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Suppression of Basil Downy Mildew Caused by *Peronospora belbahrii* using Resistance Inducers, Mineral Salts and Anti-transpirants Combined with Different Rates of Nitrogen Fertilizer under Field Conditions

Eman W.R. Ghebrial and M.G.A. Nada
Plant Pathol. Res. Inst., A. R. C., Giza, Egypt.

Field experiments were carried out under natural infection of basil downy mildew in the Experimental Farm of Sids Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate during 2015 and 2016 growing seasons in order to evaluate the efficacy of different compounds of resistance inducers, mineral salts and anti-transpirants applied on sweet basil plants (Balady variety) as foliar spray combined with two rates of nitrogen fertilizer for controlling basil downy mildew caused by *Peronospora belbahrii*. In general, all treatments had a positive effect on the reduction of disease severity with significant increase in fresh and dry weights of herb and essential oil yield at the end of experiment. The high efficacies of the tested compounds were observed when nitrogen was applied at the recommended rate. Chitosan (0.05 %), di-potassium phosphate (20 mM) and potassium silicate (20 mM) in addition to the fungicide Stone 50 % were the most effective in this concern. Furthermore, plants sprayed with each of chitosan, di-potassium phosphate and potassium silicate showed significant increases in the defence-related enzymes, peroxidase (POD) and phenylalanine ammonia-lyase (PAL) as compared with the untreated basil plants.

Keywords: Anti-transpirants, chitosan, defence-related enzymes, di-potassium phosphate, downy mildew, mineral salts, nitrogen fertilizer, potassium silicate, resistance inducers and sweet basil.

Sweet basil (*Ocimum basilicum* L., Fam. Lamiaceae) is the most commercially important annual culinary herb crop used for both fresh and dry consumption and as a source of essential oils and oleoresin for manufacturing perfumes, food flavours and aromatherapy products (Simon *et al*., 1990). Basil is an important source of antioxidants (Koroch *et al.*, 2010) and antimicrobial agents (Hussain *et al.*, 2008) with a potential use in food preservation (Suppakul *et al.*, 2003). Recently, a new disease of basil, downy mildew, incited by *P. belbahrii* Thines (Garibaldi *et al*., 2004; Belbahri *et al*., 2005 and Thines *et al*., 2009) was observed for the first time in Egypt especially in Beni-Sueif governorate in 2013 (Hilal and Ghebrial, 2014). Since then the disease has been quickly spread to all the Egyptian basil-growing areas.

The epidemiology of the pathogen is still scanty. However, it is believed that the pathogen has spread globally in recent years via the shipment of infested seed as well as through wind currents (Garibaldi *et al*., 2004 and McGrath, 2009). Under favorable conditions, the disease rate could reach 100 % within three to five days after the attack, making prevention and control extremely difficult. Chemical control is considered one of the most effective ways to protect many crops from downy mildew infection (Gullino *et al.*, 2009) but this is complicated by the very limited availability of chemicals for basil crop as medicinal, due to the risk of the presence of residues at harvest as well as the difficulty to obtain fungicide registration for minor crops such as basil (Leadbeater and Gisi, 2010). Protectant fungicides may not provide control once the pathogen has infected the plants and begins sporulating on the abaxial surface of infected leaves (Gregory, 2009). However, there are reports on the appearance of resistant isolates to the fungicide mefenoxamand some isolates showed reduced sensitivity to dimethomorph and mandipropamid (Cohen *et al.*, 2013 and Ben-Naim *et al.*, 2015). In Egypt, the unavailability of commercially resistant basil varieties intensifies the need for alternative methods for disease control. One of the potential methods of reducing the severity of the disease in an environmentally safe manner is the induction of plant resistance. Certain substances, such as salicylic acid, bion, silicon, potassium salts were reported to induce resistance in basil plants against downy mildew (Mersha *et al.*, 2012; Gilardi *et al.*, 2013, 2015; Homa *et al.*, 2014 and Patel *et al.*, 2014).

Mono- and di-potassium salts of phosphorous acid provided the best control of basil downy mildew, whereas moderate disease suppression was provided by mandipropamid, cyazofamid and fiuopicolide (Homa *et al.*, 2014). It was speculated that basic phosphates applied to plants could sequester apoplastic calcium, altering membrane integrity and influencing the activity of apoplastic enzymes like polygalacturonases, thereby releasing elicitor-active oligogalacturonides from plant cell walls (Walters and Murray, 1992). Phosphate-mediated induction of resistance was associated with increased activities of phenylalanine ammonia-lyase (PAL), peroxidase (POD) and lipoxygenase (Mitchell and Walters, 2004).

Acibenzolar-S-methyl (ASM) has been developed as a potent systemic acquired resistance (SAR) activator which does not has antimicrobial properties, but instead increases crop resistance to diseases by activation the SAR signal transduction pathway in several plant species (Lopez and Lucas, 2002 and Baysal *et al.*, 2003). The area under the disease progress curve (AUDPC) of basil downy mildew disease severity was significantly reduced compared to the non-treated control when ASM was sprayed or drenched pre-, or pre- + post-inoculation at rates of 25-400 mg/l. Basil plants treated with ASM and challenged with the pathogen showed significantly higher peroxidase activity than the non-treated control at 8 days after inoculation (Mersha *et al.*, 2013).

Although silicon (Si) is not listed among the essential elements, its role in conferring plant resistance to both abiotic and biotic stresses has received increasing attention (Liang *et al*., 2003 and Epstein, 2009). There is a cumulative body of evidence linking the presence of Si with resistance of plants against fungal pathogens (Kanto *et al.*, 2006 and Dallagnol *et al.*, 2012). However, the mechanisms by which Si provides protection against fungal plant pathogens are still unclear. Some authors believe that Si acts as a physical barrier in cell walls, preventing the penetration of fungal hyphae into host tissues (Samuels *et al*., 1991 and Bowen *et al*., 1992), while others believe that Si is related to specific plant defence reactions (Fawe *et al*., 2001and Rodrigues *et al*., 2004).

Among the most promising bioactive oligosaccharides is chitosan which has attracted attention because of its unique biological properties, including its inhibitory effect on the growth of various pathogenic fungi, its ability to be a potent elicitor of plant defense reactions and as an agent for plant growth (Prapagdee *et al*., 2007). Chitosan has been reported to protect different plant species against downy mildew (Manjunatha *et al.*, 2008 and El-Mougy *et al.*, 2014). Epidermis-coating polymers, such as film forming anti-transpirants, have been reported to provide protection against several foliar plant diseases (Nasraoui *et al.*, 1996 and Haggag, 2002). Although the effect of anti-transpirants on disease control appears similar to those of the natural cuticle layer in defending plant pathogens (Hsieh and Huang, 1999), physical effects should be also considered (Nasraoui, 1993). Nitrogen (N) is essential for plant growth and development, and crop plants will obtain it from the soil as nitrate or ammonium (Lea and Azevedo, 2006 and Lea *et al.*, 2007). Application of N fertilizer above recommended rates can lead to significantly higher downy mildew disease severity (Zarafi *et al.*, 2005 and dos Santos *et al.*, 2009).

The objective of this study was to evaluate the efficacy of different plant resistance inducers, mineral salts and film forming anti-transpirants applied on basil as foliar spray combined with two rates of nitrogen fertilizer for controlling basil downy mildew under field condition.

