

## Management of Zinnia Powdery Mildew using Safe Alternatives to Fungicides

Nour El-Houda A. Reyad and M. F. Attia

Plant Pathol. Dept., Fac. of Agric., Cairo Univ., Giza, Egypt.

**T**he use of safe alternatives to fungicides has become an important matter of urgency due to their toxicity and development of new strains have capabilities to overcome the efficiency of fungicides. In the present study, six treatments, *i.e.* the control (water only), Penconazole fungicide (5ml/20L), dipotassium phosphate (50, 75 and 100 Mm.), Nanosilica (300, 500 and 750 ppm.), Propolis extract (5, 10 and 15%), garlic extract (5 and 10 %) and Alga Grow-4 (1ml/l.) as alternative means to fungicides were investigated against zinnia powdery mildew. *In vitro* study showed that dipotassium phosphate and garlic extract were the most effective in reducing the conidial germination percentage. The reduction percentage was increased by increasing the tested concentrations. Under natural conditions, spraying plants six times with one week interval, showed that dipotassium phosphate and garlic extract, respectively, were the most superior treatments which significantly decreased disease severity and improved the number of flowers/plant, chlorophyll content, oxidative-reductive enzymes (POX and PPO enzymes) and total phenols. On the other hand, the reference fungicide showed the highest efficiency.

**Keywords:** Alga Grow-4, enzymes, garlic extract, phosphate salt, powdery mildew, nanosilica, total phenols and zinnia.

Zinnia (*Zinnia elegans* L.) is a worldwide popular annual ornamental plant belonging to the Asteraceae family. It is grown for cut flowers and flower beds (Javid *et al.*, 2005; Pinto *et al.*, 2005 and Szopińska, 2014). Zinnia, like most other plants, is subjected to attack by different pathogens which may impair the beauty of the flowers or loss its ornamental value.

Powdery mildew is a major disease on many of cultivated and wild plants worldwide, causing significant yield losses during the commercial production. Kavak (2011) reported that *Podosphaera xanthii* (*Oidium* subgenus *Fibroidium*) is the causal pathogen of zinnia powdery mildew in Turkey. Meanwhile, Hoshi *et al.* (2013) confirmed that members of the genus *Euoidium* are the causal pathogens of zinnia powdery mildew in Japan. In Egypt, literature regarding the causative of zinnia powdery mildew is relatively few. To the best of our knowledge, *Oidium* sp. was identified early in 1959 by Elarosi and Assawah as the causal of zinnia powdery mildew in Alexandria region. The occurrence and dominance of the conidial stage of *Erysiphe cichoracearum* DC. Ex Merat as the cause of zinnia powdery mildew has emerged from studies performed by El-Kot and Hegazi (2008); Hegazi and El-Kot (2010<sub>a</sub> and 2010<sub>b</sub>). The pathogen attacks all plant parts above the soil surface resulting in the appearance of powdery mildew symptoms which reduce production levels, marketability and profitability of the cut flower production by weakening the plant growth and reducing flowering (Kamp, 1985; El-Kot and Hegazi, 2008; Hegazi and El-Kot, 2010<sub>a</sub> and 2010<sub>b</sub> and Dixon, 2012).

As an alternative approach to the control of zinnia powdery mildew, Kamp (1985) successfully used a polymer-based anti-transparent. Similarly, applications of soluble silicon (Tefagiorgis, 2008), biocontrol agents (Tefagiorgis, 2008 and Hegazi and El-Kot, 2010<sub>a</sub>), essential oils and plant extracts (Hegazi and El-Kot, 2010<sub>b</sub> and Dixon, 2012) have produced promising results in managing powdery mildew of zinnia.

The entire work was carried out to evaluate the effect of dipotassium phosphate ( $K_2HPO_4$ ), Nanosilica, Propolis extract, garlic extract, a commercial algae extract (Alga Grow-4) and the commercial fungicide Penconazole against powdery mildew of zinnia under natural conditions in relation to the plant physiological changes.

### Materials and Methods

#### 1. Source and preparation of the tested treatments:

Nanosilica was pursued from Egypt. Nanotech. Company Limited, Cairo, Egypt. The size of the used nanosilica was 50nm with a purity of 99.99%. It was used as spray at concentrations of 300, 500 and 750 ppm.

