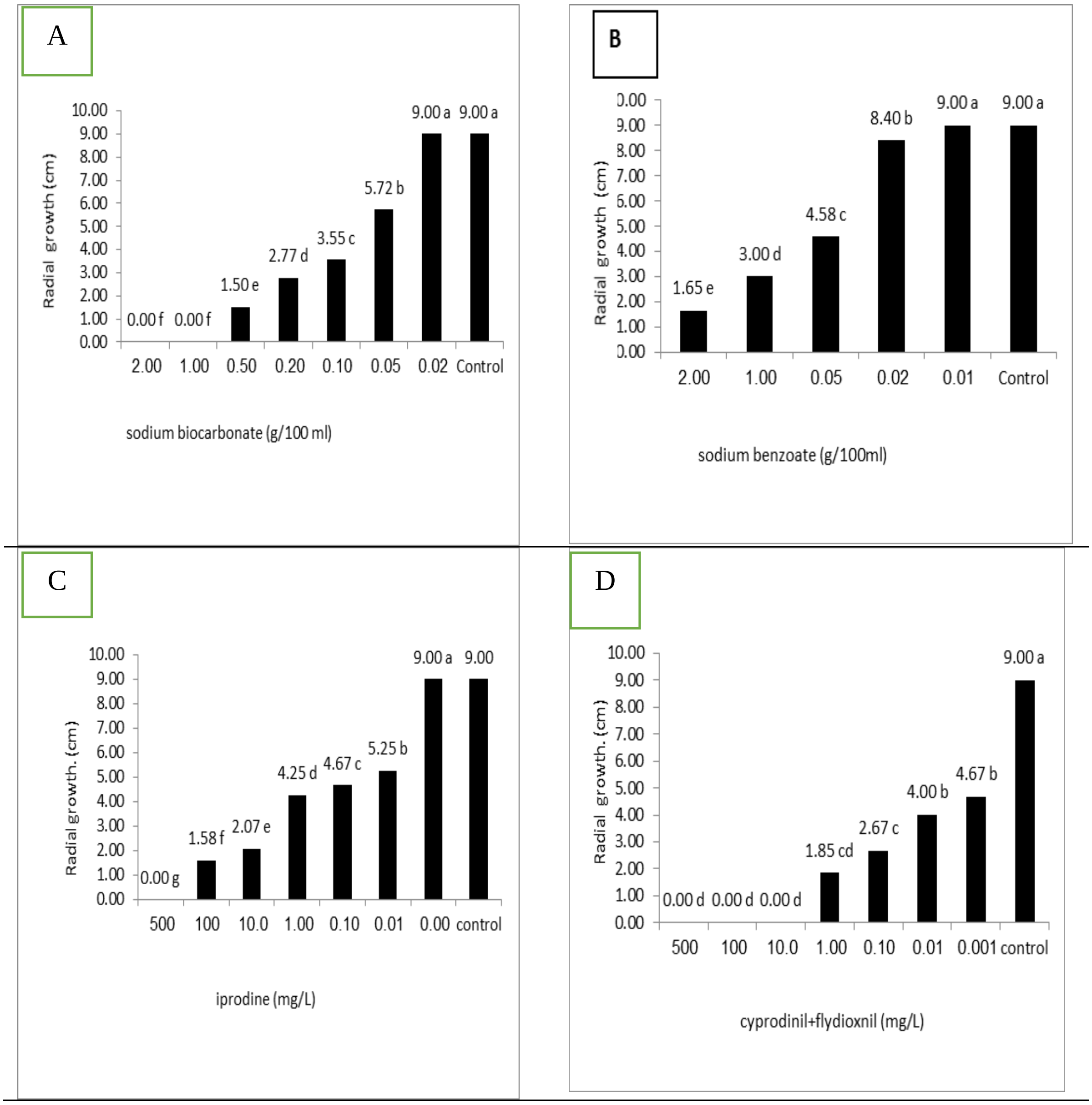


Figure 1: The *in vitro* effect of different concentrations of sodium bicarbonate (A) & sodium benzoate (B) salts and iprodione (C) and cyprodinil+flydioxonil (D) fungicides on radial growth of *B. cinerea.*

1.2. The in vitro effect of salt and fungicide mixtures to inhibit the growth of B. cinerea.

It is evident from Figure (2) that SBC supplemented with each of the two tested fungicides were effectively in suppress the in vitro *B. cinerea* radial growth, compared to the untreated control. Meanwhile, data presented in Table (2) illustrated in Figure (3) showed that mixing the tested salts with fungicides increased their inhibition effect on B. cinerea growth at the lower fungicide concentrations. SBC at 0.2 mg/100 ml mixed with iprodione at a.i. 1.00 mg/L was the most effective and decreased radial growth diameter of *B. cinerea* to 1.933 cm, compared to 9.0 cm for the untreated control, while all tested SBC and iprodione combinations *(i.e*. SBC, 0.2, 0.1, 0.05 x iprodion, 1.0, 0.1, 0.01) resulted in radial growth ranged between 1.933 cm and 5.767 cm, compared to 9.0 cm for the untreated control.

However, SBC at 0.2 g/100 ml mixed with cyprodinil+flydioxonil at 0.1mg/L completely inhibited radial growth of B. cinerea, while other combinations resulted in radial growth ranged between 0.800 cm and 2.833 cm, compared to 9.0 cm for the untreated control.

