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Suppression of Strawberry Black Root Rot Disease in the Field by Chitosan Applied as Root Dipping and Foliar Spray

Farid Abd-El-Kareem, Ibrahim E. Elshahawy and Mahfouz M. M. Abd-Elgawad

Plant Pathology Department, National Research Centre, Egypt.

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ABSTRACT

Eight concentrations, i.e., 0.0, 0.5,1,2,3,4,5 and 6 g/L of chitosan were tested to study their effect on linear growth of strawberry black root rot fungi (*F. solani, R. solani* and *M.phaseolina)* in the laboratory. Results revealed that all tested concentrations of chitosan except that 0.5 and 1 g/L significantly reduced the linear growth of all tested fungi. Complete reduction was obtained with chitosan at 6 g/L for all tested fungi. The highest reduction was obtained with chitosan at 5g/L which reduced the linear growth by 91.1, 90.3 and 88.9 % for *F. solani, R. solani* and *M.phaseolina* respectively. In field experiments, application of chitosan at concentrations of 5 & 6 g/L and at 0.5 &1g/L as root dipping and foliar spray respectively were evaluated alone or in combination to study their effect on black root rot disease, some vegetative characters, yield and enzyme activities of strawberry plants. Results revealed that all tested concentrations of chitosan applied as root dipping or foliar spray significantly reduced the black root rot disease of strawberry plants. The most effective treatments are combined treatments between root dipping and foliar spray which reduced disease incidence and severity more than 76.8 and 75.9 % respectively. Followed by root dipping treatment which reduced disease incidence and severity more than 64.3 and 61.1 % respectively. While, foliar spray was less effective. The highest increase of fresh and dry weight was obtained with combined treatments between root dipping and foliar spray which increased the fresh and dry weight more than 85.7 and 89.5 % respectively. While, single treatments showed moderate effect. Moreover, all tested concentrations of chitosan applied as root dipping or foliar spray significantly increased the strawberry yield. The most effective treatments are combined treatments between root dipping and foliar spray which increased the yield 92.0 %. Followed by root dipping treatment which increased the yield more than 58.0 %. While, foliar spray was less effective. Furthermore, combined treatments between root dipping and foliar spray caused increased the peroxidase and chitinase activities more than 172.0 and 181.3 % respectively. While, single treatments showed moderate effect.

Keywords: Strawberry, Black root rot disease, Chitosan, Vegetative characters, Yield, Enzyme activities

1. Introduction

Black root rot (BRR) is currently a pressing issue of strawberry plants in Egypt. This disease is complex as it is frequently caused by several fungal species, such as *Macrophomina phaseolina* (Hutton *et al.,* 2013), *Fusarium oxysporum* (Juber *et al.,* 2014), and *Rhizoctonia* spp. (Fang *et al.,* 2013). Therefore, efforts are ongoing for its control via a variety of measures in Egypt (El-Shemy *et al.,* 2013; Abd-El-Kareem *et al.,* 2019a, b). Our field monitoring revealed the frequent presence of *Fusarium solani*, *Rhizoctonia solani*, *M. phaseolina*, and *Pythium* sp. in Egypt as the causal agents. The symptoms begin by killing of feeder rootlets which is followed by blackening and degeneration of the whole plant roots resulting in the plant death or at the best causing considerable decrease in the strawberry fruit and foliage growth (Abdel-Sattar *et al.,* 2008; Fang *et al.,* 2012; Ceja-Torres *et al.,* 2014). Using chemical fungicides for controlling such pathogens can sometimes achieve plausible results, but many of such chemicals have been banned or restricted on the account of environmental concerns and negative impact

Corresponding Author: Farid Abd-El-Kareem Plant Pathology Department, National Research Centre, Egypt.
E-mail: ford62nrc@yahoo.com

on human health. This dilemma represents a current impetus for researchers and stakeholders to earnestly attempt to avoid these toxic chemicals and substitute them with benign and efficient control measures (Abd-El-Kareem *et al.,* 2019 a, b; 2021 and 2022).