Potassium phosphate dibasic ( $K_2HPO_4$ ) was pursued from Syngenta Crop Protection PTY Limited and used as a spray at concentrations of 50, 75 and 100mM.

The commercial algae extract (Trade name: Alga Grow-4) was pursued from Grow. Tech. for Agricultural Development. The product consists of algae extract, nitrogen, phosphorus pentoxide and potassium oxide. It was used as spray at recommended dose 1ml/l.

The Propolis grind was kindly obtained from beekeeping unit., Fac. of Agric. Cairo Univ. The ethanol extract of Propolis was prepared according to the procedures described by Guginski-Piva *et al.* (2015). The Propolis grind was mixed with ethanol 70%, in the proportion of 30% crude Propolis to 70% ethanol. The mixture was stored in dark glass vials, which were closed tightly and left at room temperature for two months; they were shaken daily. At the end of the storage period, the extract was filtrated and saved as stock. Other concentrations, *i.e.* 5, 10 and 15% were prepared from the stock by dilution with distilled water.

For garlic extract, 10 gm. frozen bulbs were mixed with 100 ml. water using an electric blender for 5 min. The extract was filtrated and centrifuged for 10 minutes at 3000 rpm. The filtrated extract was kept as a stock solution. The desired concentrations, *i.e.* 5 and 10% were prepared by dilution with distilled water.

The fungicide Penconazole with trade name Topas EC 100% purchased from Syngenta Crop Protection Pty Limited was used in this study as positive control. This fungicide was used as spray at the recommended dose (5 ml/20 l).

#### 2. Effect of the tested treatments on conidial germination:

Under aseptic conditions, freshly conidia were collected from the infected zinnia leaves showing typical symptoms of powdery mildew using sterilized brush and spread on dry clean glass slides previously received 0.1 ml. from each concentration of the tested treatments. Each slide was placed over the U shaped glass rod inside a sterilized Petri-dish containing sterilized distilled water to provide high relative

humidity. Spore suspension, in distilled sterilized water only, was used as a check treatment. Three replicated glass slides were used for each particular treatment. Treatments were then incubated at  $25\pm 1^\circ\text{C}$  for 24 h. One drop of lacto-phenol cotton blue stain was added at the time of slide examination to kill and fix the germinated conidia. The percentage of conidial germination was counted in a total of 100 conidia, according to the following formula:

$$\text{Conidial germination (\%)} = \frac{\text{No. of germinated spores}}{\text{Total No. of spores}} \times 100$$

### 3. Effect of the tested treatments on the disease severity under natural conditions:

The experiment was conducted in plots ( $1\text{m}^2$ ), located in the experimental unit of the Plant Pathol. Dept., Fac. of Agric., Cairo Univ., during 2015 growing season. A complete randomized block design with three replicates was used to study the effect of the tested treatments on zinnia powdery mildew. Each replicate consisted of 9 plants (three rows with 3 hills/row). The seedlings of zinnia plants (two months age) were transplanted in May 3<sup>rd</sup> on one side of the row. The grown plants were left to the natural infection by powdery mildew. Seven treatments were used in the entire experimental setup, *i.e.* the control (water only); Penconazole fungicide (5 ml/20 l); dipotassium phosphate (Conc. 50, 75 and 100 Mm.); Nanosilica (Conc. 300, 500 and 750 ppm.); Propolis extract (Conc. 5, 10 and 15%); garlic extract (Conc. 5 and 10%); and Alga Grow-4 (1ml/l.)

The plants were sprayed six times beginning from May 25<sup>th</sup> weekly by an atomizer just before the first signs of the symptoms were appeared. Tween-20 was used as a surfactant at the rate of 0.01%. For control treatments, plants were sprayed with distilled water plus 0.01% tween-20 only.

