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EFFECT OF PLANT EXTRACTS, ESSENTIAL OILS, GROWTH REGULATORS AND NPK FERTILIZATION ON *PSEUDOPERONOSPORA CUBENSIS* THE CAUSAL ORGANISM OF SQUASH DOWNY MILDEW DISEASE

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ABSTRACT: Downy mildew of squash caused by *Pseudoperonospora cubensis* is considered one of the serious diseases that affect cucurbits crops in Egypt and all the world. This study was conducted to find alternative safe methods for controlling downy mildew disease. The use of plant extracts, essential oils, growth regulators and foliar fertilization with nitrogen, potassium and phosphorus showed that these treatments reduce disease incidence and lead to a significant increase in yield compared to untreated plants. Growth characteristics of squash plants i.e., plant height, leaf area, root length, shoot length, plant fresh weight and plant dry weight were significantly increased in response to the application of any tested control methods compared to the untreated control plants. Total water content (TWC), relative water content (RWC) and transpiration rate of squash plants were remarkably increased in response to the application of either Gibberellin, black cumin oil, clove oil (10%), mint oil (5%), Thuja extract and/or the fungicide in comparison with the untreated control. Application of the disease control methods to squash plants increased chlorophyll, total sugars content and proline concentration compared to the untreated control plants.

Keywords: Squash downy mildew, plant water extracts, plant essential oils, growth regulators, N.P.K fertilization.

INTRODUCTION

Pseudoperonospora cubensis (Berkeley) the causal organism of squash downy mildew, is responsible for devastating losses worldwide of cucumber, cantaloupe, pumpkin, watermelon and squash. This pathogen has a wide geographical distribution and has been reported in over 70 countries, including environments ranging from semi-arid to tropical. Although downy mildew has been a major issue in Europe since the mid-1980s. *Pseudoperonospora cubensis* spores move farthest and fastest during cloudy, windy weather. Spores can be blown over 600 miles in 48 hours; squash downy mildew also can be moved on diseased transplants.

Downy mildew on squash has been successfully controlled for many years through host resistance. However, since the 2004 growing season, host resistance has been effective no longer and, as a result, Chemical control is not always feasible because of the high costs associated with fungicides and their application.

The antimicrobial chemicals such as fungicides are often used to control crop diseases. Two main concerns about chemical residues in the environment and the development of resistance to pathogens (Spotts and Cervantes 1986; Osuinde *et al*., 2001). According to this concept, all possible traces of plant pests and disease control methods should be integrated to minimize the moveruse of synthetic pesticides. Within decades, scientists began looking for non-chemical approaches to disease control (Wilson *et al*., 1987). The control of downy mildew on cucurbits now requires a new alternative biological method such as using of plant extract and essential oils.

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There are many articles indicate that some plant species have shown pharmacological and biological activities such as antimicrobial activity and fungicidal properties depends on various plant products including aquas extract, oils, alkaloids, resins, saponins, organic acids and resins (Cowan, 1999). The importance of plant extracts and essential oils in Crop Protection is increasingly recognized as part of the concept of Integrated Pest and Disease Management (IPDM).

This study aimed to find out the available environmentally friendly control methods to control downy mildew disease and have a positive physiological and productive effect. In this work, certain plant water extracts, plant essential oils, growth regulators, N.P.K fertilization were tested individually and integrated to achieve the best way for controlling squash downy mildew disease.

MATERIAL AND METHODS

Experimental design

Under field and natural infection conditions, these experiments were conducted in a private Farm at Teta, Menouf, Menoufia governorate, Egypt, in two successive growing seasons (2018 and 2019) and (2019 and 2020). The plants were subjected to different individual treatments to control squash downy mildew disease. A complete randomized block design system with five replicates per each treatment was followed. Each replicate had 15 squash well grown plants 90 cm space. flood irrigation system and the recommended fertilization were applied. The treatments were applied six times, the first treatment was applied at the first of October and repeated each 15 days up to the end of December. Each application required 20 liter of the compound per replicate (15 plants), however control treatments were sprayed with sterilized distilled water. A total of 17 treatments were carried out for squash plants.

Plant water extracts

Two hundred grams of Thuja, clove and black cumine seeds were separately boiled in enough amount of distilled water for half an hour. The obtained extracts were separately heated at 90°C for 2 hours, then filtered through filter paper, completed to be 1 Liter. It was placed in small containers inside the refrigerator until use (Awad, 2016). The tested plants used for extraction were prepared into three concentrations of 5, 10 and 15% were prepared.

Plant essential oils

Essential oils of clove, mint and black cumine were obtained from El-Gomhoria Company for oils and pharmaceutical Industries, Cairo, Egypt. The oils were emulsified with 3% Tween 20. Oil emulsions were separately sprayed six times (every 15 days) at the concentrations of 5, 10 and 15 ml/L (20 liters / replicate).

Growth regulators

Kelpak (auxin 7%), krigibb (gibberellin 10%) and caytochem (cytokinin 4%) were tested for their efficiency to control squash downy mildew disease. The used concentrations of Gibberellin were (5, 10 and 15 ml/L), auxin (5, 7.5 and 10 ml/L) and cytokinin (2, 4 and 6 ml/L).

