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Bio-Suppression of Strawberry Fruit Rot Disease Caused by *Botrytis cinerea*

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Abstract

In grade to obtain high quality and safety food along with the environment protection from pesticides hazards, four bio agents were used to control fruit rot disease caused by *Botrytis cinerea* in strawberry fruits. *Chlorella vulgris*, *Spirulina platensis*, *Azotobacter chroococcum*, *Trichoderma harizianum* and their combinations (T_1, T_2, T_3, T_4, T_{12}) were used as spray treatment in the open field and on strawberry after picking. The individual bio agents treatments decreased liner growth and spore production of *B. cinerea* in comparing with mixed bio agents. Also, all the treatments decreased Disease Severity% (DS%). The treatments of T_1, T_2, T_3 and T_4 caused 13.9, 22.3, 22.3 and 24.1 DS% during 2014; 12.26, 15.5, 17.0 and 21.86 DS% during 2015 in comparing with the mixed bio agents (T_{12}) which caused 29.3 and 29.03 DS% during 2014 and 2015, respect., after three sprayings in the field. Also, the treatments of T_1, T_2, T_3 and T_4 caused 0.00 DS% after 2nd spray through the storage at 5°C. The treatment of T_{12} caused the highest increase in the total sugar content in strawberry fruits which infected naturally with *B. cinerea* and stored at 5°C for three weeks increased with T_{12}. In addition, the treatment of T_{12} caused the highest increase in polyphenol oxidase (PPO) activity and peroxidase (PO) which were 0.459 and 0.360 U/mg fresh weight respect., in comparing with T_1, T_2, T_3 and T_4 which caused 0.278, 0.287, 0.298, 0.313 and 0.139, 0.202, 0.276, 0.302 U/mg fresh weight respect., after storage for 6 weeks. The treatment of T_1 gave the best result in K% in leaves (1.82). T_5 gave the best value of N% in soil (72.2%), T_3 gave the best one in P% in soil (36.0%) and T_4 caused the best amount of K% (16.0) in the soil.

Keywords: Bio agents; Strawberry; *Clorella sp; Spirulina sp*; *Azotobacter sp*; *Trichoderma sp*

Introduction

Strawberry (*Fragaria ananassa*) is one of the most widely grown small fruit crops in the world. Fungal diseases of strawberry, mainly caused by *Colletotrichum acutatum* and *Botrytis cinerea*, are responsible for severe economic losses [1,2]. Strawberry fruit have a very short postharvest life, gray mold caused by *B. cinerea* Infection may occur in the lower, remain quiescent until fruits mature, and then develop abundantly, causing fruit decay accompanied by profuse sporulation of the pathogen *B. cinerea* also causes significant losses during shipping and marketing making it one of the most economically important pathogens of strawberry [1].

Botrytis cinerea is an airborne and soil borne plant pathogen with anecrotophic life style attacking over 200 crop hosts worldwide over 200 mainly dicotyledonous plant species, including important protein, oil, fiber and horticultural crops, in temperate and subtropical regions. It can cause soft rotting of all aerial plant parts, and produce prolific grey conidiophores and (macro) conidia typical of disease [2] *Botrytis* fruit rot, also known as gray mold, caused by the fungus *B. cinerea* and is one of the most important diseases of strawberry worldwide. The disease affects fruit in the field resulting in severe pre-harvest losses. It also affects fruit after harvest, since infections that begin in the field continue to develop during storage and transit at refrigeration temperatures. Strawberry flowers are highly susceptible to *B. cinerea*, and may be blighted directly. However, symptoms usually are observed later on green and ripening fruit. Lesions typically develop on the stem end of the fruit and are often associated with infected stamens or dead petals adhering to the fruit or trapped beneath the calyx *B. cinerea* is a common colonizer of strawberry foliage in the nursery, and is also present on dying vegetation around strawberry fields. During the last 50 years, management of *B. cinerea* relieled heavily upon the use of synthetic chemicals [3].