### 4. Disease assessment:

Disease severity (%) was assessed according to the modified scale of 0 to 10 categories presented by Chattopadhyay *et al.* (2010) as follows:

Category	Infection (%)
0	0% ,
1	<0 to 3% ,
2	<3 to 6% ,
3	<6 to 12% ,
4	<12 to 25% ,
5	<25 to 50% ,
6	<50 to 75% ,
7	<75 to 88% ,
8	<88 to 94% ,
9	<94 to 97% ,
10	<97 to 100%

The following formula was used to calculate the disease severity (%):

$$\text{Disease severity (\%)} = \frac{\sum (nxv)}{10 N} \times 100$$

Whereas: n = Number of infected leaves in each category; v = Numerical values of each category and N = Total number of the infected leaves.

5. *The effect of the tested treatments on the number of flowers/plant and chlorophyll content:*

The flower number/plant was counted after 63 days of planting. Also, the chlorophyll content was determined using a portable chlorophyll meter (Minolta SPAD- 502, Japan), as SPAD unit; these units were transformed to  $\text{mg.m}^2$  as described by Monje and Bugbee (1992) as follows:

$$\text{Chlorophyll content (mg. m}^2\text{)} = 80.05 + 10.4 (\text{SPAD 502}).$$

Whereas: SPAD 502= chlorophyll meter reading (CMR).

6. *Effect of the tested treatments on activity of oxidative reductive enzymes:*

Activity of peroxidase (POX) and polyphenoloxidase (PPO) was determined 24h. after the following treatments: (1)  $\text{K}_2\text{HPO}_4$  at 100 mM; (2) Nanosilica at 750 ppm.; (3) Propolis extract at 15%; (4) garlic extract at 500 ppm.; (5) Alga Grow-4 at 1ml/l. and (6) check treatment.

Peroxidase activity was determined according to Allam and Hollis (1972) as the change in absorbance at 425 nm/minute/g fresh weight.

Polyphenoloxidase activity was quantitatively determined according to Matta and Dimond (1963) as the change in absorbance at 495 nm/minute/g fresh weight.

7. *Effect of the tested treatments on total phenol contents.*

Total phenols were spectrophotometrically determined by Folin Denis reagent described by Snell and Snell (1953) as mg catechol/1g fresh weight of leaves.

8. *Statistical Analysis*

Most of the data were statistically evaluated according to Snedecor and Cochran (1967). Averages were compared at 5% level of probability using the Least Significant Differences (L.S.D.) as mentioned by Fisher (1948). On the other hand, percentage data were transformed to arcsines and then subjected to statistical analysis to determine the least significant differences (L.S.D.) to compare variance between treatments (Gomez and Gomez, 1984).

## Results

According to the analysis of variance, the effect of the tested treatments on the level of this variable ( $p < .05$ ) was, in most cases, significant if compared with the control. According to the means of conidial germination (%), examining the effect of different concentrations of the tested treatments revealed that increasing the concentration of the tested treatments resulted in reducing the percentage of conidial germination (Table 1). Penconazole followed by phosphate salt (100 Mm) and garlic extract (10%) were the most effective treatments in reducing the percentage of conidia germination without significant differences. The corresponding values for conidial germination were 7.33, 10.67 and 11.67% on the average, respectively. On the other hand, Propolis extract and Alga Grow-4 were the least effective treatments in this respect (Table 1).

**Table 1. Effect of the tested treatments on conidial germination**

Treatment	Concentration	Conidia germination (%)	Reduction (%)
Dipotassium phosphate	50 Mm	16.33	52.74
	75 Mm	14.33	58.52
	100 Mm	10.67	69.12
Nanosilica	300 ppm	23.00	33.43
	500 ppm	21.67	37.28
	750 ppm	20.33	41.16
Propolis extract	5%	30.67	11.23
	10%	26.67	22.81
	15%	26.00	24.75
Garlic extract	5%	16.33	52.74
	10%	11.67	66.22
Alga Grow-4	1ml/l.	25.33	26.69
Penconazole	5 ml/20l	7.33	78.78
Distilled water (Control)		34.55	-
L.S.D at 0.05		4.86	-

Results revealed that, application of the tested treatments significantly reduced the disease severity at 5% level of significance under field conditions (Table 2). The highest reduction in disease severity was obtained due to application of Penconazole fungicide, being 69.02%, followed by dipotassium phosphate (100 Mm), 58.87% and garlic extract (10%), 46.78%, respectively. Propolis extract was the least effective in this regard (Table 2).

