

Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

Management Powdery Mildew of Marigold (*Calendula officinalis*) Using some Nanoparticles and Chitosan

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ABSTRACT

The effect of seven substances, Copper, CuO, ZnO, AlO NPs (8 mg L⁻¹), CuO, ZnO, AlO (8 mg L⁻¹) and chitosan was evaluated under both greenhouse and field conditions during two growing seasons (2017/2018 and 2018/2019) to control powdery mildew disease caused by *Erysiphe cichoracearum* on marigold (*Calendula officinalis*). Results have shown that all the treatments tested had decreased disease severity compared to the non-treated control. In this respect, chitosan showed the best effect in decreasing disease severity followed by ZnO NPs. Meanwhile, CuO gave the lowest effect in this respect. The decrease in disease severity of powdery mildew was positively expressed by an increase in crop parameters, i.e. numbers of inflorescence weight, fresh and dry weights of plants, plant height, number of branches and increased chlorophyll (a and b) and carotenoids.

Keywords: Marigold (*Calendula officinalis*), *Erysiphe cichoracearum*, chitosan, copper, zinc, aluminum, nanometal particales

INTRODUCTION

Marigold (*Calendula officinalis*), Family Asteraceae is an medicinal and ornamental plants (Garibaldi *et al.*, 2008)

Marigold (*Calendula officinalis*) is one of the most important and economic medicinal and ornamental crops in Egypt. Cultivated area of marigold in 2016 growing season reached about 550 feddan, which yielded 9397 ton fresh flowers (Hanafi and Abdel Wahab, 2016). The origin of these plants is Southern Europe and North Africa. It is cultivated in Egypt, especially in Beni-Sweif, Fayoum and Menia governorates. It used in folk medicinal treatment for the respiratory system, also it used in the treatment of stomach ulcers and gums, natural source of coloring materials, treatment of gastritis and esophagitis. As well as appetite wound treatment (Hanafi and Abdel Wahab, 2016).

Powdery mildew was detected in Argentina for the first time in 2007 (Gabriela *et al.*, 2007), in Italy (Garibaldi *et al.*, 2008). And in Turkey (Hamit, Kavak 2011).

The causal pathogen was identified as *Erysiphe cichoracearum* (Pyeatt and Kretschun, 1983) in California, in Florida, USA it was recorded in 1984 and was identified as *Erysiphe cichoracearum* (Alfieri *et al.*, 1984), in Egypt (Hilal *et al.*, 1998) found that chamomile (*Matricaria chamomilla*), Coriander (*Corianderum sativum*) and cumin (*Cuminum cymimum*) were susceptible to infect by powdery mildew incited by *Sphaerotheca fuliginea*, *E. polygoni* and *E. polygoni*, respectively, it was identified as *Podosphaera xanthii* (Garibaldi *et al.*, 2008), in Netherlands it was recorded for the first time in 2012 and was identified as *Erysiphe cichoracearum* (Broun and Cook, 2012), It was reported in India in 2019, and was identified as *Oidium* sp. And *Leveillula taurica* (Pinky *et al.*, 2019), in 2004 was also described in Egypt. Then *Erysiphe cichoracearum* was described as the causal pathogen (Madein, 2007)

Due to their high surface areas and irregular crystal structures, highly ionic nanoparticulate metal oxides such as

MgO, CaO, AlO and ZnO NPs are unique (Klabunde *et al.*, 1996). Nanoparticulate materials (NPs) have gained increasing attention because of their unusual physical and chemical properties, which vary significantly from their traditional equivalents (Wade *et al.*, 2018; Stoimenov *et al.*, 2002). Recent studies have shown antimicrobial effectiveness of various NPs materials including gold, magnesium (Gu *et al.*, 2003), copper (Cioffi *et al.*, 2005), silver (Kim *et al.*, 2008; Kumar *et al.*, 2008), and zinc oxide (Liu *et al.*, 2009).