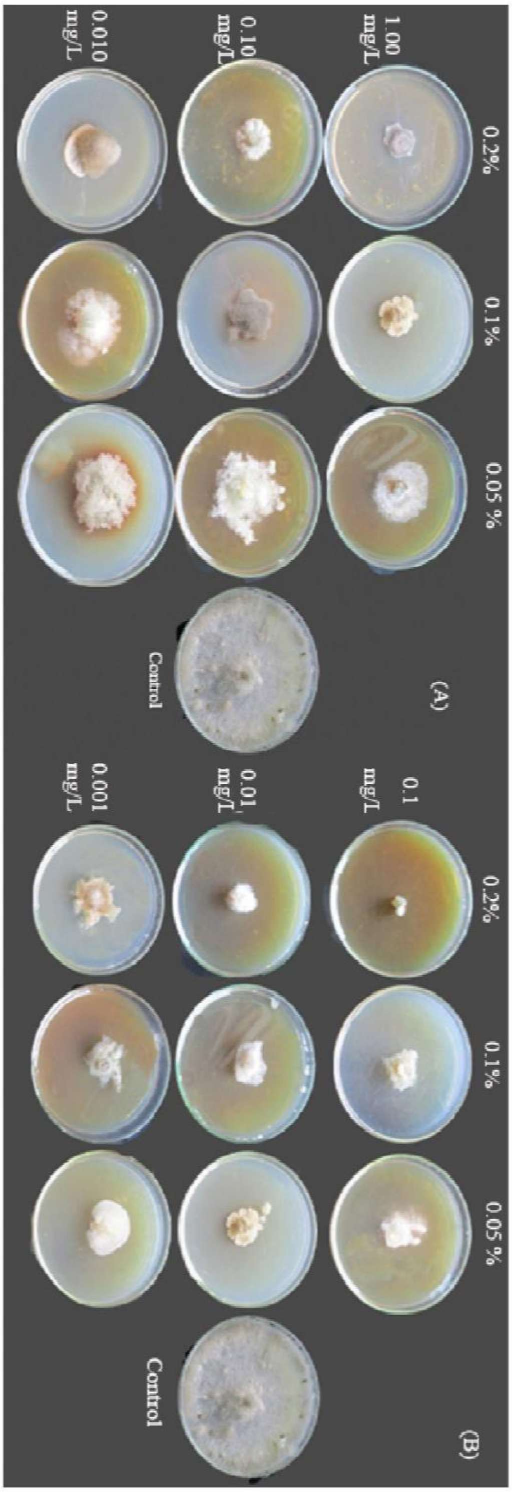


Figure 2: Radial growth of *B. cinerea* grown on PDA amended with SBC and (A) iprodione, or (B) cyprodinil+fludioxonil fungicides, at three concentrations for each, seven days after inoculation.

Table 2: The *in vitro* effect of different concentrations of fungicides (iprodione and mixture cyprodinil+flydioxonil) combined with salts (SBC and/or SBO) on radial growth of *B. cinerea*

Fungicide concentration (mg/L)	sodium bicarbonate (%)			Fungicide conc. mean	sodium benzoate (%)			Fungicide conc. mean
	0.2	**0.1**	**0.05**		**2.0**	**1.0**	**0.5**	
iprodione				iprodione				iprodione
1.000	1.933 c	2.000 d	2.917 d	2.283 d	0.000 b	0.000 c	0.000 d	0.000 d
0.100	2.033 c	3.617 c	4.350 c	3.333 b	0.000 b	0.000 c	2.133 c	0.711 c
0.010	3.967 b	5.167 b	5.767 b	4.967 c	0.000 b	2.450 b	3.200 b	1.883 b
Control	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a
LSD 5%	0.496	0.438	0.407		0.621	0.591	0.545	
Salts conc. Mean	2.644 B	3.595 A	4.345 A		0.000 C	0.817 B	1.778 A	
cyprodinil−flydioxonil				cyprodinil+flydioxonil				cyprodinil+flydioxonil
0.1	0.000 c	1.050 c	1.417 d	0.822d	0.000 b	0.000 b	0.000 b	0.000 b
0.01	0.800 c	2.050 b	2.317 c	1.456c	0.000 b	0.000 b	0.917 b	0.306 b
0.001	2.250 b	2.350 b	2.833 b	2.478 b	0.000 b	0.000 b	1.267 b	0.422b
Control	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a	9.000 a
LSD 5%	0.590	0.544	0.517		0.621	0.621	0.603	
Salt conc. Mean	1.017 B	1.817 A	2.189 A		0.000 A	0.000 A	0.728 A	

Values followed by different letter (s), for each single parameter, are significantly different at 0.05 of probability

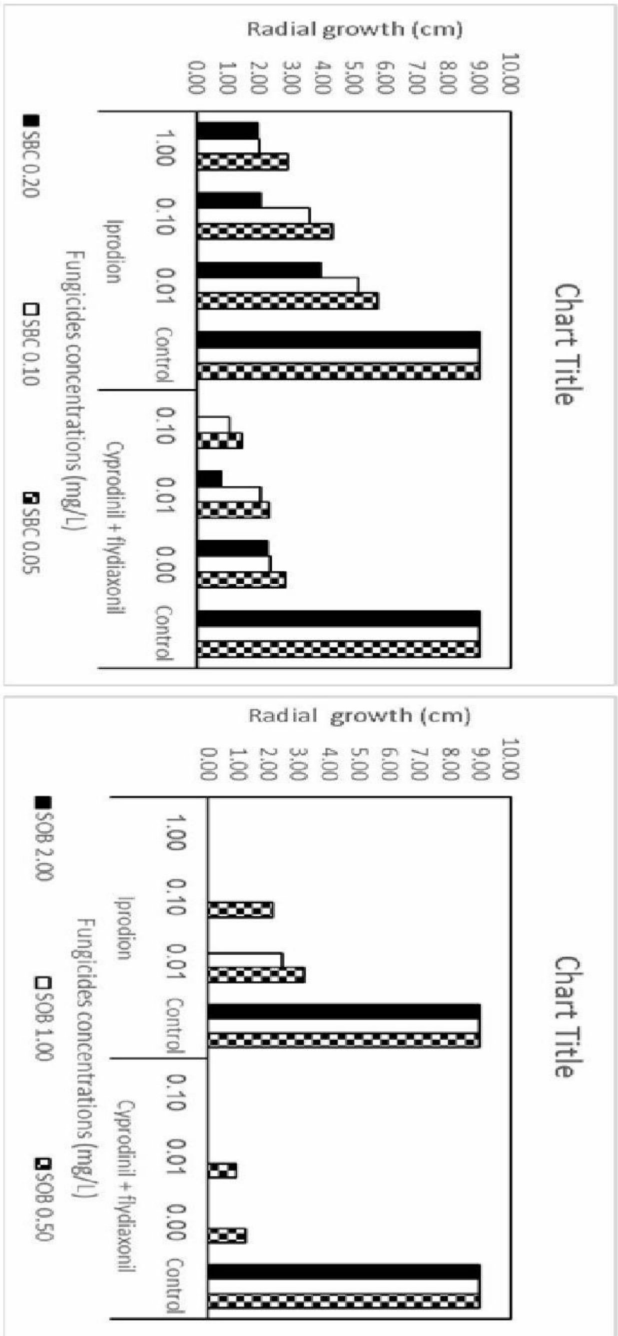


Figure 3: The *in vitro* effect of different concentrations of fungicides (iprodione and mixture cyprodinil+flydioxonil) mixed with salts (SBC and/or SBO) on radial growth of *B. cinerea*.

