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Efficacy of some plant extracts on *Botrytis cinerea*, the causal of gray mould rot of strawberry fruits

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ABSTRACT

For controlling *B. cinerea* using plant extracts *in vitro*, all tested plant extracts concentrations were effective in inhibiting fungal growth in all experimental trials in petri dishes. Galls and Cinnamon extracts were more effective in inhibiting *B. cinerea* growth than the other plant extracts. *in vivo* experiments, all plant extracts with different concentrations which applied as dipping treatment decreased disease severity % (DS %) on wounded and unwounded strawberry fruits which inoculated with *B. cinerea* when compared with control treatment. the tested concentrations of Galls, Clove and cinnamon extracts were generally more effective than other extracts in controlling strawberry fruit rot infection. There were no significant differences between the four strawberry cultivars in disease index on the same level of each plant extract concentration. In general, application with Galls and Clove extracts on strawberry fruits either by spraying or dipping was effective in decreasing DS % when compared with control of wounded or un-wounded fruits. Controlling *B. cinerea* using *Trichoderma spp. in vitro, T. harzianum* (isolate 1), *T. hamatum* (isolate 1) and *T. hamatum* (isolate 2) were the most effective bio-agents in reducing the fungal growth. With regard to controlling strawberry fruit rot infection using *Trichoderma* spp. culture filtrates and fungicides *in vivo,* spraying the wounded and un-wounded strawberry fruits in or with three sterilized culture filtrates of three *Trichoderma* isolates pre-inoculation with *B. cinerea* decreased the strawberry fruit rot infection on the four-tested strawberry cultivars.

Key words: Strawberry fruit rot disease, *Botrytis cinerea*, Plant extracts, Biological control, Fungicides.

INTRODUCTION

Botrytis cinerea Pers.Fr., causes gray mould in multi types of fruits, vegetables, field crops and ornamental flowers. The disease symptoms could notice on infects leaves, stems, flowers and fruits (Elad and Shtienberg, 1995). This necrotrophic fungus causes gray mould disease on more than 200 dicotyledonous plant species (Williamson *et al.,* 2007), and produces serious food and ornamental crop losses, especially after harvest (Jarvis, 1980). Strawberry (*Fragaria ananassa* Duchesne) transplants, cultivated for annual winter production is an herbaceous perennial member of the rose family (*Rosaceae*). It is considered one of the most important and widely distributed vegetable crops in Egypt. It is one of the major vegetable winter crops in commercial fields in Egypt. However, during the last few decades efforts were concentrated to improve strawberry production and fruit quality under Egyptian conditions in protected system under plastic tunnels to get early yield for exportation as well as in open fields for local consumption. Strawberry as other vegetable crops is considered a major source of essential nutrients such as

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vitamins, minerals, carbohydrates, antioxidants and anti-carcinogenic substances, which are important to human nutrition and health (Joseph, 1994). Strawberry plants are subjected to the attack by several diseases which are responsible for considerable losses in fruit yield. These diseases are root rot, fusarium wilt, verticillium wilt, powdery mildew, leaf spots, gray mould and fruit rots (Paulus, 1990). Fruit rot diseases are economically very important which attack strawberry plants during flowering and fruit growth stages causing destructive effect on plants and fruits which responsible for the losses in fruit yield due to diseases infection (Droby and Lichter, 2004). Antimicrobial chemicals such as fungicides are often used in control of plant diseases, thus, because of increasing concerns about fungicides usage in food and environment. In the few decades, the scientists go to search about nonchemical approaches to postharvest disease control (Wilson *et al.,* 1987). There are many records that some plant species indicated pharmacological and biological activity such as antimicrobial activity and fungicidal properties depended on various plant products including oils, alkaloids, resin, saponin, organic acids and gums (Cowan, 1999).*Trichoderma* isolates well known for their ability to control plant pathogen infection. Various isolates of *Trichoderma*, including the commercial biological control products were effective in controlling gray mould (*Botrytis cinerea*) in strawberry (Sutton, 1995). To avoid the hazards of using the fungicides and minimize the environmental pollution as well as toxic affect during human consumption. The objectives of these studies were searching about alternative control method for reducing the use of fungicides was carried out using some plant extracts and their antifungal activities, as well as biological control agents against *Botrytis cinerea*, the causal agent of fruit rot of strawberry fruit.Chemical control using the specific fungicides was subjected in these studies as comparing study.

