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EFFICACY OF CHITOSAN AND CHITOSAN NANOPARTICLES TO CONTROL CHOCOLATE SPOT DISEASE ON FABA BEAN UNDER FIELD CONDITIONS

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ABSTRACT

This study was conducted at Plant Pathology Dep., Faculty of Agriculture, Damanhour University and Etay El-Baroud Agricultural Research Station, El El-Beheira governorate during 2018 - 2019 season. The aim of this study was to examine the efficacy of Chitosan and Chitosan nanoparticles to control chocolate spot disease caused by *Botrytis fabae* on faba bean (cv. Giza 40) under field conditions. Spraying Chitosan or Chitosan NP (30, 45, 60 after sowing) significantly decreased chocolate spot disease severity to 8.7% compared to 22.3 for the untreated control which was not significantly different from the fungicide Mancozeb which exhibited 10.17% disease severity. This was accompanied with an increase in totals phenols in Chitosan NP treated plants to 4.43 mg compared to 0. 436 for the untreated control. Also, this was accompanied with an increase in polyphenol and peroxidase activity to be as high as 0.399 and 0.394 compared to 0.133 and 0.130 (absorbance/min./g f.w.), in the untreated control, respectively. Meanwhile, use of Chitosan and Chitosan NP significantly improved faba bean vegetative growth, *i.e.* plant height, number of pods, as well as seeds yield per plant compared to the untreated control with more superiority for Chitosan NP.

Keywords: *Botrytis fabae*, faba bean, chocolat spot disease, chitosan, chitosan nanoparticle, peroxidase, polyphenol oxidase, growth characteristics.

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INTRODUCTION

Faba bean (*Vica faba* L.) is a widely popular grain legume that is used as human food and animal feed for its high nutritional quality **(Yasmin *et al*., 2020).** Also, the crop adds to soil health improvement through biological N-fixation **(Rubiales and Mikic, 2015)**. Faba bean chocolate spot caused by ***Botrytis fabae*** **(Sard.)** is the most important disease throughout the world **(Tivoli *et al*., 2006; Samuel *et al*., 2008)**. Also, **Abd-El-Karem *et al*. (2013)** mentioned that chocolate spot disease caused by ***Botrytis fabae*** **(Sard.)** is the most important disease of faba bean in Egypt. The disease is routinely managed by the use of fungicides, however, the widespread use of fungicides is becoming increasingly unsatisfactory due to the development of fungicidal resistance **(Khaled *et al*., 1995),** as well as their negative effects on public health and the environment and modification of balance of beneficial microorganisms **(Tola *et al*., 2016).**

Chitosan is a natural linear biopolymer that reported to control pathogenic microorganisms by preventing growth, sporulation, spore viability, germination and disrupting cell and encouragement of different defense responses in host plant inducing and/ or inhibiting different biochemical activities during the plant-pathogen interaction **(Romanazzi *et al*., 2015; Hassan and Chang, 2017)**. Chitosan based nanoparticles provoke even higher physiological and biochemical responses in plants as compared to their bulk form because of their higher surface to volume ratio **(Saharan and Pal, 2016)**. The present study, therefore, aimed to investigate the efficacy of Chitosan and Chitosan nanoparticles to control chocolate leaf spot disease caused by ***B. fabae*** under field conditions.

MATERIALS AND METHODS

Plant materials, Chemicals, and experiment preparations

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The susceptible faba bean cv. Giza40 was tested under field conditions at Agric. Res. Station, Etay El-Baroud, El-Behera governorate during 2018-2019 growing season. Tested seeds of faba bean cv. G. 40 were obtained from Field Crop Res. Inst., Agric. Res. Centre, Giza, Egypt. Seeds were cultivated in rows, three rows of ten plants for each treatment.

Faba bean plants (cv. Giza 40) were sprayed with chitosan (8 g/L) and chitosan nanoparticles (0.08 g/L), dissolved in 1% acetic acid (AA), according to **(Sakif *et al*. (2016)** and **Ahamed (2017)** three times at 30, 45 and 60 days after sowing at the same time the fungicide (Mancozeb 80%) at the rate of 2.5 g/ L which served as positive control while a treatment sprayed with tap water as negative control. Chitosan and Chitosan NP were obtained from Nakaa Technology Company in Cairo while the fungicide was obtained from UBL Limited, India. Plants treated in the field as normal and left for the natural infection with chocolate leaf spot disease.

Disease assessment

Plants were visually rated for the developed chocolate leaf spot symptoms according to the scale reported by **Hanounik (1986)** in five ratings as follows:

1= No spots to less than 25% affected plants, 2= Spots common on leaves covering> 25 - <50% of leaf area, little defoliation and some spots on the stems, 3= very common spots on leaves covering > 50 - <75% of leaf area, some defoliation and many spots on the stems, 4= extensive spots on leaves and stems covering > 75 - <100% of leaf area., 5= dead leaves and severe defoliation.