On the other hand, SBO at 2.0 g/100 ml mixed with iprodione at 1.00, 0.100 and 0.010 a.i mg/L and SBO at 1.0 g/100 ml mixed with iprodione at 1.00 and 0.10 mg/L in addition to SBO at 0.5g/100 ml mixed with the same fungicide at 1.00 mg/L were the most effective and completely inhibited radial growth of *B. cinerea,* while the combinations of SBO at 1.0- 0.5 g/100 ml with iprodione 0.10 and 0.01mg/L resulted in radial growth ranged between 2.133 cm and 3.200 cm, compared to 9.0 cm for the untreated control. However, SBO with mixture cyprodinil+flydioxonil combinations were more effective, where most treatments completely inhibited *B. cinerea* radial growth with the best treatment for 2.0 and 1.0% SBO with 0.1, 0.01 and 0.001 a.i mg/L cyprodinil+flydioxonil fungicide, SBO (0.5 g/100 ml) mixed with 0.1 mg/L of the previous fungicide.

According to these results, it can be concluded that the highest concentration of SBO increased the efficacy of the least concentrations of fungicide in reducing the mycelial growth of *B. cinerea* (Table 2).The obtained results are in harmony with those mentioned by Zamani et al. (2008) and Helalia and Sameer (2014), who showed that the potency of the tested fungicides against Penicillium digitatum markedly increased by adding sodium bicarbonate imazalil had EC50 value of 0.148 µg / ml when used separately but recorded EC50 values of 0.08, 0.053 and 0.04 µg / ml and fungitoxic activity increments of 1.85, 2.79 and 3.70 folds when mixed with 1000, 2000 and 3000 µg / ml sodium bicarbonate, respectively.

The results in Figure (4) summarized the activity of the tested salts with fungicides mixtures in reducing radial growth of *B. cinerea*. Significant reductions in radial growth were detected by SBC + iprodione or cyprodinil+flyudioxonil mixtures at the averages of 3.528 and 0.865cm, respectively. On the other hand, SBO mixed with cyprodinil+flyudioxonil had the highest effective inhibition of linear growth with an average of 0.321 cm, compared with the other treatments. Generally, the fungicidal activity of the tested fungicides was increased by adding salts. These results were in agreement with those reported by other investigators (Smilanick *et al*., 2008, Dore *et al*., 2009, Montesinos-Herrero *et al*., 2011 and McKay *et al*., 2012).

2- Field Experiments

2.1. Effect of preharvest spray with certain salts and fungicides on controlling Botrytis fruit rot of strawberry:

Data in Table (3) showed that all treatments significantly decreased percentage of infection with Botrytis fruit rot on the stored strawberry fruits, harvested 1, 3, 7, 10 and 15 days after treatment, compared with the untreated control. However, there were insignificant differences in infection percentages among most of the tested treatments for fruits harvested 1 or 3 days after treatment and stored at 5˚C for seven days. Fruits harvested after 7, 10 and 15 days of treatment, the 50% cyprodinil+flydioxonil + SBC 2% treatment was the most effective where mean percentage of infection was as low as 4%, compared to 23.867 % for the untreated control. This was followed by 50% iprodione +SBC 2% treatment, 25% iprodione + SBC 2%, 25% cyprodinil+flydioxonil + SBC 2%, cyprodinil+flydioxonil alone and iprodione alone with percentages of infection being 7%, 8%, 8.5%, 8.5%, and 9%, respectively, while treatment with SBC 2% showed the least infection level with 12.93% (Table 3). In this respect, Palou *et al*. (2001) and Smilanick *et al*. (2005) reported that sodium and ammonium bicarbonate inhibited fungal pathogens of fruits, field crops, vegetables and ornamentals and in addition, SBC applied at room temperature at 2 to 4% reduced blue mold caused by Penicillium italicum. Embaby *et al*. (2016) found that switch and Bio-Arc fungicides were better than others; they can reduce infection percentage of strawberry fruit decay caused by Alternaria sp, R. stolonifera and B. cinerea from 26.4 to 9.6%.

Concerning the sodium benzoate (SBO) in combination with the tested fungicides, data in Table (4) showed that 50% cyprodinil+flydioxonil + SBO 2%, 25% cyprodinil+flydioxonil + SBO 2% and 50% iprodione +SBO 2% treatments were the most effective, as mean percentages of fruit infection were as low as 3%, 4.5%, and 6.43%, respectively, compared to 23.86% for the untreated control. This was followed by cyprodinil+flydioxonil then; iprodione with 8.5%, and 9.0% percentage of infection, respectively. However, SBO 2%, and 25% iprodione + SBO 2% showed the lowest effect with 17% and 19% mean percentage of infection, respectively, seven days after storage at 5˚C (Table 4).The obtained results are in agreement with Adaskaveg *et al*. (2005), who evaluated potassium sorbate, SBO and other food additives should be evaluated in combination with low doses of the fungicide propiconazole (Menthor 45 WP, Syngenta) for their activity against sour rot as an additional strategy for managing postharvest application of this active ingredient and reducing the risk of proliferation of resistant strain of G. candidum. The present findings are in harmony with those obtained by Palou *et al*. (2009), who reported that both incidence and severity of brown rot on summer fire nectarines wound inoculated with Monilinia fructicola and incubated at 20 °C for 5 days were reduced by about 100% after packing line application with a commercial CDA containing 20% fruit coating amended with half or full doses of fludioxonil and different mixtures of potassium sorbate, sodium benzoate, or 2-deoxy-D-glucose.

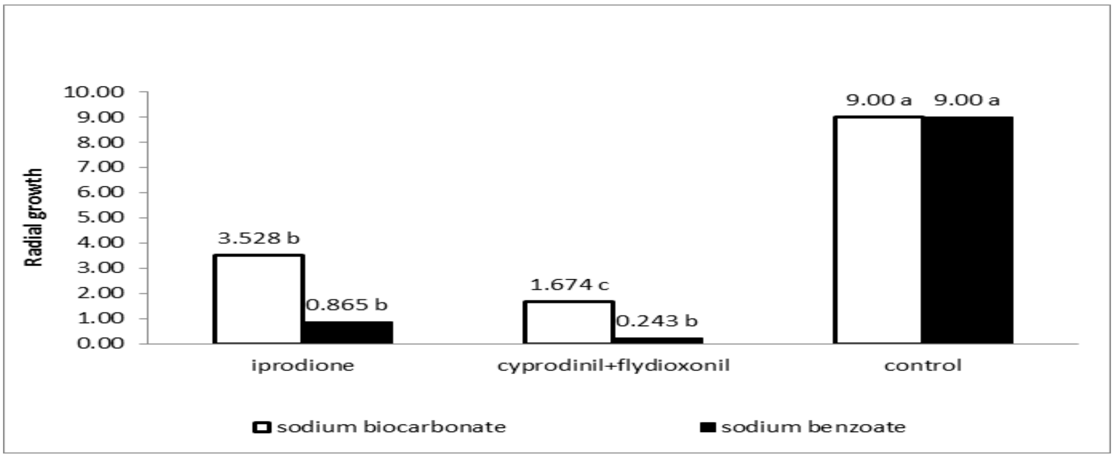


Figure 4: The *in vitro* effect of fungicides combined with salts on radial growth of *B. cinerea*

Table 3: Effect of pre-harvest treatments with iprodione or cyprodinil+flydioxonil fungicides alone or their mixture with SBC on percentage of infection with gray mould of strawberry (cv. Festival) on fruits harvested at different periods after treatment and stored at 5 ˚C for seven days.