MATERIALS AND METHODS

Source of *Botrytis cinerea* as a causal pathogen of strawberry fruit rot disease:

Botrytis cinerea, the causal pathogen of strawberry fruit rot disease was isolated from diseased samples of naturally infected strawberry fruits showing rot symptoms which collected from one locality of strawberry fields (Shebin El-kom) in Minoufia governorate. The infection percentage was estimated as diseased fruits in relation to the total healthy ones. The strawberry fruit rot pathogen (*Botrytis cinerea*) isolates infecting strawberry fruits were isolated on Potato Dextrose Agar (PDA) medium. The resultant cultures were purified using the single spore culture or hyphal tip techniques according to Dhingra and Sinclair, (1985). The growing fungal colonies were transferred to slant tubes of PDA medium and then incubated for 7 days at 24ºC. The pure cultures of *Botrytis cinerea* isolates were examined microscopically and identified based on their morphological features at Agricultural Botany Department, Faculty of Agriculture, Minufiya University using the methods adopted by Neergard, (1945); Barnett, (1960) and Domsch *et al*., (1980) in addition to the key of imperfect fungi of Barnett and Hunter, (1972).

Source of *Trichoderma* isolates as antagonists:

Trichoderma fungi were isolated from soil and rhizosphere samples of grown strawberry in the previously mentioned fields by uprooting the infected plants with great care to obtain most of the intact root system. The dilution plate method (DPM) was used for isolation of *Trichoderma* spp. The isolated *Trichoderma* fungi were cultured onto 20% malt extract agar, incubated for two days at 25°C then, identified according to Rifai, (1969) and Bissett, (1991). Stock cultures of isolated *Trichoderma* spp. were maintained on PDA slants then kept in a refrigerator at 5°C and they repeatedly sub-cultured every 4 weeks on fresh PDA slants.

Preparation of *Trichoderma* culture filtrates:

Trichoderma culture filtrates were prepared by inoculating disk of the fungus onto liquid potato dextrose medium in flasks (100/200 ml), then incubated by shaking at 25°C for 3 days then incubated for 7 days. *Trichoderma* culture filtrates

were prepared by eliminating the mycelial mates, then the filtrates were centrifuged at 8000g for 10 min., and filtered through a Hydrophobic filter (type A/E, Gelman Sciences, Ann Arbor, M1) (Harman *et al.,* 2004).

Plants extract preparation:

In this study, different preparations of plant extracts were used. All powder plant materials involved in this study were collected and identified (English name, scientific name, Arabic name and used parts) in Table (1). Powders of six plant samples were used in this study. 100 gram of dry powder of each plant material was added to 1000 ml distilled water and mixed thoroughly then autoclaved with steam under pressure at 90° C for 1 hour. Three concentrations of each aqueous extracts *i.e*. 2.5, 5 and 10 % were used. The aqueous extracts were kept in dark glass bottled in refrigerator for further studies. (Metwally *et al.,* 2010) Used in 3 replicates for each concentration provide with 5 cm disc sprayed with plant extract. Another replicate for each concentration was left as control provided with clear disc sprayed with water.

Table (1): Plant materials used in aqueous extracts for control of cucumber fruit rot postharvest diseases.

Number	English name	Scintefic name	Used part
1	Galls	*Thuja standishii*	Buds
2	Clove	*Eugenia caryophyllus*	Buds
3	Mustard	*Brassica hirta*	Seeds
4	Visagna	*Ammi visnaga*	Fruits
5	Cinnamon	*Cinnamun zeylanicum*	Cortex
6	Marjoram	*Majorana hortensis*	Leaves

Using plant extracts *in vitro* to Control *B. cinerea*:

In this trail, the six plant extracts were used with 3ml of each one. They were applied at different concentrations *i.e*., 2.5, 5 and 10 %. The used plant extract were dropped onto the surface of poured solid PDA medium in petri dish (9cm), and then the drops were spread well till the complete absorption into media. The plates were inoculated with the inoculum disk (4mm) of the tested fungal pathogen at the center of the dish.