Effect of NPK fertilization on the incidence of downy mildew disease of cucurbits (squash and cucumber)

Effect of different rates of fertilizers on the downy mildew severity and yield production of squash under natural infection conditions in the field during 2018-2019 season was studied.

Fertilizers used were as follows:

NPK (15-5-5), NPK (10-10-40) and NPK (10-52-10) at three levels, i.e., 2.5, 5.0 and 7.5 g/L were individually sprayed.

Squash cultivars were subjected for control method experiment five plants of squash cv. assembled the replicates of each treatment in a complete randomized block design system and LSD at 5% was estimated.

Data recorded

The results of percentage of infection (P.I) was recorded six times after 15 days of the treatment application. Plants showed any downy mildew symptoms considered diseased. Severity of infection (D.S) was determined six times using the scale of 0-9 according to the area of the leaves covered with the disease symptoms where: (0: healthy plants and 9: up to 100% infection). Percentage of infection was calculated according to the formula of Soliman *et al.* (1988):

$$\text{Severity of infection} = \frac{(a \times b))}{(N \times K)} \times 100$$

Where: a: Number of the diseased plants.
b: Infection rate (0-9).
N: Total number of the plants / plot.
K: Total infection rates.

Physiological aspects

The following plant characteristics were studied: Vegetative growth characters: Root length (cm), Plant height (cm), Dry weight of roots, shoots, and total dry weight / plant (g). Plant materials were dried in an electric oven at 70°C for 72 hours then used for chemical analysis.

Water relations

Total water content determination (TWC): The total water content (TWC, %) in the fresh leaves (4^{th} upper leaf) were determined according to the methods described by Gosev (1960) and Kreeb (1990). Relative water content determination (RWC): was determined according to the method of Barrs and Weatherley (1962). Transpiration rate: was determined according to the plant weight method described by Kreeb (1990).

Photosynthetic pigments

The photosynthetic pigments were extracted from fresh leaf samples (fourth upper leaf) by 85% acetone according to the method described by Fadeels (1962) which cited in A.O.A.C. (2005). The absorbance was determined using spectrophotometer model 390 at the wave lengths of 662, 644 and 440 nm.

Chemical analysis in physiology

Determination of total sugars: Total sugars were determined spectro-colorimetricaly (at the wavelength of 490 nm) according to the method of Doubois *et al.* (1956) cited in A.O.A.C. (2005). All estimations were expressed as (mg/g D.Wt.).

Proline concentration: Proline concentration was measured in fresh leaves after 60 days from sowing by using 5-sulphosalysilic acid solvent and acid ninhydrin reagent. The red color intensity was determined at 520 nm using colorimeter according to the method of Bates *et al.* (1973).

Chemical analysis

LC-MS/MS instrumentation and analytical conditions: Analyses were carried out using LCMS/MS 8050 system with a triple quadrupole mass spectrometer. The sample solutions were injected into a reversed phase column (BEHC 8, 1.7 mm, 2.1 mm × 150 mm, waters, Milford, MA, USA) with appropriate pre-columns. The column was maintained at 40°C. The mobile phase consisted of the mixture of aqueous solutions of 10 mM formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.25 ml/minutes. The linear gradient and isocratic flowers of the mobile phase were slightly modified according to the method of (Daniel and George 1972).

Sample preparation for LC/MS-MS analysis: The analysis of phenolic compounds by LC-MS/MS method was done with extracts prepared from plant material according to slightly modified methods. Plant material (100 mg) was homogenized and sonicated for 15 minutes with 800 ml of 80% methanol. After centrifugation for 10 minutes at 17,000 g and collection of the supernatant, the extraction was repeated with the new amount of 80% methanol.

The supernatants were collected and evaporated to the dryness, while the residue was kept for isolation of non- the volume of.

Results

Effect of plant extract on downy mildew disease incidence and squash yield production of Azyaad cultivar (2018-2019) and (2019-2020) seasons.

Data in Table (1) illustrate that, the most effective used plant extract was thuja at all tested concentrations, and it was the best for reducing the disease severity (6.2, 5.3 and 4.3%), while (P.I.) recorded 12.40, 10.60 and 8.60% respectively at 5, 10 and 15% concentrations. Clove plant extract came in the second rank which scored 9.40, 8.40 and 7.30% (D.S.) and 18.80, 16.80 and 14.60% (P.I.) during 2018-2019 season.

Also, Table (1) indicate that the most effective used plant extract was thuja at all tested concentrations. Where the best for reducing the disease severity which recorded 5.50, 5.20 and 4.70% (D.S.) and infection percentages were 11.00, 10.40 and 9.40% (P.I.) in the same respect, clove plant extracts came in the second rank which scored 9.40, 8.30 and 7.50% (D.S.) and were 18.80, 16.60 and 15.00% (P.I.), respectively. The least effective plant extract was black cumine in all applied concentration, where it recorded 18.90, 17.90 and 16.90% (D.S.) and 37.80, 35.80 and 33.80% (P.I.), respectively during 2019-2020.

The least effective plant extract was black cumine in all applied concentrations, where it recorded 16.00, 13.20 and 11.20% (D.S.) and 31.40, 26.60 and 22.00% (P.I.), respectively.