**Table 2. Effect of the tested treatments on disease severity under natural conditions**

Treatment	Con.	Disease severity (%) after 49 days	Reduction (%)
Dipotassium phosphate	50 Mm	28.13	32.59
	75 Mm	24.93	40.26
	100 Mm	18.00	58.87
Nanosilica	300 ppm	31.23	25.16
	500 ppm	29.73	28.76
	750 ppm	29.20	30.03
Propolis extract	5%	40.67	2.54
	10%	37.47	10.21
	15%	35.07	15.96
Garlic extract	5%	28.13	32.59
	10%	22.21	46.78
Alga Grow-4	1ml/l.	32.67	21.71
Penconazole	5 ml/20 l.	12.93	69.02
Distilled water (Control)		41.73	-
L.S.D 0.05		2.81	-

Data in Table (3) indicate that all treatments increased the flowers number and chlorophyll content compared to control treatment. The maximum values of chlorophyll content were obtained due to application of Penconazole fungicide followed by dipotassium phosphate (100 Mm) and garlic extract (10%), respectively. Meanwhile, Propolis extract and Alga Grow-4, respectively, were the least effective. The same trend was also true for the number of flowers/plant. Penconazole fungicide followed by dipotassium phosphate (100 Mm) and garlic extract (10%), respectively, gave the highest number of the flowers/plant without significant differences. Meanwhile, Propolis extract (15%) and Alga Grow-4 (1 ml/l), respectively gave the lowest number of the flowers/plant, being 9.33 and 9.20 on the average, respectively.

**Table 3. Effect of the tested treatments on the number of flowers/plant and total chlorophyll content.**

Treatment	Conc.	Total chlorophyll (SPAD)	Total chlorophyll (mg.m <sup>2</sup> )	Number of flowers/plant*
Dipotassium phosphate	50 Mm	32.5	418.05	11.93
	75 Mm	33.6	429.49	12.13
	100 Mm	35.4	448.21	12.73
Nanosilica	300 ppm	25.8	348.37	10.40
	500 ppm	26.4	354.61	11.07
	750 ppm	26.5	355.65	11.20
Propolis extract	5%	25.2	342.13	9.13
	10%	25.3	343.17	9.27
	15%	25.4	344.21	9.33
Garlic extract	5%	31.6	408.69	11.47
	10%	35.0	444.05	12.60
Alga Grow-4	1ml/l.	26.0	350.45	9.20
Penconazole	5ml/20l	37.3	467.97	12.87
Distilled water (Control)		24.8	337.97	9.00
L.S.D 0.05		-	2.66	0.36

\* Mean of 5 plant/plot (three replicated plots each 1 m<sup>2</sup>)

Results in Table (4) indicate that application of the tested treatments significantly increased the levels of oxidative reductive enzymes, *i.e.* Peroxidase and Polyphenoloxidase. The maximum activity of peroxidase enzyme was obtained due to application of dipotassium phosphate (100 Mm) followed by garlic extract (10%). Meanwhile, maximum activity of Polyphenoloxidase was obtained by using dipotassium phosphate (100 Mm) and garlic extract (10%), respectively, without significant differences. The lowest activity of Peroxidase and Polyphenoloxidase enzymes was obtained due to spraying plants with either Propolis extract or Alga Grow-4.

**Table 4. Effect of different treatments on the activity of oxidative reductive enzymes in the tissues of zinnia leaves**

Treatment	Peroxidase (POX) activity (absorbance at 430 nm)		Polyphenoloxidase activity (PPO) (absorbance at 495nm)	
	Activity	Increasing over control (%)	Activity	Increasing over control (%)
Dipotassium phosphate (100 Mm)	1.181	60.899	0.133	68.354
Nanosilica (750 ppm)	1.047	42.643	0.113	43.038
Propolis extract (15%)	0.939	27.929	0.104	31.646
Garlic extract (10 %)	1.142	55.586	0.127	60.759
Alga Grow-4 (1 ml/l)	1.033	40.736	0.108	36.709
Distilled water (Control)	0.734	-	0.079	-
L.S.D at 0.05	0.031	-	0.018	-

The content of total phenols was greatly increased due to the tested treatments compared with the untreated control (Table 5). The maximum increase in total phenolic compounds was recorded due to application with dipotassium phosphate (100 Mm), being, 53.16% over check treatment followed by garlic extract (10%), being 30.29% over check treatment. However, Alga Grow-4 (1 ml/l) and Propolis extract (15%) showed slight increases in phenolic contents in comparison with the control treatment without significant differences.

