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Using Some Natural Substrates to Enhance the Growth, Yield and the Tolerance of Strawberry Plants to Rhizoctonia Root Rot Disease

S. M. Rizk[1] and Maha H. Mohamed[2]

[1]Potato and Vegetatively Propagated Vegetables Res. Dept., Hort. Inst., Agric. Res. Centre, Giza, Egypt.
[2]Plant Pathology Dept., Fac. Agric., Ain Shams Univ., Cairo, Egypt

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ABSTRACT

The experiments were carried out during 2019/2020 and 2020/2021 seasons. Greenhouse experiments carried out at Department of Plant pathology, Faculty of Agriculture, Ain Shams University. While, the field experiments were carried out at El-Kanater Research Station, Qaluobia Governorate, to study the effect of some natural compounds (chitosan, methionine, and ascorbic acid) on the tolerance of strawberry plants to Rhizoctonia root rot disease, as well as the vegetative growth, chemical composition of the plant, fruit yield and its components, as well as the quality of the yield of strawberry Festival Cultivar compared to untreated infected strawberry plants.

Obtained results showed that applications with chitosan at 500 ppm, methionine at 20 ppm and ascorbic acid at 20 mM via transplant root soaking, foliar spray, and combining the root soaking with foliar spray, or transplant root soaking with Rhizolex (1g/L) showed significant decrease in root rot incidence caused by *Rhizoctonia solani*. The combining of root soaking with foliar spray of chitosan or methionine resulted the highest significant decreases of disease incidence under greenhouse and field conditions. However, all applications except Rhizolex resulted in significant increases in phenolic compounds, oxidative enzymes activity *i.e.,* PPO, SOD and CAT in roots during the two growing seasons.

Obtained results showed also that soaking plus foliar spraying with chitosan at 500ppm, methionine at 20 ppm and ascorbic acid at 20 mM significantly increased all vegetative growth characters, N, P, K and total carbohydrates in plant foliage, fruit yield and its components as well as physical and chemical fruit quality.

KEYWORDS: Strawberry, *Rhizoctonia solani*, Chitosan, Methionine, Ascorbic acid, Antioxidant enzymes, Growth, Yield.

1. INTRODUCTION

Strawberry (*Fragaria X anannasa* Duch.) is one of the most important vegetable crops in Egypt for fresh consumption, processing and exportation. Strawberry plant is affected by many soil borne fungi causing root rot disease. *Rhizoctonia* spp. are among them that causing sever root rot symptoms. The disease cause a deterioration of the main root system, declines the vigor and productivity of the plant causing reduction in the yield (Awad 2016; Errifi *et al.*, 2019).

Several new natural substrates have an activity on the plant diseases control. Such materials also play an important role in improving plant growth characteristics and crop productivity.

Chitosan and its derivatives have been studied as an antimicrobial against a wide range of microorganisms including bacteria and fungi (Liman *et al.,* 2011). However, several studies have proved the preventing effectiveness of chitosan against fungal growth by directly interfering or by activating certain vitality processes (El Ghaouth *et al.,* 1992a and b). Chitosan is also known to be eliciting the plant defense responses by activating pathogenesis-related gene functions such as chitosanase, chitinase and β-glucanase (Mauch *et al*., 1984; Benhamou and Theriault, 1992; Notsu *et al*., 1994).

Numerous studies have reported the ability of chitosan to increase plant growth (height, leaf number, fresh and dry weight), yield components (fruit number/plant, fruit weight and total yield) and plant contents of N, P and K in different plant species cultivated under diverse growth conditions on strawberry El-Miniawy *et al*. (2013), Shams *et al.* (2014), Nithin *et al*. (2020) and Nithin *et al.* (2020).

Shafshak *et al*. (2011), Manal *et al*., (2019) and Fatma and Hanaa (2020) found that amino acids increased vegetative growth and chemical constituents of strawberry plant foliage. However, L-Methionine is a precursor of ethylene and growth factors such as spermine and spermidine (Singh, 1999). It also has a role in the revitalization of rooting.

Previous studies revealed that amino acids play an effective role in inducing plant systemic resistance against fungal plant diseases (Kadotani *et al.,* 2016). Foliar spray with different concentrations (0, 5, 10, 25, and 50 mM) of L-arginine, L-methionine, L-ornithine decreased the severity of canker disease caused by *Xanthomonas citri* subsp. *citri* as a result of induction of plant resistance due to increases of *β*-1,3-glucanase and activity of antioxidant enzymes, catalase, peroxidase, and phenylalanine ammonia-lyase (Hasabi *et al.,* 2014). Also, potato tuber slice treatment with L-methionine significantly decreased dry rot caused by *Fusarium solani* (Mohamed and Mostafa, 2021).

Ascorbic acid (vitamin C) is an essential antioxidant in the ascorbate-glutathione pathway in plant. It is a cofactor for many enzymes, including those involved in cell wall synthesis, most notably in the hydroxylation of proline residues (Smith *et al.*, 2007; Abo-Hinna and Merza, 2012). Moreover, Gad El-Hak *et al*. (2012) reported that foliar spraying of ascorbic acid improved vegetative growth and yield as well as pod soluble solid substances and pigments constituents of leaves and pods of pea plants. Also, Anany and Ismail (2020) on common bean found that, foliar spray of vitamin C significantly increase growth and total yield and leaf contents of nutritional values. Also, ascorbic acid is considered the first line of plant defense against damaging reactive oxygen species (ROS), that protecting plant cells from any factors that induce oxidative stress, such as salinity, wounding (Boubakri, 2017), and plant pathogens attacks (Somai-Jemmali *et al*., 2015 ; Boubakri, 2017). Foliar application of pepper with ascorbic acid, chitosan and saccharin significantly reduced of powdery and downy mildew comparing with fungicide treated plants (Abdel-Kader and El-Mougy, 2014).

The aim of the current study was to investigate the effect of chitosan, methionine and ascorbic acid by different application methods (soaking the transplant roots, foliar spraying and soaking plus foliar spraying) on root rot disease caused by *Rhizoctonia solani*, vegetative growth, yield, and quality characteristics of strawberry fruits.

2. MATERIAL AND METHODS

2.1. Isolation and identification of the pathogen

Samples of strawberry plants exhibit symptoms of Rhizoctonia root rot disease were collected from Al Qalyubia Governorate fields. The isolation of the casual fungi was carried out as usual. For purification *Rhizoctonia*-like mycelia, hyphal-tip of developed mycelium growth were picked up then sub-cultured on Petri dishes contained PDA media. Plates were incubated as above for 5 days until isolates grow well. Identification of isolates was carried out according to Parmeter and Whitney (1970).

2.2. Fungal inoculum preparation

Sand was washed with a hydrochloric acid and dried then distribution in 9 cm diameter Petri dishes (120 g/dish). Sandy dishes were wetted by pouring Czapek Dox broth (25 ml/plate) then autoclaved at 121°C for 30 min. After cooling, sandy dishes were infected by putting pieces of actively fungal growth. The plates were incubated at 25±1˚C for 7 days. Sandy dishes containing the fungal growth were used for infestation of pots. To infestation the pots, two dishes were used for every earthen pot (20 cm diameter, each pot contains approximately 3000 g sandy loam soil). Infected pots were left for 7 days with daily irrigation follow-up before transplanting (Mohamed *et al.*, 2015).

2.3. Pathogenicity tests

Three isolates were obtained of genus *Rhizoctonia solani.* The isolates were tested for their pathogenicity on strawberry (Festival cv.) in greenhouse. Soil infestation was performed 7 days before transplanted as described above. One transplant was transferred in earthen pots (20 cm in diameter) containing infected or non-infected (control) sterilized sandy loam soil (1:1) with 18 replicates for each isolate. Pots were kept under greenhouse conditions. Pots were irrigated daily with water, and every 7 days by NPK fertilizer. Data were recorded as percentage of disease incidence and disease severity of infected root areas to determine the virulence of tested isolates.

2.4. Effect of some natural substrates on Rhizoctonia root rot disease, total phenols, oxidative enzymes and parameters of strawberry plant growth

All experiments were conducted during two successive seasons of 2019/2020 and 2020/2021. Greenhouse experiments were carried out at Department of Plant Pathology, Faculty of Agriculture, Ain Shams University. Field experiments were carried out at the experimental farm at El-Kanater Horticulture Research Station (El-Qaluobia Governorate), Agricultural Research Center (ARC), Egypt.

Tested substrates including chitosan at 500 ppm, methionine at 20 ppm and ascorbic acid at 20 mM were applied by different methods as: soaking the roots of transplants before planting for 15 minutes in substrate, foliar spray, combined treatments of root soaking and foliar spraying as well as, soaking the roots of transplants before planting for 15 minutes in the Rhizolex at 1 g/L and untreated plants as control. Foliar spray treatments were started after 25 days from transplanting and every 15 days for 5 times through the growing season. All experiments were arranged in a randomized complete blocks design in three replicates.