Treatment	Percentage of fruit infection & Days after treatment					
	1 Day	3 Day	7 Day	10 Day	15 Day	Mean
iprodione alone	0.00 b	2.50 b	7.50 c	17.50 bc	17.50 d	9.00cd
50% iprodione +SBC 2%	0.00 b	0.00c	2.50 e	10.00 e	22.50 cd	7.00 e
25% iprodione + SBC 2%	0.00 b	0.00c	5.00 d	10.00 e	25.00 c	8.00 de
cyprodinil+flydioxonil alone	0.00 b	0.00 c	10.00 b	15.00 de	17.50c	8.50 c
50% cyprodinil+flydioxonil + SBC 2%	0.00 b	0.00c	5.00 d	5.00 f	10.00 e	4.00 f
25% cyprodinil+flydioxonil + SBC 2%	0.00 b	0.00c	10.00 b	15.00 cd	17.500 d	8.50 c
SBC 2%	0.00 b	2.50 b	7.50 c	14.66d	40.00 b	12.93 b
Control (sprayed with tap water)	2.50 a	7.50 a	15.00 a	32.50 a	61.83 a	23.86 a
LSD 5%	0.278	0.816	1.244	2.595	5.168	1.890
(Mean)	0.313 D	1.56 D	7.81 C	14.95 B	26.479A	

SBC, sodium bicarbonate, 50%= 45.0 to iprodione and 37.5 to cyprodinil+flydioxonil mg/L, 25%= 22, 5 to iprodione and 18.75 to cyprodinil+flydioxonil mg/L

Values followed by different letter(S), for each parameter, are significantly different at 0.05 of probability.

Table 4: Effect of pre-harvest treatments with iprodione or cyprodinil+flydioxonil fungicides alone or their mixture with sodium benzoate on percentage of infection with gray mould of strawberry (cv. Festival) on fruits harvested at different periods after treatment and stored at 5 ˚C for seven days.

Treatment	Percentage of fruit infection & Days after treatments					
	1 Day	3 Day	7 Day	10 Day	15 Day	Mean
iprodione	0.00 b	2.50 b	7.50 d	17.50 c	17.50 b	9.00 c
50% iprodione +SBO 2%	0.00 b	0.00 c	2.50 e	9.66 d	20.00 b	6.43 de
25% iprodione + SBO 2%	0.00 b	2.50 b	12.50 b	22.50 b	57.50 a	19.00 b
cyprodinil+flydioxonil	0.00 b	0.00 c	10.00 c	15.00 c	17.50 b	8.50 cd
50% cyprodinil+flydioxonil + SBO 2%	0.00 b	0.00 c	0.00 f	2.50 e	12.50 c	3.00 f
25% cyprodinil+flydioxonil + SBO 2%	0.00 b	0.00 c	0.00 f	5.00 e	17.50 b	4.50 ef
SBO 2%	0.00 b	0.00 c	7.50 d	22.50 b	57.50 a	17.50 b
Control sprayed with tap water	2.50 a	7.50 a	15.00 a	32.50 a	61.83 a	23.86 a
LSD 5%	0.278	0.834	1.781	3.159	6.862	2.395
Mean	0.313 D	1.563 D	6.875 C	15.896 B	32.729 A	

SBO, sodium benzoate, 50%= 45.0 to iprodione and 37.5 to cyprodinil+flydioxonil mg/L, 25%= 22, 5 to iprodione and 18.75 to cyprodinil+flydioxonil mg/L

Values followed by different letter(S), for each parameter, are significantly different at 0.05 of probability.

2.2. Correlation between postharvest botrytis fruit rot incidence and harvest interval after treatments.

Positive strong correlations coefficient (r = 0.943 - 0.981) were depicted between postharvest Botrytis fruit rot increase and harvest intervals. The strength of these relationships were shown again in the sharpness of the regression slops were revealed between postharvest botrytis fruit rot incidence and harvest interval periods, as increasing harvest interval periods increased percentage of natural infection of strawberry fruit with botrytis gray mould (Figure 5).

2.3-Effect of the field salt and fungicide treatments on enzymes activity of strawberry fruits after storage.

Data illustrated in Figure 6 (a, b, c and d) showed the effect of field application with sodium bicarbonate (SBC) or sodium benzoate (SBO), iprodione and cyprodinil+fludioxonil fungicides singly or in combination, on pectinase (PC) and celluloses (CX) activity of strawberry fruits harvested at different periods after treatments and stored at 5˚C for seven days. It is evident that obvious increase in PC and Cx activities were revealed in fruits harvested after 10 and 15 days of salt and fungicide preharvest treatments and stored for 7days at 5 ˚C. Meanwhile, considerable lowest activities were recorded for the tested enzymes in fruits harvested 1, 3 and 7 days after preharvest treatments and stored for 7days at 5˚C.

Increases in the activity levels of pectolytic and cellulytic enzymes associated with fruit rots, incited by B. cinerea were reported by several investigators in strawberry (Berto *et al*., 2001, Brummell *et al*., 2002 and El-Habbaa, 2003).