Kaur and Dhillon (2014) characterized chitosan as a linear polymer. Partially deacetylated chitin can generate chitosan (Kumar 2000). As natural chitin is relatively cheap and available polysaccharide, chitosan use for the disease control can lend itself as a cost-effective approach (Elieh-Ali-Komi and Hamblin 2016). Chitin is a main material to form the cuticle of many organisms such as exoskeleton of crustaceans, insects, and fungal cell walls. Chitosan is a promotor of plant defence (Yin *et al.,* 2016; Suarez-Fernandez *et al.,* 2020) and a hormone in major food crops (Iriti and Faoro 2008; El-Tantawy 2009). It helps in accumulating auxin in roots of many plants (Lopez-Moya *et al.,* 2017). It is well-known that the high molecular weight chitosan is viscous at a concentration of 5 and 6 g/L. This attribute voluntarily facilitates treating the roots of seedlings and soil. Yet, it cannot be used in spraying due to the high viscosity. Therefore, the use of chitosan at a concentration of 0.5 and 1.0 g/L as foliar application is the readily concentration used in the present study. While chitosan is known at high concentrations (5 and 6 g/l) to have a direct anti-fungal effect and a stimulus activity to induce resistance in the plant, its role at low concentrations (0.5 and 1.0 g/ l) is to activate the induced resistance in the plant against several diseases (Abd-El-Kareem and Haggag 2015).

The objective of this investigation was to evaluate treatment with chitosan via root dipping and/or foliar spray of strawberry plants on BRR disease and yield parameters in the field.

2. Material and Methods

2.1. Pathogens causing BRR disease of strawberry

Local isolates of *Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina*, the causal agents of BRR disease of strawberry plants were provided by the authors' collection (Abd-El-Kareem *et al.,* 2019a,b; 2021 and 2022), Plant Pathology Department NRC Giza, Egypt.

2.2. Strawberry cultivar

Certified strawberry seedlings of cultivar Festival was utilized in the field trials.

2.3. Chitosan

Chitosan with high molecular weight (HMW~600000 Dalton) produced by Sigma-Aldrich Chemicals Company were used in the present work.

2.4. Bioassays for the linear growth of the obtained fungi

Chitosan concentrations at 6, 5, 4, 3, 2, 1, 0.5, and 0 g chitosan /l of water were formed separately using sterilized potato dextrose agar (PDA) before solidification. Then, they were added to Petri-plates as described by Abd-El-Kareem *et al.* (2019b) to evaluate their impact on the growth of the targeted fungi (*F. solani*, *R. solani*, and *M. phaseolina*). After solidification, 6-mm fungal disc was centered at each plate and incubated (25±1.5°C). Plates were replicated five times per a concentration for each species. The decrease in linear growth of each species was calculated relative to the check (without chitosan).

2.5. Field trials

Experiments were completed in a field with light loamy soil and natural infestation at Eldeer hamlet, Toukh county, Qalyubia Governorate, Egypt for seasons 2019/20 and 2020/21. At each season, seedlings were transplanted on the 3rd of November and fruit harvest was completed after 5 months. The target area was divided into plots (4×8 m), each with 8 rows. Thirty two holes per row were used to transplant the strawberry seedlings. We applied a randomized complete block design. Three replicates (plots) per treatment were furnished; a replicate had 100 strawberry transplants. All seedlings were awarded the same production practices such as soil fertilizers, aerial spray, and irrigation (El-Shemy *et al.,* 2013).

2.6. Chitosan and fungicide applications

Root dipping: Ninety-day-old strawberry seedlings were used. This treatment was executed using chitosan solutions of 5 or 6 g/l for 5 minutes before transplanting. Additional strawberry roots were similarly dipped but in water only to function as check.

I. Foliar spray: Thirty days after transplanting, seedlings were sprayed with 0.5 or 1 g/l of chitosan solutions, 10 ml of a solution per plant.

II. Combined treatments: The plant roots were dipped in chitosan solutions as. Then, 30 days after transplanting, the same plants were treated again with chitosan but as a spray with its aforementioned solutions. The fungicide actamyl (3 g/l) was used as a check too. Seedlings were dipped in the fungicide solution for 5 minutes before transplanting and then sprayed with the solution one month after cultivation.

2.7. Assessment of disease incidence and severity

Symptoms begin on plants after 30 days of transplanting in the form of stunting and yellowing of plants and may progress until the end of the season. So, the disease incidence [(Number of infected plants/ Total number of plants) X 100] was assessed 100 days after transplanting. The disease severity was evaluated at the end of the trials (5 months after transplanting). We adopted a 0–5 scale for the evaluation as illustrated by Morocko (2006) where the severity % = [(Σ Disease grade x number of plants in each grade)/(Total number of plants x highest disease grade)] x 100.