The first season plant water extracted decreased disease parameters and increased fruit yield compared to control treatment significantly where they resulted 26.00% (D.S.) and 52.00% (P.I.). Data in Table (6) illustrate the results of spraying squash plants with plant water extracts during 2018-2019 season. Thuja extract was more efficient than those of clove and black cumine ones. The same treated of results that obtained during 2018-2019 season was confirmed the obtained during 2019-2020 season.

Table (1). Effect of some plant extracts and on downy mildew disease incidence and squash yield production of Azyaad cultivar grown during (2018-2019) and (2019-2020) seasons.

Treatment	Conc. (ml/L)	Season (2018 – 2019)			Season (2019 – 2020)		
		P.I. (%)	D.S. (%)	Average yield (kg/plant)	P.I. (%)	D.S. (%)	Average yield (kg/plant)
Thuja extracts	5	12.40	6.20	37.70	11.00	5.50	33.02
	10	10.60	5.30	38.30	10.40	5.20	34.28
	15	8.60	4.30	37.18	9.40	4.70	35.34
Clove extracts	5	18.80	9.40	33.80	18.80	9.40	30.48
	10	16.80	8.40	33.50	16.60	8.30	32.90
	15	14.60	7.30	33.98	15.00	7.50	32.76
Black cumine extracts	5	31.40	16.00	25.40	37.80	18.90	26.30
	10	26.60	13.20	25.78	35.80	17.90	27.64
	15	22.00	11.20	26.00	33.80	16.90	29.00
Control (-)	–	52.00	26.00	16.74	71.40	35.70	16.22
Control blank	–	33.40	16.60	19.66	47.40	23.70	18.48
L.S.D at 0.05	–	2.749	1.390	0.635	1.101	0.550	1.439

P.I.: Percentage of infection. D.S.: Disease severity (%).

The second season plant water extracts decreased disease parameters and increased fruit yield compared to control treatment significantly where they resulted 35.70% (D.S.) and 71.40% (P.I.). Data in Table (14) illustrate the results of spraying squash plants with plant water extracts during 2019-2020 season. Thuja extract was more efficient than those of clove and black cumine ones. The same treated of results that obtained during 20190-2020 season was confirmed the obtained during 2018-2019 season.

In general, increasing the concentration of any tested plant extract showed significant efficiency in reducing the disease and increasing the yield.

Effect of plant essential oils on downy mildew disease incidence and squash yield production of Azyaad cultivar (2018-2019 and 2019-2020 seasons)

Data in Table (2) indicate that spraying squash plants with the tested plant essential oils affected both disease severity and percentage of infection during 2018-2019 season.

Also, Table (2) indicate that spraying squash plants with the tested plant oils affected both disease severity and percentage of infection during 2019-2020 season. The most effective plant oil was clove oil. Where the recorded disease severity was zero, zero and zero% (D.S.) and were zero, zero and zero% (P.I), respectively followed by mint oil which scored 12.50, 11.60 and 10.30% (D.S.) and were 25.00, 23.20 and 20.60% (P.I.), respectively with the oil concentration (5, 10 and 15%).

The most effective plant oil was clove oil, where the recorded disease severity was zero% (D.S.) and were zero, zero% (P.I.), respectively, followed by mint oil which scored 9.00, 7.80 and 6.29% (D.S.) and 18.60, 15.60 and 12.18% (P.I.), respectively with the oil concentration (5, 10 and 15%). The least effective plant oil was black cumine oil at all tested concentrations, where it gave 16.30, 15.70 and 14.90% (D.S.) and 33.80, 31.40 and 29.80% (P.I.), respectively with the oil concentration.

Table (2). Effect of some plant essential oils on downy mildew disease incidence and squash yield production of Azyaad cultivar grown during 2018-2019 and 2019-2020 seasons.

Treatment	Conc. (ml/L)	Season (2018 – 2019)			Season (2019 – 2020)		
		P.I. (%)	D.S. (%)	Average yield (kg/plant)	P.I. (%)	D.S. (%)	Average yield (kg/plant)
Clove oil	5	0.0	0.0	39.30	0.0	0.0	36.58
	10	0.0	0.0	38.98	0.0	0.0	35.78
	15	0.0	0.0	38.80	0.0	0.0	36.68
Mint oil	5	18.60	9.00	26.80	25.00	12.50	24.40
	10	15.60	7.80	25.80	23.20	11.60	25.98
	15	12.18	6.29	24.40	20.60	10.30	27.44
Black cumine oil	5	33.80	16.30	22.40	35.40	17.70	20.18
	10	31.40	15.70	21.98	32.60	16.30	22.40
	15	29.80	14.90	27.40	19.60	9.80	23.92
Control (-)	–	52.0	26.0	16.74	71.40	35.70	16.22
Control blank	–	33.40	16.60	19.66	47.40	23.70	18.48
L.S.D at 0.05	–	2.701	1.868	0.858	1.084	0.542	1.163

P.I.: Percentage of infection. D.S.: Disease severity (%).

Generally, plant essential oils decreased disease parameters and increased fruit yield compared to control treatment which resulted 26.00% (D.S. and 52.00% (P.I.). Data in Table (2) illustrate the results of spraying squash plants with plant oils during 2018-2019 season. Clove oil was more efficient than those of mint and black cumine ones.