Chitosan is a non-toxic compound and was reported to be resistance inducer against some fungal pathogens (Lafontaine and Benhamou, 1996)

This study was carried out in Beni-Sweif governorate to investigate the powdery mildew pathogen affecting marigold and its control. The aims of this study are; (1) Evaluation the nanoparticles and chitosan activity of marigold powdery mildew, (2) Estimation of the incidence and disease severity of the powdery mildew, (3) Determination the growth parameters of marigold plants in greenhouse and field condition.

MATERIALS AND METHODS

Survey of marigold powdery mildew at Beni-Sweif governorate:

Assessment of naturally infected marigold plants with powdery mildew for disease incidence and disease severity was conducted during 2017/2018 and 2018/2019 in Beni-Sweif governorate located at Beba, Ehnasea and Somosta. The survey was done three times during 2017/2018 and 2018/2019.

Artificial inoculation technique :

The local variety of marigold plants were obtained from the Medicinal & Aromatic Plants Res. Sta., Hort. Res. Inst., Agric. Res. Center, Beni-Sweif governorate. Four pots 25 cm were sown each with five seedlings (45-days-old) / pot were transplanted for each treatment. After two weeks of transplantation, marigold plants were inoculated with the conidia by shaking or gently dusting contaminated specimens with

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DOI: 10.21608/jppp.2020.117989

powdery mildew conidia over the healthy plants, inoculation was achieved for 24 hours, inoculated plants were covered with polyethylene bags to retain adequate moisture needed for infection, and kept under greenhouse conditions, a collection of uninoculated plants were kept as control care. Inoculated plants were examined regularly, and the incidence and severity of disease symptoms were determined according to the method defined by Ali and Ayoub (2017) after 10 days of inoculation.

Disease control.

Preparation of Aluminum oxide nanoparticle conc. (mg L-1)

Aluminum oxide nanoparticle were obtained from the Faculty of Graduate Studies and Advanced Science (Nanotechnology Department) at Beni-Sweif University. The nanoparticles were prepared according to Mohammad *et al.* (2014).

Preparation of ZnO and CuO nanoparticle conc. (mg L-1)

Zinc oxide nanoparticle and CuO nanoparticle were obtained from the Faculty of Graduate Studies and Advanced Science (Nanotechnology Department) at Beni-Sweif University. The nanoparticles were prepared according to Sawai and Yoshikawa (2004).

Greenhouse experiment:

The experiments were carried out in order to control powdery mildew of marigold seedlings and plants. For all experiments, the following procedures and experimental materials were applied as follows: For each particular treatment, 4 pots (30-cm / diam) and five seedlings (45-days-old) / pot were transplanted for each treatment. Prepared concentrations were sprayed in advance to inoculation of marigold seedlings transplanted in 30 cm diameter pots. All the treatments were triple sprayed on marigold plants grown under greenhouse conditions, three weeks after transplanting, and repeated two times every two weeks. Disease severity (DS) (%) was recorded 10 days after each spray and the average of (DS %) was recorded according to Ali and Ayoub (2017). For all treatments, randomized complete block design was implemented and statistical analysis for reported data was performed. The control treatment was sprayed exclusively with water.

Table 1. Copper oxide, ZnO and AlO metals, nanoparticles and chitosan, their commercial name, composition and concentrations used.

Commercial Names	Composition	Used rate in orchard	% Active Ingredient	The Producing company
1-CuO	CuO	8mg –L water	100 % Cuo	
2-ZuO	ZuO	8mg –L water	100 % Zno	Faculty of Graduate Studies and
3-AIO	AIO	8mg –L water	100% AIO	Advanced Science, Beni-Sweif-Egypt
4-Zno-NPs	Zno-NPs	8mg L-L water	100 % Zno-NPs	
5-Cuo-NPs	Cuo-NPs	8mg L-L water	100 % Cuo-NPs	
6-AIO-NPs	AIO-NPs	8mg L-L water	100 % AIO-NPs	
7- Chitosan	chitin, which is a chain of carbohydrates (sugars)	50 mg-L water	chitin	Chitosan•Egypt, Jiangsu, China