2.8. Evaluating growth and fruit yield of strawberry

Dry and fresh weights of the plants at each plot were recorded at season-end. Also, accumulated yield of fruit (Ton/Feddan) in the field trials was calculated.

2.9. Enzyme activities

Homogenization, centrifugation, and getting the liquid lying above the solid residue of the freshly collected plant leaves after centrifugation were done as described by Goldschmidt *et al.,* (1968). This liquid, supernatant, was used to measure activities of two separate enzymes.

2.10. Peroxidase assay

Peroxidase activity was measured via incubation of enzyme extract with guaicol solution using the technique reported by Abeles *et al.,* (1971). The activity was identified, via spectrophotometer (Spectronic 20-D), as the raise in absorbance at 470 nm/gram fresh weight/1 min.

2.11. Chitinase assay

The chitin powder was utilized to furnish the substrate of colloidal chitin (Ried and Ogryd-Ziak 1981), and then measuring the activity of the enzyme as described by Monreal and Reese (1969) using spectrophotometer (Spectronic 20-D) where optical density was determined at 540 nm. Its activity was named in the character of mM N-acetylglucoseamine equivalent released/gram fresh weight tissue/60 min.

2.12. Statistical analysis

Data were exposed to statistical analysis and treatment means were compared utilizing Duncan's New Multiple Range Test (DNMRT).

3. Results

3.1. Chitosan efficacy on growth of the pathogenic fungi

Except chitosan concentrations at 0.5 and 1g/l, all tested concentrations of chitosan could significantly lower the linear growth of the three tested fungi (Table 1, Fig. 1). At 6 g/l, chitosan worked out full inhibition in the growth of the 3 tested fungal species. This was followed by their growth inhibition at 5g/l, linear growth reduced by 91.1, 90.3 and 88.9% for *F. solani, R. solani* and *M.phaseolina* respectively.

Table 1: Antagonistic effect of chitosan solution on the linear growth of strawberry BRR fungi

Chitosan	Average of fungal growth area (cm^2)*					
g/l	*F. solani*	Reduction %	*R. solani*	Reduction %	*M. phaseolina*	Reduction %
0.5	90.0 a	0.0	90.0 a	0.0	90.0 a	0.0
1.0	90.0 a	0.0	90.0 a	0.0	90.0 a	0.0
2.0	45.0 b	50.0	40.0 b	55.6	55.0 b	38.9
3.0	35.0 c	61.1	32.0 c	64.4	37.0 c	58.9
4.0	27.0 d	70.0	24.0 d	73.3	24.0 d	73.3
5.0	8.0 e	91.1	6.0 e	90.3	10.0 e	88.9
6.0	0.0 f	100.0	0.0 e	100.0	0.0 f	100.0
Control	90.0 a	00.0	90.0 a	00.0	90.0 a	0.0

* Figures followed by different letters are significantly ($P \leq 0.05$) different from each other according to DNMRT

3.2. Field trials

3.2.1. Chitosan application

All tested chitosan treatments and concentrations significantly lowered the rot disease in terms of both its incidence and severity (Table 2, Fig. 2). The most favorable results were given when root dipping was followed by foliar spray as combined treatments. Such combinations decreased disease incidence and severity by more than 76.8 and 75.9%, respectively. Only root dipping treatment could decrease incidence and severity of the disease by more than 64.3 and 61.1%, respectively. Foliar spray alone was less effective.

Table 2: Effect of chitosan on strawberry BRR disease under field conditions

Chitosan treatment		Strawberry BRR disease*			
		Disease incidence	Reduction %	Disease	Reduction
Root dipping	Foliar spray			severity	%
		Single treatments			
5.0	**0.0**	20.0c	64.3	21.0 c	61.1
6.0	**0.0**	18.0c	67.9	19.0 c	64.8
0.0	**0.5**	32.0 b	42.9	30.0 b	44.4
0.0	**1.0**	30.0 b	43.2	29.0 b	46.3
		Combined treatments			
5.0	**0.5**	13.0 de	76.8	13.0 d	75.9
	1.0	12.0de	78.6	13.0d	75.9
6.0	**0.5**	12.0de	78.6	12.0 d	77.8
	1.0	10.0 e	82.1	11.0 d	79.6
Actamyl 3 g/l (fungicide)		14.0 d	75.0	14.0 d	74.1
Control		56.0 a	0.0	54.0 a	0.0