Using plant extracts *in vivo* to Control *B. cinerea*:

The six plant extracts were applied by dipping and spraying at different concentrations *i.e.* 2.5, 5 and 10 % on wounded (W) and un-wounded (UW) strawberry fruits. In case of dipping method, the fruits were soaked individually in the targeted concentration for five minutes, then raised and leaved for air drying. Then, the fruits (wounded and unwounded) were inoculated at the surface with an equal disk (4mm) of the pathogen and left in foam plates then covered with stretch film till appearance of fruit rot symptoms. The developed symptoms were daily investigated.

Using *Trichoderma* spp. *in vitro* to Control *B. cinerea:*

The antagonistic ability of tested six isolates of *Trichoderma* spp. was assessed against the pathogenic isolate *B. cinerea* that isolated from strawberry rotted fruits in dual culture according to the method described by Fokkema (1973). Three days old cultures of *Trichoderma harzianum* (2 isolates)*, T. hamatum* (2 isolates) and *T. viride* (2 isolates) were used as sources of antagonistic inocula. An equal disc of each one of tested Trichoderma isolates (4mmØ) was placed at 20 mm far from the edge of PDA plates (9 cmØ). A disc of pathogen was placed 50 mm away from the biocontrol fungal disc. Cultures were incubated in the dark at 25ºC until the growth of the pathogen covered completely the check plates. The biological control

agent inhibits the pathogen growth as a result of producing antagonistic metabolites, which decrease the rate of the pathogen growth.

The growth reduction percentage that pooled out was calculated using the following formula:

$$\% \text{ Growth reduction} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Controlling strawberry fruit rot infection using *Trichoderma* spp. and fungicides *in vivo:*

Three culture filtrates of tested *Trichoderma* isolates were assessed against the pathogenic isolate *B. cinerea* by one method (spraying) on wounded and un-wounded strawberry fruits. The treated fruits (wounded and unwounded) were inoculated on surface with disk of the pathogen inoculum and left in foam plates then covered with stretch film till appearance of fruit rot symptoms. The developed symptoms were daily investigated.

Two different fungicides i.e., Switch 62.5% WG (Cyprodinil-Fludioxonil) and Dichlofluanid (dichloro methyl sulfanyl) were used at three different concentrations *i.e.,* 100, 150 and 200 ppm based on their active ingredients for controlling the fruit rot infection on wounded and un-wounded strawberry fruits in case of spraying application only. Additionally, using results of the tested fungicidal treatments in comparison with those of plant extracts and Trichoderma treatments.

Disease assessment

Disease parameters were determined on rotted fruits according to the disease index rating which was made to determine the average diameter of the infected areas on fruit surface after seven days of inoculation. The following numerical rates were suggested to facilitate visual determination and to give a satisfactory comparison:

0 = No rot.

1 = Scattered small rot.

2 = Rots coalescing and including about 25-50 % fruit area.

3 = More than 50% of the fruit area was infected.

Readings were converted to disease index according to the equation suggested by (Townsend and Heuberger, 1943) as follows:

$$\text{Disease Severity \%} = \frac{\sum(n \times r1) + (n \times r2) + (n \times r3)}{3N \times 100}$$

Where (n) is the number of fruits in each numerical rate; r1, r2 and r ç are ratings and (N) is the total number of inoculated fruits multiplied by the maximum numerical rate 3.

Also the percentage of infected fruits was estimated.