Field experiment:

Field experiments were carried out at Sids Research Station at two different locations of Beni-Sweif governorate, Egypt during two successive growing seasons of 2017/2018 and 2018/ 2019. All the treatments were triple sprayed on marigold plants grown under open field conditions, three weeks after transplanting, and repeated two times every two weeks. Control treatment sprayed with water only. Marigold seedlings, however, were transplanted in the chosen field. Four replicates per each treatment. Seedlings were transplanted in October. Each plot (6X7 m; 42 m2) with 7 rows was transplanted with 112 seedlings, 16 ones per each row, and 30 cm between them. However, 100 healthy seedlings were obtained from planting 112.

Growth and yield parameters for Marigold.

In order to study the effect of powdery mildew on the growth of treated and untreated marigold plants grown under field conditions, morphological parameters include: plant height (cm), number of branches, fresh and dry weight (g) per plant.

Effect on yield inflorescence :

The effect of the tested treatments on powdery mildew disease severity affected the inflorescence yield of marigold plants was assessed. Also, different parameters of inflorescence yield represented by the number of inflorescences/plant as well as fresh and dry weight of inflorescences/plant.

Disease incidence and severity assessment:

1- Percentage of disease incidence (DI %):

The percentage of powdery mildew disease incidence was calculated as follows:-

$$DI (\%) = \frac{\text{No. of infected plants}}{\text{Total number of inspected plants}} \times 100$$

2- Percentage of disease severity (DS %)

Using disease scale levels from 0 to 5 classes according to infected plants by powdery mildew, *i.e.* 0 = 0 %, 1 = 0 – 5 %, 2 = 5 – 25 %, 3 = 25 -50 %, 4 = 50 -75 %, 5 = 75 – 100 % the percentage of disease severity was calculated as mentioned by Ali and Ayoub (2017) as follows:

$$D.s. = \frac{\sum (n \times c)}{N} \times 100$$

n = number of infected plants per category

c = category number

N = total examined plants

The determination of the content of chlorophyll a, b and carotenoids.

Carotenoids and chlorophyll a, b contents according to the method described by (Kumari *et al.*, 2018), flowers were determined. The tow gram of dry flowers were ground using acid-washed sand in a porcelain mortar with a pinch of calcium carbonate. It was then extracted with small quantities of 80% aqueous acetone and filtered through a sintered glass funnel (363) and washed several times with 80 % acetone until the tissues became colourless. The extract amount was composed of up to 100 ml of 80 percent acetone. Using the pye unicam SP 1800 recording spectrophotometer, the absorption of the extract was measured against 80 % acetone in 1 cm curets at 663, 645 and 452 nm. According to the following equations given by Kumari *et al.*, (2018), the content of chlorophyll a, b and carotenoids was expressed as mg / g dry weight of flowers:

$$\text{Chlorophyll A mg /g} = 12.7 \times \text{reading at 663 nm} - 2.69 \times \text{reading at 645 nm.}$$

$$\text{Chlorophyll B mg /g} = 22.4 \times \text{reading at 645 nm} - 4.68 \times \text{reading 663 nm.}$$

Carotenoids mg / L = 4.57 x (O.D) 452 – (0.226 x (chlorophyll a+b mg/L)

Statistical analysis:

Data obtained in these experiments were statistically analyzed using the factorial design suggested by Snedecor and Cochran (1982). Least significant difference (LSD) at 5% probability was used to compare treatment averages (Fisher, 1984).