The least effective plant oil was black cumine oil at all tested concentrations, where it gave 17.70, 16.30 and 9.80% (D.S.) and 35.40, 32.60 and 19.60% (P.I.), respectively with the oil concentrations.

Generally, plant oils decreased disease parameters and increased fruit yield compared to control treatment which resulted 35.70% (D.S.) and 71.40% (P.I.).

Data in Table (2) illustrate the results of spraying squash plants with plant oils during 2019-2020 season. Clove oil was more efficient than those of mint and black cumine ones. The same treated of results that obtained during 2019-2020 season was confirmed the obtained during 2018-2019 season. Nearly similar results of the disease incidence and yield production were observed during the second season (Table 2). In general, increasing the concentration of any tested plant oils showed significant efficiency in reducing the disease and increasing the yield.

Effect of some growth regulators on downy mildew disease incidence and squash yield production of Azyaad cultivar (2018-2019 2019-2020 seasons)

Data in Table (3) indicate that spraying squash plants with all concentrations of each tested growth regulators affected disease severity of downy mildew and percentage of infection (%) during 2018-2019 season.

The most effective growth regulators were cytokinin, where it resulted 7.00, 3.50 and zero% (D.S.) and 14.00, 70.00 and zero% (P.I.), respectively.

Table (3). Effect of some growth regulators on downy mildew disease incidence and squash yield production of Azyaad cultivar grown during 2018-2019 and 2019-2020 seasons.

Treatment	Conc. (ml/L)	Season (2018 – 2019)			Season (2019 – 2020)		
		P.I. (%)	D.S. (%)	Average yield (kg/plant)	P.I. (%)	D.S. (%)	Average yield (kg/plant)
Gibberellin	5	16.20	7.40	43.50	10.2	5.10	39.00
	10	12.00	6.00	42.28	9.40	4.70	40.28
	15	7.00	3.50	44.14	7.80	3.90	41.00
Cytokinin	2	14.00	7.00	42.56	6.60	3.3	41.50
	4	7.00	3.50	43.88	0.0	0.0	42.68
	6	0.0	0.0	46.32	0.0	0.0	44.00
Auxin	5	23.00	11.40	34.48	20.60	10.30	34.14
	7.5	20.60	10.30	32.88	17.20	8.60	36.003
	10	19.40	9.70	35.52	15.00	7.50	36.88
Control (-)	–	52.00	26.00	16.74	71.40	35.70	16.22
Control blank	–	33.40	16.60	19.66	47.40	23.70	18.48
L.S.D at 0.05	–	2.415	1.404	1.354	1.032	0.516	1.239

P.I.: Percentage of infection. D.S.: Disease severity (%).

Gibberellin came in the second rank where it scored 7.40, 6.00 and 3.50% (D.S.) and 16.20, 12.00 and 7.00% (P.I.), respectively with the used concentrations. The least effective growth regulators were Auxin at all applied concentration, where it gave 11.40, 10.30 and 9.70% (D.S.) and 23.00, 20.60 and 19.40% (P.I.), respectively with the concentrations.

Generally, growth regulators decreased disease parameters and increased fruit yield compared to control treatment that resulted disease severity 26.00% and percentage of infection 52.00%.

Nearly similar results of the disease incidence and yield production were observed during the second season (Table 3). In general, increasing the concentration of any tested some growth regulators showed significant efficiency in reducing the disease and increasing the yield.

Effect of NPK fertilization on downy mildew disease incidence and squash yield production of Azyaad cultivar (2018-2019 and 2019-2020 seasons)

Data in Table (4) illustrate that, spraying squash plants with NPK fertilization greatly affected downy mildew disease incidence compared to the untreated squash plants (control).

There were clear significant differences among the levels of nitrogen. Increasing nitrogen doses increased the disease severity and percentage of infection too, while increasing potassium and phosphorus doses decreased the same mentioned disease parameters, significantly.

The most effective growth regulators were cytokinin, where it recorded were 3.30, zero and zero% (D.S.) and 6.60, zero and zero% (P.I.), respectively. Gibberellin came in the second scored 5.10, 4.70 and 3.90% (D.S.) and where were 10.20, 9.40 and 7.80% (P.I.), respectively with the used concentrations.

Table (4). Effect of NPK fertilization on downy mildew disease incidence and squash yield production of Azyaad cultivar grown during 2018-2019 and 2019-2020 seasons.

Treatment	Conc. (g/L)	Season (2018 – 2019)			Season (2019 – 2020)		
		P.I. (%)	D.S. (%)	Average yield (kg/plant)	P.I. (%)	D.S. (%)	Average yield (kg/plant)
N - P - K 15 - 5 - 5	2.5	34.00	16.90	21.86	10.2	5.10	39.00
	5.0	52.40	26.20	20.76	9.40	4.70	40.28
	7.5	62.60	31.40	18.18	7.80	3.90	41.00
N - P - K 10 -10 - 40	2.5	24.20	12.10	27.98	6.60	3.3	41.50
	5.0	16.20	8.10	31.00	0.0	0.0	42.68
	7.5	13.20	6.60	32.56	0.0	0.0	44.00
N - P - K 10 - 52 -10	2.5	32.40	15.60	24.12	20.60	10.30	34.14
	5.0	24.00	12.00	26.90	17.20	8.60	36.00
	7.5	16.40	8.20	27.72	15.00	7.50	36.88
Control (-)	–	52.00	26.00	16.74	71.40	35.70	16.22
Control blank	–	33.40	16.60	19.66	47.40	23.70	18.48
L.S.D at 0.05	–	3.208	1.544	1.090	1.032	0.516	1.239

P.I.: Percentage of infection. D.S.: Disease severity (%).