* Figures followed by different letters are significantly ($P \leq 0.05$) different from each other according to DNMRT

Also, all tested chitosan treatments and concentrations significantly raised the fresh and dry weights of the plants in the treated plots (Table 3). The best weights were given when root dipping was followed by foliar spray as combined treatments. Such combinations lessened incidence and severity of the disease by more than 85.7 and 89.5%, respectively. Likewise, **r**esults in Table (4) revealed that all treatments significantly enhanced fruit yield of the strawberry, combined treatments (root dipping + foliar spray) could attain 92% yield increase. Root dipping treatments boosted the yield more than 58%. Foliar spray was less effective.

3.2.2. Application impact of chitosan on enzyme activities

All chitosan treatments could boost the activities of peroxidase and chitinase in the fresh leaves of the strawberry plants (Table 5). The combined treatments (root dipping + foliar spray) were the most effective as they raised the peroxidase and chitinase activities by more than 172 and 181.3%, respectively. Single treatments have not as high effects as combined treatments.

Table 3: Effect of chitosan on weights of strawberry plants at season-end.

Chitosan treatment		Weight (g)/plant*			
Root dipping	**Foliar spray**	**Fresh**	**Increase %**	**Dry**	**Increase %**
		Single treatments			
5.0	**0.0**	200.0 c	42.9	32.0 b	68.4
6.0	**0.0**	220.0b	57.1	30.0b	57.9
0.0	**0.5**	220.0 b	57.1	32.0b	68.4
0.0	**1.0**	210.0 b	50.0	30.0b	57.9
		Combined treatments			
5.0	**0.5**	260.0 a	85.7	36.0a	89.5
	1.0	265.0 a	89.3	36.0a	89.5
6.0	**0.5**	270.0 a	92.9	38.0a	100.0
	1.0	270.0 a	92.9	39.0a	105.3
Actamyl 3 g/l (fungicide)		210.0 b	50.0	31.0b	63.2
Control		140.0 d	0.0	19.0 c	0.0

* Figures followed by different letters are significantly ($P \leq 0.05$) different from each other according to DNMRT.

Table 4: Effect of chitosan on strawberry yield in the field.

Chitosan treatments		Strawberry yield	
Root dipping	**Foliar spray**	**Tons/Feddan***	**Increase %**
		Single treatments	
5.0	**0.0**	16.0 b	60.0
6.0	**0.0**	16.5 b	65.0
0.0	**0.5**	15.8 c	58.0
0.0	**1.0**	15.8 c	58.0
		Combined treatments	
5.0	**0.5**	19.2 a	92.0
	1.0	19.0 a	90.0
6.0	**0.5**	20.0 a	100.0
	1.0	20.2 a	102.0
Actamyl 3 g/l (fungicide)		15.2 c	52.0
Control		10.0 e	0.0

* Figures followed by different letters are significantly ($P \leq 0.05$) different from each other according to DNMRT

Table 5: Effect of different chitosan treatments on peroxidase and chitinase activities in fresh leaves of strawberry plants.

Chitosan treatment		Enzyme activities*			
		Peroxidase		**Chitinase**	
Root dipping	**Foliar spray**	**Activity**	**Increase %**	**Activity**	**Increase %**
		Single treatments			
5.0	**0.0**	4.5 c	80.0	6.0b	87.5
6.0	**0.0**	4.3 c	72.0	6.2b	93.8
0.0	**0.5**	4.4 c	76.0	6.3b	96.9
0.0	**1.0**	4.6 c	84.0	6.5b	103.1
		Combined treatments			
5.0	**0.5**	6.8 a	172.0	9.0a	181.3
	1.0	6.9 a	176.0	9.0a	181.3
6.0	**0.5**	7.1 a	184.0	9.3a	190.6
	1.0	7.3 a	192.0	9.4a	193.8
Actamyl 3 g/l (fungicide)		5.5 b	120.0	6.0b	87.5
Control		2.5 c	0.0	3.2 c	0.0

* Figures followed by different letters are significantly ($P \leq 0.05$) different from each other according to DNMRT