RESULTS AND DISCUSSION

Results

1- Survey of marigold powdery mildew

Data presented in Table (2) and Fig. (1) indicate that among the three locations surveyed, Somosta county ranked the highest average for powdery mildew disease incidence percentage (80.1%) followed by 75.2 % in Ehnasea county while the lowest was Beba county which recorded disease incidence percentage 67.0 % . Meanwhile, the highest values of disease severity, being 60.2 and 50.5 % were recorded from

Somosta and Ehnasea counties, on the average, respectively. Meanwhile, the lowest average of disease severity value was 42.3 % in Beba County. The averages of disease incidence (DI %) and disease severity (DS %) values were increased gradually with increasing plant age from 2, 4 5 months and reached the maximum as shown in Table (2).

Table 2. Survey of marigold powdery mildew incidence and severity in the different growing areas at Beni-Sweif governorate during 2017/2018.

Inspected counties	Diseases incidence %				Disease severity %			
	Two-month-old*	4-month-old*	5-month-old*	over all month-mean	Two-month-old*	4-month-old*	5-month-old*	over all month-mean
Beba	45.5	70.0	85.5	67.0	30.80	40.6	55.4	42.3
Ehnasea	50.0	85.5	90.0	75.2	35.5	45.6	70.5	50.5
Somosta	55.3	90.0	95.0	80.1	40.0	65.5	75.0	60.2
Mean	50.3	81.8	90.2		35.4	50.6	67.0	
L.S.D.at 5%	5.0	3.50	4.50		3.50	4.50	5.5	



Fig. 1. Marigold plants showing powdery mildew symptoms

Effects of different treatments on powdery mildew severity in greenhouse of marigold plant.

The effect of the tested treatments AIO-Nps, CuO-Nps, ZnO-Nps, AIO, CuO, ZnO and Chitosan at three different concentrations (4 mg L-1 water, 6 mg L-1 and 8 mg L-1) on the percentage of disease severity of marigold powdery mildew 10 days of inoculation with *Erysiphe cichoracearum* under greenhouse conditions was shown in Table (3). All the tested concentrations caused a significant decrease in disease severity compared to control. Increasing concentration of the tested inducers led to a decrease in disease severity. In addition, the highest decreasing obtained when plants sprayed with Chitosan (18.0 %) compared to control (45.60 %) while, the lowest effect was recorded when plants were treated with CuO then followed by AIO.

Table 3. Effect of different treatments on powdery mildew disease severity of marigold under greenhouse conditions after 10 days from inoculation during 2017/2018.

Treatments	Concentrations (%)		
	4 mg L-1	6 mg L-1	8 mg L-L water
	Disease severity (%)		
	32.50	25.40	23.50
	35.50	27.60	25.50
	25.60	22.30	20.50
	37.40	30.70	26.50
	38.60	31.50	27.40
	30.20	26.50	24.30
	25.40	20.20	18.0
	55.20	50.50	45.60

Least significant difference (L.S.D.) at: 5%

Treatments (T) = 3.20

Concentrations (C) = 2.50

Interaction (T X C) = 5.20

The effect of different treatments on powdery mildew disease severity of marigold under field conditions .

The effect of the treatments tested, AIO-Nps, CuO-Nps, ZnO-Nps, AIO, CuO, ZnO and Chitosan, on the

incidence and severity of marigold powdery mildew during the two successive seasons, is shown in Table (4). The treatments used caused a significant reduction in the percentage of disease incidence and severity compared to control treatments under field conditions. Chitosan was the most effective in reducing powdery mildew during the two seasons, followed by ZnO-Nps, while CuO was the lowest.

Table 4. Effect of various treatments on disease incidence and disease severity of powdery mildew on marigold during two successive growing seasons under field conditions.