The least effective growth regulators were Auxin at all applied concentration, where it gave 10.30, 8.60 and 7.50% (D.S.) and 20.60, 17.20 and 15.00% (P.I.), respectively with the concentrations.

Generally, growth regulators decreased disease parameters and increased fruit yield compared to control treatment that resulted disease severity 35.70% and percentage of infection of 71.40%. Nearly similar results of the disease incidence and yield production were observed during the second season (Table 4).

Also, Table (4) revealed that the lowest disease severity and percentage of infection were obtained with (10-10-40 NPK) at the rate of 7.5, 5 and 2.5 g/L, respectively. On the contrary, the highest disease severity and percentage of infection were obtained when (15-5-5 NPK) at the rate of 7.5, 5 and 2.5 g/L were applied, respectively. The highest yield production was obtained when the highest level of potassium, 7.5 g/L was applied followed by 5 and 2.5 g/L treatment, respectively. Data also show that when disease severity was increased the yield losses were also increased. As well as disease severity and percentage of infection were highly in 2018-2019 than in 2019-2020 season.

In general, increasing the concentration of potassium and phosphor fertilizations showed significant efficiency in reducing the disease and increasing the yield.

Physiological studies

Growth characters of squash during 2018/2019 and 2019/2020 seasons.

Data in Table (5) in growing season 2018/2019 show that, there was remarkable increase in root length, plant height, leaf area, dry and fresh weight of root and shoot system in response to the application of clove oil, black cumine oil, mint oil, thuja extract, clove extract, black cumine extract, cytokinin and gibberellin treatments. In the meantime, the higher doses of potassium, phosphorus, nitrogen and cabriodio fungicide also gave good efficiency as compared to the untreated control plants. Meanwhile, there was decrease in the aforementioned characters at treatments of thuja extract 5%, thuja extract 10%, clove extract 10%, clove extract 15%, thuja extract 10%, clove extract 10%, clove extract 15%, black cumine 5%, mint oils 5%, clove oils 10%, black cumine 5%, black cumine oil 15%, gibberellin, high potassium and high phosphorus the highest value was recorded at the treatment of black cumine extracts 15%, mint oil 15%, black cumine oil 10%, cytokinin, high nitrogen and cabriodio fungicide, which reached about, black cumine extract 15% (8.2, 20.5, 6.4, 210.0 and 133.0%), mint oil 15% (9.0, 18.4, 75.3, 67.7 and 106.7%), black cumine oil 10% (11.48, 2.31, 40.0, 92.3 and 4.28%), cytokinin (4.9, 22.49%, 4.34, 73.85 and 156.0%) and high nitrogen (13.12, 16.3, 15.8, 123.0 and 220.0%), significantly.

Also, in 2019/2020 data in Table (5) reported that there was remarkable increase in root length, plant height, leaf are, dry and fresh weight of root, shoot at the treatment of gibberellin, black cumin 10%, clove extract 15%, thuja extract 15%, cytokinin, mint oil 10%, thuja extract 10%, clove extract 5%, high potassium and mint oil 5% when compared with the untreated plants, meanwhile, there was decrease in the aforementioned characters at treatments of black cumin 15%, high phosphorus and clove oil 10%. The highest value was recorded at the treatment of gibberellin, black cumin 10%, clove extract 15%, thuja extract 15% and cytokinin, which reached about gibberellin (3.73, 0.236, 2.088, 2.88, 1.85), black cumin 10% (2.059, 0.157, 1.552, 1.212, 2.020), clove extract 15% (1.926, 0.288, 0.919, 02.20, 20.077) and cytokinin (1.198, 0.026, 0.283, 1.812, 1.946), significantly. The results at seasons one and two were same.

Table (5). Growth characters of squash during two successive 2018/2019 and 2019/2020 seasons.