Materials and Methods

A field experiment was carried out under natural infection of basil downy mildew in the Experimental Farm of Sids Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate during the two growing seasons of 2015 and 2016. The soil texture is clay (16.5 % sand, 30.1% silt, 53.4 % clay, pH of 8.1, EC 1.2 dSm^{-1}; 1.3 % organic matter and 26.2, 10.1 and 176 ppm available N, P and K, respectively). During both trial years, the following treatments were applied as foliar spray:

- Chitosan at a concentration of 0.05 % dissolved in 0.01M acetic acid and adjusted pH to 5.6 using 1N NaOH.
- Salicylic acid at the rate of 25 mML^{-1}, added with 0.01 % tween 80.
- Bion (Acibenzolar-S-methyl) at the rate of 50 mg L^{-1}.
- Mono-potassium phosphate K_2HPO_4 at the rate of 20 mML^{-1}.
- Di- potassium phosphate KH_2PO_4 at the rate of 20 mML^{-1}.
- Potassium carbonate K_2CO_3 at the rate of 20 mML^{-1}.
- Potassium silicate K_2O_3Si at the rate of 20 mML^{-1}.
- Silicon dioxide SiO_2 at the rate of 10 mML^{-1}.
- Kaolin (Aluminum silicate, Al_2SiO_5) at the rate of 1 %.
- The commercial antitranspirant product Green miracle at the rate of 3 mlL^{-1} water.
- The fungicide Stone 50% (Dimethomorph 50 %) at the rate of 50 gm/100 L water.
- Control (untreated).

In each season, the soil was mechanically ploughed and planked twice. During the preparation for cultivation, calcium super-phosphate (15.5 % P_2O_5) was added at the rate of 200 kg/fed. The uniform healthy basil seedlings (Balady variety), 10-15 cm length were transplanted on rows at 15 cm spacing between plants. Weeds were removed by manual operations as needed and plants were irrigated regularly as necessary, throughout the growing season in order to maintain constant growth.

Nitrogen was applied in the form of ammonium sulphate (20.6 % N), at the rate of 400 (recommended rate), 500 kg/fed. (High rate) as follow: the first one was added after 21 days from transplanting and the second after 30 days from the first application. The remainder amounts were added after each cut. Potassium sulphate (48 % K_2O) was added at the rate of 75 kg/fed. The plants were sprayed weekly, always performed early in the morning, with the tested compounds after 21 days from transplanting (before the appearance of first symptoms) until run off.

The experiment was arranged in a split-split plot design, with three replications. Monitoring and scouting the plants weekly for downy mildew and disease incidence and severity were estimated as follows:

Disease incidence:

Percentage of disease incidence was recorded as the number of diseased plants relative to the number of growing plants for each treatment, and then the average of disease incidence was calculated. Protection was calculated using the formula:

$$\text{Reduction of downy mildew disease incidence (\%)} = [C-T]/C \times 100$$

Whereas: C and T are percentage of downy mildew incidence in control and treated plants, respectively.

Disease severity:

Disease severity was measured according to (Abd-Alla, 2004). Percentage of disease severity was recorded according to the following equation:

$$\text{Disease severity \%} = [\sum (n \times c)]/ (N \times C) \times 100$$

Whereas: n = Number of infected leaves, c = Category number, N = Total number of examined leaves and C = The highest category number of infection.

The plants were harvested three times (first, second and third cuttings) when the basil plants had full flowers by cutting plants 10 cm above the soil surface with three replications in each cut. Fresh and dry weights of herb yield (ton/fed.) were determined.

To determine the yield of essential oil, the fresh plants (leaves and flowers) were collected from chitosan, di-potassium phosphate, potassium silicate and fungicide treatments during the three cuts and weighted (100g/treatment) representing each replicate then subjected to steam distillation and determined according to Guenther (1961).

Peroxidase activity was determined using the method described in the Worthington enzyme manual (Worthington, 1971). Phenylalanine ammonia-lyase enzyme was determined according to Zucker (1965).

Data were statistically analyzed for computing L.S.D. test at 5 % probability according to the procedure outlined by Snedecor and Cochran (1989).

Results

According to the results obtained of the two growing seasons it was found that the three tested elicitors (bion, chitosan and salicylic acid) in addition to the fungicide Stone 50 % significantly provided protection against downy mildew infection and affected disease severity at an important level at the two nitrogen rates compared to the untreated control for the three cuts of plants (Tables, 1 and 2). In general, the high efficacy values of the tested elicitors were observed when the nitrogen was applied at the recommended rate (400 kg/fed.). The highest reduction in disease incidence was resulted from using any of the fungicide Stone 50% or chitosan treatments with significant differences between them at the two nitrogen rates, followed by salicylic acid treatment. The reduction in disease incidence and disease severity percentages obtained with the fungicide Stone 50% in the first season (2015) were 97.8 & 1.2 %for the first cut, 89.2 & 8.1% for the second cut and 91.3& 2.1% for the third cut, respectively at the recommended rate of nitrogen (400 kg/fed.). Meanwhile application with fungicide in replicates gave high nitrogen fertilizer rate (500 kg/fed.) resulted in 95.0 & 2.5%, 85.5 & 14.0% and 91.0 & 3.9%, respectively in percentage of reduction in disease incidence and disease severity. While, they recorded (100.0 & 0.0 %), (92.5 & 7.0 %) and (96.03 & 1.57 %), respectively at the recommended rate of nitrogen and (98.7 & 1.2%), (87.3 &10.7%) and (93.80 & 2.00%), respectively at the high rate of nitrogen fertilizer during the second growing season (2016). On the other hand, reduction in percentages of disease incidence and value of disease severity were (95.8 & 3.1%), (85.0 & 12.6%), (89.3 & 5.9%) and (93.70 & 4.0%), (83.5 & 14.8 %), (89.0 & 7.2%) in the first growing season and (99.03 & 2.5 %), (89.9 & 9.4 %), (95.0 & 3.1 %) and (96.2 & 2.9 %), (85.7 & 11.5 %), (92.4 & 5.9 %) in the second growing season due to chitosan treatment for the three plant cuts, respectively at the two nitrogen levels. Bion showed relatively less effect in this concern.

Results presented in Tables 3 and 4 exhibit that the three elicitors tested improved plant growth as shown by the significant increments in fresh and dry weights of herb (ton/fed.) in the two experimental seasons compared to the untreated control. Significant differences among treatments were found for plant weights. In general, the plant weights were significantly affected by the rate of nitrogen fertilizer added. Application of high amount of nitrogen fertilizer significantly increased fresh weight of herb but decreased dry weight of herb.

Table 1. Reduction in percentage of disease incidence due to different elicitors applied as foliar spray at two nitrogen rates against downy mildew of basil for three plant cuts during the two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Season 2015					Season 2016				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Bion	400	60.2	46.5	52.5	53.1	52.5	64.5	48.8	54.7	56.0	54.8
	500	59.8	44.3	51.9	52.0		62.1	46.7	52.0	53.6	
Mean		60.0	45.4	52.2	---		63.3	47.7	53.3	---	
Chitosan	400	95.8	85.0	89.3	90.0	89.4	99.1	89.9	95.0	94.6	93.0
	500	93.7	83.5	89.0	88.7		96.2	85.7	92.4	91.4	
Mean		94.7	84.3	89.1	---		97.6	87.8	93.7	---	
Salicylic acid	400	87.2	78.6	83.0	82.9	80.5	91.4	82.6	86.5	86.8	83.6
	500	85.1	72.7	76.5	78.1		87.0	75.9	78.2	80.3	
Mean		86.1	75.7	79.7	---		89.2	79.2	82.3	---	
Stone 50%	400	97.8	89.3	91.3	92.8	91.7	100.0	92.5	96.0	96.2	94.7
	500	95.1	85.5	91.0	90.5		98.7	87.3	93.8	93.3	
Mean		96.4	87.4	91.2	---		99.3	89.9	94.9	---	
Control	400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00
	500	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
Mean		0.0	0.0	0.0	---		0.0	0.0	0.0	---	
Mean of nitrogen rates	400	68.2	59.9	63.2	63.8	---	71.0	62.8	66.4	66.7	---
	500	66.7	57.2	61.7	61.9		68.8	59.1	63.3	63.7	
Overall mean		67.5	58.5	62.4	---		69.9	60.9	64.9	---	
L.S.D. at 5 % for: Treatments (T) = 0.55, Nitrogen rates (N) = 0.15, Cuttings (C) = 0.18, T x N = 0.33, T x C = 0.41, N x C = 0.26, T x N x C = 0.57							T = 0.56, N = 0.14, C = 0.17, T x N = 0.31, T x C = 0.38, N x C = 0.24, T x N x C = 0.54				