Treatments	Growing seasons			
	2017/2018		2018/2019	
	Disease Incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
1- AIO-Nps	20	32.50	25	34.40
2-CuO-Nps	15	33.0	20	33.60
3-ZnO-Nps	10	30.0	10	32.30
4-AIO	30	35.40	30	36.50
5-CuO	35	36.50	40	37.50
6-ZnO	45	34.20	50	35.50
7-Chitosan	5	27.60	10	26.50
Control	60	59.20	65	62.50
(L.S.D.) at 0.05	3.00	2.50	4.50	3.60

All the treatments tested significantly reduced the severity of the disease compared to control treatment. The highest disease severity (36.50 %) and (37.50 %) were resulted from treated with CuO in 2017/2018 and 2018/2019, Chitosan treatment proved to be the superior treatment, since it significantly decreased disease severity followed by ZnO-Nps (26.50 %) and (32.30 %), respectively comparing with the other treatments. While, the lowest disease incidence (5 %) and (10%) were resulted from treated with Chitosan and ZnO-Nps.

Effect of different treatments on some growth parameters of marigold during two successive growing seasons under conditions on the field.

Data shown in table (5) indicate that all treatments led to significant increase in plant height, number of branches, fresh and dry weights of marigold plants compared to control treatment. In the first season, the tallest plants, being 90.60 cm on the average were recorded due to using Chitosan followed by 89.0 cm for ZnO-Nps, respectively, as compared to control (50.50 cm.). The lowest effect on plant height was recorded on plants treated with ZnO, being 60.0 cm. The greatest numbers of branches, being 20.50 and 19.20 were obtained when plants were treated with Chitosan and ZnO-Nps, on the average, respectively as compared to the control (15.0). The lowest mean number of branches, being 11.30 was recorded after plant treatment with ZnO. In addition, the highest values of fresh and dry weights of plants were 1300 g and 250.36 g, respectively for plants treated

with Chitosan. The corresponding figures recorded for control treatment were 800 g and 156.60 g, respectively. Compared to control treatment, all tested treatments significantly increased the plant height (cm). The lowest Plant height(cm) 65.40 cm and (65.20 cm) were resulted from treated with CuO in 2017/2018 and 2018/2019, Chitosan treatment proved to be the superior treatment, since it significantly increased Plant height (cm) followed by ZnO-Nps (90.60 cm) and (89.0 cm), respectively comparing with the other treatments.

Effect of different treatments on yield components of marigold grown under field conditions during two successive growing seasons.

Data shown in Table (6) and Figs. (2, 3 and 4) reveal that all treatments caused a significant increase in marigold inflorescences yield including inflorescence number, and inflorescence fresh and dry weights compared to control treatments.

Table 5. The effect of different treatments on growth parameters of marigold plants grown under field conditions during 2017/2018 and 2018/2019 growing seasons.

Treatment	Plant height (cm)			Branches NO.			Plant fresh weight (g)			Plant dry weight (g)		
	2017/2018	2018/2019	Mean	2017/2018	2018/2019	Mean	2017/2018	2018/2019	Mean	2017/2018	2018/2019	Mean
1-AIO-Nps	85.20	80.50	82.85	17.60	17.0	17.3	1200	1190	1195	235.20	230.30	232.75
2-CuO-Nps	80.40	78.60	79.5	15.60	15.0	15.3	1150	1140	1145	220	218.30	219.15
3-ZnO-Nps	89.0	88.20	88.6	19.20	18.0	18.6	1250	1240	1245	244	236	240
4-AIO	70.10	70.0	70.05	14.20	14.0	14.1	1100	1050	1075	230	228	229
5-CuO	65.40	65.20	65.3	13.0	12.5	12.75	1000	990	995	220	200	210
6-ZnO	60.0	58.20	-59.1	11.30	11.0	11.15	900	890	-895	200	190	-795
7-Chitosan	90.60	86.80	88.7	20.50	19.0	19.75	1300	1250	1275	250.36	240.5	245.4
Control	50.50	45.0	47.75	15.0	13.0	14.0	800.0	780.4	790.2	156.60	150.40	153.5
(L.S.D.) at 0.05	3.5	2.7		3.20	1.5		6.60	3.40		7.6	7.0	

Table 6. Effect of different treatments on marigold inflorescence yield, plant fresh and dry weights during two successive growing seasons (field experiment).