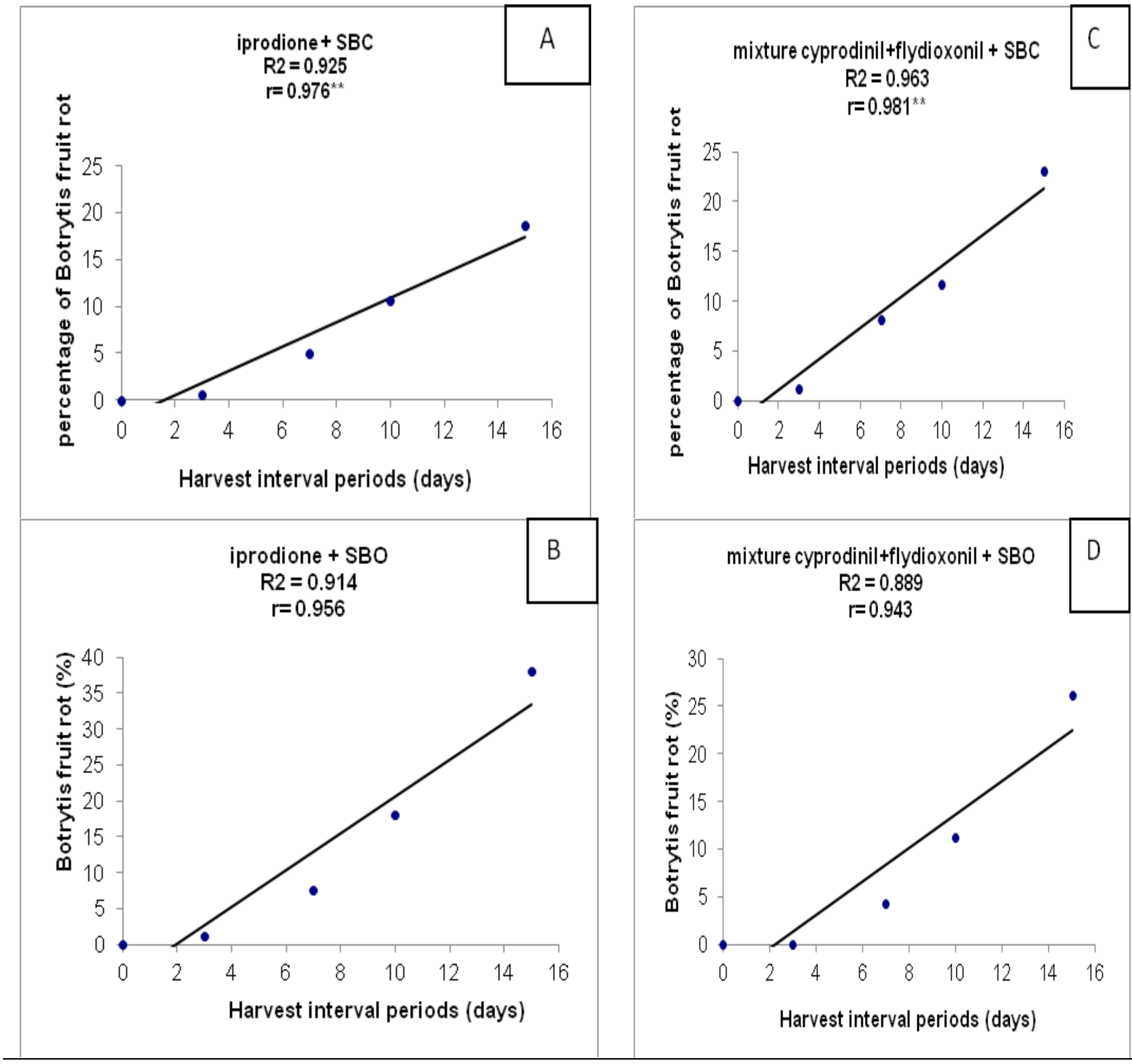


Figure 5: Correlation between harvest intervals, after treatments with two salts and iprodione and cyprodinil+fludioxonil fungicides, and percentage of infection with Botrytis fruit rot developed in strawberry fruits stored at 5 C for seven days

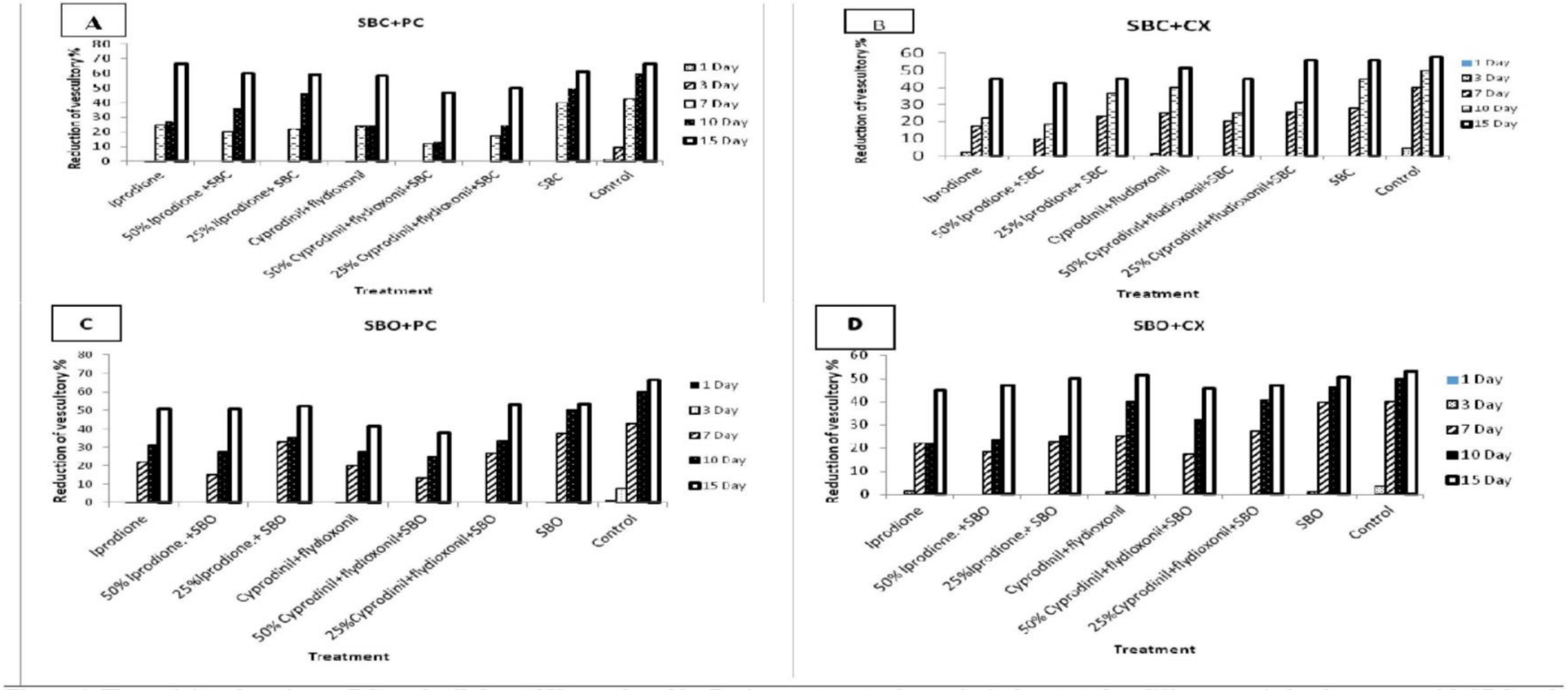


Figure 6: The activity of pectinase (PC) and cellulase (CX), produced by B. cinerea on strawberry fruits harvested at different periods after spray with SBC and SBO singly or mixing with fungicides and stored at 5°C for seven days