RESULTS

Controlling *B. cinerea* using plant extracts *in vitro*:

Data in Table (2) indicate that all tested plant extracts concentrations were effective in inhibiting fungal growth of *B. cinerea* in all experimental trials in petri dishes. In this respect, the Galls and Cinnamon extracts were more effective in inhibiting the growth of *B. cinerea* than the other plant extracts. The highly effective concentration of all plant extracts *in vitro* was 10% while, the least effective concentration was 2.5%. On the other hand, all tested concentrations of plant extracts were effective in inhibiting the growth of *B. cinerea* compared with control treatment. There was a clear gradually increase in inhibiting the growth of *B. cinerea in vitro* for all plant extracts with increasing the tested concentrations.

Table (2): Effect of plant extracts in vitro on growth of *B. cinerea* the causal organism of strawberry fruit rot disease.

Plant extract	Cocentration %	Linear Growth (mm)			Mean
		T1	T2	T3	
Galls	2.5	44	43	39	42
	5	21	19	23	21
	10	12	12	9	11
Clove	2.5	48	51	50	49.7
	5	33	28	27	29.3
	10	20	22	23	21.7
Mustard	2.5	55	53	53	53.7
	5	48	45	44	45.7
	10	41	40	41	40.7
Visagna	2.5	80	82	80	80.7
	5	75	75	75	75
	10	61	66	70	65.7
Cinnamon	2.5	39	35	37	37
	5	25	23	20	22.7
	10	17	17	15	16.3
Marjorma	2.5	51	47	53	50.3
	5	41	40	41	40.7
	10	25	24	27	25.3
Conrtol		90	90	90	90

*T= Trial

Cotrolling strawberry fruit rot infection using plant extracts *in vivo*:

Data in Table (3) show that all plant extracts with different concentrations which applied as dipping treatment decreased disease severity% on wounded and unwounded strawberry fruits which inoculated with *B. cinerea* when compared with control treatment (fruits treated with pathogenic fungi only). In this respect, increasing the concentration of plant extracts decreased gradually the determined disease severity%. However, the tested concentrations of Galls, Clove and cinnamon were generally more effective than other extracts in controlling strawberry fruit rot infection. Also, no one of the four-tested strawberry cultivars exhibited clear resistant to fruit rot infection more than others with all plant extracts at all tested concentrations on wounded and un-wounded fruits. There were no significant differences between the four strawberry cultivars in disease index on the same level of each concentration of plant extract.

As for the sprayed strawberry fruits with the six plant extract types, data in Table (4) indicate that the sprayed strawberry fruits with Galls and Clove extracts were more effective in reducing the strawberry fruit rot infection for the un-wounded at all tested concentrations than the other plant extracts. The highest effective concentration of Galls extract in

reducing the fruit rot infection was 10%. The effect of Galls extract at 10% concentration was better on un-wounded fruits than wounded for all strawberry cultivars. In general, application with Galls and Clove extracts on strawberry fruits either by spraying or dipping was effective in decreasing DS% when compared with control of wounded or un-wounded fruits.

Table (3): Effect of plant extracts as dipping application on strawberry fruit rot infection caused by *B. cinerea* on wounded (W) and un-wounded (UW) strawberry fruits of different cvs.

		Cultivars / D.s %							
Plant extract	concentration %	Sana		Fertona		Floreda		Festival	
		W	UW	W	UW	W	UW	W	UW
Galls	2.5	29.2	22.3	33.4	21.4	34.5	20	32.7	23.1
	5	15.8	11.2	17.2	11.1	18.5	13	13.3	12.1
	10	0	0	0	0	0	0	0	0
Clove	2.5	33.1	20.4	35.2	25.1	32.1	24.2	32	21.9
	5	14.5	9.4	14.7	12.2	16.2	13.3	17.2	11.1
	10	0	0	1.1	0	1	0	0	0
Mustard	2.5	60.4	43.5	55.4	44.2	57.2	50	54.3	42.3
	5	40.1	32.1	42.2	29.2	39.1	33.5	45.5	30
	10	18.1	12.4	22.4	13.1	19.2	13.2	17.1	12.2
Visagna	2.5	69.8	56.1	66.4	49.2	65.5	53.6	68.8	51.4
	5	48.8	40.1	49.2	37.8	48.9	38.2	51.2	39
	10	33.2	17.5	29.4	19.2	31.6	16.3	32.1	15.7
Cinnamon	2.5	31.1	26.2	29.4	24.3	33.3	21.7	30	24.2
	5	19.5	14.1	17.5	14.1	21.2	12.7	19.4	13.2
	10	2.1	0	1	0	0	0	1.3	0
Marjoram	2.5	58.5	45.2	57.6	43.4	57.2	48.8	59	45.7
	5	40.2	35.7	39.9	33.2	41.4	33.7	42.1	34.2
	10	15.5	11.2	17.2	13.4	17.6	15.1	18.4	15.8
Conrtol		86.5	75.3	82.4	76.2	87.3	75.2	86.2	77.1