Red and brown algae are mainly used as human healthy food sources, due to their high concentration in polysaccharides, natural richness in minerals, polyunsaturated fatty acids and vitamins. Macroalgae (seaweeds) are rich sources of structurally new and biologically active metabolites. In recent years, there have been many reports of macro algae derived compounds that have a broad range of biological activities, such as antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic and antimitotic activities Special attention has been reported for antiviral, antibacterial and/or antifungal activities against human pathogens and biostimulant properties of seaweeds are explored for use in agriculture [4-10]. Biochemical resistance through the accumulation of various phenolic compounds and phytoalexins and the activation of peroxidases, polyphenol oxidases and key enzymes in the phenylpropanoid and isoflavonoid pathways may play a crucial role in the biological control of and resistance to pathogenic attack in plants. The defense strategy of plants consists of two stages. The first stage is assumed to involve the rapid accumulation of phenols at the infection site, which function to slow the growth rate of the pathogen and to allow for the activation of "secondary" strategies that will more thoroughly restrict the pathogen. The secondary responses involve the activation of specific defense mechanisms, such as the synthesis of molecules related to pathogen stress [11]. However, it is still not easy and costly in application. It can serve as the best control

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measure under green-house conditions. The concern of pesticide use with respect to human health and environment has brought increasing interest in alternatives by avoiding negative effects on the environment. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market.

Trichoderma harzianum isolates were also reported to control strawberry grey mold [12]. Various isolates of *Trichoderma spp.*, that was effective in controlling anthracnose and grey mold in strawberry, under laboratory and greenhouse conditions [13,14]. It has been reported on various occasions that the combination of a number of biological control agents (BCAs) can further improve grey mold control [13,14]. Also, it was reported that co-application of T-39 of *T. harzianum* and AQ10 of *Ampelomyces quisqualis* resulted in improved control of grey mould in cucumber than with each organism alone [15].

Calcium is one of the main components in the plant cell wall and, it causes the cell wall solidity [16]. Calcium addition showed positive effects at fruit density and fruit nutrition value growth and crop yields [17,18]. Nitrogen assisted in amino acids and enzymes of the plant and this element participate at contain of the fruit taste and lignin. Also it effects on plant vegetative growth, leave and stems. Increasing of N causes yield enchasing, fruit size and TSS addition. Of course extra Nitrogen causes to decline the fruit density in ripening proses by function of cell wall dissolution enzymes [19].

Khafagi, [20] found that sugar contents increased in extract of strawberry fruit inoculated with *phytophthora cactorum* as compared with uninoculated fruits However, the least susceptible cultivar (Aliso) showed the lowest amount of reducing and total sugars as compared with the most susceptible one (Baugar). El-neshway [21] reported that sugar contents of strawberry extracts increased as a total and reducing sugars in the least susceptible cultivars. However, reverse trend was noticed with the non- reducing sugars. Generally, the amount of reducing and total sugars varied from one cultivar to another regardless of the infection percentage or level of susceptibility.

The present study is directed for testing some bio agents against strawberry fruit rot disease and its effect on N, P and K contents of strawberry leaves, the activities of polyphenol oxidase and peroxidase and fruits sugar content.

Materials and Methods

Source of fungal pathogen

In these experiments; four bio agents were used alone or as mixtures besides the fungicide (switch) and untreated one as controls. Diseased fruits of strawberry showing various types of rots collected from field (fresh planting system) at EI-Tahreer city regions. Fruit samples were rinsed several times in sterilized water and surface sterilized by using 70% ethyl alcohol for two minutes and dried between sterilized filter papers. They were then cut into small pieces and placed in petri- dishes containing potato dextrose agar medium (PDA). The dishes were incubated for 7 days at 25°C. Developed mycelium was transferred and kept on PDA slants.

Preparation of bio agents

Trichoderma harizianum: In order to isolate from healthy plants, it collected from different cultivated areas at Sadat City. Roots and stem bases of the obtained samples were washed tap water, surface sterilized by 70% ethanol and then left to dry on sterilized filter papers. The samples were cut into small pieces. Plated on PDA medium and incubated at 25°C. Petri dishes were examined daily. Isolates were purified and identified according to Barnett and Hunter [22]. The spores of 7 days old culture was collected and suspended in water and adjusted at 2×10^7 spore/ml water using haemocytometer, then applied as foliar spray at the rate of 5 ml/plant.

Azotobacter chroococcum: *Azotobacter chroococcum* isolate was grown on Jensen medium for 48 hrs in a rotary shaker at 25°C. The bacterial inoculum was applied as a foliar treatment at the rat of 5 ml bacterial suspension per plant (1×10^8 cfu/ml).

Algae extract: *Spirulina platensis* and *Chlorella vulgris* extract were obtained from International Research Center, Giza, Egypt as digested solution; the extract was suspended by the rate of 50 g/L water. The algal extract was applied as foliar spray at the rate of 5 ml/plant [23].