Treatment	Conc. (%)	2018/2019					2019/2020				
		Root length (cm)	Plant height (cm)	Leaf area (cm^2)	Dry weight (g)		Root length (cm)	Plant height (cm)	Leaf area (cm^2)	Dry weight (g)	
					Root	Shoot				Root	Shoot
Thuja extract	5	18.62	14.48	39.95	0.50	5.29	57.93	26.38	189.83	1.27	21.02
	10	24.31	11.38	51.21	0.57	12.23	68.28	26.90	55.86	1.51	16.17
	15	33.62	13.97	126.85	0.60	15.46	77.59	27.93	193.45	1.33	24.31
Clove extract	5	31.03	15.52	89.74	0.73	7.74	75.52	20.69	191.38	1.50	15.60
	10	28.45	13.97	63.88	0.71	4.29	66.21	21.72	165.52	1.47	10.32
	15	27.41	12.16	71.25	0.79	16.66	102.93	25.34	177.93	2.56	40.59
Black cumine	5	23.79	10.86	43.06	0.82	7.78	56.38	17.07	114.83	1.74	19.03
	10	32.59	14.48	81.47	0.64	7.82	107.59	22.76	224.48	1.77	20.15
	15	34.14	15.26	94.66	2.02	14.72	34.14	15.26	94.66	2.02	14.72
Mint oil	5	26.38	11.12	49.91	0.73	3.96	60.00	18.10	271.03	1.53	18.77
	10	33.62	11.90	99.31	0.89	5.05	68.79	20.69	206.90	1.75	10.34
	15	34.40	15.00	155.95	1.09	13.02	67.25	22.24	170.17	1.97	14.66
Clove oil	5	32.07	12.41	95.82	1.34	6.59	72.93	18.10	229.66	2.75	15.09
	10	22.76	11.90	55.60	1.07	3.87	54.83	17.07	315.00	2.23	19.05
	15	31.55	15.78	105.00	1.07	16.58	63.10	15.52	92.07	1.77	14.77
Black cumine	5	22.76	15.78	59.22	0.90	4.00	57.93	19.14	235.86	1.80	13.61
	10	35.17	12.93	116.90	1.25	6.57	65.69	18.10	372.41	2.17	19.77
	15	25.60	10.34	48.23	1.09	7.81	58.45	24.83	151.03	2.52	23.46
Gibberellin	15 ml/L	32.07	13.45	60.26	0.74	4.75	166.55	24.31	271.55	3.11	19.05
Cytokinin	4 ml/L	33.10	15.52	92.84	1.13	16.14	77.33	19.14	112.86	2.25	19.65
Auxin	10 ml/L	32.07	12.41	160.34	1.34	14.85	59.48	16.81	178.97	2.29	16.83
High potassium	7.5 gm/L	25.34	13.45	94.91	1.33	14.16	66.72	22.50	118.97	3.05	25.56
High phosphorus	7.5 gm/L	28.71	12.93	55.86	1.64	22.17	36.47	15.26	83.79	2.04	31.92
High nitrogen	2.5 gm/L	35.69	14.74	103.06	1.45	20.17	51.72	19.40	140.17	2.05	27.32
Control	–	31.55	12.67	88.97	0.65	6.30	35.17	19.66	87.93	0.80	6.67
L.S.D at 0.05	–	0.248	0.141	1.853	0.057	0.285	0.229	0.154	1.564	0.049	0.197

Characteristics of water relations on squash

The data in Table (6) clear that, there was remarkable increase in total water content (T.W.C.), relative water content determination (R.W.C.) and transpiration rate at the treatments of gibberellin, black cumine oil, clove oil 10%, mint oil 5%, black cumin extract 10%, clove extract 15%, clove oil 5%, black cumin oil 5%, thuja extract 15%, thuja extract 10%, mint oil 10% and clove extract 5% when compared to the untreated plants, meanwhile, the treatments of thuja extract 10%, black cumin extract 5%, black cumin extract 15%, clove oil 15% and high phosphorus caused decrease in T.W.C, R.W.C and trans rate. The highest value was recorded at the treatments of gibberellin, black cumine oil 10%, clove oil 10%, black cumine 10%, mint oil 5%, clove extract 15%, which reached about, gibberellin (0.0828, 0.164 and 0.16), black cumine oil 105 (0.076, 0.160 and 0.12), clove oil 10% (0.075, 0.157 and 0.12), black cumine extract 10% (0.074, 0.132 and 0.12), clove extract 15% (0.069, 0.118 and 0.12) and clove oil 5% (0.069, 0.117 and 0.12), significantly. The results at seasons one and two were same.

Table (6). Water relations of squash during 2018/2019 – 2019/2020 seasons.

Treatment	Conc. (%)	2018/2019 season			2019/2020 season		
		TWC (%)	RWC (%)	Trans rate (mg/g fw.h)	TWC (%)	RWC (%)	Trans rate (mg/g fw.h)
Thuja extract	5	73.30	65.99	0.023	73.80	66.34	0.022
	10	71.56	63.05	0.025	72.137	63.55	0.025
	15	73.73	66.67	0.022	4.33	67.21	0.022
Clove extract	5	73.47	66.15	0.022	74.20	66.81	0.022
	10	72.51	65.21	0.023	73.22	65.85	0.023
	15	76.23	67.59	0.022	77.02	68.29	0.022
Black cumine	5	71.93	63.66	0.024	72.75	64.39	0.024
	10	76.61	68.44	0.022	77.25	69.01	0.022
	15	71.50	62.88	0.025	73.52	64.66	0.026
Mint oil	5	76.46	68.08	0.022	77.19	68.73	0.022
	10	73.51	66.22	0.022	74.40	67.02	0.022
	15	72.90	65.85	0.023	73.99	66.83	0.023
Clove oil	5	76.23	67.54	0.022	77.57	68.73	0.022
	10	76.65	69.95	0.022	77.72	70.93	0.022
	15	71.84	63.63	0.024	72.91	64.58	0.024
Black cumine	5	73.95	67.26	0.022	74.85	68.08	0.023
	10	76.74	70.15	0.022	77.99	71.29	0.022
	15	72.19	65.02	0.023	73.28	66.00	0.023
Gibberellin	15 ml/L	77.21	70.41	0.021	77.95	71.08	0.021
Cytokinin	4 ml/L	72.10	64.28	0.024	73.23	65.29	0.024
Auxin	10 ml/L	72.66	65.66	0.023	74.00	66.87	0.023
High potassium	7.5 gm/L	72.16	64.79	0.024	73.49	65.98	0.023
High phosphorus	7.5 gm/L	71.77	63.35	0.025	73.41	64.80	0.026
High nitrogen	2.5 gm/L	72.17	64.83	0.023	73.62	66.13	0.023
Control	–	71.30	60.44	0.025	71.95	60.95	0.026
L.S.D at 0.05	–	1.058	0.824	N.S	1.102	0.698	N.S

N.S: Non significant.