Table (4): Effect of plant extracts as spraying application on strawberry fruit rot infection caused by *B. cinerea* on wounded (W) and un-wounded (UW) strawberry fruits of different cvs.

Plant extract	concentration %	Cultivars / D.s %							
		Sana		Fertona		Floreda		Festival	
		W	UW	W	UW	W	UW	W	UW
Galls	2.5	38.6	28.4	24.6	27.6	43.6	26.4	41.8	29.8
	5	24.7	17.6	26.8	16.9	27.6	19.4	22.4	17.8
	10	8.3	0	6.2	0	7.2	0	7.8	0
Clove	2.5	42.1	26.4	43.9	30.9	41.2	31.5	41.1	24.9
	5	22.8	16.5	25	18.7	25.3	17.9	26.3	18.6
	10	11.3	2.1	11.6	3.5	9.9	3.5	11.6	2.3
Mustard	2.5	68.9	48.6	64.7	51.1	66.3	66.1	63.4	48.5
	5	49.3	38.7	50.8	35.4	48.2	39.8	54.6	35.7
	10	26.4	17.6	33.1	18.7	28.3	18.9	26.2	18.5
Visagna	2.5	78.6	62.7	74.3	54.6	74.6	60.1	77.9	65.9
	5	56.9	45.9	56.9	44.3	58	44.7	60.3	45.3
	10	42.8	23.7	38.5	25.5	40.7	23.5	41.2	22.4
Cinnamon	2.5	40.6	32.4	39.4	30.5	42.4	28.4	39.1	30.8
	5	27.9	20.8	25.8	20.9	30.3	19.3	28.5	18.5
	10	12.3	4.2	10.1	4.6	9.1	3.7	10.4	4.6
Marjoram	2.5	67.6	51.7	65.8	48.9	66.3	55.2	68.1	52.3
	5	49.6	42.1	49.3	38.7	50.5	40.3	51.2	40.6
	10	23.9	17.6	26.7	19.5	26.7	21.4	27.5	21.7
Conrtol		86.5	75.3	82.4	76.2	87.3	75.2	86.2	77.1

Severe damage can occur due to gray mould epidemics, because of these damage , the control of *B. cinerea* are based on multiple applications of fungicides during the flowering and fruiting periods. To avoid the hazards of using the fungicides and minimize the environmental pollution as well as toxic affect during human consumption, furthermore, the use of some synthetic chemicals to control fungal diseases is restricted due to their high toxicity, long degradation periods and environmental pollution. From these current study, the use of natural compounds as plant extracts may be an alternative to fungicides to control plant pathogens (Tsair-Bor & Shang-Tzen, 2008).

Controlling *B. cinerea* using *Trichoderma spp. in vitro*

Data in Table (5) indicate that *T. harzianum* (isolate 1), *T. hamatum* (isolate 1) and *T. hamatum* (isolate 2) were the most effective bio-agents in reducing the growth of *B. cinerea* comparing with others. On the other hand, the highest growth reduction% was recorded by *T. harzianum* (isolate 1) and *T. hamatum* (isolate 1) with clear inhibition zone and appearance of over their growth on the growth of *B. cinerea* followed by *T. hamatum* (isolate 2) which exhibited wide inhibition zone comparing with the two isolates of *T. viride* and *T. harzianum* (isolate 2) . *harzianum* (isolate 1) and *T. hamatum* (isolate 1) were the two isolates which exhibiting over growth on *B. cinerea* growth without appearance of inhibition zone.