In greenhouse experiments, the isolate of *Rhizoctonia solani* that appeared highest degree of infection in pathogenicity test was used for artificial soil infestation as described above. Seedlings were cultivated and all treatments were applied as mentioned above with 18 replicates for each treatment.

In field experimental the soil was sandy loam in texture with PH of 7.28 Soil mechanical and chemical analyses are shown in Table (1).

Table 1. The chemical properties of pot sand soil

Physical analysis		Chemical analysis			
		Cations meq/l		Anions meq/l	
Sp %	58.00%				
Coarse sand	9.56%	Ca^{+2}	5.27	CO_3	0
Fine sand	17.93%	Mg^{+2}	2.38	HCO_3	2.17
Silt	33.84%	Na+	3.78	Cl^-	3.46
Clay	38.67%	K+	0.17	SO_4^{-2}	5.97
Soil pH	7.28	Available N	22.5 mg/kg		
E.C, dS/m	1.18	Available P	16.2 mg/kg		
		Available K	209 mg/ kg		

The area of the experimental plot was 12.25 m^2 included one bed each seven meters in length and 1.75 meters in width. Each bed included four rows and the transplanting was done at 25 cm apart between transplants in the same row. Transplanting was done on 1 and 3 of October in 2019/2020 and 2020/2021, respectively. Sprinkler irrigation was used in the first month after transplanting, after that, the beds were covered with 40 micron black plastic mulch. After planting the drip irrigation was used after mulching until the end of the growing season.

Random samples of three plants of strawberry root from each greenhouse experimental were uprooted after 72 hr of transplanting to determine phenolic compounds and antioxidant defensive enzymes activities.

2.4.1. Disease assessment

Disease incidence was determined 20 days after transplanting on 18 and 147 plants in greenhouse and field experiments, respectively. In greenhouse, plants were uprooted after130 days from transplanting to determined disease severity of Rhizoctonia root rot by using scale (0 to 5) according to Fang *et al*. (2013).

2.4.2. Determination of total soluble phenols

Total soluble phenols in strawberry roots (grown under greenhouse conditions) were determined using the method described by Shahidi and Naczk (1995). The concentration of total soluble phenols was calculated using the standard curve of catechol. Total soluble phenols concentration was expressed as µg equivalents of catechol per g FW of the sample.

2.4.3. Enzymes assay

For enzyme extraction frozen tissues (one gram) of root (grown under greenhouse conditions) were ground and homogenized with 4 ml cold sodium phosphate buffer (100 mM, pH=7) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 0.1 mM EDTA by using cold mortar and pestle. The homogenate was centrifuged at 10000 rpm, at 4º C for 15 min. The supernatant was used for determination the activity of polyphenol oxidase (PPO), superoxide dismutase (SOD) and catalase (CAT). The activity of PPO was measured based on the method described by Oktay *et al*. (1995). The activity of SOD was according to Beyer and Fridovich (1987). The activity assay of CAT was determined according to Sumner and Somers (1947). All the enzymes activity was expressed as unit.mg^{-1} protein. Protein concentration was determined in the crude extract according to Bradford (1976) by using bovine serum albumin as a standard curve.

Fig 1. The degree of rot severity on strawberry roots using scoring from 0 to 5 according to (Fang *et al.*, 2013) as follows 0 = healthy root, no discoloration; 1: <25% root discolored; 2: ≥ 25% < 50% root discolored; 3: ≥ 50% <75% root discolored; 4: ≥75% root discolored; 5: all root rotted.

2.4.4. Vegetative growth characteristics

Three plants (in greenhouse experiments) and five plants (in field experiments) were taken from each experimental unit after 130 days from planting to determine plant height (cm), fresh and dry weight/plant shoots (g), number of leaves/plant, crown diameter (cm), root fresh and dry weight (g).

In field experiments, chemical composition of plant foliage, *i.e.*, total carbohydrates, nitrogen, phosphorus and potassium were determined according to A. O. A. C. (1990), Pregl (1945), John (1970) and Brown and Lilleland (1946), respectively.

2.4.5. Fruit yield and its components

Early fruit yield was determined as weight of all harvested fruits at the ripe stage during December and January. Exportable yield was calculated as weight of harvested fruits at the 3/4 color stage during December and January after discarding the misshaped fruits. Also, total fruit yield per plant and feddan. Marketable yield was calculated after discarding the infected fruits. Un-marketable yield was calculated as weight of infected fruits during the harvesting season.

2.4.6. Physical and chemical fruit quality

A random samples of 10 fruits at marketable stage from each experimental plot was taken to determine the following properties. Average fruit weight, fruit length, fruit diameter and fruit firmness. Total soluble solids (T.S.S.), total titratable acidity, ascorbic acid and anthocyanin as the method described in A. O. A.C. (1990). Total sugars were determined as the method described by Nelson (1974).

2.5. Statistical analysis

All obtained data were analyzed by analysis of variance (ANOVA). Least significant differences at p=0.05 and Duncan's multiple range test was applied to compare between means (Gomez and Gomez, 1984).

3. RESULTS AND DISCUSSION

3.1. The pathogen and pathogenicity test

Three isolates of *Rhizoctonia* spp. (RS1, RS2 and RS3) were isolated from strawberry root showed typical symptoms of Rhizoctonia root rot disease. Identification of the isolates ensured that all of them have identical specifications of *R. solani*. Isolate RS1 showed the highest percentage of disease incidence and disease severity Figs (2and3).

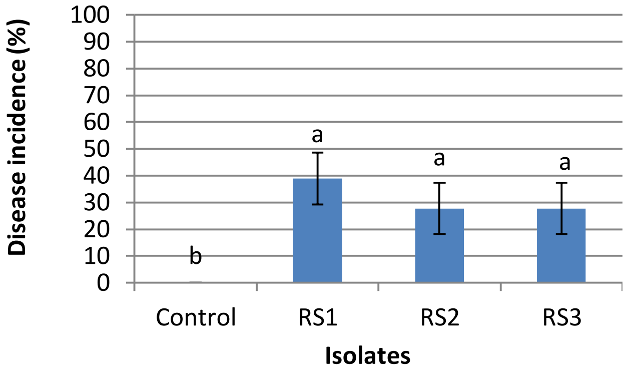

Fig 2. Root rot incidence on strawberry by *Rhizoctonia* isolates under greenhouse conditions.

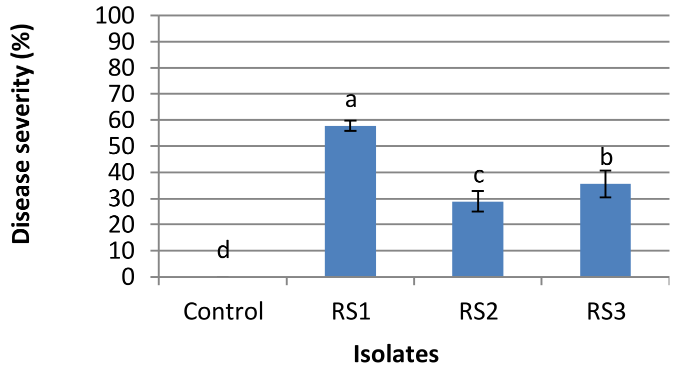

Fig 3. Disease severity of *Rhizoctonia* isolates on strawberry root under greenhouse conditions.

3.2. Effect of some natural substrates on Rhizoctonia root rot disease, total phenols, oxidative enzymes, and parameters of strawberry plant growth

Effect of natural substrates or Rhizolex on the incidence and severity of root rot under greenhouse conditions were illustrated in Figs. (4,5,6 and7) and on the incidence in field experiments were shown in Figs. (8and9). Data in Fig. (4) clarify that all treatments resulted in clear significant decrease in disease incidence except foliar spray with ascorbic acid compared with control. Meanwhile, the foliar spray plus seedling root soaking in all substrates (chitosan, methionine and ascorbic acid) recorded the highest reduction in the percentage of plants death than control or than other treatments. Data in Fig. (5) showed that all treatments resulted in clear significant decrease in disease severity. The foliar spray plus seedling root soaking of chitosan and methionine gave the highest values of reduction in disease severity, followed by the foliar spray with chitosan. In addition, the results of both incidence and severity of root rot disease tended in similar trend in the second season (Figs 6 and7). Also, the similar results of the disease incidence in field experiments were recorded (Fig. 8).