Among elicitors tested with recommended rate of nitrogen fertilizer (400 kg/fed.), chitosan was the most effective treatment in this respect. It yielded the highest mean of fresh and dry weight of herb through the three cuts during the season 2015, being 9.78and 2.12 tons/fed., respectively. Meanwhile, the same trend was also noticed through the three cuts determined in the growing season 2016, being 10.84 and 2.35 tons/fed. on the average, respectively. Increasing rate of nitrogen fertilizer to 500 kg/fed. caused significant increases in herb fresh weight with all elicitors tested. The highest means of herb fresh weight were recorded from chitosan treatment. The corresponding means were 10.31 and 11.08 tons/fed. during the two successive seasons (2015 and 2016), respectively. On the other hand, salicylic acid treatment showed moderate effect whereas; bion treatment gave the lowest increase in herb fresh and dry weights during the two growing seasons.

Table 2. Evaluation of different elicitors applied as foliar spray at two nitrogen rates on disease severity of basil downy mildew for three plant cuts during the two growing seasons 2015 and 2016 under field conditions

Treatment	Nitrogen rates (kg/fed.)	Disease severity %									
		Season 2015					Season 2016				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Bion	400	19.3	36.9	22.7	26.3	27.3	16.1	35.8	20.3	24.1	25.2
	500	20.6	37.9	26.4	28.3		18.0	36.2	24.8	26.3	
Mean		19.9	37.4	24.5	---		17.0	36.0	22.5	---	
Chitosan	400	3.1	12.6	5.9	7.2	7.9	2.5	9.4	3.1	5.0	5.9
	500	4.0	14.8	7.2	8.7		2.9	11.5	5.9	6.8	
Mean		3.6	13.7	6.6	---		2.7	10.5	4.5	---	
Salicylic acid	400	4.3	17.9	10.1	10.8	11.5	3.0	15.2	7.8	8.7	9.6
	500	5.3	20.0	11.1	12.1		3.9	18.0	9.8	10.6	
Mean		4.8	18.9	10.6	---		3.5	16.6	8.8	---	
Stone 50%	400	1.2	8.1	2.2	3.8	5.3	0.0	7.0	1.6	2.9	3.8
	500	2.5	14.0	3.9	6.8		1.2	10.7	2.0	4.6	
Mean		1.9	11.0	3.0	---		0.6	8.9	1.8	---	
Control	400	48.6	63.1	54.3	55.3	57.5	50.9	66.5	55.2	57.5	60.3
	500	51.7	70.8	56.5	59.7		55.2	74.3	59.7	63.1	
Mean		50.2	66.9	55.4	---		53.1	70.4	57.4	---	
Mean of nitrogen rates	400	15.3	27.7	19.0	20.7	---	14.5	26.8	17.6	19.6	---
	500	16.8	31.5	21.0	23.1		16.2	30.2	20.4	22.3	
Overall mean		16.1	29.6	20.0	---		15.4	28.5	19.02	---	

L.S.D. at 5 % for: Season 2015		Season 2016	
	T x N = 0.29		T x N = 0.29
Treatments (T) = 0.28	T x C = 0.36	T = 0.36	T x C = 0.35
Nitrogen rates (N) = 0.13	N x C = 0.23	N = 0.13	N x C = 0.22
Cuttings (C) = 0.16	T x N x C = 0.51	C = 0.16	T x N x C = 0.49

Table 3. Effect of three elicitors applied as foliar spray at two nitrogen rates on fresh weight of herb (ton/fed.) for three cuts of basil plants naturally infected by downy mildew during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Fresh weight of herb (ton/fed.)									
		Season 2015					**Season 2016**				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Bion	400	4.7	6.2	7.3	6.1	6.3	5.2	6.6	8.0	6.6	6.8
	500	4.9	6.6	7.9	6.5		5.5	7.1	8.4	7.0	
Mean		4.8	6.4	7.6	---		5.4	6.8	8.2	---	
Chitosan	400	7.8	9.7	11.8	9.8	10.0	8.7	10.7	13.1	10.8	11.0
	500	8.3	10.2	12.4	10.3		9.0	11.0	13.3	11.1	
Mean		8.0	10.0	12.1	---		8.8	10.8	13.2	---	
Salicylic acid	400	7.0	8.9	11.0	9.0	9.3	7.8	10.1	12.1	10.0	10.2
	500	7.6	9.5	11.6	9.6		8.2	10.6	12.5	10.4	
Mean		7.3	9.2	11.3	---		8.0	10.3	12.3	---	
Stone 50%	400	8.1	10.2	12.4	10.2	10.5	8.9	11.0	13.4	11.1	11.3
	500	8.7	10.6	12.9	10.7		9.5	11.6	13.6	11.6	
Mean		8.4	10.4	12.7	---		9.2	11.3	13.5	---	
Control	400	3.2	3.7	4.4	3.8	3.9	2.9	3.4	4.1	3.5	3.7
	500	3.4	3.9	4.7	4.0		3.3	3.8	4.4	3.8	
Mean		3.3	3.8	4.5	---		3.1	3.6	4.3	---	
Mean of nitrogen rates	400	6.1	7.7	9.4	7.7	---	6.7	8.3	10.1	8.4	---
	500	6.9	8.2	9.9	8.2		7.1	8.8	10.4	8.8	
Overall mean		6.4	8.0	9.6	---		6.9	8.6	10.29	---	

Season 2015	Season 2016
L.S.D. at 5 % for: T x N = ns	T x N = ns
Treatments (T) = 0.17 T x C = 0.39	T = 0.19 T x C = 0.37
Nitrogen rates (N) = 0.14 N x C = ns	N = 0.14 N x C = ns
Cuttings (C) = 0.18 T x N x C = ns	C = 0.17 T x N x C = ns

Table 4. Effect of three elicitors applied as foliar spray at two nitrogen rates on dry weight of herb (ton/fed.) for three cuts of basil plants naturally infected by downy mildew during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Herb dry weight (ton/fed.)									
		Season 2015					**Season 2016**				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Bion	400	1.1	1.3	1.5	1.3	1.25	1.2	1.5	1.7	1.5	1.4
	500	1.0	1.2	1.3	1.2		1.1	1.3	1.5	1.3	
Mean		1.1	1.2	1.4	---		1.2	1.4	1.6	---	
Chitosan	400	1.9	2.1	2.4	2.1	2.06	2.1	2.3	2.6	2.3	2.3
	500	1.8	2.0	2.2	2.0		2.0	2.1	2.3	2.2	
Mean		1.8	2.0	2.3	---		2.2	2.2	2.5	--	
Salicylic acid	400	1.7	2.0	2.2	2.0	1.89	1.9	2.2	2.4	2.2	2.1
	500	1.6	1.8	2.0	1.8		1.7	2.0	2.2	2.0	
Mean		1.6	1.9	2.1	---		1.8	2.1	2.3	---	
Stone 50%	400	2.0	2.3	2.5	2.3	2.16	2.2	2.4	2.7	2.4	2.4
	500	1.8	2.1	2.3	2.0		2.0	2.3	2.5	2.3	
Mean		1.9	2.1	2.4	---		2.1	2.4	2.6	---	
Control	400	0.8	1.0	1.1	1.0	0.86	0.8	0.9	1.1	0.9	0.8
	500	0.7	0.8	0.8	0.7		0.6	0.7	0.7	0.7	
Mean		0.7	0.9	0.9	---		0.7	0.8	0.9	---	
Mean of nitrogen rates	400	1.5	1.7	1.9	1.7	---	1.6	1.9	2.1	1.9	---
	500	1.4	1.6	1.7	1.5		1.5	1.7	1.8	1.7	
Overall mean		1.4	1.7	1.8	---		1.6	1.8	1.98	---	