Determining liner growth of *Botrytis cinerea*

Effect of the tested antagonists on the mycelia growth of *B. cinerea* was carried out by inoculating PDA culture with 5 mm. discs of the tested pathogens against discs of the *T. harizianum* and against streak of the *Azotobacter* and algae, 6 cm apart. The tested antagonistic bio agents were inoculated at the same time with the pathogen. Four replicates were used for each treatment. Petri-dishes were kept at 20 ± 2°C until the mycelia growth covered the entire surface on the control plates, and the percentage of the mycelia growth inhibition was calculated as the following formula:

$$\text{Percentage of mycelia growth inhibition}=\frac{dc-dt}{dc}\times 100$$

Where:

dc=average diameter of fungal colony in the check.

dt=average diameter of fungal colony in the treatment.

Treatments

Four bio agents *i.e., Chlorella vulgris, Spirulina platensis, Azotobacter chroococcum*, *Trichoderma harizianum* and their combinations were applied in 12 treatments (from T_1 to T_2) in comparing with chemical control (T_{13}) and untreated control (T_{14}) as shown in Table 1.

Effect of tested bio agents on *Botrytis cinerea* spore production: After determining linear growth of the pathogen, spores were collected and transferred to sterilized water (50 ml/treatment). Spores were then counted by using haemocytometer and spore concentration/ml was determined according to the following equation:

Spore conc.=total spores count four squares × 2500 × dilution factor.

Filed experiment: Experiment was carried out at El-Tahreer city regions. Healthy strawberry transplants cv. Festival were used for fresh planting system. Treatments were mentioned in Table 1: (T_1, T_2, T_3, T_4, T_5, T_6 T_7, T_8, T_9, T_{10}, T_{11}, T_{12} T_{13}, T_{14}) and sprayed in January, 2014 and 2015 three times a week interval with four replicates; each replicate contained 50 plants. The percentage of fruit rot severity caused by *B. cinera* was estimated by the equation adopted by Hanounik.

$$\text{Disease severity } (DS)\% = \frac{\sum(NPC \times CR)}{NIP \times MSC} \times 100$$

Where:

CR= Class rate

NPC=No of plants in each class rate

NIP=No of infected fruits

MSC=Maximum severity class rate

Treatment	Codeessese
Chlorella vulgris	T_1
Spirulina platensis	T_2
Azotobacter chroococcum	T_3
Trichoderma harizianum	T_4
Trichoderma harizianum+Chlorella vulgris	T_5
Trichoderma harizianum+Spirulinaplatensis	T_6
Trichoderma harizianum+Azotobacter chroococcum	T_7
Azotobacter chroococcum+Chlorella vulgris	T_8
Azotobacter chroococcum+Spirulinaplatensis	T_9
Azotobacterchroococcum+Chlorellavulgris+Trichoderma harizianum	T_{10}
Azotobacter chroococcum+Spirulina platensis+Trichoderma harizianum	T_{11}
Azotobacter chroococcum+Chlorellavulgris+Spirulina platensis+Trichodermaharizianum	T_{12}
Chemical control (switch)	T_{13}
Untreated control	T_{14}

Table 1: The experimental treatments codes.

Effect of bio agents on the incidence of fruit rot in laboratory: Healthy mature fruits of cv. Festival were sprayed with different bio agents as follow:

1- Sprayed on laboratory.

2- Sprayed once only in the field.

3- Sprayed two times in the field, one week interval.

4- Sprayed three times in the field, one week interval.

All the previous treatments were collected after a week from the last spray and stored six weeks at 5°C.

Determination of sugar contents: Quantitative analysis of total, reducing and non- reducing sugars were determined, according to Tomas and Ducher [23] by using picric acid method.

The determinations were calculated as milligrams glucose per gram fresh weight.

Determination of nitrogen, phosphorous and potassium: Contents in strawberry leaves, soil, *Azotobacter chroococcum* and algae were determined in the central laboratory of soil fertility, Agriculture ministry, Giza, Egypt using the methods described by Awad [24] (Table 2).

Determination of enzymes activity: The enzymes activity of polyphenol oxidase and peroxidase were determined. Samples of 100 gm of strawberry of each treatment were blended in 200 ml distilled water for 2 min. The mixture was squeezed through several layers of cheese cloth and centrifuged at 3000 rpm for 20 minutes. Supernatants were kept at 5°C until assaying. The measurement of enzyme activity was carried out in the prepared filtrates by measuring according to the method of Aneja [25] using spectrophotometric procedure; as described by Matta and Dimond [26].