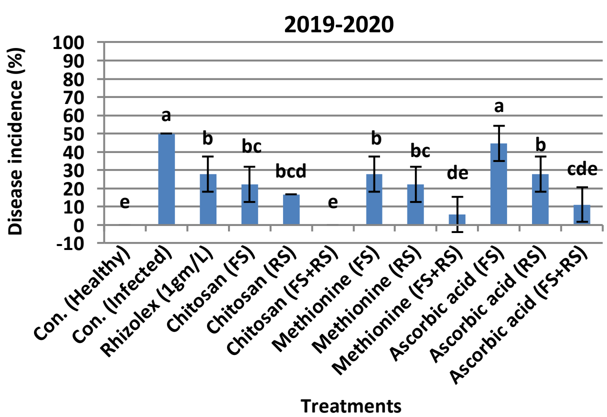

Fig 4. Effect of some natural substrates on the incidence of root rot disease on strawberry under greenhouse conditions during 2019/2020

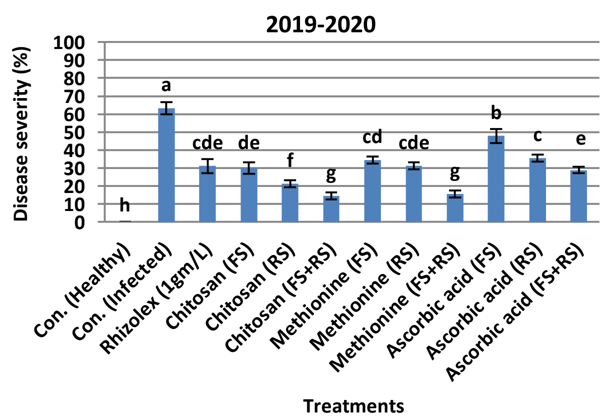

Fig 5. Effect of some natural substrates on the severity of root rot diseases on strawberry under greenhouse conditions during 2019/2020.

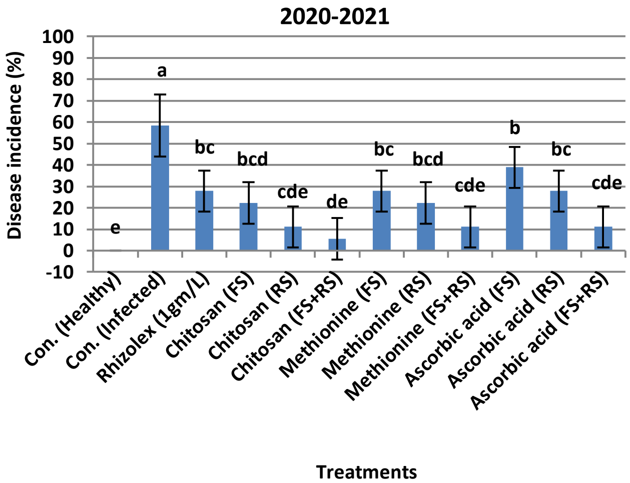

Fig 6. Effect of some natural substrates on the incidence of root rot disease on strawberry under greenhouse conditions during 2020/2021.

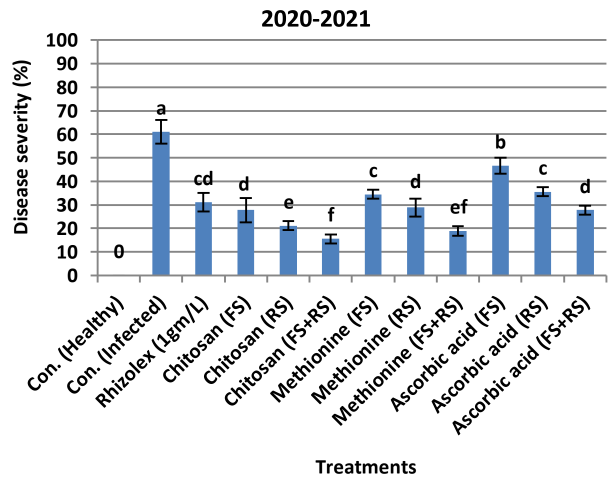

Fig 7. Effect of some natural substrates on the severity of root rot diseases on strawberry under greenhouse conditions during 2020/2021.

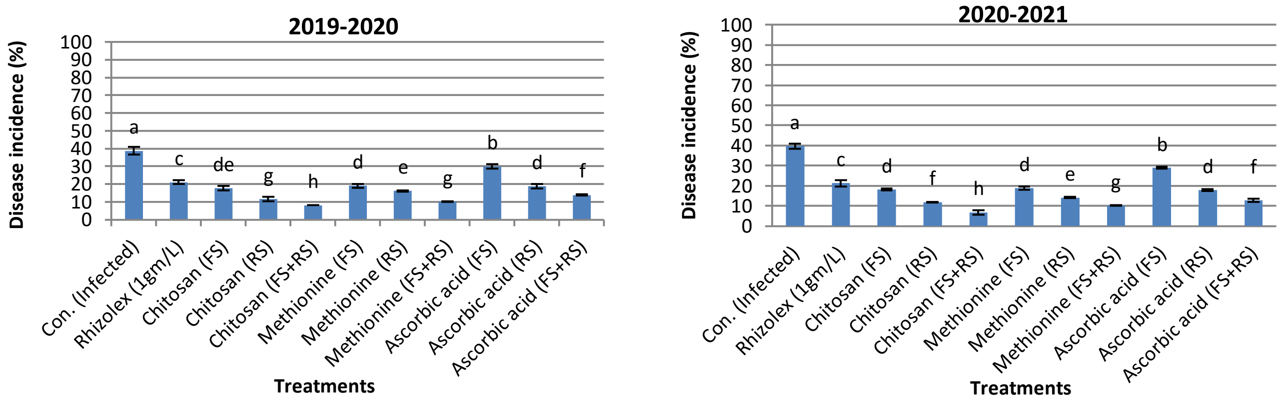

Fig 8. Effect of some natural substrates on the incidence of root rot disease on strawberry under field conditions during seasons 2019/2020- 2020/2021.

3.2.1. Total phenolic compounds

Data in Fig. (9) indicated that the level of total phenolic compounds was significantly increased in all applications with chitosan, methionine and ascorbic acid compared with untreated infected plants. no significant effect was found in root application with Rhizolex compared with control or other applications in both seasons. In the first season, foliar spray plus transplant root soaking of chitosan and methionine recorded the highest phenolic compounds 780.4 and 779.4 µg/g FW, respectively followed by foliar spray plus transplant root soaking of ascorbic acid 684.4 µg/g FW compared to untreated infected plants 320.5 µg/g FW. Also, the same trend in the second season, data showed that the maximum level of phenols was detected in application with foliar spray plus transplant root soaking of chitosan and methionine were obtained 516.0 and 512.8 µg/g FW, respectively followed by seedling root soaking of methionine was recorded 494.2 µg/g FW compared to untreated infected plants 239.2 µg/g FW. Phenols are considered the components of lignin which increase the plant cell wall resistance against cell wall degrading enzymes (Bhuiyan *et al.*, 2009). It can be conclude that chitosan, methionine, and ascorbic acid can induce strawberry resistance response to *R. solani* by increasing the concentrations of phenolic compounds, which restrict or weaken pathogen growth throw forming around wounds or pathogen infected areas (Reimers and Leach, 1991).

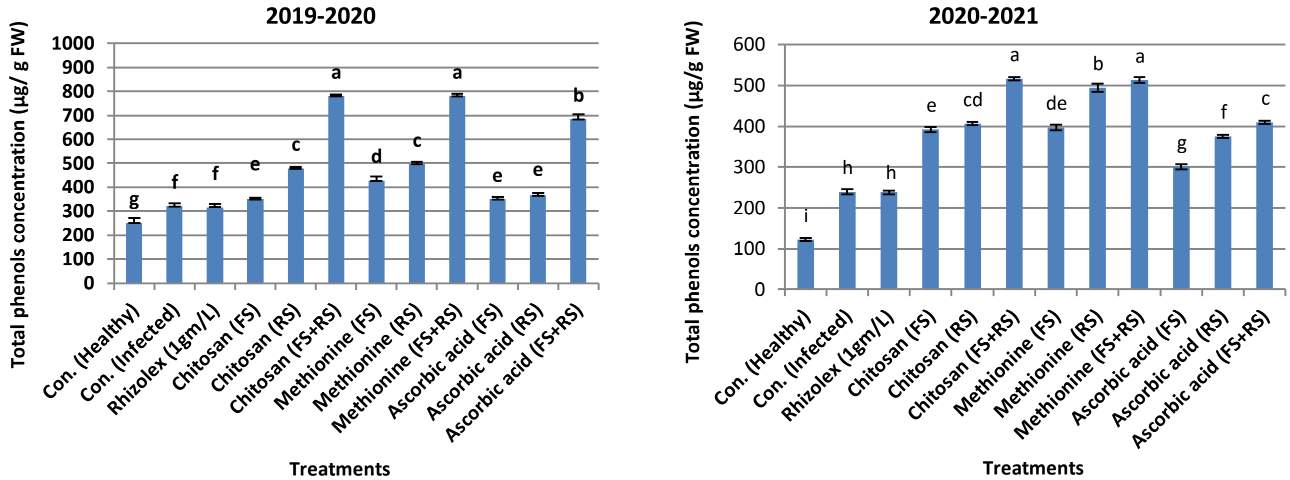

Fig 9. Effect of some natural substrates on phenolic compounds in infected strawberry with *Rhizoctonia solani* during two seasons 2019/2020 and 2020/2021.