Season 2015		Season 2016	
L.S.D. at 5 % for:	T x N = ns		T x N = 0.03
Treatments (T) = 0.10	T x C = ns	T = 0.04	T x C = 0.04
Nitrogen rates (N) = 0.06	N x C = ns	N = 0.01	N x C = 0.02
Cuttings (C) = 0.07	T x N x C = ns	C = 0.02	T x N x C = 0.05

Data of field experiments shown in Tables 5 and 6 indicate that using any of the tested anti-transpirants applied as foliar spray treatments at two nitrogen rates caused a significant protection against downy mildew infection during the two seasons. It is evident that between the anti-transpirants tested, potassium silicate showed superiority in reducing downy mildew infection through the two successive seasons when applied on plants given recommended rate of nitrogen (400 kg/fed.). The corresponding mean values of reduction in downy mildew incidence were 70.2 and 73.9 % and disease severity, being 19.9 and 16.7 %, through 2015 and 2016 growing seasons, respectively. It is worthy to note that treatment with fungicide Stone 50% gave the highest protection against downy mildew of basil when applied on plants received the recommended rate of nitrogen fertilizer (400 kg/fed.). Green miracle came in the second rank and showed moderate plant protection against downy mildew infection followed by silicon dioxide. The least effective treatment was aluminum silicate. Generally, the severity of downy mildew was increased when nitrogen was added at a rate above the recommended and showed the maximum increase in the second plant cut.

Table 5. Reduction in percentage of disease incidence due to different anti-transpirant treatments applied as foliar spray at two nitrogen rates against downy mildew of basil for three plant cuts during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Season 2015					Season 2016				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Green miracle	400	53.5	42.1	48.3	48.0	46.5	55.3	43.7	50.8	49.9	48.1
	500	51.7	40.9	42.0	45.0		52.3	42.0	44.7	46.3	
Mean		52.6	41.5	45.2	---		53.8	43.0	47.8	---	
Aluminum silicate (Al_2SiO_5) (Kaolin)	400	21.4	12.7	16.3	16.7	15.8	22.9	13.3	17.5	17.9	17.1
	500	19.5	10.0	15.3	14.8		21.1	11.5	16.6	16.4	
Mean		20.5	11.4	15.3	---		22.0	12.4	17.0	---	
Potassium silicate (K_2O_3Si)	400	78.3	63.0	69.0	70.2	69.5	82.9	65.7	73.1	73.9	72.6
	500	78.0	60.2	68.0	68.7		81.2	62.9	70.0	71.4	
Mean		78.1	61.6	68.5	---		82.1	64.3	71.6	---	
Silicon dioxide (SiO_2)	400	29.9	19.5	26.0	25.1	24.1	31.0	21.9	28.9	27.3	26.1
	500	28.0	18.3	22.0	23.0		30.7	20.0	24.1	24.9	
Mean		29.0	19.0	24.0	---		30.8	21.0	26.5	---	
Stone 50%	400	97.8	89.3	91.0	92.8	91.7	100.0	92.5	96.0	96.2	94.7
	500	95.1	85.5	91.3	90.5		98.7	87.3	93.8	93.3	
Mean		96.4	87.4	91.2	---		99.3	89.9	94.9	---	
Control	400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
Mean		0.0	0.0	0.0	---		0.0	0.0	0.0	---	
Mean of nitrogen rates	400	46.8	37.8	41.8	42.1	---	48.7	39.5	44.4	44.2	---
	500	45.4	35.9	39.9	40.4		47.3	37.3	41.5	42.0	
Overall mean		46.1	36.8	40.8	---		48.0	38.4	43.0	---	

L.S.D. at 5 % for: Season 2015		Season 2016	
	T x N = 0.26		T x N = 0.27
Treatments (T) = 0.52	T x C = 0.32	T = 0.54	T x C = 0.33
Nitrogen rates (N) = 0.11	N x C = 0.18	N = 0.11	N x C = 0.19
Cuttings (C) = 0.13	T x N x C = 0.45	C = 0.14	T x N x C = 0.47

Table 6. Evaluation of different anti-transpirant treatments applied as foliar spray at two nitrogen rates on disease severity of basil downy mildew for three plant cuts during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed)	Disease severity %									
		Season 2015					Season 2016				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Green miracle	400	23.6	38.4	25.0	29.0	29.8	21.6	36.5	22.1	26.7	28.1
	500	23.9	38.8	28.9	30.5		23.5	37.2	27.8	29.5	
Mean		23.7	38.6	26.9	---		22.6	36.8	24.9	---	
Aluminum silicate (Al_2SiO_5) (Kaolin)	400	29.1	41.1	31.0	33.7	35.1	27.3	40.2	30.5	32.7	34.2
	500	29.5	42.9	37.0	36.5		28.5	42.0	36.4	35.6	
Mean		29.3	42.0	34.0	---		27.9	41.1	33.5	---	
Potassium silicate (K_2O_3Si)	400	9.9	28.0	21.7	19.9	20.4	6.5	25.7	18.0	16.7	17.4
	500	11.3	28.9	22.5	20.9		8.0	26.0	20.5	18.2	
Mean		10.6	28.4	22.1	---		7.2	25.8	19.3	---	
Silicon dioxide (SiO_2)	400	22.9	38.6	30.1	30.5	31.4	20.6	37.9	28.0	28.8	29.3
	500	25.2	39.0	32.4	32.2		21.9	38.9	28.4	29.8	
Mean		24.0	38.8	31.2	---		21.3	38.4	28.2	---	
Stone 50%	400	1.2	8.1	2.2	3.8	5.3	0.0	7.0	1.6	2.9	3.8
	500	2.5	13.9	3.9	6.8		1.2	10.7	2.0	4.6	
Mean		1.9	11.1	3.0	---		0.6	8.9	1.8	---	
Control	400	48.6	63.1	54.3	55.3	57.5	50.9	66.5	55.2	57.5	60.3
	500	51.7	70.8	56.5	59.7		55.2	74.3	59.7	63.1	
Mean		50.2	66.9	55.4	---		53.1	70.4	57.4	---	
Mean of nitrogen rates	400	22.5	36.2	27.4	28.7	---	21.2	35.6	25.9	27.6	---
	500	24.0	39.0	30.2	31.1		23.1	38.2	29.1	30.1	
Overall mean		23.3	37.6	28.8	---		22.1	36.9	27.5	---	

L.S.D. at 5 % for:	Season 2015		Season 2016	
Treatments (T)	= 0.82	T x N = 1.38	T = 0.45	T x N = 0.28
Nitrogen rates (N)	= 0.56	T x C = 1.69	N = 0.11	T x C = 0.34
Cuttings (C)	= 0.69	N x C = 0.98	C = 0.14	N x C = 0.19
		T x N x C = 2.39		T x N x C = 0.48

The tested anti-transpirants significantly affected the growth of basil plants by increasing the fresh and dry weights of herb than the untreated control (Tables 7 and 8). It is clear that maximum mean values of fresh and dry weights of herb in ton per fed. for the three cuts were obtained due to using of the potassium silicate treatment applied at the two rates of nitrogen fertilizer. The corresponding mean values of fresh and dry weights in the first season (2015) were 7.9 & 1.7 ton/fed. respectively, when the plants received the recommended rate of nitrogen fertilizer (400 kg/fed.), being 8.6 & 1.6 ton/fed. when plants received high rate of nitrogen fertilizer (500 kg/fed.), followed by green miracle and silicon dioxide treatments. A similar trend was obtained in the second season (2016). The lowest values of these plant growth parameters were found in kaolin (Aluminum silicate) treatment.