Determination of total sugars and proline concentration

Data in Table (7) indicate that there was increase in chlorophyll, total sugars (mg/g d.wt) and proline concentration (mg lucine/gmd.wt) at treatments of gibberellin, black cumine oil 10%, black cumine extract 10%, clove oil 10%, mint oil 5%, clove oil 5% and black cumine oil 5%, when compared to the untreated control plants. However, the treatments of black cumine extract 15%, thuja extract 10% and high phosphorus caused decrease in chlorophyll and total sugars and increased proline concentration.

Table (7). The chemical content of squash during 2019/2020 season.

Treatment	Conc. (%)	Chlorophyll (mg/g d.wt)			Total sugars (mg/g d.wt)	Proline conc. mg lucine/gmd.wt
		A	B	Carotenoids	Root	Shoot
Thuja extract	5	3.60	1.99	2.87	14.94	215.96
	10	2.81	1.56	1.71	8.89	325.09
	15	3.96	2.15	3.16	17.16	171.31
Clove extract	5	3.69	2.01	3.00	15.05	201.69
	10	3.49	1.89	2.58	11.76	238.85
	15	4.03	2.31	3.24	17.89	135.15
Black cumine	5	3.14	1.68	2.28	10.18	290.43
	10	4.33	2.41	3.39	18.07	111.24
	15	2.77	1.49	1.56	8.61	327.31
Mint oil	5	4.16	2.36	3.32	18.25	112.54
	10	3.76	2.04	3.10	16.13	198.55
	15	3.56	1.95	2.70	14.74	233.19
Clove oil	5	4.12	2.31	2.31	18.26	114.14
	10	4.51	2.46	3.54	19.11	111.18
	15	3.09	1.61	2.05	9.79	301.67
Black cumine	5	4.00	2.17	3.18	17.68	162.58
	10	4.61	2.53	3.71	19.16	109.68
	15	3.49	1.89	2.53	11.46	268.43
Gibberellin	15 ml/L	4.76	2.66	4.04	19.20	107.98
Cytokinin	4 ml/L	3.16	1.75	2.30	10.32	276.90
Auxin	10 ml/L	3.54	1.90	2.66	14.20	233.19
High potassium	7.5 gm/L	3.35	1.77	2.34	10.64	274.71
High phosphorus	7.5 gm/L	2.91	1.59	1.73	9.15	312.82
High nitrogen	2.5 gm/L	3.36	1.84	2.46	11.43	274.28
Control	–	2.49	1.37	1.54	8.30	344.63
L.S.D at 0.05	–	0.034	0.039	0.082	0.406	1.087

The highest values were recorded at the treatments of gibberellin [(0.911, 0.941, 1.62), (1.31) and (-0.686)], black cumine oil 10% [(0.851, 0.846, 1.54), (1.30) and (-0.681)], clove oil 10% [(0.811, 0.795, 1.29), (1.302) and (-0.677)], black cumine extract 10% [(0.738, 0.759, 1.201), (1.177) and (-0.677)], mint oil 5% [(0.670, 0.38, 1.155), (1.198) and (-0.673)], clove oil 5% [(0.654, 0.686, 1.149), (1.2) and (-0.668)] and clove extract 15% [(0.618, 0.686, 1.103), (1.155) and (-0.607)], significantly.

Chemical analysis of plant essential oils

Table (8) showed that *Nigella sativa* oil mainly contained of gallic acid (29.59 mg/ml) followed by cinnamic acid (4.98 mg/ml). Peppermint oil mainly contained from gallic acid (18.6 mg/ml) and caffeic acid (4.8 mg/ml). Clove oil has high amount of eugenol (72.72 mg/ml), eugenol acetate (2.1 mg/ml). It also has guaiacol (4.16 mg/ml) and methyl guaiacol (9.79 mg/ml).

Table (8). Chemical analysis of essential *N. sativa*, Peppermint oil and Clove oil

Essential oil	Phenolic compounds	Content
Black cumine oil	Gallic acid	29.59
	trans-Cinnamic acid	4.98
Peppermint oil	Gallic acid	18.60
	Caffeic acid	4.80
Clove oil	Eugenol	72.72
	Methyl guaiacol	9.79

Chemical analysis of plant water extracts

Table (9) showed that Thuja plant extract contained tannic acid (38.16 mg/100 gm sample) and catechin (33.54 mg/100 gm). However, phenolic compounds included in clove plant extract are given eugenol (79.72 mg/100 gm sample) followed by limonin (3.52 mg/100 gm sample). Nigella sativa plant extract as a high amount of vanillic acid (68.94 mg/100 gm sample) and gallic acid (29.59 mg/100 gm sample).