3.2.2. Antioxidant defensive enzymes

Data presented in Fig. (10) showed that all applications with chitosan, methionine and ascorbic acid led to significant increase in polyphenol oxidase (PPO) activity compared with untreated plants in both seasons. Application with the foliar spray plus transplant root soaking of chitosan followed by the same application with methionine attained the highest PPO activity in strawberry root compared with untreated control. On the otherwise, all applications with ascorbic acid recorded least PPO activity. Otherwise, there were no significant differences in PPO activity between root application with Rhizolex and untreated infected plants in first season. However, in second season compared with control, Rhizolex application led to decrease in PPO activity.

The activity of superoxide dismutase (SOD) was significantly affected by all applications of chitosan, methionine and ascorbic acid. Application with the foliar spray plus seedling root soaking of chitosan attained the highest SOD activity in strawberry root followed by the same application with methionine compared with untreated control. Otherwise, root application with Rhizolex decreased SOD activity compared with untreated infected plants in both seasons (Fig. 11).

The enzyme activities of catalase (CAT) were significantly increased in all applications with chitosan, methionine and ascorbic acid compared to untreated control as shown in Fig. (12) in two successive seasons. However, in the first season, the maximum increase in CAT activity was resulted with the foliar spray plus seedling root soaking of chitosan followed by transplant root soaking with chitosan compared to untreated infected plants. Furthermore, in the second season, the maximum increase in CAT activity was resulted with the foliar spray plus transplant root soaking of chitosan followed by the same application with methionine compared with untreated infected plants. On the other hand, there were no significant differences in CAT activities between root application with Rhizolex and untreated infected plants in both seasons.

Increasing the activities of three antioxidant enzymes *i.e.*, PPO, SOD and CAT ensured the role of theses substrates (chitosan, methionine and ascorbic acid) in induction of strawberry resistance against *R. solani*. These findings agree with a numerous studies on the role of these enzymes in plant resistance (Oliveira *et al.*, 2016). Also, these results are in agreement with previous findings that chitosan was reported to induce resistance against several soil borne fungi (Ashley *et al.*, 1998; Benhamou and Theriault, 1992; Abd-El-Kareem *et al.*, 2002). In this regard, soil amended with chitin and chitosan resulted significant reduction of disease incidence and disease severity of *Fusarium* yellows disease of celery (Ashley *et al.*, 1998). Otherwise, the reduction of strawberry Rhizoctonia root rot disease with chitin treatments may have been due to the direct effect on *Rhizoctonia solani* via chitin decomposition releases volatiles such as ammonia which suppress several soil borne fungi (Sneh and Henis, 1972; Bell *et al.*, 1998; Stamford *et al.*, 2010; Kim *et al.*, 2016). In present study, the observed reduction in strawberry root rot incidence or severity may be attributed to indirect effect of chitin and its defense response in plants. In this respect, several studies reported that various plants treatment with chitosan induced plant resistance by increasing antioxidant enzymes activities (Matta *et al.*, 1988; Siddaiah *et al.*, 2018).

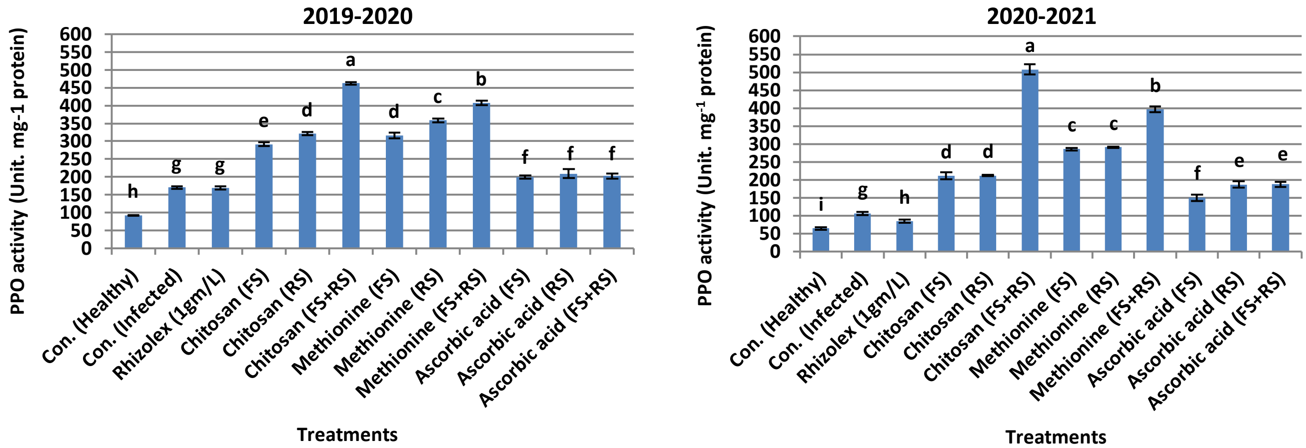

Fig 10. Effect of some natural substrates on PPO activity in infected strawberry with Rhizoctonia solani during two seasons 2019/2020 and 2020/2021.

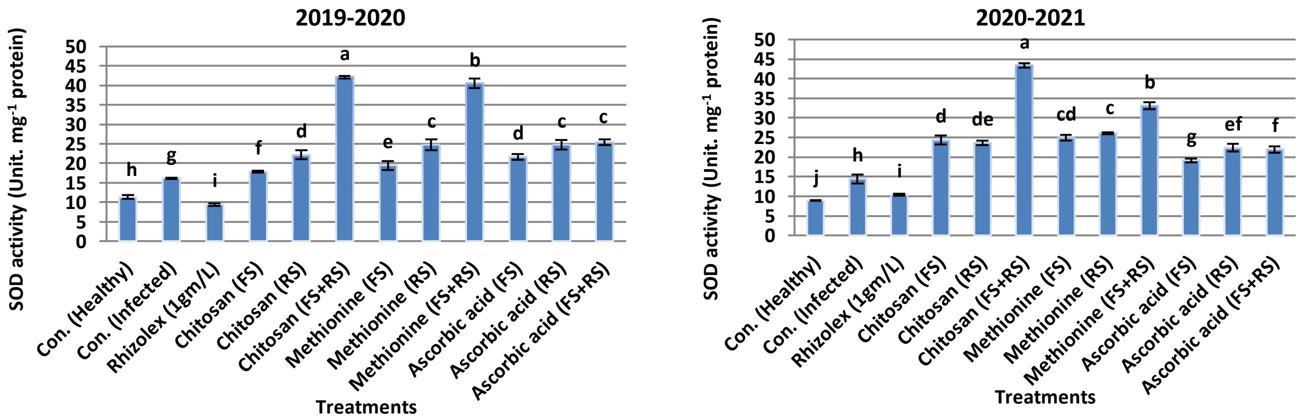

Fig 11.Effect of some natural substrates on SOD activity in infected strawberry with Rhizoctonia solani during two seasons 2019/2020 and 2020/2021.