Table 7. Effect of anti-transpirant treatments applied as foliar spray at two nitrogen rates on fresh weight of herb (ton/fed.) for three cuts of basil plants naturally infected by downy mildew during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Fresh weight of herb (ton/fed.)									
		Season 2015					**Season 2016**				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Green miracle	400	4.1	5.3	6.0	5.1	5.3	4.7	5.7	6.6	5.7	5.8
	500	4.3	5.5	6.6	5.5		5.0	6.0	7.0	6.0	
Mean		4.2	5.4	6.3	---		4.8	5.8	6.8	---	
Aluminum silicate (Al_2SiO_5) (Kaolin)	400	3.4	4.2	4.9	4.2	4.4	3.9	4.4	5.5	4.6	4.8
	500	3.7	4.6	5.4	4.6		4.2	5.0	6.0	5.1	
Mean		3.5	4.4	5.2	---		4.0	4.7	5.8	---	
Potassium silicate (K_2O_3Si)	400	6.10	8.1	9.6	7.9	8.3	6.6	9.8	10.5	9.0	9.4
	500	6.8	8.8	10.1	8.6		7.1	10.8	11.3	9.7	
Mean		6.5	8.9	9.8	---		6.8	10.3	10.9	---	
Silicon dioxide (SiO_2)	400	3.9	4.7	5.7	4.8	4.9	4.1	5.1	6.0	5.1	5.3
	500	4.2	4.9	6.0	5.0		4.6	5.3	6.5	5.5	
Mean		4.1	4.8	5.8	---		4.3	5. 2	6.3	---	
Stone 50%	400	8.1	10.2	12.4	10.2	10.5	8.9	11.0	13.4	11.1	11.3
	500	8.7	10.6	12.9	10.7		9.5	11.6	13.6	11.6	
Mean		8.4	10.4	12.7	---		9.2	11.3	13.5	---	
Control	400	3.2	3.7	4.3	3.8	3.9	2.9	3.4	4.1	3.5	3.7
	500	3.4	3.9	4.7	4.0		3.3	3.8	4.4	3.8	
Mean		3.3	3.8	4.5	---		3.1	3.6	4.3	---	
Mean of nitrogen rates	400	4.8	6.0	7.2	6.0	---	5.1	6.6	7.7	6.5	---
	500	5.2	6.4	7.6	6.4		5.6	7.1	8.1	6.9	
Overall mean		5.0	6.2	7.4	---		5.4	6.8	7.9	---	

Season 2015		Season 2016	
L.S.D. at 5 % for:	T x N = ns		T x N = ns
Treatments (T) = 0.22	T x C = 0.39	T = 0.27	T x C = 0.38
Nitrogen rates (N) = 0.13	N x C = ns	N = 0.13	N x C = ns
Cuttings (C) = 0.16	T x N x C = ns	C = 0.15	T x N x C = ns

Table 8. Effect of anti-transpirant treatments applied as foliar spray at two nitrogen rates dry weight of herb (ton/fed.) for three cuts of basil plants naturally infected by downy mildew during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Herb dry weight (ton/fed.)									
		Season 2015					**Season 2016**				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Green miracle	400	1.0	1.1	1.3	1.1	1.1	1.1	1.3	1.6	1.3	1.3
	500	0.9	1.0	1.2	1.0		1.1	1.2	1.4	1.2	
Mean		0.9	1.0	1.3	---		1.1	1.2	1.5	---	
Aluminum silicate (Al_2SiO_5) (Kaolin)	400	0.8	1.0	1.1	1.0	0.9	0.8	1.1	1.4	1.1	1.0
	500	0.7	0.8	0.9	0.8		0.7	0.9	1.0	0.9	
Mean		0.7	0.9	1.0	---		0.7	1.0	1.2	---	
Potassium silicate (K_2O_3Si)	400	1.5	1.7	1.9	1.7	1.6	1.6	2.0	2.2	2.0	1.8
	500	1.4	1.6	1.7	1.6		1.4	1.7	2.0	1.7	
Mean		1.4	1.6	1.8	---		1.5	1.9	2.10	---	
Silicon dioxide (SiO_2)	400	0.9	1.0	1.23	1.0	1.0	0.9	1.2	1.5	1.2	1.1
	500	0.8	0.9	1.1	0.9		0.9	1.0	1.20	1.03	
Mean		0.8	0.9	1.2	---		0.9	1.1	1.4	---	
Stone 50%	400	2.0	2.3	2.5	2.3	2.2	2.2	2.4	2.7	2.4	2.4
	500	1.8	2.1	2.3	2.0		2.0	2.3	2.5	2.3	
Mean		1.9	2.2	2.4	---		2.1	2.4	2.6	---	
Control	400	0.8	1.0	1.1	1.0	0.9	0.8	0.9	1.1	0.9	0.8
	500	0.7	0.8	0.8	0.7		0.6	0.7	0.7	0.7	
Mean		0.7	0.9	0.9	---		0.7	0.8	0.9	---	
Mean of nitrogen rates	400	1.2	1.4	1.5	1.3	---	1.2	1.5	1.8	1.5	---
	500	1.0	1.2	1.3	1.2		1.1	1.3	1.5	1.3	
Overall mean		1.1	1.3	1.4	---		1.18	1.4	1.6	---	
L.S.D. at 5 % for: Treatments (T) = 0.02, Nitrogen rates (N) = 0.01, Cuttings (C) = 0.01		T x N = 0.03, T x C = 0.04, N x C = 0.02, T x N x C = 0.05					T = 0.04, N = 0.01, C = 0.01		T x N = 0.03, T x C = 0.04, N x C = 0.02, T x N x C = 0.05		

The obtained data Tables, 9 and 10 show that spraying any of mineral salts tested, *i.e.* di-potassium phosphate, mono-potassium phosphate and potassium carbonate significantly affected the disease compared to the untreated control. Di-potassium phosphate applied weekly provided the most efficacious control of basil downy mildew in both seasons at the two rates of nitrogen fertilizer. The reduction in disease incidence reached 73.6 and 77.8 % on plants given the recommended rate of nitrogen fertilizer (400 kg/fed.) through the two growing seasons, respectively. While, it reached 72.2 and 75.9 % at the high rate of nitrogen fertilizer (500 kg/fed.), respectively. On the other hand, the disease severity value was significantly lower compared to the other treatments which recorded 16.2 and 13.8 % at the recommended rate of nitrogen and 19.5 and 17.5 % at the high rate of nitrogen fertilizer in both experimental seasons, respectively. Moderate disease suppression was provided with potassium carbonate. Meanwhile mono-potassium phosphate was the lowest efficient salt in controlling basil downy mildew. In general, the fungicide Stone 50 % was more effective than the other treatments in minimizing the disease incidence and severity in both seasons.