**Table 5. Effect of different treatments on the total phenol contents in the tissues of zinnia leaves**

Treatment	Total phenols (mg/g fresh weight)	Increase over control (%)
Dipotassium phosphate (100 Mm)	59.06	53.16
Nanosilica (750 ppm)	47.74	23.81
Propolis extract (15%)	43.46	12.71
Garlic extract (10%)	50.24	30.29
Alga Grow-4 (1 ml/l)	43.81	13.62
Distilled water (Control)	38.56	-
L.S.D 0.05	5.79	-

### Discussion

Powdery mildew is one of the most important diseases affecting zinnia plant resulting in reducing its ornamental value. The present work was planned to reduce the using of chemical fungicides in the agricultural ecosystem. In addition, an attempt was investigated to find out the most effective natural compounds that have the ability to protect zinnia plants from powdery mildew attacking.

The entire work indicated that dipotassium phosphate and garlic extract, respectively, were the most superior alternative treatments in reducing the disease severity and improving the number of flowers/plant, chlorophyll content, activity of oxidative reductive enzymes and total phenols under natural conditions. Meanwhile, Propolis extract and Alga Grow-4 were the least effective in this regard. These results might be due to activation the resistance mechanisms in the treated plants.

Dipotassium phosphate is a highly water-soluble salt which is often used as a fertilizer, food additive and preservative agent. It is considered a source of phosphorus and potassium. In general, phosphate salts exhibit efficacy for resistance induction, especially systemic acquired resistance (SAR) in a number of plant species against various pathogens. In this respect, Orober *et al.* (2002) suggested that spraying foliar of cucumber plants by dipotassium phosphate resulted in activation of mechanisms like those initiated by necrotizing microbes and viruses which trigger SAR. On the other hand, a collapse of fungal cell walls and shrinkage of conidia and conidiophores were observed by Homma *et al.* (1981) due to potassium salts application. These observations may clear the positive effect of spraying the zinnia plant against powdery mildew under natural conditions and its inhibitory properties against conidial germination obtained in this study.

Garlic is a natural food additive, it has a unique flavor and ranks highly among health, protecting foods due to its antioxidant components. Many investigations confirmed and revealed its antifungal and antimicrobial properties to allicin. Allicin is a membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins (Gurjar *et al.*, 2012). Several workers cleared the efficiency of garlic extract against many plant pathogens, including powdery mildew on different crops (Hegazi and El-Kot, 2010<sub>b</sub> and Moharam and Obiadalla, 2012). These findings are in harmony with those obtained in the present study.

Spraying plants with silicon (Si) produced promising results in controlling powdery mildew of different crops (Derbalah *et al.*, 2012; Tesfagiorgis and Laing, 2013; El-Sharkawy *et al.*, 2015; Dallagnol *et al.*, 2015; Mohaghegh *et al.*, 2015 and Vivancos *et al.*, 2015) However, most of these researchers indicated that the level of control has been usually low. Based on the viewpoint of Tesfagiorgis (2008) the reason for the low level of protection caused by Si application was usually due to the limited number of applications per week. On the other hand, Shen *et al.* (2010) demonstrated that silicon application to plants reduced disease incidence due to its act as a physical barrier against pathogen penetration or by activating defense response in plants. They also excluded the direct effect of silicon on the pathogen. These findings explain the low efficacy of nanosilica in reducing the conidia germination as well as disease severity observed during our study.