Results and Discussion

Data in Table 3 show that reduction in mycelia growth was noticed with all bio agents and the highest inhibition of *Botrytis cinerea* mycelia growth was exhibited in case of T_3 and T_1 followed by T_4 and T_2 with averages of 61.11, 58.30, 55.50 and 50.00 mm., respectively. Moreover, inhibition were moderately with the other mixed bio agents; T_5, T_9, T_6, $T_{11,}$ T_7 and T_8 Sporulation of *B. cinerea* showed significant reduction with all the tested bio agents (*Chlorella vulgaris*, *Spirulina platensis*, *Azotobacter chroococcum* and *Trichoderma harizianum*) alone or as mixture. Regardless of the sequence of application rates differed according to the potency of the tested bio agents and application sequence**.**

The highest reduction in sporulation of *B. cinerea* were obtained with T_1 treatment (2.2%), compared with those of the other tested bio agents, followed by T_3 and T_4 (3.3%).

In this respect, cyanobacteria (blue-green algae) and eukaryotic algae produce biologically active compounds that have antifungal activity antibiotic and toxic activity (Bonjouklian and Kiviranta [27,28]. Also Hussien [29] evaluated the effect of culture filtrates of nine algal strains at concentrations of 10, 20, 30, and 40% on mycelium growth and spore production of *Cercospora beticola* causing leaf spot disease in sugar beet. Generally, they found that all the algal culture filtrates reduced the fungal mycelium growth, but the best results were obtained by *Spirulina platensis*, *Oscillatoria* sp. and *Nostoc muscorum*; the highest fungal mycelium growth inhibition percentage was achieved by the concentrations of 30% (100, 100, and 82%, respectively.) and at 40% (100, 100, and 100%, respectively). Fungal spore production (number of spores) was completely inhibited by the previous three algal cultural filtrates particularly at the concentration of 40%. They conclude that the antifungal activity of the algal culture filtrates has been attributed to the presence of bioactive compounds, that is, total phenolic compounds, total saponins, and alkaloids in the algal culture filtrates. Also, several workers reported that the extracts of *Nostoc muscorum* significantly inhibited the growth of *Candida albicans* and *Sclerotinia sclerotiorum* and [30] *a*Bis (2, 3-dibromo-4, 5-dihydroxybenzyl) ether (BDDE) is a bromophenol isolated from marine algae which possesses cytotoxic and antibacterial activity. BDDE inhibits the growth of *B. cinerea* cultured on a solid medium

Bio agents	N%	P%	K%
Chlorella vulgaris	1.62	0.39	3.96
Spirulina platensis	1.17	0.19	2.09
Azotobacter chroococcum	3.2	0.28	2.25

Table 2: Nitrogen, phosphorous and potassium contest of some bio agents contents.

Treatment	Linear growth (mm)		Spores production x10^3
	Reduction	Mycelia growth (mm)	
T_1	58.3	37.5	2.2
T_2	50.1	45.0	4.5
T_3	61.1	35.0	3.3
T_4	55.5	40.0	3.3
T_5	25.0	67.5	5.5
T_6	23.3	72.5	5.5
T_7	22.2	70.0	6.1
T_8	20.5	71.5	6.2
T_9	25.0	67.5	5.5
T_{10}	30.5	62.5	13.6
T_{11}	23.3	72.5	14.0
T_{12}	19.4	74.0	14.9
Switch (2200 ppm)	100.0	0.0	0.0
Untreated Control t	0.0	90.0	100
L.S.D at 5%	0.23		0.60

Table 3: The effect of different bio agents on the liner growth and spores production of *B. cinerea*.

of potato dextrose agar (PDA) as well as on the potato dextrose broth (PDB) medium. Further studies have revealed that BDDE decreases the germination rate and inhibits the mycelia growth of *B. cinerea.* The inhibition mechanisms are related to the disruption of the cell membrane integrity in *B. cinerea* spores and newly formed germ tubes. *Spirulina platensis* – green algae (*Chlorophyta*); *Fucus vesiculosus* , inhibited the mycelia growth *Botrytis cinerea* Bc 2107 with at 50% 0.5% and 90% at 2.02% algae the presence of bioactive molecules, as phenolic compounds (phlorotannins, terpenes, alkaloids), polysaccharides or fatty acids, many of these structures being identified as antimicrobials [31]. *Azotobacter* is able to inhibit the growth of phytopathogenic fungi species such as *Alternaria*, *Venturia*, *Sclerotinia*, *Rhizoctonia*, and *Pythium* [32]. The isolates of *Trichoderma* which chose possessed variety of potential modes of action *in vitro* [33]. Ap-PCR indicated that isolate T-105 survived better than the other isolates in the mix [34].