4. Discussion

Many reports have documented the importance and growing occurrence of BRR in strawberry plants in Egypt and elsewhere. The disease is caused by one or several fungal species including the encountered fungi in our field trials (e.g., Fang *et al.,* 2013, Hutton *et al.,* 2013, Juber *et al.,* 2014, Abd-El-Kareem *et al.,* 2019a, b). However, favorable conditions for the abundant and profitable production of strawberry plants in Egypt regarding the geographical location, the ecological factors, and the labor force have sparked wide interest to expand its cultivation and created worldwide markets for export (Abd-Elgawad 2019). Moreover, the successful establishment of several US strawberry elite varieties in Egypt (El-Borai and Whitaker 2021) helped to enhance strawberry fruit production. Clearly, all such factors called to avoid the increased losses caused by the spread of this disease in Egypt **(**Abd-El-Kareem *et al.,* 2019a, b). The disease control via chemical fungicides has been discouraged. Some of these chemicals have been banned or restrictions have been imposed on their use due to ecological issues and health hazards (Abd-El-Kareem *et al*., 2019 a, b and 2021). Hence, the importance of the chitosan impact in managing this disease is apparent as both safe and effective alternative to unsafe chemicals. Furthermore, chitosan has been characterized as general elicitor of plant defenses (Yin *et al.,* 2016, Suarez-Fernandez *et al.,* 2020). So, fungicidal activity of chitosan has been documented against many economically significant species of fungi and oomycetes (Ait Barka *et al.,* 2004, Vasyukova *et al.,* 2005, Abd-El-Kareem *et al.,* 2021). In addition to the treatments followed herein, chitosan may be applied as seed or soil treatments to control diseases of many plant species (Badawy *et al.,* 2005).

A few hypotheses have been speculating the mechanism by which chitosan affects the growth of the targeted pathogenic fungi. The polycationic quality of chitosan may intervene with macromolecules residues that have negative charge and are found on the cell surface of the targeted fungus. El Hassni *et al.,* (2004) assumed that such a reaction drives to infiltration of proteinaceous constituents and intracellular electrolytes. Another hypothesis presumes that the reaction of hydrolysis components with microbial DNA can command the inhibition of mRNA (Vasyukova, *et al.,* 2005). Also, chitosan may function as a chelating of essential nutrients, spore elements, and metals (Rabea *et al.,* 2005). Other assumptions postulate modified functions of chitosan with either topical impact or genetic deformation of the target fungi (Pitta-Alvarez and Giulietti 1999, Palma-Guerrero *et al.,* 2008, Pandey 2017). He *et al.,* (2009) suggested chitosan as a possible boosting material for cell penetration.

Increased doses of chitosan can account for gathering of fluorescence materials corresponding to hormones and phenolics. These latter groups are related to both plant defense against stressed conditions and growth (Colman *et al.,* 2019, Park *et al.,* 2019, Samari *et al.,* 2020). Chitosan boosts metabolic pathways comprising the biosynthesis of these materials (Fooladi vanda *et al.,* 2019, Iglesias *et al.,* 2019, Singh *et al.,* 2020). A small amount of chitosan can raise plant immunity via piling up of plant hormones especially salicylic acid and jasmonic acid in the root systems (Lopez-Moya *et al.,* 2017, Singh *et al.,* 2020). However, this effect has not yet been detected in tomato root exudates. Additionally, chitosan proved to replace recurrently utilized growth factors, e.g. auxins, methyl jasmonate, or cytokinins (Ahmed *et al.,* 2014, Acemi 2020). All of the above-mentioned benefits of various chitosan applications must be taken seriously for earnest attempts on broader scale for prospective and promising agriculture in Egypt.

Conclusions

Heightened concern for the dangers of chemical fungicide has positively re-directed research for benign alternatives such as chitosan. As it raised strawberry fruit and yield parameters herein, chitosan has commendable effect on the BRR disease of strawberry.

List of abbreviations

BRR: Black root rot; DNMRT: Duncan's New Multiple Range Test; PDA: Potato Dextrose Agar.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication
Not applicable

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions
All authors equally participated in the development and implementation of the reviewing plan. Subsequently, they worked it out wrote the manuscript; the first author FA wrote and discussed the different parts of the article with IE and MA and all together finalized the manuscript. All authors have read and approved the final manuscript.

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