Table 9. Reduction in percentage of disease incidence due to different mineral salts applied as foliar spray at two nitrogen rates against downy mildew of basil for three plant cuts during two growing seasons 2015 and 2016 under field conditions

Treatment	N.rates (kg/fed)	Season 2015					Season 2016				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Di-potassium phosphate (K_2HPO_4)	400	81.7	67.5	71.6	73.6	72.9	86.5	71.0	75.9	77.8	76.8
	500	80.6	65.7	70.4	72.2		85.0	68.9	73.7	75.9	
Mean		81.1	66.6	71.0	---		85.7	70.0	74.8	---	
Mono-potassium phosphate (KH_2PO_4)	400	62.3	48.9	53.5	54.9	53.6	65.0	51.5	55.7	57.4	56.0
	500	60.2	44.3	52.2	52.2		63.0	46.5	54.5	54.7	
Mean		61.2	46.6	52.9	---		64.0	49.0	55.1	---	
Potassium carbonate (K_2CO_3)	400	67.6	56.1	60.5	61.4	59.1	70.8	59.0	63.5	64.4	62.0
	500	64.1	50.4	56.1	56.9		66.9	53.0	58.9	59.6	
Mean		65.8	53.2	58.3	---		68.8	56.0	61.2	---	
Stone 50%	400	97.8	89.3	91.3	92.8	91.7	100.0	92.5	96.0	96.2	94.7
	500	95.1	85.5	91.0	90.5		98.7	87.3	93.8	93.3	
Mean		96.4	87.4	91.2	---		99.3	89.9	94.9	---	
Control	400	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	500	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
Mean		0.0	0.0	0.0	---		0.0	0.0	0.0	---	
Mean of nitrogen rates	400	61.9	52.3	55.4	56.5	---	64.4	54.8	58.2	59.2	---
	500	60.0	49.2	53.9	54.4		62.7	51.1	56.2	56.7	
Overall mean		60.9	50.8	54.7	---		63.6	53.0	57.2	---	

Season 2015		Season 2016	
L.S.D. at 5 % for:	T x N = 0.31		T x N = 0.33
Treatments (T) = 0.42	T x C = 0.37	T = 0.51	T x C = 0.41
Nitrogen rates (N) = 0.14	N x C = 0.24	N = 0.15	N x C = 0.26
Cuttings (C) = 0.17	T x N x C = 0.53	C = 0.18	T x N x C = 0.57

Table 10. Evaluation of different mineral salts applied as foliar spray at two nitrogen rates on disease severity of basil downy mildew for three plant cuts during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Disease severity %									
		Season 2015					Season 2016				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Di-potassium phosphate (K_2HPO_4)	400	6.6	27.4	14.7	16.2		5.5	24.5	11.5	13.8	
	500	8.8	28.3	21.5	19.5	17.9	6.9	26.0	19.5	17.5	15.7
Mean		7.7	27.8	18.1	---		6.2	25.2	15.5	---	
Mono-potassium phosphate (KH_2PO_4)	400	15.6	33.3	20.9	23.3		13.5	32.0	18.5	21.4	
	500	15.7	34.9	26.8	25.	24.5	13.9	33.0	25.7	24.2	22.8
Mean		15.6	34.1	23.8	---		13.7	32.5	22.1	---	
Potassium carbonate (K_2CO_3)	400	11.1	32.8	24.9	22.9		9.0	30.6	22.7	20.8	
	500	14.7	36.9	28.6	26.7	24.8	12.6	35.9	26.3	24.9	22.8
Mean		12.9	34.8	26.7	---		10.8	33.2	24.5	---	
Stone 50%	400	1.2	8.1	2.2	3.8		0.0	7.0	1.6	2.9	
	500	2.5	13.9	3.9	6.8	5.3	1.3	10.7	2.0	4.6	3.8
Mean		1.9	11.0	3.1	---		0.6	8.9	1.8	---	
Control	400	48.6	63.1	54.3	55.3		50.9	66.5	55.2	57.54	
	500	51.7	70.8	56.5	59.7	57.5	55.2	74.3	59.7	63.1	60.3
Mean		50.2	66.9	55.4	---		53.1	70.4	57.4	---	
Mean of nitrogen rates	400	16.6	32.9	23.4	24.3		15.8	32.1	21.9	23.3	
	500	18.7	36.9	27.44	27.8	---	18.0	36.0	26.6	26.9	---
Overall mean		17.7	34.9	25.42	---		16.9	34.5	24.3	---	

L.S.D. at 5 % for: (Season 2015)		L.S.D. at 5 % for: (Season 2016)	
	T x N = 0.34		T x N = 0.35
Treatments (T) = 0.27	T x C = 0.42	T = 0.28	T x C = 0.42
Nitrogen rates (N) = 0.15	N x C = 0.26	N = 0.16	N x C = 0.27
Cuttings (C) = 0.19	T x N x C = 0.59	C = 0.19	T x N x C = 0.60

The efficacy of all tested mineral salts in disease control was reflected on the plant yield, causing significant increase in the fresh and dry weights of herb (Tables 11 and 12). The pronounced increase in this respect was observed with di-potassium phosphate treatment which resulted in the highest mean values of herb fresh weight (8.6 ton/fed.) as well as dry weight of herb (1.8 ton/fed.) when nitrogen fertilizer was applied at the recommended rate (400 kg/fed.) and 9.0 and 1.6 ton/fed., respectively when nitrogen fertilizer was applied at the high rate (500 kg/fed.) in the first season 2015 with significant differences with the other treatments. The corresponding mean values for the second season in this respect were 9.3 and 2.01ton/fed. at the recommended rate of nitrogen fertilizer and 10.0 and 1.8 ton/fed. at the high rate of nitrogen fertilizer. Potassium carbonate gave higher values than those recorded due to using mono-potassium phosphate which scored the lowest plant yields.

Table 11. Effect of mineral salts applied as foliar spray at two nitrogen rates on fresh weight of herb (ton/fed.) for three cuts of basil plants naturally infected by downy mildew during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Fresh weight of herb (ton/fed.)									
		Season 2015					**Season 2016**				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Di-potassium phosphate (K_2HPO_4)	400	6.8	8.7	10.3	8.6	8.8	7.4	9.2	11.3	9.3	9.7
	500	7.2	9.0	10.7	9.0		7.9	10.2	11.8	10.0	
Mean		7.0	8.8	10.5	---		7.7	9.7	11.6	---	
Mono-potassium phosphate (KH_2PO_4)	400	5.3	6.8	8.1	6.7	6.9	5.8	7.8	8.5	7.4	7.6
	500	5.5	7.1	9.0	7.2		6.2	8.1	9.2	7.8	
Mean		5.4	6.9	8.5	---		6.0	8.0	8.9	---	
Potassium carbonate (K_2CO_3)	400	5.7	7.7	8.9	7.4	7.6	6.1	8.0	9.7	7.9	8.2
	500	6.0	8.0	9.5	7.8		6.6	8.7	9.9	8.4	
Mean		5.9	7.8	9.2	---		6.3	8.3	9.8	---	
Stone 50%	400	8.1	10.2	12.4	10.2	10.5	8.9	11.0	13.4	11.1	11.3
	500	8.7	10.6	12.9	10.7		9.5	11.6	13.6	11.6	
Mean		8.4	10.4	12.7	---		9.2	11.3	13.5	---	
Control	400	3.2	3.7	4.4	3.8	3.9	2.9	3.4	4.1	3.5	3.7
	500	3.4	3.9	4.7	4.0		3.3	3.8	4.4	3.9	
Mean		3.3	3.8	4.5	---		3.1	3.6	4.9	---	
Mean of nitrogen rates	400	5.8	7.4	8.8	7.2	---	6.2	7.9	9.4	7.8	---
	500	6.2	7.7	9.3	7.7		6.7	8.5	9.8	8.3	
Overall mean		6.0	7.6	9.1	---		6.5	8.2	9.6	---	

Season 2015		Season 2016	
L.S.D. at 5 % for:	T x N = ns		T x N = ns
Treatments (T) = 0.25	T x C = 0.36	T = 0.28	T x C = 0.34
Nitrogen rates (N) = 0.13	N x C = ns	N = 0.12	N x C = ns
Cuttings (C) = 0.16	T x N x C = ns	C = 0.15	T x N x C = ns