Data presented in Table 4 indicate that percentage of disease severity of fruit rot decreased by spraying strawberry fruits with all the different bio agents in field and laboratory after cold storage at 5°C for six weeks. Results showed that the lowest disease severity were (0.0) with (T_1, T_2, T_3, and T_4) with two, three and four spray treatments compared with untreated control (100%), where at the first treatment, disease severity were 0.3, 0.6, 0.3 and 1.6 with T_1, T_2, T_3, and T_4, respectively. Therefore, the highest reduction rate (40.3%) was obtained on spraying strawberry fruits, growing on field with mixed bio agents (T_{12}) and stored at 5°C for six weeks.

These compounds could be environmentally friend means of plant disease control and could be utilized in organic farming and for vegetable cropping systems where application of synthetic fungicides or chemicals needs to be avoided [24]. Antifungal compounds have been detected in different algal species. For example, extracts of the brown alga *Cystoseira tamariscifolia* showed *In Vitro* fungal activity against the plant pathogens *B. cinerea* , *F oxysporum* and *Verticillium album-atrum* [35].

Application of biological control using antagonistic microorganisms has proved to be successful for controlling various plant diseases [36]. Recently algae are one of the chief biological agents that have been studied for the control of plant pathogenic fungi [37].

Data in Table 5 show the effect of sprayed strawberry by some bio agents on the disease severity% (DS%) of fruit rot under field conductions. It is clear that at the first season (2014) T_1, T_7, T_9, T_2 and T_3 had the best effect in reducing DS% on the other hand the same treatment was cleared in the second season (2015) with light differences in arrangement where T_1 had the first grade followed by T_{10}, T_2, T_3 and T_7, respect., and generally the fungicide Switch decreased DS% more than any other treatment. Generally disease severity decreased when sprayed strawberry fruity under filed conditions by *Clorella vulgaris* (T_1) compared with (T_2, T_5, T_6, T_8, and T_9) during (2014&2015). The results indicated that the application of bio agents (T_1, T_2, T_3 and T_7) decreased disease severity during season 2014 and 2015 control.

This result is in agreement with Edra et al., [38]. who found that the brown-alga *Lessonia trabeculata* inhibited bacterial growth and reduced both the number and size of the necrotic lesions in tomato leaves following infection with *Botrytis cinerea.* Marine algae represent a great source of a vast variety of complex natural products and could be a promising source of a novel bioactive compound that can help plant survival by offering protection against stress imposed by pathogens. Aqueous and ethanolic extracts from the red-alga *Gracillaria chilensis* prevent the growth of *Phytophthora cinnamomi.* Similarly, aqueous and ethanolic extracts from the brown-alga *Durvillaea antarctica* were able to diminish the damage caused by tobacco mosaic virus (TMV) in tobacco leaves. These results suggest that macro-algae contain compounds with different chemical properties which could be considered for controlling specific plant pathogens. On the other hand, *Trichoderma* isolates, as well as T-39 and TRICHODEX were used effectively, for the control of *Colletotrichum* and *Botrytis* on strawberry petioles and leaves [14]. Isolates T-115, T-161, T-166, T-39 and TRICHODEX were effective in reducing *Botrytis* leaf grey mould incidence whereas T-118, T-165, T-166, T-39 and TRICHODEX were effective in reducing anthracnose in petioles [14].