Disease severity (DS) percentage was calculated using the following equation:

Disease severity % = Σ (n X v)/9N) X 100,

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Where: (n) = Number of plants in each category; (v) = Numerical values of symptoms category; (N) = Total number of plants; (4) = Maximum numerical value of symptom category.

Determination of the oxidative enzymes and total phenols

After 90 days from sowing, three faba bean leaves from each treatment were taken randomly for the determination of total phenols, oxidative activities (peroxidase and polyphenol oxidase). Leaves were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine enzyme activities **(Tuzun *et al.*, 1989).**

a. Determination of peroxidase (PO)

Peroxidase activity was expressed as the increase in absorbance at 425 nm/g fresh weight/minute and determined according to the method described by **Kar and Mishra (1976).**

b. Determination of Polyphenoloxidase (PPO)

Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/minute and determined according to the method described by **Matta and Dimond (1963).**

c. Determination of Total phenols

Total phenols were determined using Spectrophotometer (SPECTRONIC 20-D) at 520 nm according to the method of **Bary and Thorpe (1954).**

Growth characters and yield determination

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Plant height (cm), number of pods / plant, seed weight / plant (g) were determined as the average of five plants chosen randomly from the center of the three rows of each replicate at harvest time.

Statistical analysis

All data were subjected to the analyses of variance (ANOVA) for split-plot design followed by compared means with LSD at level probability 5% according to **(Gomez and Gomez, 1984).**

RESULTS

1. Effect of spraying Chitosan and Chitosan nanoparticles (NP) on faba bean (cv.Giza 40) plants on chocolate spot disease severity, total phenols and oxidative enzymes activity of leaves

Data presented in Table (1) showed that foliar spraying of faba bean leaves (cv. Giza 40) with Chitosan or Chitosan NP significantly decreased the developed Chocolate spot disease severity (%). However, Chitosan NP treatment was the most effective as decreased disease severity to be as low as 8.71% compared to 22.23% for the untrated control. This effect was even more effective than the fungicide Mancozeb at the tested concentrations. This was accompanied with an increase in totals phenols in Chitosan NP treated plants to 4.43 mg/g f.w.) compared to 0. 436 for the untreated control. Also, this was accompanied with an increase in polyphenol and peroxidase activity to be as high as 0.399 and 0.394 compared to 0.133 and 0.130 (absorbance /min./g f.w.) in the untreated control, respectively.

Table (1): Effect of foliar spray with Chitosan and Chitosan nanoparticles (NP) on chocolate leaf spot disease severity, total

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phenols and the oxidative enzymes activity of faba bean leaves (cv. Giza 40) under field conditions.

Parameter / Treatments & Concentration	Disease severity (%)	Total phenols (mg/g f.w.)	Peroxidase activity (Absorbance / min./g f.w.)	Polyphenol oxidase activity (Absorbance/ min./g/f.w.)
Chitosan (0.80 g/L)	9.97	1.13	0.189	0.134
Chitosan NP (0.08 g/L)	8.73	4.43	0.354	0.399
Acetic acid (1%)*	10.71	1.69	0.129	0.131
Mancozeb (2.5g/L)	10.17	0.670	0.172	0.183
Untreated Control	22.23	0.436	0.130	0.133
LSD 5%	1.05	0.347	0.019	0.012

***Solvent of Chitosan and Chitosan NP as a control.**

2- Effect of spraying Chitosan (Ch) and Chitosan NP on faba bean (cv. Giza 40) plant growth characteristic and yield.

Data presented in Table (2) showed that spraying faba bean (Giza 40) with Chitosan and Ch NP significantly increased seed weight/ plant compared to the untreated control. This was most pronounced with Chitosan NP where 21.6 seed weight g./plant was recorded compared to 11.89 g for the untreated control. This effect was even not significantly different from the fungicide Mancozeb effect which exhibited 19.08 g./plant. Meanwhile, similar trend was obtained for plant height while for No. of pods/ plant Chitosan NP was more effective to increase number of pods (Table 2).

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Table (2): Effect of spraying chitosan and chitosan NP on vegetative growth and yield of faba bean (cv. Giza 40) under field conditions.

Parameter / Factor	Plant height (cm)	No. pods/ plant	Seed weight (g/plant)
Chitosan (0.80 g/L)	65.23	10.00	20.6
Chitosan NP (0.08 g/L)	68.10	11.87	21.6
Acetic acid *(1%)	60.37	6.53	7.87
Mancozeb (2.5 g/L)	66.43	7.87	19.08
Untreated Control	63.80	4.40	11.89
LSD 5% Treatment (T)	3.02	1.83	3.45

***solvent of Chitosan and Chitosan NP as a control.**

DISCUSSION

Due to the high environmental risks of chemical pesticides, the use of biological pesticides for biological control of plant diseases is strongly encouraged and recommended. Chitosan and chitosan oligosaccharides have become well-known biological control agents due to their non-toxic, and biodegradable properties **(El-Mohamedya, 2019).**