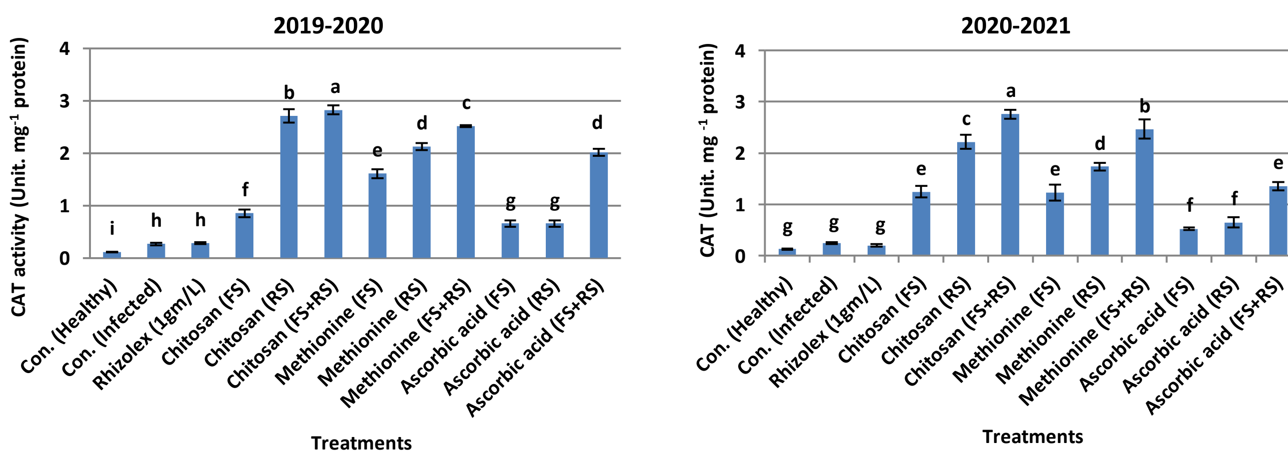

Fig 12. Effect of some natural substrates on CAT activity in infected strawberry with *Rhizoctonia solani* during two seasons 2019/2020 and 2020/2021.

Our results are supported by previous studies reported that L-methionine and riboflavin induced the plant resistance against powdery mildew infection in strawberry, squash, melon, cantaloupe and pea (Tzeng *et al.,* 1996; Wang and Tzeng, 1998; Sarosh *et al*., 2005). another study indicated that L-methionine induced plant resistance against *Xanthomonas citri* sub.sp. *citri* which led to significant reduction of disease severity by changes in β-1,3-glucanase transcript levels and increasing the activity of antioxidant enzymes *i.e.,* catalase, peroxidase, and phenylalanine ammonia-lyase (Hasabi *et al.,* 2014).

Ascorbic acid (AA) is an antioxidant molecule and acts as substrate for the detoxification of reactive oxygen species (Akram *et al.,* 2017) that protecting plant cells from plant pathogens attacks (Somai-Jemmali *et al*., 2015; Boubakri, 2017). Pre-planting seedlings of marjoram plants treated with some mineral compounds and antioxidants including ascorbic acid reduced the disease incidence of root rot and wilt diseases caused by *Rhizoctonia solani, Sclerotinia sclerotiorum and Fusarium xysporum* (Ahmed *et al.,* 2017). Besides its AA role in inducing plant resistance, also it has a direct effect as antifungal. In this regard, Botanga *et al.* (2012) reported that AA has direct effect on the hyphal development of *Alternaria brassicicola*. Overall, our results indicated that AA have a role in induced plant resistance.

3.2.3. Vegetative growth characteristics

In greenhouse experiments, data in Fig. (13) and Table (2), clarified that all applications with chitosan, methionine and ascorbic acid or application with Rhizolex significantly improved all growth characters (plant high, fresh and dry weight of shoot or root, number of leaves, crown diameter) compared with untreated (infected or not infected) plants. The combined between foliar spray and seedling root soaking of chitosan or methionine have the highest growth characters.

In the field experiments, it is clear from data in Table (3) that soaking the transplants roots, foliar or both together with safety compounds (chitosan at 500 ppm, methionine at 20 ppm and ascorbic acid at 20 mM) significantly enhanced all studied morphological traits of strawberry plants i.e., plant height, plant fresh weight, plant dry weight, number of leaves, number of crown per plant and crown diameter during the first and second seasons of study compared with the control and fungicide (Rhizolex 1 g/L). In this regard, soaking plus foliar spraying with chitosan at 500 ppm gave the highest value for plant height, plant fresh weight, plant dry weight, number of crown and crown diameter in two seasons while, methionine at 20 ppm (soaking plus foliar spraying) reflected the highest values for number of leaves in both seasons. Also, ascorbic acid (soaking plus foliar spraying) increased the root fresh and dry weight per plant in first and second seasons. The increase in growth due to the application of tested stimulants may be attributed to the role of such materials as antioxidants which contract the free radical (O_2) which affect the activity and viability of plant cells. Furthermore, increasing the growth duration and retardation of plant senescence. In addition, the superiority of chitosan, methionine and ascorbic acid may be due to its role as a source of growth promoting substances which affect plant cells division and elongation and in turn increased plant growth. Chitosan, plays main role in enhancement plant metabolism phytoprotection and maintain plant health and consequently increase plant growth. Also, the effect of chitosan on increasing key enzymes activity of nitrogen metabolism, which enhanced plant growth and development (Mondal *et al*., 2012). The amino acids which play an important role in plant metabolism and protein assimilation necessary for cells formation and consequently increased fresh and dry matter of plant which are good indicator for plant growth. Ascorbic acid shares in regulation of cell division and growth involving in signal transduction in plants through using multiple pathways for to synthesizing ascorbic acid, which reflects the importance of this molecule for plant health. This result agree with those of El-Miniawy *et al*. (2013), Shams *et al.* (2014) and Nithin *et al*. (2020) on strawberry; they found that foliar concentration of chitosan increased vegetative growth characteristics (plant height, number of leaves/ plants, leaf area and fresh and dry weight per plant) compared with control. Also, Gad El-Hak *et al*. (2012) stated that foliar spray with 20 mM ascorbic acid increased pea plant height, number of branches and plant dry weight. In addition, Manal *et al*. (2019) reported that amino acids application increased strawberry leaves number and shoot dry weight/plant. Also, Fatma and Hanaa (2020) on strawberry found that spraying strawberry plants with mixture of tryptophan and methionine led to an increase in vegetative growth characteristics.

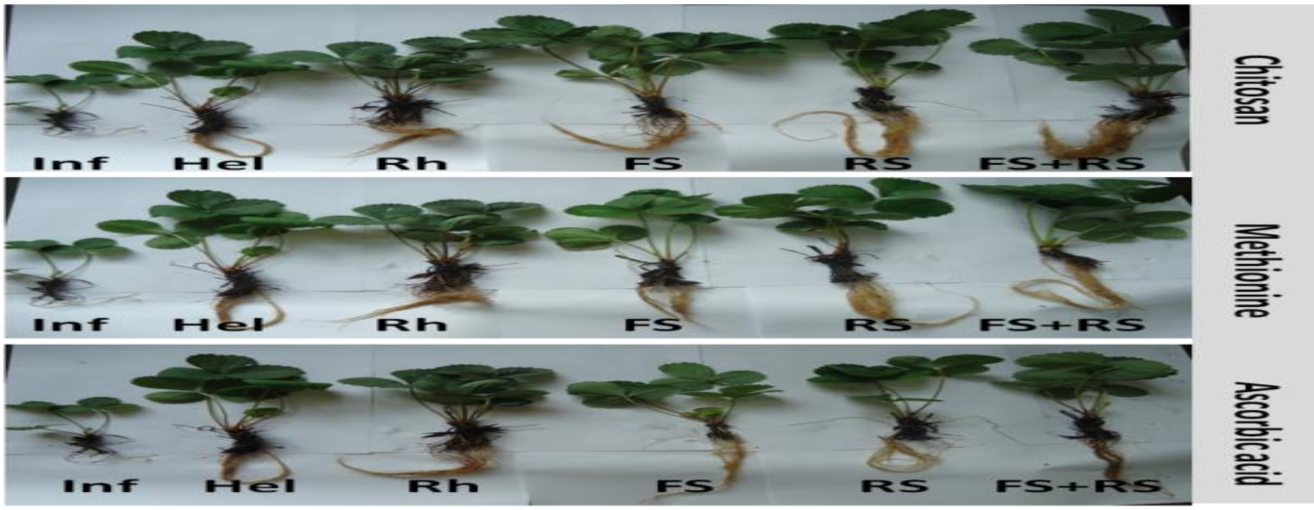

Fig 13. Effect of different abiotic inducer treatments on growth parameters of strawberry infected with *Rhizoctonia solani.* Inf: infected plants, Hel: healthy plants, Rh: Rhizolex, FS: foliar spray, RS: root soaking.

Table 2. Effect of different abiotic inducer treatments on growth parameters of strawberry infected with *Rhizoctonia solani* during (2019-2020/2020-2021) seasons.