Data in Table 6 summarize the amounts of reducing, non-reducing and total sugars in naturally infected fruits with *Botrytis cinerea* and treated by different bio agents, after 1, 2, 3 weeks of storage at 25°C. Reducing sugars increased with increasing incubation periods from one to three weeks. The highest value of reducing sugar treated with mixed

Treatment	Disease severity%			
	1st spray*	2nd spray**	3rd spray**	4th spray**
T_1	0.3	0.0	0.0	0.0
T_2	0.6	0.0	0.0	0.0
T_3	0.3	0.0	0.0	0.0
T_4	1.6	0.0	0.0	0.0
T_5	24.0	20.0	9.0	3.0
T_6	22.3	14.3	8.6	2.6
T_7	27.3	13.0	7.6	3.3
T_8	19.9	12.6	9.0	6.6
T_9	19.3	13.6	6.7	2.6
T_{10}	36.6	20.8	13.0	8.2
T_{11}	33.6	19.7	13.5	7.0
T_{12}	40.3	25.0	20	9.0
Switch (T_{13})	0.0	0.0	0.0	0.0
Untreated control (T_{14})	100	100	100	100
L.S.D at 5%	0.48	0.47	0.47	0.46

* In the Lab.
**In the field.

Table 4: Effect of some bio agents on disease severity percentage of strawberry fruits after storage for six weeks at 5°C.

Treatment	Disease severity							
	2014				2015			
	1st spray	2nd spray	3rd spray	Mean	1st spray	2nd spray	3rd Spray	Mean
T_1	19.6	13.3	9.0	13.9	17.5	12.3	7.0	12.2
T_2	21.0	18.0	11.6	22.3	20.0	17.0	9.6	15.5
T_3	32.0	19.0	16.0	22.3	19.0	18.0	14.0	17.0
T_4	35.3	20.3	17.0	24.1	30.3	20.3	15.0	21.8
T_5	38.3	23.6	20.0	27.3	38.3	22.6	19.0	26.6
T_6	28.3	32.6	16.3	25.7	26.3	30.6	15.3	24.6
T_7	23.8	23.0	15.3	20.0	22.6	20.0	14.3	18.9
T_8	29.0	27.0	20.3	25.4	28.0	26.6	19.3	24.0
T_9	23.3	22.0	17.3	20.8	22.6	21.0	16.3	19.9
T_{10}	35.3	23.6	20.6	26.4	18.3	15.6	12.6	15.4
T_{11}	40.3	29.0	20.6	29.9	39.6	28.0	18.3	28.6
T_{12}	41.6	27.0	19.3	29.3	40.0	26.6	20.5	29.0
Switch (T_{13})	13.6	8.6	5.3	9.16	13.6	7.6	4.3	8.5
Untreated control (T_{14})	55.7	53.3	54.0	54.3	56.o	53.3	50.0	53.1
L.S.D at 5%	0.58	0.92	1.29		0.60	0.95	1.30	

Table 5: Effect of some bio agents on disease severity percentage (DS%) of Strawberry fruits in field experiment during two successive seasons 2014 and 2015.

Treatment	Sugar content (mg/g fresh weight of fruit)								
	1 week			2 weeks			3 weeks		
	R	NR	T	R	NR	T	R	NR	T
T1	73.1	11.6	84.7	83.5	11.7	95.2	93.2	11.7	210.2
T2	88.3	11.7	100	93.5	21.4	114.9	93.6	26.6	120.2
T3	86.3	12.1	98.4	92.5	20.4	112.9	92.6	25.5	118.1
T4	86.3	12.1	98.4	92.3	27.3	119.6	104.2	22.5	126.4
T5	93.2	22.2	115.4	98.4	27.3	125.7	100.1	27.3	127.4
T6	93.1	21.3	114.4	98.1	31.2	129.3	104	27.2	131.2
T7	94.4	22.5	116.9	99.3	31.2	130.5	108	32.4	140.4
T8	98.2	27.0	125.2	102.1	46.5	148.6	109	46.4	155.4
T9	99.3	27.6	126.9	104.0	51.5	155.5	118.5	47.2	165.7
T10	102.1	31.2	133.3	116.4	67.4	183.4	128.1	67.4	195.5
T11	109.8	50.4	160.2	114.9	77.3	192.2	134.8	82.6	217.4
T12	118.7	62.3	181	120.2	83.2	203.4	138.7	95.6	234.3
Switch(T_{13})	73.1	11.6	84.7	78.7	21.2	99.9	93.1	11.7	104.8
Untreated control(T_{14})	128.1	64.3	192.4	130.3	85.2	215.5	140.3	97.5	237.8
L.S.D at 5%	0.49	0.54	0.60	0.49	0.59`	0.44	0.57	0.4	0.59

R: Reducing sugars; NR: Non-reducing sugars T: Total sugars.

Table 6: Sugar content mg/g fresh weight of strawberry fruits and naturally infected with *B. cinerae* treated with different bioaganets after storage one, two and three weeks.