Treatment	Averages of some yield components								
	Inflorescences No./ plant			Fresh weight (g)/ plant			Dry weight (g)/ plant		
	2017/18	2018/19	Mean	2017/18	2018/19	Mean	2017/18	2018/19	Mean
1- AIO-Nps	370.59	365.54	368.06	190.20	190.0	190.10	35.69	34.60	35.14
2-CuO-Nps	350.20	350.0	350.1	180.30	179.23	179.76	30.62	30.0	30.31
3-ZnO-Nps	390.64	390	390.32	195.30	190.60	192.95	39.54	38.40	38.97
4-AIO	330.45	325.64	328.04	170.69	165.30	167.99	25.60	25.0	25.30
5-CuO	320.96	310.20	315.58	160.45	155.90	158.17	24.20	24.0	24.10
6-ZnO	310.45	310	310.22	155.89	154.60	155.24	23.40	23.0	23.20
7-Chitosan	402.60	400	401.3	200.0	199	199.5	41.20	40.60	40.90
Control	300.23	290.90	295.56	100.0	95.60	97.80	21.64	20.10	20.87
L.S.D. at 5%	15.70	9.50		8.0	9.40		5.0	2.60	

In the first season, the highest number of inflorescences was 402.60 and 390.64 for plants treated with Chitosan and ZnO-Nps, respectively. The lowest number recorded was 310.45 when plants were treated with ZnO. On the other hand, the highest fresh and dry weights of inflorescences were 200.0 g and 41.20 g, respectively when plants were treated with chitosan compared to the control that recorded 100.0 and 21.10 g, respectively, 135.89 g and 23.40 g were the lowest fresh and dry inflorescence weights for plants treated with ZnO.

The Effect of different treatments on chlorophyll a, b and carotenoids of produced marigold yield.

Data presented in Table (7) indicate that chlorophyll (a and b) were increased using treatments (600.8 and 620.8) for ZnO-Nps and (550.9 and 640.8) for AIO-Nps Similar results were obtained during the estimation of carotenoids (1801.4 and 2030.4) for ZnO-Nps and AIO-Nps.

Table 7. The effect of different treatments on chlorophyll a, b and carotenoids of produced marigold yield.

Treatments	Chlorophyll a	Chlorophyll b	Carotenoids
1- AIO-Nps	550.9	640.8	2030.4
2-CuO-Nps	401.3	640.7	1890.4
3-ZnO-Nps	600.8	620.8	1801.4
4-AIO	350.2	580.7	1840.6
5-CuO	310.4	540.8	1811.5
6-ZnO	240.0	430.8	1080.4
7-Chitosan	730.2	610.8	1940.02
Control	150.4	230.2	156.42
L.S.D. at 5%	2.44	2.50	50.4

Discussion

The causal organism of marigold powdery mildew was identified as *Erysiphe cichoracearum*; according to the observed disease symptoms and morphological characteristics of the conidial stage. Our results are in consistent with those

recorded by Garibaldi *et al.*, (2008); Broun and cook (2012) and Madian, 2007 .

Survey conducted at three counts in Beni-sweif governorate indicated that marigold powdery mildew was more severe in Somosta County followed by Ehnasea County. The lowest severity of the disease was reported in county Beba. The recorded differences on the disease spread may attribute to the differences in climatic conditions among these counties. Results of the greenhouse experiments revealed that using 8 mg / L water concentration of the tested nanoparticles as spraying treatment can control powdery mildew of marigold. Zinc oxide NPs was the best among the tested treatments to control powdery mildew on marigold. All treatments reduced the disease incidence. These findings are consistent with those stated by Brayner *et al.* (2006) and Zhang *et al.* (2007) and Sharma and Sharma (2008), who indicated that ZnO NPs might cause structural changes in the membrane of the microbial cell , causing leakage of cytoplasm and eventually cell death.