Table (5): Effect of *Trichoderma* spp. on growth of *B. cinerea*, the causal organism of strawberry fruit rot *in vitro*.

Bioagents	*Botrytis cinerea*		Bio-interaction	
	linear growth (mm)	Growth reduction %	Over growth (mm)	Inhibition zone (mm)
Trichderma harzianum Iso.1	15	83.3	10	–
Trichderma harzianum Iso.2	27	70	–	13
Trichderma hamatum Iso.1	18	80	6	–
Trichderma hamatum Iso.2	22	75.6	–	4
Trichoderma viridi Iso.1	28	68.9	–	3
Trichoderma viridi Iso.2	32	64.4	–	6

Controlling strawberry fruit rot infection using *Trichoderma* spp. and fungicides *in vivo*

Data in Table (6) show that spraying the wounded and un-wounded strawberry fruits in or with three sterilized culture filtrates of three *Trichoderma* isolates pre-inoculation with *B. cinerea* in vivo affected greatly the strawberry fruit rot infection on the four-tested strawberry cvs. where the recorded DS% were lesser than those recorded with control treatment.

On the other hand,*T. harzianum* (isolate 1) and *T. hamatum* (isolate 1) treatment were the most effective one followed by *T. hamatum* (isolate 2) with the four-tested strawberry cvs. Furthermore, the least DS% was recorded on treated strawberry fruits cv. Florida on un-wounded and wounded fruits respectively. Whereas, the highest DS% was recorded in cv. Fertona with culture filtrates of by *T. hamatum* (isolate 2).

Data in Table (6) also show that the two tested fungicides with different concentrations as spraying treatment decreased clearly the determined DS% on wounded and unwounded strawberry fruits infected with *B. cinerea* comparing to control (inoculated fruits with *B. cinerea* only). Also, results indicate that increasing the concentration of each one of the tested fungicides decreased gray mold infection on treated fruits. The best concentration for use was 200 µg/L where the recorded DS% of Switch and Dichlofluanid fungicides was 0.0% on all wounded and un-wounded fruits of the four-tested strawberry cvs. except the wounded fruits for cultivars Floreda (1.1% and 1.1%) and Festival (1.3% and 0.9%) respectively.

Generally, spraying strawberry fruits with fungicides pre-inoculation with *B. cinerea* was more effective in controlling the strawberry fruit rot infection on un-wounded fruits than wounded ones. There were slight differences between the four strawberry cultivars in the determined DS% at the same level of each concentration .

Table (6): Effect of Trichoderma culture filtrates and fungicides as spraying applications on strawberry fruit rot infection caused by *B. cinerea* on wounded and unwounded fruits.

Bioagents / fungicides		Cultivars / D.s %							
		Sana		Fertona		Floreda		Festival	
		W	UW	W	UW	W	UW	W	UW
Trichderma harzianum Iso.1		68.8	45.3	63.3	41.2	59.7	40.1	65.7	44.7
Trichderma hamatum Iso.1		66.4	43.1	70.4	46.3	65.7	42.8	68.3	45.5
Trichderma hamatum Iso.2		70.2	49.6	71.2	49.4	69.8	47.2	69.5	48.7
Switch	100 (ppm)	22.4	13.5	21.8	12.7	28.2	13.7	29.5	13.6
	150 (ppm)	12.6	7.4	11.4	5.1	15.7	6.8	14.9	4.2
	200 (ppm)	0	0	0	0	1.1	0	1.3	0
Dichlofluanid	100 (ppm)	25.4	11.2	26.8	13.5	27.9	11.7	27.6	11.5
	150 (ppm)	11.6	5.2	14.7	5.5	16.3	5.3	17.5	5.2
	200 (ppm)	0	0	0	0	1.1	0	0.9	0
Conrtol		86.5	75.3	82.4	76.2	87.3	75.2	86.2	77.1

Sommer *et al.,*(1973) found that most Botrytis fruit rot that developed in cross-country shipments arose from quiescent infections initiated in the field before harvest, because of this , the effective control of this disease must begin in the field. Instead of spraying fungicides in the field preharvest, we recommended spraying plants before harvest fruits using plant extracts of Galls, Clove and cinnamon with 10% concentration, and/or culture filtrate of *T. harzianum* or *T. hamatum ,* its will be must effective in controlling this disease on fruits of strawberry.