Alga Grow-4 and Propolis extract, respectively, were the least effective treatments in reducing conidial germination and disease severity of zinnia powdery mildew. For algae, they have different active elicitor compounds mainly saccharides such as laminarin and ulvan. Jaulneau *et al.* (2010) reported that crude extract of the green macroalga, *Ulva armoricana* has promising results in protecting crop plants against powdery mildew diseases. The activity of algae extract in protecting plants



against diseases may be due to activation of plant pathogenesis related protein. During the progress of the present work, the low activity of Alga Grow-4 in controlling zinnia powdery mildew in the entire work may be due to the tested product itself or the experimental conditions. Additional studies are required on this product in relation to powdery mildew pathosystem.

The known antimicrobial and pharmaceutical properties of Propolis resulted in consuming it by man for millennia. Recently, several researchers confirmed the antifungal properties of Propolis against different plant pathogenic fungi including powdery mildew pathogens. Hegazi and Abd El Hady (2002); Ahmed (2004); Mahdy *et al.* (2006) and Guginski-Piva *et al.* (2015). Although the capability of the Propolis to activate plant defense mechanisms was reported by several investigators as highly promising due to the large number of substances in its composition, the obtained results of the present study were not compatible with most of these investigators. On the other hand, Guginski-Piva *et al.* (2015) reported that comparing of Propolis extract to other alternative means such as milk, plant extract or aqueous extracts of organic material are considered low. They determined the factors that affect the efficiency of Propolis extract against pathogens as the time of collection and the local seasonality. Castro *et al.* (2007) reported that seasonality influenced the antibacterial activity of Propolis extract through changes in the concentration of bioactive compounds.

Phenols and oxidizing enzymes such as peroxidase and polyphenoloxidase have an active role in the resistance mechanism against plant diseases. In this regard, several researchers have reported that treatment plants with various biotic and abiotic inducers increased activities of phenols and oxidative enzymes (Arun *et al.*, 2010). This is in line with the findings of this study.

In conclusion, our work confirmed the possible use of phosphate salts and garlic extract in the management of zinnia powdery mildew to overcome the hazards caused by extensive use of fungicides in the agrosystems.

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استخدام بدائل آمنة للمبيدات في مكافحة  
مرض البياض الدقيقي في الزينيا  
نورالهدى عبد التواب رياض و محمد فاروق  
عطية

قسم أمراض النبات ، كلية الزراعة ، جامعة القاهرة.

تم دراسة تأثير ستة معاملات مختلفة هي الشاهد (الماء فقط) و مبيد البيكونازول (5 مل / 20 لتر ماء) وملح ثنائي البوتاسيوم فوسفات بتركيزات 50 و 75 و 100مليمول والنانوسيليكات بتركيزات 300 و 500 و 750 جزء في المليون و مستخلص البروبوليس بتركيزات 5 و 10 و 15% ومستخلص الثوم بتركيزات 5 و 10% ومستخلص الطحالب المعروف تجارياً بالألاجارو-4 بتركيز 1مل / لتر علي انبات الجراثيم الكونيدية وعلي شدة الإصابة بمرض البياض الدقيقي في الزينيا تحت ظروف العدوي الطبيعية.

اثبتت الدراسات المعملية ان كل من ملح الفوسفات ثنائي البوتاسيوم ومستخلص الثوم علي التوالي هما الأكثر فاعلية في خفض النسبة المئوية لإنبات الجراثيم الكونيدية. كما زاد هذا التأثير بزيادة التركيزات المستخدمة. وجاءت نتائج الدراسات الحقلية متوافقة الي حد كبير مع الدراسات المعملية ، حيث أدي رش النباتات بالمعاملات السابقة ستة مرات بمعدل رشة واحدة كل سبعة أيام الي حدوث انخفاض معنوي في شدة الإصابة بالمرض وصاحبه زيادة في عدد الأزهار لكل نبات وزيادة ملحوظة في محتوى الكلورفيل ونشاط انزيمات الأكسدة والإختزال (البيروكسيديز والبولي فينول اكسيديز) ومحتوي النبات من الفينولات الكلية ، وتفوق كل من ملح الفوسفات ثنائي البوتاسيوم ومستخلص الثوم علي التوالي في هذا الشأن الا أن المبيد الفطري بينكونازول تفوق علي جميع المعاملات في معظم هذه التأثيرات المختبرة.