2.4. Degradation of fungicidal residues:

Data in Table (5) showed that residues of all tested fungicides in treated strawberry fruits decreased with decreasing percentage of the fungicide in the salt+fungicide mixture. cyprodinil 25% + SBO, and cyprodinil 25% + SBC treatments exhibited the lowest residue values being 1.420 and 1.680 mg/kg fruit one hour after treatment, while iprodione 25% + SBO, and iprodione 25% + SBC showed 8.430 and 9.650 mg/kg fruit, respectively. However, iprodione 100%, cyprodinil 100%, flydioxonil 100% showed the highest residue values being 29.810, 8.400, 7.300 mg/kg fruit, respectively. Also, residues decreased with increasing time after preharvest application with fungicides and storage for seven days at 5˚C where fruits harvested after 15 days and stored for 7 days at 5˚C showed the lowest residue values for all treatments. Generally, iprodione treatments showed the highest fungicidal residues, followed by cyprodinil and flydioxonil (Table 5 and figure 7). However, addition of any of the two salts to any of the tested fungicides did reduce the residue values compared to the single fungicide.

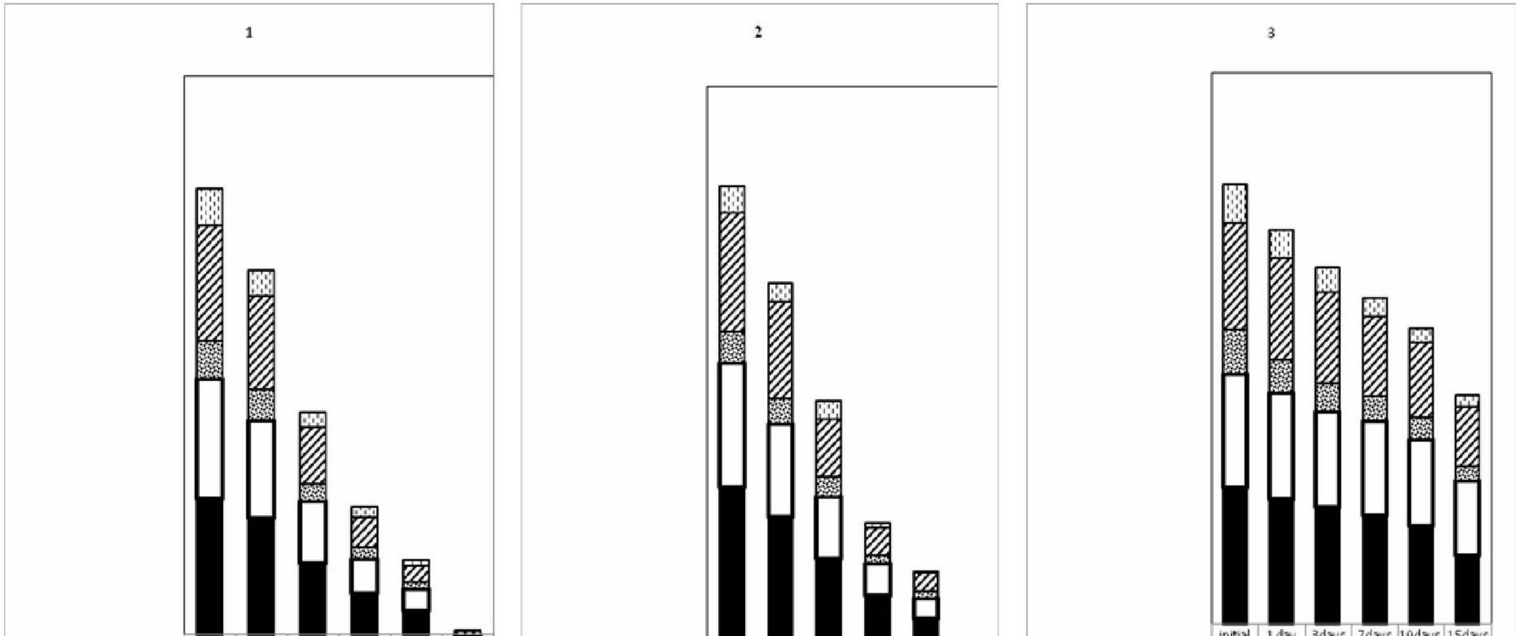
On the other hand, a strong negative correlations were revealed between time after treatment and residues in strawberry fruits of the three fungicides with r = 0.976, r = 0.949, and r = 0.946, for iprodione, cyprodinil, and flydioxonil, respectively (Figure 8). The obtained results are in agreement with Abada et.al (2005) as indicated that residues of the fungicides in the harvested fruits gradually decreased by increasing the intervals after spraying.

Consequently, as the use of fungicides is still an important component of any integrated pest management program (IPM) as the last resort, search for more effective fungicides at low concentrations and lower number of application with the aid of such salt applications is conducted and approved in the present study for a safer control of postharvest gray mold of strawberry caused by *B. cinerea.* However, effect of such salts on market proprieties of fruits and their physical and chemical characteristics and acidity is still in need for further studies indeed.

Table 5: Fungicide residues in strawberry fruits sprayed preharvest with flydioxonil, cyprodinil and iprodione fungicides individually or in combination with sodium bicarbonate or sodium benzoate and harvested at different intervals after treatment and stored at 5˚C for seven days.