Treatments	Plant height (cm)	Shoot weight (g)		No. of leaves/plant	Crown diameter (cm)	Root weight (g)	
		fresh	dry			fresh	dry
			2019-2020				
Control (Healthy plants)	16.50d	38.13ef	8.06de	10.50cde	1.26f	16.65e	3.78d
Control(Infected plants)	10.85f	21.76i	5.73g	7.33h	0.96h	10.10g	2.21f
Rhizolex (1g/L)	15.06e	30.06h	6.41f	9.16fg	1.08g	14.11f	2.91e
Chitosan (FS)	18.21bc	38.91e	8.48d	11.16c	1.61bc	21.60c	4.30c
Chitosan (RS)	18.36bc	48.98b	10.81b	12.00b	1.65b	23.11b	4.26c
Chitosan (FS+Rs)	21.00a	53.50a	11.4a	13.16a	1.81a	23.86a	4.96a
Methionine (FS)	17.86c	42.63d	9.28c	9.66ef	1.45de	21.36c	4.18c
Methionine (RS)	18.50bc	43.20d	9.46c	10.16de	1.50cde	21.35c	4.66b
Methionine (FS+RS)	19.00b	45.73c	9.76c	10.83cd	1.60bc	22.71b	4.75ab
Ascorbic acid (FS)	15.81de	35.91g	7.71e	8.66g	1.40e	20.35d	4.40c
Ascorbic acid (RS)	16.45d	36.60fg	8.23d	9.00fg	1.50cde	21.53c	4.83ab
Ascorbic acid (FS+RS)	16.31d	37.38efg	8.18de	9.16fg	1.55bcd	23.95a	4.86ab
			2020-2021				
Control (Healthy plants)	16.33e	32.56e	8.13e	10.33b	1.23e	14.46e	3.20d
Control(Infected plants)	10.16g	19.00g	5.63g	6.33d	1.00f	9.20f	2.03e
Rhizolex (1g/L)	14.16f	28.66f	6.86f	8.00c	1.00f	14.10e	2.30e
Chitosan (FS)	18.66bc	35.16de	8.16e	10.33b	1.63ab	19.13cd	4.00c
Chitosan (RS)	19.66b	37.80bcd	10.63bc	11.00b	1.63ab	20.96bc	4.50ab
Chitosan (FS+Rs)	21.33a	47.40a	13.53a	12.33a	1.73a	24.20a	4.86a
Methionine (FS)	18.00cd	38.96bc	9.70cd	10.33b	1.46c	19.03cd	4.00c
Methionine (RS)	18.66bc	38.53bcd	9.76cd	10.66b	1.46c	19.53cd	4.06bc
Methionine (FS+RS)	19.00bc	39.80b	11.00b	10.66b	1.53bc	22.43ab	4.86a
Ascorbic acid (FS)	16.00e	32.96e	7.86ef	10.33b	1.26de	17.16d	3.60cd
Ascorbic acid (RS)	16.16e	34.90de	8.30e	11.00b	1.26de	17.63d	3.83c
Ascorbic acid (FS+RS)	17.16de	35.86cde	8.70de	10.33b	1.40cd	18.30cd	3.96c

Within a column, means followed by the same letter are not different at P< 0.05

Table 3. Effect of some natural compounds (chitosan, methionine and ascorbic acid) on vegetative characteristics of strawberry plants cv. Festival during 2019/2020 and 2020/2021 seasons.

Treatments	Plant height (cm)	Plant weight (g)		Number		Crown diameter (cm)	Root weight (g)	
		Fresh	Dry	Leaves	Crowns		Fresh	Dry
				2019/2020				
Control	19.67 d	54.55 e	12.27 h	18.50 d	2.57 d	2.43 c	18.29 f	4.45 c
Rhizolex (1g/L)	20.17 d	55.20 e	12.94 gh	18.50 d	3.00 cd	2.50 bc	18.61 ef	4.67 bc
Chitosan (FS)	21.66 abc	57.89de	14.51 fg	20.33 bc	3.50 abc	2.63 abc	18.79def	4.73 bc
Chitosan (RS)	20.83bcd	68.38bc	17.49 bc	19.67bcd	3.70 abc	2.60 bc	19.40 c	5.11 ab
Chitosan (FS+Rs)	22.50 a	73.95 a	20.39 a	20.68 b	3.93 a	2.87 a	21.29 a	5.22 a
Methionine (FS)	21.83 abc	65.56 c	16.27cde	19.33bcd	3.33 a-d	2.53 bc	20.07 b	5.05 ab
Methionine (RS)	21.67 abc	65.80 c	17.03bcd	18.66 d	3.51 abc	2.60 bc	19.00cde	4.85 abc
Methionine (FS+RS)	22.16 a	70.65ab	19.66 a	22.67 a	3.96 a	2.63 abc	21.29 a	5.19 a
Ascorbic acid (FS)	20.66 cd	65.47 c	15.49def	19.00 cd	3.03 bcd	2.70 ab	19.33 cd	4.90 ab
Ascorbic acid (RS)	21.50 abc	60.95 d	14.86 ef	19.34bcd	3.57 abc	2.50 bc	19.41 c	4.96 ab
Ascorbic acid (FS+RS)	22.00 ab	71.73ab	18.73 ab	20.67 b	3.83 ab	2.67 abc	21.48 a	5.26 a
				2020/2021				
Control	24.00 c	64.96 g	16.09 f	19.67 e	2.25 c	2.05 e	19.62 d	4.51 e
Rhizolex (1g/L)	24.33 bc	80.96 f	19.62 e	21.00 e	3.17 b	2.23de	19.67 d	4.90 de
Chitosan (FS)	25.17abc	86.11de	24.81ab	24.99 bc	3.16 b	2.43 cd	20.67 bc	5.06 bcd
Chitosan (RS)	25.00 abc	90.14 c	24.68 b	24.66 bc	3.40 ab	2.65 abc	21.40 b	5.36 abc
Chitosan (FS+Rs)	26.16 a	97.70 a	26.98 a	25.60 ab	3.70 ab	2.86 a	22.29 a	5.46 ab
Methionine (FS)	24.33 bc	83.25 ef	22.08 cd	24.53 bc	3.43 ab	2.54 bc	20.70 bc	5.11 bcd
Methionine (RS)	24.67 abc	87.04cd	24.40 bc	23.00 d	3.57 ab	2.45cd	20.62 c	4.97 cd
Methionine (FS+RS)	26.00 a	97.57 a	26.58 ab	26.90 a	3.90 a	2.75 ab	22.29 a	5.46 ab
Ascorbic acid (FS)	24.33 bc	81.20 f	20.05 de	24.07 cd	3.33 ab	2.57bc	20.12 cd	4.96 cd
Ascorbic acid (RS)	25.17 abc	93.98 b	25.68 ab	25.00 bc	3.50 ab	2.40 cd	19.77 d	5.06 bcd
Ascorbic acid (FS+RS)	25.83 ab	96.51ab	26.29 ab	25.03 bc	3.67 ab	2.65 a-c	22.81 a	5.68 a

Within a column, means followed by the same letter are not different at $P< 0.05$

3.2.4. Chemical composition of plant foliage

As for the effect of tested natural compounds (chitosan, methionine and ascorbic acid) on total nitrogen, phosphorus, potassium and carbohydrates concentration in plant foliage of strawberry plant, data in Table (4) indicate that (soaking plus foliar spraying) strawberry plants with such safety compounds significantly affected total phosphorus, potassium both seasons. However, it did not significantly affect total nitrogen content during both seasons and total carbohydrates only during the second one. In this regard, soaking and foliar together application of methionine, chitosan and ascorbic acid at 20 ppm, 500 ppm and 20 mM respectively, exhibited the highest values of N, P, K and carbohydrates in plants compared with the control. Such results are true during the two seasons of growth. In this connection, the promoting effects of growth stimulants on the concentration of macro- elements was connected with its positive effects on vegetative growth parameters (Table 1). Moreover, such increasing tendency of N, P, K and carbohydrates as a result of studied growth stimulants may be attributed to its main role on the enzymes which may control the active absorption of nutrients and water from the soil. Obtained results are in the same line with those reported by Ramadan and Mohamed (2015) on okra and Dawa *et al*. (2017) on

Table 4. Effect of some natural compounds i.e. chitosan, methionine and ascorbic acid on chemical composition of plant foliage of strawberry plants cv. Festival during 2019/2020 and 2020/2021 seasons.