Clove oil has been reported to possess fungicidal properties against several pathogenic fungi, such as *Botrytis cinerea* Antonov, *et al.,* (1997). These findings confirmed our results on plant extracts and their effect s on *B.cinerea.*

Trichoderma harzianum is an efficient biocontrol agent that is used against *Botrytis cinerea* under commercial conditions. The mechanisms have been suggested for the control of plant diseases by *Trichoderma* spp. Chet, (1987). Competition for space and nutrients is thought to control *B.cinerea* , and many other biocontrol actions .

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الملخص العربى

فعالية بعض المستخلصات النباتية على فطر البوترايتس سيناريا المسبب لمرض العفن الرمادى على ثمار الفراولة

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تمت هذه الدراسة على مقاومة *فطر Botrytis cinerea* بإستخدام المستخلصات النباتية معمليا ، حيث ثبت فعالية جميع التركيزات المختبره من المستخلصات النباتية وكان مستخلصى نباتى العفص والقرفه أكثر المستخلصات فعالية فى تثبيط نمو الفطر المختبر فى أطباق البترى وكان أكثر التركيزات فعالية 10% واقلها تأثيرا إثنان نصف % وإرتبط تأثير المستخلصات تدريجيا بزيادة تركيزها – وفى الحقل ثبت بإستخدام تقنية الغمر للثمار بالمستخلصات فعاليتها فى تقليل شدة الإصابة بالمرض سواء كانت الثمار سليمه أو مجروحة والتى حقنت بجراثيم الفطر المختبرمقارنة بالثمار المحقونة بجراثيم الفطروالغير معاملة بالمستخلصات النباتية . أيضا بزيادة تركيز المستخلصات النباتية المعامل بها ثمار الفراوله سواء السليمه او المجروحه زياده تدريجية لوحظ تقليل شدة الإصابة على الثمار تدريجيا وكان أكثر المستخلصات النباتية تأثيرا العفص والقرفه والقرنفل – وكان مستخلص العفص 10% أكثرها تأثيرا على الثمار السليمة والغير مجروحه أكثر منه على الثمار المجروحه. وكانت المعاملة بالمستخلصات النباتية فعاله فى مقاومة حدوث المرض سواء رشا على الثمارأو غمرا للثمار. وفى دراسة المقاومة للمرض بإستخدام راشح فطريات Trichoderma تم إختبار تأثير راشح تلك الفطريات تحت ظروف المعمل على نمو الفطر *B. cinerea* فى أطباق بترى , كانت العزلة (1) من فطر التضاد الحيوى *T. harzianum* والعزلتين (1 ،2) من الفطر *T. hamatum* أكثر عزلات الترايكودرما مقدرة على تثبيط نمو الفطر الممرض معمليا مع تواجد مناطق تثبيط النمو وظهور النمو الفوقى over growth وعند تطبيق معاملات الرش براشح فطريات التضاد الحيوى على الثمار فى الحقل ثبت فعالية تلك المعاملات فى تقليل شدة الإصابة على الثمار سواء كانت المعاملة بالرش أو بالغمر فى راشح فطريات التضاد الحيوى . وفى دراسة مقارنة على إستخدام المبيدات الفطرية (الموصى بها) تم إستخدام مبيدين هما سويتش وديكلوفلونيد فى ثلاث تركيزات هى 100 و 150 و 200 جزء فى المليون وقد ثبت من البحث أن أعلى التركيزات المستخدمة رشا على الثمار كانت مانعة تماما للإصابة ومثبطة لنمو الفطر الممرض على الثمار.