Treatment	Polyphenoloxidas (U/mg fresh weigh)			Peroxides(U/mg fresh weigh)		
	1st spray	2nd spray	3rd spray	1st spray	2nd spray	3rd spray
T1	0. 278	0.309	0.320	0. 139	0. 231	0. 267
T2	0. 287	0.310	0.325	0. 202	0. 305	0. 325
T3	0. 298	0.314	0.330	0. 276	0. 312	0.368
T4	0. 313	0.330	0.350	0. 302	0. 313	0.370
T5	0. 325	0.452	0.520	0. 304	0. 345	0.375
T6	0.367	0.509	0.624	0.314	0. 391	0.400
T7	0. 375	0.596	0.600	0. 326	0.401	0.435
T8	0.329	0.620	0.630	0. 335	0. 423	0.450
T9	0. 363	0.724	0.730	0. 345	0. 444	0.455
T10	0.379	0.858	0.860	0.350	0. 445	0.456
T11	0. 380	0.870	0.880	0. 358	0.488	0.470
T12	0. 459	0.897	0.910	0.360	0.490	0.475
Switch(T_{13})	0. 220	0.310	0.310	0.150	0. 228	0.270
Untreated control(T_{14})l	0. 410	0.911	0.920	0.360	0.495	0.500
L.S.D at 5%	0.012	0.003	0.82	0.45	0.04	0.29

Table 7: Effect of different bio agents on fruits Polyphenol oxidase and Peroxidase activities in treated strawberry fruits after storage six weeks at 5°C.

bio agents treatment (T_{12}) after storage periods 1, 2 and 3 weeks with averages 118.7, 123.0 and 138.7 mg/g fresh weight of fruit, respectively. Generally, reducing, non-reducing and total sugars increased sharply in case of treatments T_{10}, T_{11} and T_{12} compared with the other treatments but the treatment (T_{14}) had the highest values.

Early studies have reported that germination in most *B. cinerea* isolates is dependend on the presence of nutrients, mainly sugars, and that this process can be stimulated by exudates from mature fruits and [39-41]. Fouri and Holzm showed that in *B. cinerea* fructose at micro molar concentration was a more potent germination inducer than glucose. Glucose, fructose and sucrose were the better inducers of germination.

Data presented in Table 7 indicate that the enzyme activities increased with treatments of mixed bio agents. The least activities of Polyphenol oxidase and Peroxidase cleared with T_1, T_2, T_3 and T_4 after spraying three times with different bio agents and stored for six weeks under 5°C. At the same time treatment with the fungicide (switch) led to decrease in the enzymes activity, in contrast control treatment had the highest values. Generally, mixing the bio agents resulted excess in the activity for both enzymes compared to the treatment separately. Disease reduction was accompanied with a gradual increase in peroxidase activity during experiment period (Table 7).

The compared works showed that applying chemical inducers resulted in a gradual increase in peroxidase activity in *faba bean* plants pre-sprayed and inoculated with *Botrytis fabae* or *Botrytis cinerea* during examination periods. Increases in peroxidase activities were higher compared with untreated inoculated plants (control). Maximum increase in peroxidase activity was detected after 24 h. in *B. fabae* and 48 h. in *B. cinerea* , resulted in remarkable increase in disease reduction resulted from the inoculation of both pathogens [42]. Also Vance et al. [43] and Fry, [44]. Fry stated that peroxidase is known to be involved in the oxidation of polymerization of hydroxycinnamyl alcohols to yield lignin and crosslinking isodityrosine bridges in cell wall. Also, Ride [45] and Tarrad [46] reported that increase in peroxidase activity enhance lignification in response to chocolate spot infection which may restrict the fungal penetration. These findings indicate a positive relationship between resistance and peroxidase activity. Peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms [47] Another supportive suggestion was brought by Nawar and Kuti [48] who stated that an increase in peroxidase activity is considered as a preliminary indicator for. Laccase (PPO), a fungal