Several studies **(Reglinski, *et al.*, 2010, Ahmed, 2017, and El-Mohamedya *et al.*, 2019)** indicated that Chitosan has antifungal activity against a wide range of plant pathogens e.g. *Rhizoctonia solani* and *Fusarium solani, F. oxysporum, Macrophomina phaseolina, Alternaria solani, Sclerotium rolfsii, Phytophthora infestance* and *B. fabae.* Furthermore, recent reports indicated that Chitosan in the nanoformulation was even more effective in this respect **(Kheiri *et al.*, 2017; El-Mohamedya *et al.*, 2019).** This was explained in view that Chitosan and its derivatives induce hosts to produce and increased activity of defense-related enzymes such peroxidase and polyphenol

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oxidase **(Xing *et al.*, 2015; Li *et al.*, 2016)**, also, total phenolics accumulation was also reported **(Abd-El-Hack *et al*., 2020).**

The present study supported these results as foliar spraying of faba bean leaves (cv. Giza 40) with Chitosan or Chitosan NP under field conditions significantly decreased the developed Chocolate spot disease severity (%). However, Chitosan NP treatment was the most effective as decreased disease severity to be as low as 8.71% compared to 22.23% for the untreated control. This effect was even more effective than the fungicide Mancozeb at the tested concentrations. This was accompanied with an increase in totals phenols in Chitosan NP treated plants to 4.43 mg/g f.w.) compared to 0.436 for the untreated control. Also, this was accompanied with an increase in polyphenol and peroxidase activity to be as high as 0.399 and 0.394 compared to 0.133 and 0.130 (absorbance /min./g f.w.) in the untreated control, respectively.

The superior effect of Chitosan NP is expected due to its features based on its specific properties such as size, distribution, penetration, and shape which made it even more effective at lower concentration being 0.08g/L compared to 0.8 g/L for bulk Chitosan. These results are in harmony with **(Xing *et al.*, 2015), Li *et al*. (2016), Abd-El-Hack *et al*. (2020), and Khairy *et al*. (2021).**

Meanwhile, Chitosan was found to increases photosynthesis, promotes and enhances plant growth, stimulates nutrient uptake, increases germination and sprouting, and boosts plant vigor **(Zakiullah, 2019)**. In the present study data showed that spraying faba bean (Giza 40) with Chitosan and Ch NP significantly increased seed weight/ plant compared to the untreated control. This was most pronounced with Chitosan NP where 21.6 seed weight g./plant was recorded compared to 11.89 g for the untreated control. This effect was even not significantly different from the fungicide Mancozeb effect which exhibited 19.08 g/plant. Meanwhile, similar trend was obtained for plant height while

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for No. of pods/ plant Chitosan NP was more effective to increase number of pods. These findings are in harmony with other investigators **(Malerba and Cerana , 2016, Rahman, 2018, Zakiullah, 2019).**

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فاعلية الكيتوزان والكيتوزان النانو على مرض التبقع الشيكولاتى على الفول البلدى تحت الظروف الحقلية

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تم أجراء هذه الدراسة فى قسم أمراض النبات بكلية الزراعة جامعة دمنهور و المحطة البحثية بإيتاى البارود ، بمحافظة البحيرة ، خلال الفترة 2018-2019 بهدف إختبار فاعلية الكيتوزان وكذا الصورة النانوية للكيتوزان فى مقاومة مرض التبقع الشيكولاتى فى الفول تحت ظروف العدوى الطبيعية فى الحقل على صنف جيزة 40 الحساس للمرض. وأظهرت الدراسة أن رش النباتات (30 ، 45 ، 60 يوم بعد الزراعة) بتركيز 8.0ج/لتر كيتوزان أو الرش بالكيتوزان النانو بتركيز 0.08ج/لتر قلل بشكل معنوى شدة المرض على النباتات وكانت الصورة النانوية هى الأكثر فاعلية إذ انخفضت شدة المرض إلى 8.7% مقارنة ب 22.3% للمقارنة الغير معاملة. وكان هذا التأثير لا يختلف معنويا عن مبيد المانكوزيب الذى خفض شدة المرض بنسبة 10.17%. وكان تأثير الصورة النانوية للكيتوزان مصحوبا بزيادة فى الفينولات بالأوراق المعاملة حيث كانت 4.43 mg/g.f.w. /جم وزن طرى مقارنة ب 0. 436 للكنترول الغير معامل ، كذلك كان ذلك مصحوبا بزيادة فى نشاط انزيمات الاكسدة ، البولى فينول أوكسيديز والبيروكسيديز، حيث أظهرت 0.399 ، 0.394 مقارنة بـ 0.133 ، 0.130 (absorbance/min./g. f.w.) على التوالى ، كذلك فإن المعاملة بالكيتوزان أو الصورة النانوية حسن من صفات نمو نباتات الفول (طول النبات ، عدد القرون ، وزن البذور للنبات) بالمقارنة بالكنترول الغير معامل مع تفوق معنوى لتأثير الصورة النانوية بالمقارنة بالصورة العادية.