Treatments	2019/2020				2020/2021			
	N%	P%	K%	Carbohydrates (g/100g d.w)	N%	P%	K%	Carbohydrates (g/100g d.w)
Control	2.61a	0.43c	1.43 d	13.36 d	2.44a	0.32 d	1.22 c	14.23 a
Rhizolex (1g/L)	2.62a	0.55b	1.43 d	13.98 d	2.44a	0.34cd	1.26bc	14.32 a
Chitosan (FS)	2.63a	0.55b	1.45 cd	15.40 bc	2.47a	0.37cd	1.27bc	14.38 a
Chitosan (RS)	2.62a	0.57b	1.46 cd	15.18 bc	2.46a	0.41abc	1.33ab	14.45 a
Chitosan (FS+Rs)	2.67a	0.68a	1.58 ab	16.12 ab	2.50a	0.47 a	1.37 a	14.80 a
Methionine (FS)	2.63a	0.56b	1.49 cd	15.89 abc	2.47a	0.38bcd	1.26bc	14.36 a
Methionine (RS)	2.65a	0.57b	1.47 cd	15.069 c	2.46a	0.42abc	1.27bc	14.40 a
Methionine (FS+RS)	2.67a	0.73a	1.63 a	16.735 a	2.54a	0.48 a	1.38 a	14.89 a
Ascorbic acid (FS)	2.64a	0.55b	1.47cd	15.27 bc	2.46a	0.36 cd	1.32ab	14.44 a
Ascorbic acid(RS)	2.66a	0.57b	1.46cd	14.98 c	2.45a	0.41abc	1.31ab	14.60 a
Ascorbic acid (FS+RS)	2.66a	0.68a	1.51bc	16.10 ab	2.48a	0.46 ab	1.37 a	14.71 a

Within a column, means followed by the same letter are not different at $P< 0.05$

tomato, they found that foliar spray of chitosan resulted in increasing the percentages of N, P and K in leaves. Also, Abo Sedera et al. (2010) and Mohamed (2015) on strawberry found that foliar application of amino acid resulted in increasing the percentages of N, P, K and carbohydrates in leaves. However, Shadia (2017) on potato and Anany and Ismail (2020) on common bean reported that ascorbic acid foliar application increased N, P, K and carbohydrates in plant.

3.2.5. Fruit yield and its components

Concerning the effect of soaking transplants roots before planting and spraying strawberry plants with natural compounds *i.e.*, chitosan، methionine and ascorbic acid on total produced fruit yield and its components expressed as early yield, exportable, marketable and total yield either per plant or feddan as well as unmarketable yield.

Data recorded in Table (5) show that soaking transplants roots before planting for 15 minutes plus foliar spraying the plants five times during the growing seasons chitosan at 500 ppm exhibited the highest values in all determined yield parameters compared with all treatments during both seasons of this experiment. However, no significant differences were noticed among the used levels of chitosan, methionine and ascorbic acid (soaking plus foliar spraying) in all measured yield parameters during both seasons of study. In this regard, the highest values of un marketable fruit yield was recorded in case of the control treatment. However, the increments in total produced yield and its components as a result of using chitosan، methionine and ascorbic acid were connected with the increase in vegetative growth parameters (Table 3). In addition, it's effect on increasing macro- nutrients (Table 4) which affected plant growth and in turn increased its productivity. Also, using such growth enhancers reduce the proportion of infected fruit and the number of days to reach flower synthesis, resulting in an increase in the early and marketable yield. The obtained results are similar to those reported by El-Miniawy *et al*. (2013), Shams *et al.* (2014) and Nithin *et al*. (2020) on strawberry, they found that the foliar application of chitosan increased the yield and also, Shadia (2017) and Anany and Ismail (2020) on common bean, they found that the use of ascorbic acid led to an increase in the yield.. However, Manal *et al*. (2019) on strawberry and Fatma and Hanaa (2020) on potato reported that spraying strawberry plants with amino acids increased yield.

Table 5. Effect of some natural compounds, i.e., chitosan, methionine and ascorbic acid on total fruit yield and its components of strawberry plants cv. Festival during 2019/2020 and 2020/2021 seasons.

Treatments	Early yield (ton/fed)	Exportable yield (ton/fed)	Marketable yield (ton/fed)	Unmarketable yield(kg/fed)	Total yield (g/plant)	Total Yield (t/fed)
			2019/2020			
control	4.384 e	2.83 e	16.113 c	766.50 a	421.99 c	16.879 c
Rhizolex (1g/L)	5.438 d	3.02 d	17.184 bc	720.67 b	447.62 bc	17.905 bc
Chitosan (FS)	5.846 bc	3.72 c	18.308 ab	571.83 g	472.00 ab	18.880 ab
Chitosan (RS)	5.841 bc	3.84 b	17.371bc	585.37 fg	448.91 bc	17.956 bc
Chitosan (FS+Rs)	6.902 a	4.66 a	19.253 a	566.90 g	495.50 a	19.820 a
Methionine (FS)	6.614 a	3.71 c	18.239 ab	655.93 cd	472.37 ab	18.895 ab
Methionine (RS)	5.654 bcd	3.71 c	17.371 bc	668.27 c	450.98 bc	18.039 bc
Methionine (FS+RS)	6.725 a	4.61 a	18.398 ab	617.33 ef	475.38 ab	19.015 ab
Ascorbic acid (FS)	6.008 b	3.70 c	18.289 ab	659.90 cd	473.72 ab	18.949 ab
Ascorbic acid (RS)	5.605 cd	3.66 c	17.274 bc	680.80 c	448.87 bc	17.955 bc
Ascorbic acid(FS+RS)	6.713 a	4.65 a	18.766 a	629.74 de	484.89 a	19.396 a
			2020/2021			
control	4.196 e	2.81 d	15.183 d	687.37 a	396.76 d	15.870 d
Rhizolex (1g/L)	5.266 d	2.97 c	16.894 c	669.27 ab	439.07 c	17.563 c
Chitosan (FS)	5.408 bc	3.59 b	17.948 abc	558.73 f	462.67 abc	18.507 abc
Chitosan (RS)	5.367 cd	3.64 b	17.059 c	572.00 ef	440.82 c	17.633 c
Chitosan (FS+Rs)	5.725 a	3.84 a	19.227 a	550.40 f	494.43 a	19.777 a
Methionine (FS)	5.505 b	3.65 b	17.544 bd	626.33 bcd	454.26 bc	18.170 bc
Methionine (RS)	5.419 bc	3.62 b	17.056 c	677.47 ab	443.33 c	17.733 c
Methionine (FS+RS)	5.699 a	3.79 a	18.119 abc	577.07 def	467.39 abc	18.696 abc
Ascorbic acid (FS)	5.438 bc	3.61 b	17.276 c	626.57 bcd	447.57 c	17.903 c
Ascorbic acid (RS)	5.378 c	3.60 b	16.999 c	654.67 abc	441.35 c	17.654 c
Ascorbic acid(FS+RS)	5.668 a	3.79 a	18.775 ab	614.00 cde	484.72 ab	19.389 ab

Within a column, means followed by the same letter are not different at P< 0.05

3.2.6. Physical Fruit quality

Data presented in Table (6) show that soaking transplants roots before planting and spraying strawberry plants with natural compounds (chitosan at 500 ppm, methionine at 20 ppm and ascorbic acid at 20 mM), significantly increased all measured fruit physical quality (average fruit weight, length, diameter and firmness) compared to the control in both seasons. In this respect, using soaking plus foliar spraying with chitosan at 500 ppm ranked first followed by methionine at 20 ppm (soaking plus foliar spraying) without significant differences among them. Obtained results are true during both seasons of study. Such increment in fruit parameters due to chitosan and methionine treatment may be attributed to the main role from chitosan and methionine on increasing the uptake of NPK (Table 4) which in turn increased fruit size. Similar observations were recorded by Abdel-Mawgoud *et al.* (2010) and Shafshak *et al.* (2011) for chitosan, El-Awadi *et al*. (2011), Manal *et al.* (2019) and Fatma and Hanaa (2020) for amino acids and Gad El-Hak *et al*. (2012), Shadia (2017) and Anany and Ismail (2020) for ascorbic acid.

3.2.7. Chemical Fruit quality

Data presented in Table (7) show that total soluble solids (T.S.S), vitamin C., total acidity, total sugars and anthocyanin content of fruits were significantly affected due to soaking the transplant roots, foliar spraying or both together in strawberry plants with natural compounds (chitosan at 500 ppm, methionine at 20 ppm and ascorbic acid at 20 mM) compared with the control treatment in two seasons. In this connection, the highest value in all determined chemical constituents were recorded in case of soaking and foliar application together of ascorbic acid, methionine and chitosan at 20 mM, 20 ppm and 500 ppm respectively. Obtained results were true during both seasons of study. Such results are coincided with those reported by Abdel-Mawgoud *et*

Table 6. Effect of some natural compounds *i.e.* chitosan, methionine and ascorbic acid on physical fruit quality of strawberry plants cv. Festival during 2019/2020 and 2020/2021 seasons.