He *et al.* (2011) reported that ZnO NPs could significantly inhibit the growth of *Botrytis cinerea* and *P. expansum* at concentrations greater than 3 mMol L⁻¹. Sawai and Yoshikawa, (2004) reported that the minimum inhibitory concentration of ZnO bulk powder against *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger* and *Rhizopus stolonifer* was more than 100 mg ml⁻¹ (~1.2 mol L⁻¹) by an indirect Leittimetric assay. Due to their unique properties, such as wide surface area, ZnO NPs showed a great improvement in antimicrobial activity.

Chitosan was tested individually, as resistance inducer in controlling marigold powdery mildew at three different concentrations before and after inoculation with the pathogen. The results showed that all inducers decreased disease severity as compared to the check treatment. Chitosan was highly effective treatment against the disease compared to the check treatment. These positive effects of chitosan in controlling powdery mildew were reported on other crops (El Ghaouth *et al.*, 1992 and Fajardo *et al.*, 1995)

In addition to its direct impact on microbial behaviour, other studies have strongly indicated that a series of defence reactions induced by chitosan correlate with enzymatic activity. It has been shown that chitosan increases the production of glucan-hydrolases, phenolic compounds and the synthesis of unique antifungal phytoalexins, and also decreases macerating enzymes such as polygalacturonases, Methyl esterase pectin, etc (Ben-Shalom *et al.*, 2003). Chitosan also causes structural barriers, such as inducing lignin-like material synthesis. In some horticultural and ornamental products, because of its ability to shape a semi-permeable coating Borkowski and Kowalczyk (1999), chitosan extends the shelf life of treated fruits and vegetables by Borkowski *et al.* (2004) decreased the rate of respiration and decreased water loss. Chitosan has the potential to become a new type of plant protector as a non-toxic biodegradable material, as well as an elicitor, contributing towards the aim of sustainable agriculture. These findings are in accordance with those that several researchers have obtained (El-Ghaouth *et al.*, 1992 and Moret *et al.*, 2009). In the current research, it was decided that, in addition to preventing environmental contamination, the compounds used in therapies could be used as an efficient and safe technique for disease control. In the meantime, further alternative control trials mean that the use of

the therapies tested and their compounds can be expanded in the future. In addition, because of the current plant approach, In order to promote the use of alternative chemicals for disease prevention, safety would consider maintaining environmental conditions free from toxic contamination as a priority for further studies .

In addition, field tests reported that, due to the effects of the tested inducers, the reduction in disease intensity was reflected in a substantial increase in plant height, number of branches and fresh and dry weight of the treated plants relative to control treatments. (Borkowski and Kowalczyk, 1999 and Borkowski *et al.* (2004). Further trials with the nonspecific disease resistance compounds may be advised.

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مكافحة مرض البياض الدقيقي على الأقحوان باستخدام جزيئات النانو المعدنية والشيتوزان

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تم دراسة تأثير سبعة مواد هي الشيتوزان وأكاسيد النحاس والزنك والألمونيوم النانوي والمعني تحت ظروف الصوبة خلال عام (2017/2018) وتحت ظروف الحقل في موسمي (2017/2018 و2018/2019) لمكافحة مرض البياض الدقيقي على نباتات الأقحوان. أظهرت النتائج المتحصل عليها أن المواد المستخدمة للمقاومة أدت إلى خفض الإصابة مقارنة بالنباتات غير المعاملة. بالإضافة إلى ذلك فإن الشيتوزان كان الأكثر كفاءة في خفض شدة الإصابة يليه أكسيد الزنك (نانوي) بينما كان التأثير الأقل لمركب أكسيد النحاس المعني. جدير بالذكر أن خفض شدة الإصابة بالمرض كان مصحوبا بزيادة في قياسات الصفات النباتية للنباتات المختبرة مثل عدد ووزن النورات الناتجة وكذا طول وعدد أفرع النبات والوزن الطازج والجاف للنباتات وزيادة نسبة الكاروتينات وكلوروفيل (أ.ب).