polyphenol oxidase from *Botrytis cinerea*, has been widely studied in relation to its effect on the composition and quality of must and wine [49-51]. In particular, PPO has been shown to be the main enzyme involved in oxidation of red and white wines from rotten grapes. This involves oxidation at all stages the winemaking process, ageing, and while stored in bottles. This is largely because PPO is ubiquitous enzyme in the plant world, requiring a polyphenol substrate, and is well adapted to the physicochemical conditions of wine (pH, ethanol, SO_2) [52-55]. Peroxidase also functions in the metabolism of reactive oxygen species (ROS) and reactive nitrogen species (RNS), thus activating the hypersensitive response (HR), a form of programmed cell death at the infection site that is associated with limiting pathogen development [56]. Peroxidase is thought to be involved in the polymerization of phenolic monomers to generate the aromatic matrix of suberin. In addition, polyphenol oxidase and peroxidase are activated by pathogens, resulting in the oxidation of phenolics to form quinones, which are effective inhibitors of phytopathogens. In addition, a large number of toxic phytoalexins can be derived from phenolic compounds. Peroxidases, polyphenol oxidases and key enzymes in the phenylpropanoid and isoflavonoid pathways may play a crucial role in the biological control of and resistance to pathogenic attack in plants. The defense strategy of plants consists of two stages. The first stage is assumed to involve the rapid accumulation of phenols at the infection site, which function to slow the growth rate of the pathogen and to allow for the activation of "secondary" strategies that will more thoroughly restrict the pathogen. The secondary responses involve the activation of specific defense mechanisms, such as the synthesis of molecules related to pathogen stress [12].

Nitrogen, phosphorous and potassium contents were determined in soil, strawberry leaves and algae extracts (*Chlorella vulgaris and Spirulina platensis*) and *Azotobacter chroococcum* after spraying strawberry plants with different bio agents. Data presented in Table 8 cleared that, N content in strawberry leaves increased in the treatments T_3, T_4, T_{12} andT_{11} compared to the other treatments except T_{13} (Fungicide switch) which had the highest value (1.41%). From the same table, P content showed an increase with the treatments especially in case of T_{12}, T_{10}, T_1, T_5 and T_6 with the average of 0.35, 0.35, 0.34, 0.32 and 0.32% respectively. In this respect K content increased with the treatments T_1, T_2, T_3, T_7 and T_8 with averages of 1.82, 1.79, 1.77, 1.69 and 1.62%, respectively. Generally, leaves, algae, *Azotobacter* and Soil contented of calcium is the highest than nitrogen and phosphorous. Data in Table 8 indicate that soil content of nitrogen, phosphorous and calcium is the highest compared with leaves, algae and *Azotobacter* contents of nitrogen, phosphorous and potassium caused by the highest fertilization the Soil. When compared leaves, algae, *Azotobacter* and soil contents of potassium noted that leaves contents of potassium is agree with algae and *Azotobacter* contents.

Treatment	In Leaves of strawberry			In soil		
	N%	P%	K%	N%	P%	K%
T_1	1.21	0.34	1. 82	27.7	15.0	112.0
T_2	1.31	0.32	1. 79	37.8	20.0	120.0
T_3	1.41	026	1. 77	36.1	36.0	120.0
T_4	1.33	0.29	1. 54	34.4	10.0	160.0
T_5	1.32	0.32	1. 51	72.2	8.0	128.0
T_6	1.24	0.32	1.44	47.0	8.0	72.0
T_7	1.17	0.30	1.69	42.0	10.0	88.0
T_8	1.23	0.31	1. 62	61.3	9.0	96.0
T_9	1.24	0.28	1.45	56.3	8.0	80.0
T_{10}	1.30	0.35	1. 46	52.1	8.0	80.0
T_{11}	1.32	0.29	1.47	60.5	9.0	88.0
T_{12}	1.33	0.35	1.43	50.0	10.0	80.0
Switch(T_{13})	1.41	0.27	1.62	27.7	15.0	112.0
Untreated control(T_{14})l	1.28	0.38	1.54	37.8	20.0	120.0
L.S.D at 5%	0.49	0.01	0.54	0.63	0.57	0.54

Table 8: Effect of some bio-agents on NPK% contents of Strawberry after harvesting.

Therefor algae and *Azotobacter* contents of potassium decreased strawberry fruit rot, when nitrogen is deficit in plants it inhibits the synthesis of 6-phosphogluconate and glucose-6-phosphate dehydrogenase and enhances the synthesis and builds up of phenolic compounds which gives rise browning of curds. In the other hand Eman and Abd-Allahwhen, found that using algal extract at concentrations above 50% caused a progressive increase in N, P and K% in the leaves which was observed as a result of increasing concentration of algal extract till 50% while with concentrations above 50% the increase was slightly appeared.

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