Treatments	2019/2020				2020/2021			
	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit firmness (g/cm2)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit firmness (g/cm2)
Control	18.69 f	4.10 d	3.30 d	155.00 f	24.74 d	4.87 d	3.40 e	161.87 h
Rhizolex (1g/L)	21.14 e	4.17cd	3.46 cd	160.00 c-f	25.22 d	4.90 cd	3.61 de	165.00gh
Chitosan (FS)	22.97de	4.57bc	3.67 abc	168.57 ab	26.27cd	5.07 bcd	3.85 a-d	177.33 a-d
Chitosan (RS)	25.33cd	4.83ab	3.83 ab	168.60 ab	27.39bc	4.90 cd	3.67 cde	178.64 ab
Chitosan (FS+Rs)	28.67 a	5.10 a	4.00 a	171.67 a	30.92 a	5.53 a	4.10 a	179.71 a
Methionine (FS)	24.38cd	4.67 b	3.63 bcd	163.53 b-e	25.65cd	4.90 cd	3.86 a-d	171.77 ef
Methionine (RS)	25.55 c	4.86ab	3.70 abc	158.33def	26.74cd	5.03 bcd	3.70 bcd	167.50 fg
Methionine (FS+RS)	28.36ab	4.97ab	3.97 ab	165.13 a-d	29.26ab	5.30 ab	3.93 abc	178.40abc
Ascorbic acid (FS)	24.91cd	4.80ab	3.87 ab	164.93 a-d	26.46cd	5.16 bc	3.73 bcd	172.37def
Ascorbic acid (RS)	25.51 c	4.63 b	3.73 abc	156.67 ef	26.01cd	5.13 bcd	3.90 abc	173.33cde
Ascorbic acid (FS+RS)	26.13bc	4.96ab	3.93 ab	166.67abc	29.37ab	5.20 b	3.97 ab	173.47 b-e

Within a column, means followed by the same letter are not different at P< 0.05

Table 7. Effect of some natural compounds i.e. chitosan, methionine and ascorbic acid on chemical fruit quality of strawberry plants cv. Festival during 2019/2020 and 2020/2021 seasons.

Treatments	TSS%	Vit.C (mg/100g f.w)	Acidty (mg/100g f.w)	Total sugars%	Anthocyanin (mg/100g f.w)
			2019/2020		
Control	9.90 d	51.93 c	0.93 d	5.40 d	92.17 c
Rhizolex (1g/L)	10.53 cd	53.66 bc	0.97 cd	5.85 cd	93.82 c
Chitosan (FS)	10.53 cd	56.00 bc	1.04 bcd	6.19 bc	97.29 b
Chitosan (RS)	10.70 cd	56.96 b	1.08 abc	6.22 bc	98.89 b
Chitosan (FS+Rs)	11.50 abc	64.43 a	1.20 a	6.62 ab	102.30 a
Methionine (FS)	10.86 bcd	66.33 a	1.13 ab	6.36 ab	98.93 b
Methionine (RS)	11.10 bc	54.00 bc	1.09 ab	6.47 ab	97.97 b
Methionine (FS+RS)	11.77 ab	56.00 bc	1.15 ab	6.74 a	102.37 a
Ascorbic acid (FS)	11.40 abc	57.50 b	1.15 ab	6.52 ab	98.63 b
Ascorbic acid (RS)	11.20 abc	64.46 a	1.14 ab	6.52 ab	98.80 b
Ascorbic acid (FS+RS)	12.17 a	67.70 a	1.20 a	6.81 a	102.85 a
			2020/2021		
Control	9.80 d	55.33 e	1.00 d	6.52 f	81.96 e
Rhizolex (1g/L)	10.70 cd	57.50 de	1.03 cd	6.64 ef	83.79 de
Chitosa9n (FS)	11.06 cd	59.66 cd	1.03 cd	6.96 de	85.00 cd
Chitosan (RS)	11.50 bc	60.33 cd	1.04 cd	7.10 cd	87.27 bc
Chitosan (FS+Rs)	11.93 abc	69.03 ab	1.16 a	7.51 ab	92.45 a
Methionine (FS)	11.33 bc	61.80 c	1.05 bcd	7.18 bcd	85.79 cd
Methionine (RS)	11.56 bc	58.50 cde	1.13 abc	7.13 cd	89.23 b
Methionine (FS+RS)	12.41 ab	69.83 ab	1.18 a	7.59 a	92.63 a
Ascorbic acid (FS)	11.60 bc	68.53 ab	1.15 ab	7.30 abc	87.29 bc
Ascorbic acid (RS)	11.76 abc	67.33 b	1.15 ab	7.25 bcd	88.70 b
Ascorbic acid (FS+RS)	12.93 a	72.00 a	1.19 a	7.63 a	92.97 a

Within a column, means followed by the same letter are not different at P< 0.05

al. (2010), Shafshak *et al.* (2011), El-Miniawy *et al.* (2013) and Shams *et al.* (2014) in case of using chitosan, Manal *et al.* (2019) in case of using amino acids and Shadia (2017) in case of using ascorbic acid indicated that applying such forgoing natural compounds reflected positive effect on all measure chemical fruit quality for such tested vegetable crops.

Rhizoctonia root rot disease in strawberry is one of serious soil borne diseases that threatens production. Using of eco-compatible materials may be an alternative to harmful chemicals. The present study had been conducted to evaluate the natural materials root rot caused by *Rhizoctonia solani*. The present results indicated that applications of chitosan, methionine or ascorbic acid induced resistance against root rot disease and increased strawberry yield. The observed increase in strawberry yield in this study might be due to the reduction of disease incidence, disease severity and promotion of plant growth as a result of using this safety compounds.

It could be suggested that combined applications between foliar spray and root seedling soaking with chitosan, methionine or ascorbic acid might be used commercially as safely and applicable method for controlling strawberry root rot disease and enhancement of growth parameters and increasing fruit yield.

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الملخص العربى

استخدام بعض المركبات الطبيعية لتحسين النمو والمحصول وتحمل نباتات الفراولة لمرض عفن الجذور الريزوكتونى

سيد منصور رزق [١] و مها حلمي محمد [٢]

[١] قسم بحوث البطاطس والخضر خضرية التكاثر – معهد بحوث البساتين– مركز البحوث الزراعية – الجيزة – مصر

[٢] قسم أمراض النبات – كلية الزراعة – جامعة عين شمس – مصر

جميع التجارب تم اجرائها خلال الموسمين الزراعين٢٠١٩ /٢٠٢٠ و٢٠٢٠/٢٠٢١. تم اجراء تجارب الصوب بقسم أمراض النبات، كلية الزراعة، جامعة عين شمس. بينما تم اجراء التجارب الحقلية بمحطة بحوث البساتين بالقناطر الخيرية بمحافظة القليوبية لدراسة تأثير بعض المركبات الطبيعية (الشيتوسان والمثيونين وحمض الاسكوربيك) على تحمل نباتات الفراولة لمرض عفن الجذور الريزوكتونى وكذلك النمو الخضرى والتركيب الكيماوى للنبات والمحصول الثمرى ومكوناته وكذلك جودة المحصول الناتج من الفراولة صنف فيستيفال مقارنة بنباتات الفراولة المصابة غير المعاملة.

وقد أوضحت النتائج ان استخدام كلاً من الشيتوزان بتركيز ٥٠٠ جزء في المليون، والميثيونين بتركيز ٢٠ جزء في المليون وحمض الأسكوربيك بتركيز ٢٠ ملليمولر من خلال نقع جذور الشتلات، أوالرش الورقي، أومعاملة الدمج بين نقع الجذور والرش الورقي للمركبات السابقة، أومعاملة نقع جذور الشتلات في المبيد الفطري ريزوليكس بمعدل ١جم/لتر، أدت الى حدوث انخفاض معنوى في حدوث اصابة عفن الجذور المتسبب عن فطر ريزوكتونيا سولانى. وبالاضافة لذلك، فإن معاملة الدمج بين نقع الجذور والرش الورقي بأستخدام الشيتوزان أو الميثيونين أدت إلى حدوث أفضل انخفاض معنوي في حدوث المرض تحت كل من ظروف الصوبة وظروف الحقل.

أدت جميع المعاملات باستثناء المبيد الفطري ريزوليكس إلى حدوث زيادة معنوية في الفينولات الكلية ونشاط الإنزيمات المؤكسدة مثل بولي فينول اكسيديز و سوبر اكسيد ديسميوتيز و الكتاليز خلال موسمي النمو.

كما أظهرت النتائج المتحصل عليها أن معاملة الدمج بين النقع مع الرش الورقي بالشيتوزان بتركيز ٥٠٠ جزء في المليون والميثيونين بتركيز ٢٠ جزء في المليون وحمض الأسكوربيك بتركيز ٢٠ ملليمولر أدت إلى حدوث زيادة معنوية في جميع صفات النمو الخضرى ومحتوى النبات من النيتروجين والفوسفور والبوتاسيوم والكربوهيدرات الكلية في المجموع الخضري للنبات وانتاجية وجودة ثمار نبات الفراولة.