Table 12. Effect of mineral salts applied as foliar spray at two nitrogen rates on dry weight of herb (ton/fed.) for three cuts of basil plants naturally infected by downy mildew during two growing seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Herb dry weight (ton/fed.)									
		Season 2015					**Season 2016**				
		1st cut	2nd cut	3rd cut	Mean	Overall mean	1st cut	2nd cut	3rd cut	Mean	Overall mean
Di-potassium phosphate (K_2HPO_4)	400	1.6	1.8	2.0	1.8	1.7	1.8	2.1	2.3	2.1	1.9
	500	1.5	1.7	1.8	1.6		1.6	1.8	2.1	1.8	
Mean		1.5	1.7	1.9	---		1.7	1.9	2.2	---	
Mono-potassium phosphate (KH_2PO_4)	400	1.2	1.4	1.7	1.4	1.3	1.3	1.7	1.8	1.6	1.5
	500	1.1	1.3	1.40	1.3		1.2	1.5	1.7	1.5	
Mean		1.2	1.3	1.5	---		1.3	1.6	1.7	---	
Potassium carbonate (K_2CO_3)	400	1.3	1.6	1.8	1.6	1.5	1.4	1.8	2.0	1.7	1.7
	500	1.2	1.5	1.6	1.4		1.3	1.6	1.8	1.6	
Mean		1.3	1.5	1.7	---		1.3	1.7	1.9	---	
Stone 50%	400	2.0	2.3	2.5	2.2	2.2	2.2	2.4	2.7	2.4	2.4
	500	1.8	2.1	2.3	2.0		2.0	2.3	2.5	2.3	
Mean		1.9	2.2	2.4	---		2.1	2.4	2.6	---	
Control	400	0.8	1.0	1.1	1.0	0.9	0.8	0.9	1.1	0.9	0.8
	500	0.7	0.7	0.8	0.7		0.6	0.7	0.7	0.7	
Mean		0.7	0.9	0.9	---		0.7	0.8	0.9	---	
Mean of nitrogen rates	400	1.4	1.6	1.8	1.6	---	1.5	1.8	2.0	1.8	---
	500	1.2	1.5	1.6	1.4		1.3	1.6	1.8	1.6	
Overall mean		1.32	1.53	1.69	---		1.4	1.7	1.9	---	

L.S.D. at 5 % for:	Season 2015		Season 2016	
		T x N = 0.03		T x N = 0.04
	Treatments (T) = 0.02	T x C = 0.04	T = 0.02	T x C = 0.05
	Nitrogen rates (N) = 0.01	N x C = 0.02	N = 0.02	N x C = 0.03
	Cuttings (C) = 0.02	T x N x C = 0.05	C = 0.02	T x N x C = 0.07

Among all compounds used in this study, chitosan, di-potassium phosphate and potassium silicate in addition to the fungicide Stone 50 % were selected due to their higher effect in controlling basil downy mildew to evaluate their effect on essential oil yields and induction of enzymes activities. Data in Table 13 indicate that all the selected treatments significantly increased essential oil yield/ 100 gm fresh herb than the untreated control at the two rates of nitrogen fertilizer during the two experimental seasons. Among the three tested compounds, chitosan was the most effective treatment yielding the highest increases in the total oil yield by 55.1 & 38.6 % in the first season and 65.3 & 57.7 in the second season at the two rates of nitrogen fertilizer, respectively, followed by di-potassium phosphate salt and the antitranspirant potassium silicate which provided the least oil yields.

Table 13. Effect of chitosan, di-potassium phosphate, potassium silicate and Stone 50 % as foliar spray at two nitrogen rates on essential oil yield (ml)/100 gm fresh herb during two seasons 2015 and 2016 under field conditions

Treatment	N. rates (kg/fed.)	Essential oil yield (ml)/100 gm fresh herb									
		Season of 2015					Season of 2016				
		1st cut	2nd cut	3rd cut	Total	Increase %*	1st cut	2nd cut	3rd cut	Total	Increase %*
Chitosan	400	0.2	0.2	0.3	0.7	55.1	0.2	0.3	0.3	0.7	65.3
	500	0.1	0.1	0.1	0.4	38.6	0.1	0.1	0.2	0.5	57.7
Di-potassium phosphate	400	0.2	0.2	0.2	0.6	49.1	0.2	0.2	0.3	0.7	62.3
	500	0.1	0.1	0.1	0.4	27.0	0.1	0.1	0.2	0.5	52.1
Potassium silicate	400	0.1	0.1	0.1	0.4	22.5	0.1	0.1	0.2	0.5	44.7
	500	0.1	0.1	0.1	0.3	12.9	0.1	0.1	0.1	0.4	40.5
Stone 50 %	400	0.2	0.2	0.3	0.7	59.2	0.3	0.3	0.3	0.8	69.0
	500	0.1	0.1	0.2	0.5	42.5	0.1	0.2	0.2	0.6	60.7
Control	400	0.1	0.1	0.1	0.3	---	0.1	0.1	0.1	0.3	---
	500	0.1	0.1	0.1	0.3	---	0.0	0.1	0.1	0.2	---

L.S.D. at 5 % for:	2015		2016	
		T x N = 0.012		T x N = 0.016
Treatments (T)	= 0.003	T x C = 0.012	T = 0.015	T x C = 0.026
Nitrogen rates (N)	= 0.009	N x C = 0.012	N = 0.009	N x C = 0.016
Cuttings (C)	= 0.002	T x N x C = 0.029	C = 0.006	T x N x C = 0.036

*Increase relative to the control.

Results in Table 14 reveal that all treatments increased the enzymes activities at the two nitrogen rates. The highest increase in PAL and POD activity was obtained with fungicide Stone 50 % followed by chitosan treatment, being 55.7 and 94.8 % & 49.7 and 76.9 % at the recommended rate of nitrogen and reached 54.8 and 85.9% & 22.6 and 71.1% at the high rate of nitrogen, respectively. Di-potassium phosphate treatment showed moderate effect. Meanwhile, potassium silicate was the least effective one.

Table 14. Enzyme activities in basil plants as affected by chitosan, di-potassium phosphate, potassium silicate and Stone 50% treatments applied as foliar spray at two nitrogen rates under field conditions

Treatment	N. rates (kg/fed.)	Phenylalanine ammonia-lyase (PAL)		Peroxidase (POD)	
		Activity	* % Increase	Activity	* % Increase
Chitosan	400	9.925	49.7	0.052	76.9
	500	4.657	22.6	0.038	71.1
Di-potassium phosphate	400	7.701	35.2	0.043	72.1
	500	3.838	6.1	0.031	64.5
Potassium silicate	400	5.853	14.7	0.038	68.4
	500	3.721	3.1	0.017	35.3
Stone 50 %	400	11.263	55.7	0.232	94.8
	500	7.968	54.8	0.078	85.9
Control	400	4.992	---	0.012	---
	500	3.604	---	0.011	---

* Increase relative to the control.

Discussion

Plants respond to pathogen attack or elicitor treatments by activating a wide variety of protective mechanisms designed to prevent pathogen replication and spreading. The defense mechanisms include the fast production of reactive oxygen species (De Gara *et al*., 2003), alterations in the cell wall constitution, accumulation of antimicrobial secondary metabolites known as phytoalexins (Agrios, 2005), activation and/or synthesis of defense peptides and proteins (Castro and Fontes, 2005). In various plant species, resistance can be induced with elicitors such as salicylic acid, bion and chitosan against a wide range of pathogens (Sharathchandra *et al*., 2004 and Amin *et al*., 2007). In the present study, it was found that treatment of basil plants with these inducers as foliar spray efficiently reduced susceptibility to downy mildew caused by *P. belbahrii* under field conditions, in a way similar to that of Stone 50%. Applications of chitosan at 0.05% followed by salicylic acid at 25 mM were the most effective in this concern. The least effect was found with bion (50 mg L^{-1}) treatment.