Treatments	Residue in mg/kg fruits Time harvest after treatment (days)						
	initial	**1 day**	**3days**	**7days**	**10days**	**15days**	**Mean ±SE**
flydioxonil (100%)	7.300 de	6.200 cde	3.800 de	2.200 fg	1.300 f	0.050 e	3.475 ef±0.473
flydioxonil(50%) + SBC	6.440 e	5.270 de	3.360 e	1.850 f-i	1.120 f	0.042 e	3.014 f±0.413
Flydioxonil (25%) + SBC	2.040 f	1.670 f	0.950 f	0.640 g-j	0.377 f	0.014 e	0.949 g±0.129
flydioxonil(50%) SBO	6.160 e	5.010 e	3.020 e	1.610 f-j	0.920 f	0.038 e	2.793 f±0.400
flydioxonil(25%)+ SBO	2.010 f	1.360 f	0.820 f	0.530 h-j	0.260 f	0.003 e	0.831 g±0.124
cyprodinil (100%)	8.400 cd	6.800 cd	4.550 cde	2.560 ef	1.320 f	0.040 e	3.945 ef±0.540
cyprodinil (50%) + SBC	6.720 de	5.060 e	3.370 e	1.740 f-j	1.150 f	0.042 e	3.014 f±0.421
cyprodinil (25%) + SBC	1.680 f	1.360 f	1.070 f	0.440 ij	0.340 f	0.011 e	0.817 g±0.109
cyprodinil (50%) + SBO	6.410 e	5.210 de	3.120 e	1.530 f-j	1.040 f	0.030 e	2.890 f±0.417
cyprodinil (25%) + SBO	1.420 f	1.020 f	0.970 f	0.210 j	0.060 f	0.010 e	0.615 g±0.099
iprodione (100%)	29.810 a	27.130 a	25.410 a	23.670 a	21.220 a	15.020 b	23.710 a±0.861
iprodione (50%) + SBC	24.740 b	23.150 b	21.010 b	20.590 b	18.880 b	16.160 b	20.755 b±0.507
iprodione (25%) + SBC	9.650 c	7.330 c	6.020 c	5.320 d	4.920 d	3.150 d	6.065 d±0.371
iprodione (50%) + SBO	23.260 b	22.030 b	19.920 b	17.350 c	16.280 c	13.000 c	18.640 c±0.639
iprodione (25%) + SBO	8.430 cd	6.250 cde	5.330 cd	4.110 de	3.030 e	2.550 d	4.950 de±0.366
LSD 5%	1.737	1.644	1.592	1.557	1.449	1.218	1.627
Time mean ± SE	10.538[a] ± 3.150	9.12b ± 2.98	7.57c ± 2.88	6.25d ± 2.83	5.323e ± 2.628	3.809f ± 2.209	0.724

Values followed by different letter(S), for each parameter, are significantly different at 0.05 of probability.

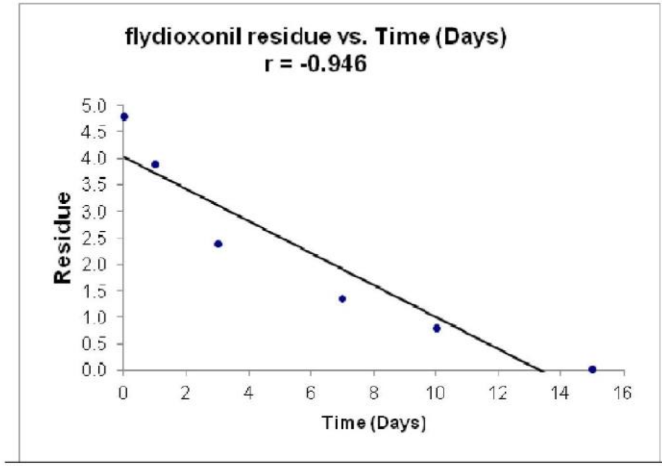


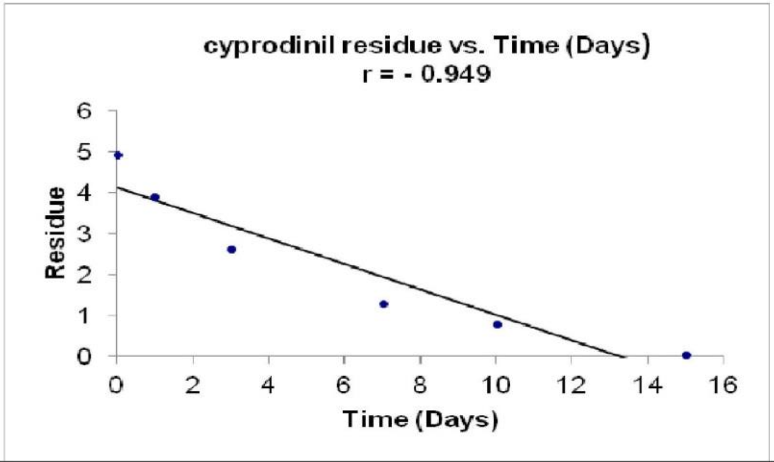
	initial	1 day	3days	7days	10days	15days
Flydioxonil (25%)+SBO	2.01	1.36	0.82	0.53	0.26	0.003
Flydioxonil (50%)+SBO	6.16	5.01	3.02	1.61	0.92	0.038
Flydioxonil (25%)+SBC	2.04	1.67	0.95	0.64	0.377	0.014
Flydioxonil(50%)+SBC	6.44	5.27	3.36	1.85	1.12	0.042
Flydioxonil (100%)	7.3	6.2	3.8	2.2	1.3	0.05

	initial	1 day	3days	7days	10days	15days
Cyprodinil (25%)+SBO	1.42	1.02	0.97	0.21	0.06	0.01
Cyprodinil (50%)+SBO	6.41	5.21	3.12	1.53	1.04	0.03
Cyprodinil (25%)+SBC	1.68	1.36	1.07	0.44	0.34	0.011
Cyprodinil (50%)+SBC	6.72	5.06	3.37	1.74	1.15	0.042
Cyprodinil (100%)	8.4	6.8	4.55	2.56	1.32	0.04

	initial	1 day	3days	7days	10days	15days
Iprodione (25%)+SBO	8.43	6.25	5.33	4.11	3.03	2.55
Iprodione (50%)+SBO	23.26	22.03	19.92	17.35	16.28	13
Iprodione (25%)+SBC	9.65	7.33	6.02	5.32	4.92	3.15
Iprodione (50%)+SBC	24.74	23.15	21.01	20.59	18.88	16.16
Iprodione (100%)	29.81	27.13	25.41	23.67	21.22	15.02

Figure 7: flydioxonil, cyprodinil and iprodione residues applied individually or in combination with sodium bicarbonate or sodium benzoate in treated strawberry plants at different harvest intervals.





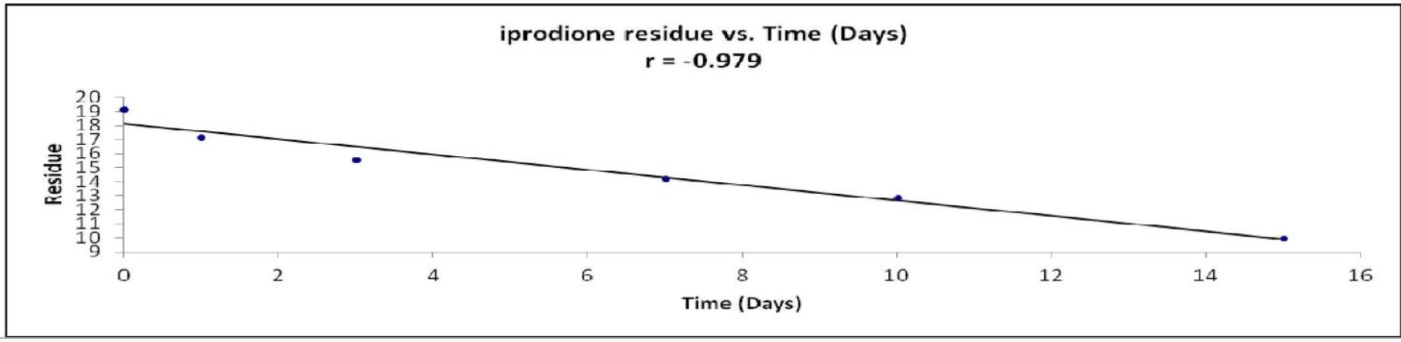


Figure 8: Relationships between residues mg/kg fruits and harvest interval periods of strawberry plants sprayed with three fungicides singly or mixed with SBC and SBO under field conditions.

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الملخص العربى

فاعليه المعامله ببعض المبيدات والأملاح لمكافحه عفن الثمار الرمادى(بوتريتس سينيريا) فى الفراولة

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[٣]قسم مبيدات الأفات – كليه الزراعه شبين الكوم – مصر

تم دراسه التأثير التثبيطى للمبيدين(iprodione and mixture of cyprodinil+flydioxonil) والملحين (بيكربونات وبنزوات الصوديوم) وذلك منفردين أو مخاليطهم فى المعمل وكذلك فى الحقل لمكافحه عفن الثمار الرمادى فى الفراولة. وجد أن كلا من المبيدين والملحين ومخاليطهم لهم تأثير مثبط على النمو الميسليومى للفطر بوتريتس سينريا. حيث حدث التثبيط الكامل للنمو الفطرى عند إستخدام الملح بيكربونات صوديوم عند التركيزات ١,٠٠ و٢,٠٠% جم / ١٠٠ مل ماء. وجد أن المبيد iprodione عند التركيز ٥٠٠ مليجرام/ لتر يحدث التثبيط الكامل للنمو الميسليومى للفطر حدث عند التركيز ١٠ مليجرام/لتر للمبيد cyprodinil+flydioxonil. وجد أن خلط المبيد iprodione بتركيز ٠,١ مليجرام/لترمع بنزوات الصوديوم بتركيز ٠,٢ جم/١٠٠ مل فى البيئه، حدث تثبيط كامل للنمو الفطرى. وكذلك وجد أنه عند خلط المبيد cyprodinil+flydioxonil بتركيز ٠,١ مليجرام/لتر مع الملح بيكربونات الصوديوم عند تركيز٠,٢ جم/١٠٠، حدث تثبيط كامل للنمو الميسليومى للفطر. وعلى العكس من ذلك وجد أن خلط المبيد cyprodinil+flydioxonil بتركيز ٠,٠٠١ مليجرام/لتر مع الملح بنزوات صوديوم بتركيز ٢,٠ جرام/١٠٠، حدث تثبيط كامل للنمو الميسليومى للفطر.

تم دراسة تأثير معامله الثمار تحت ظروف العدوى الطبيعيه فبل الحصاد بأملاح الصوديوم ٢% وبالمبيد الفطرى بالتركيزات المختلفه سواء كمعامله منفرده أو مختلطه وتأثير ذلك على فترات الحصاد المختلفه (١٥،١٠،٧،٣،١ يوم) بعد المعاملة والتخزين لمده ٧ أيام عند درجه ٥ ْم. وقد وجد أن أفضل المعاملات هى ٥٠% cyprodinil+flydioxonil+ بيكربونات الصوديوم ٢% حيث تقل النسبه المئوية لعفن الثمارالمتسبب عن الفطر البوتريتس بمتوسط ٤% مقارنه بالمعامله الكنترول ٢٣,٨٦%. يليها فى التأثير المعاملات ٢٥% iprodione +

بيكربونات الصوديوم ٢%، ٢٥% iprodione + بيكربونات الصوديوم ٢%، ٥٠% iprodione + بيكربونات الصوديوم ٢%، والمبيدين iprodione و cyprodinil+flydioxonil كمعامله منفرده بمتوسط نسبه إصابه بأعفان الثمار٧، ٨، ٨,٥، ٨,٥ و ٩ % على الترتيب كما وجد أن المعامله بالملح بيكربونات الصوديوم ٢% كمعامله منفرده سجلت أعلى نسبه إصابه وهى ١٢,٩٣%. ولقد وجد أنه توجدعلاقه إيجابيه عند معامل لإرتباط (r = ٠,٩٤٣ و٠,٩٨١) وهى تعكس العلاقه مابين نسبه الإصابه وفترات الحصاد عند التخزين لمده ٧ أيام عند درجه ٥ ْم. وكذلك وجد أن نشاط الانزيمين بكتينز وسليوليز مرتبط بزياده نسبه لإصابه بعفن الثمار. حيث وجد أن متبقى المبيدات المعامله بها ثمار الفراوله يقل بخلط المبيدات+الملح بالجرعات المختلفه. حيث وجد أن المعامة ٢٥% cyprodinil + بنزوات الصوديوم و٢٥% cyprodinil + بيكربونات صوديوم يقلل متوسط متبقى المبيد فى الثماربنسبه ١,٤٢٠ و١,٦٨٠ مليجم مبيد/كجم قبل حصاد الثمار بساعه مقارنه بالمعامله بالمبيد١٠٠% iprodion بالتركيز الموصى به حيث كان المتبقى بنسبه (٢٩,٨١ مليجم مبيد/كجم ثمار). وجد كذلك أنه توجد علاقه عكسيه بين تقليل متبقى المبيد فى الثمار وزياده الفترة الزمنيه قبل الحصاد بالمبيدات المستخدمة.