There are numerous reports concerning the protective effects of chitosan against downy mildew infection in a range of crops (Manjunatha *et al.*, 2008 and El-Mougy *et al.*, 2014). The reduction of disease severity in plants by chitosan application may be referred to its property as a hydrophobic material, thus creating a low water potential in infected leaves which prevented spore germination, infection and growth when applied before infection as well as, its ability to reduce esterase secretion by pathogens (Hsieh and Huang, 1999). Southerton and Deverall (1990) added that application of elicitors like chitosan resulted in accumulation of osmiophilic masses in the intercellular spaces of reacting host cells. So, the invading fungal cells coated by this osmiophilic material causing frequently pronounced disorganization such as plasmalemma retraction thus preventing pathogen penetration. Another explanation may be referred to the role of elicitors in induction of local and/or SAR in plants against invasion of the pathogen. In this concern, SAR was reported in a number of crops due to chitosan application (Bhaskarareddy *et al*., 1999 and Barka *et al*., 2004). Several reports indicated also that application of elicitors as chitosan, salicylic acid stimulates the accumulation of signal molecule as jasmonic acid, salicylic acid, hydrogen peroxide, reactive oxygen species and protein kinases, all of which play crucial role in intracellular signalling pathways (Atia *et al*., 2005). A pioneering study demonstrated that the application of exogenous salicylic acid or chitosan induces the synthesis of pathogenesis related proteins (PRs) and partial resistance to pathogens such as β-1,3-glucanase, chitinase and PR proteins (Raucher *et al*., 1999 and Atia *et al*., 2005).

Exogenous application of salicylic acid has been shown to move systemically through plants, resulting in the expression of a set of defense genes that are activated by pathogen infection (Lu *et al*., 2006). The development of acquired resistance by salicylic acid may be attributed at least partly, to the SA-induced phenylalanine ammonia lyase (PAL) gene expression and activation (Wen *et al*., 2005).

On the other hand, application of mineral salts (mono- & di-potassium phosphate and potassium carbonate) and anti-transpirants (green miracle, potassium silicate, aluminum silicate (kaolin) and silicon dioxide) to the highly susceptible sweet basil plants as foliar spray at weekly intervals before infection by downy mildew significantly controlled the disease and decreased its severity at an unimportant level under field conditions, which consequently improved plant growth and increased fresh and dry weights of basil herbs as well as essential oil yield. Di-potassium phosphate and potassium silicate appeared to be the most effective in this respect. Studies on other plants support our results (Mosa, 1997 and Yildirim *et al.*, 2002). Di-potassium phosphate penetrates the fungal cell and disturbs the balance of potassium and causes the disintegration of conidial walls (Ziv and Zitter, 1992). In addition, it could provide systemic resistance by forming crystallized insoluble compounds with Ca^{2+} inside the healthy plant cells (Gottstein and Kuc, 1989). Orober *et al.* (2002) showed that phosphate-mediated resistance induction in cucumber was associated with localized cell death, preceded by a rapid generation of superoxide and hydrogen peroxide. They also detected local and systemic increases in levels of free and conjugated salicylic acid following phosphate application. In barley, phosphate-mediated induction of resistance was associated with increased activities of phenylalanine ammonia-lyase, peroxidase and lipoxygenase (Mitchell and Walters, 2004).

For the protective efficacy of silicates, formation of thick and wide cells as a result of silica accumulation in the epidermal cells plays a role in resistance of the plant against the causal agent (Kuc and Hammerschmidt, 1995). Heintz and Blaich (1990) showed that silicate could accumulate inside the healthy neigh boring cells infected with *U. necator* in such a quantity that they can provide resistance against it. In addition to its protective efficacy, silicates also showed post infection inhibitive efficacy. In this case, silicates inhibit the conidial dispersion and as a result the conidial germination, by covering the colony present on the surface of the plant. Furthermore, silicates can inhibit conidial germination by increasing the pH (pH= 10)

(Yildirim and Onogur, 2001). Oh (1997) reported that the required pH for germination of conidia of *U. necator* was 5 in laboratory conditions.

Various mechanisms for the protected plants with coating polymers have been suggested (Han, 1990 and Zekaria-Oren *et al.*, 1991). The effect of film forming anti-transpirants may be similar to those of the natural cuticle layer in defence against pathogen. In this respect, Zekaria-Oren *et al.* (1991) mentioned that polymers of film forming anti-transpirants provide either an impenetrable surface associated with their thickness or are resistance to enzymatic degrading.

In this study, application of nitrogen fertilizer above the recommended rates significantly increased disease severity of basil downy mildew. This result is in line with Hoffland *et al.* (2000); Zarafi *et al.* (2005) and dos Santos *et al.* (2009). The N supply influences branching and leaf expansion, which together determine the size of the canopy produced. Large canopies with high shoot densities may be more conducive to spore transfer and pathogen infection than sparse canopies (Walters and Bingham, 2007). Olesen *et al.* (2003a) found that the severity of both powdery mildew and septoria leaf spot was enhanced by increasing rates of N fertilizer that associated with an increased leaf N concentration at flag leaf emergence (Olesen *et al.*, 2003b). These workers also obtained positive, but weaker, correlations between disease severity and crop canopy size, suggesting that leaf N concentration was more important for disease development than canopy size (Olesen *et al.*, 2003b). According to Hoffland *et al.* (2000), susceptibility to disease is the outcome of the interaction between two factors: (a) the value of the plant as a source of nutrients and energy to the pathogen and (b) the presence of secondary host metabolites (defense compounds) that prevent pathogen growth, development or multiplication. These factors are affected differentially by N supply.

In conclusion, the incorporation of chitosan, di-potassium phosphate and potassium silicate in an integrated management programs should bring positive results, both in terms of reducing disease intensity and consequently, the amount of fungicides used.

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مقاومة مرض البياض الزغبى على نبات الريحان الناتج عن *Peronospora belbahrii* باستخدام محفزات المقاومة، الأملاح المعدنية ومضادات التعرق مجتمعة مع معدلات مختلفة من الأسمدة النيتروجينية تحت ظروف الحقل

ايمان وجيه راغب غبريال و محمد جودة عبدالحق ندا
معهد بحوث أمراض النباتات، مركز البحوث الزراعية، الجيزة

تم إجراء تجارب فى الحقل تحت ظروف العدوى الطبيعية بمرض البياض الزغبى فى الريحان خلال عامى ٢٠١٥ و ٢٠١٦ فى المزرعة البحثية بمحطة البحوث الزراعية بسدس-مركز البحوث الزراعية - محافظة بنى سويف من اجل تقييم فعالية مركبات مختلفة لمحفزات المقاومة، الاملاح المعدنية ومضادات التعرق والتى تستخدم كرش ورقى على نباتات الريحان الحلو (الصنف البلدي) مع استخدام معدلين من الاسمدة النيتروجينية للسيطرة على مرض البياض الزغبى الذى يسببه *Peronospora belbahrii*. بشكل عام، كل المعاملات كان لها تأثير إيجابى على الحد من شدة المرض عند إضافة المعدلات الموصى بها من الأسمدة النيتروجينية مع زيادة معنوية فى الوزن الطازج والجاف للعشب وكمية الزيت فى نهاية التجربة. لوحظ ارتفاع كفاءة هذه المركبات عند إضافة الأسمدة النيتروجينية عند المعدلات الموصى بها. وكانت معاملات الشيتوزان (٠,٠٥ %) وفوسفات ثنائى البوتاسيوم (٢٠ ملليمولر) وسيليكات البوتاسيوم (٢٠ ملليمولر) بالإضافة إلى المبيد الفطري استون ٥٠ % هى الأكثر فعالية فى هذا المجال. علاوة على ذلك، أظهر رش النباتات بالشيتوزان وفوسفات ثنائى البوتاسيوم وسيليكات البوتاسيوم حدوث زيادة معنوية فى انزيمات المقاومة مثل انزيمات بيروكسيدز وفينيل الانين امونيا لايز بالمقارنة مع نباتات الريحان غير المعاملة.