Discussion

Different control methods were applied, but to avoid the harmful of chemical fungicides, some other safe methods could be useful for the disease management. In this investigation, it was found that spraying squash plants with plant water extracts reduce the percentage and severity of infection, significantly, in comparison with the untreated control plants. Such results were also obtained by Abdel-Megid (2001), Afifi and Sahar (2009), Kabli (2009), Jargees (2010), Derbalah *et al*., (2012), Adriano-Anaya *et al*., (2018) and Islam *et al*., (2019). The application of plant essential oils greatly reduced downy mildew disease of squash and significantly increased yield production; compared to control. Clove essential oil gave the best results and was followed by mint essential oil. These results were also observed by Shukla *et al*., (2000), Torre *et al*., (2014), Mohamed *et al*., (2016), Deweer *et al*., (2017), Fialho *et al*., (2017), Ozer and Ktircioglu (2020) and Al-Hashmi *et al*., (2020).

However, chemical analysis of the used plant extracts and/or essential oils in this work; cleared that thuja plant extract mainly contained tannic acid and catechin. Clove plant extract has a high amount of eugenol (79.72 mg/100 g sample). In the meantime, *N. sativa* essential oil contained vanillic acid (89.94 mg/ml) and gallic acid (29.59 mg/ml). Peppermint oil contained gallic acid (18.6 mg/ml) and caffeic acid (4.8 mg/ml). Such compound may act as fungitoxic or fungi stasis. They also may induce systemic resistance into the host plant.

The obtained results showed that application of the growth regulators reduced the disease incidence and improved yield production, significantly. The best results were obtained when cytokinin and/or gibberellin were individually applied. Growth regulators improved growth parameters and may increase photosynthetic pigments, water content and transpiration rate as obtained in this investigation and by Daunde *et al*., (2000), El-Desouky (2006), Suzuki *et al*., (2006), Prokopova *et al*., (2010), Gokdere and Ates (2014), Eshragh *et al*., (2014), Morrison *et al*., (2015) and Silva *et al*., (2019).

Table (9). Chemical analysis of Plant extract of Thuja, Clove, and *Nigella sativa*

Plant Extracts	Phenolic compounds	Content
Thuja plant extract	Tannic acid	38.16
	Catechin	33.54
clove plant extract	Eugenol	79.72
	Limonin	3.52
Black cumine plant extract	Vanillic acid	68.94
	Gallic acid	29.59

Foliar application of NPK fertilizers greatly affected the disease incidence and improved yield production of squash plants. No doubt, that such treatments improve plant growth characteristics which consequently affect the pathogen spread and increase yield production. However, increasing nitrogen fertilization doses led to more disease incidence and this could be due to the thin cell walls of the host, as reported by Smith (1980). In the meantime, increasing potassium fertilization levels showed best results of decreasing the disease incidence which could be attriputed to the thick cell wall as reported also by Barka *et al.*, (1989), Khafagi (1989) and Farahat *et al.*, (1990).

The plant growth characteristics, photosynthetic pigments and water relations were positively responded by the variable tested. Control methods as present in this investigation and observed by El-Sayed and Hafez (2013), El-Beshehy (2017) and El-Sayed *et al.*, (2021).

From the obtained results in this research, especially in Table (5), which presents the results of vegetative growth traits. During the two seasons of this study, an increase in the vegetative growth characteristics of squash plants was shown as a result of the treatments used in this research. This increase is explained by reference to Table (6), which presents the internal level of the plant from the water relations (total and relative water content) due to the treatments under study. Also, by referring to the chemical content (Table 7) (total soluble sugars, proline, and photosynthetic plant pigments), it is clear from the present results that there was a significant positive effect of the parameters on the chemical content and photosynthetic pigments of the treated plants under study. Each of the aforementioned reasons resulted in an improvement in the treated plants under study compared to the control plants. Plant oils and extracts are plant nutrient materials that contain many nutrients, organic and mineral elements, which are easy to enter into the plant, as well as enter into the processes of metabolism, and this results in increasing the efficiency of the metabolism of treated plants. With increasing the natural extract rate Oligo-X (an algae extract containing immunity and internal resistant stimulants, as well as N, P, K, Fe, Zn, Mn and some growth regulators), a similar increasing in plant pigments contents, the percentage of total carbohydrates, but the percentage of proline content was reduced with raising natural extracts (El-Sayed *et al.*, 2018).

Cytokinin and auxin are growth regulators for all metabolism processes within the plant cell, which in turn positively affect plant growth. Auxin plays a key role in shaping plant architecture, and it mediates responses to a broad range of external signals (Van Den Bussche and Van Der Straeten, 2004). However, the literature contains numerous examples of other hormones involved in the response to specific stress conditions.

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تأثير استخدام المستخلصات النباتية والزيوت الأساسية ومنظمات النمو والتخصيب بالنيتروجين والبوتاسيوم على فطر *Pseudoperonospora cubensis* المسبب لمرض البياض الزغبي في القرعيات

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[٣] تخصص الكيمياء الحيوية - قسم الكيمياء الحيوية – كلية الزراعة – جامعة المنوفية

الملخص العربى

يعتبر مرض البياض الزغبي في القرعيات والمتسبب عن فطر *Pseudoperonospora cubensis* من الأمراض الخطيرة التي تصيب محاصيل القرعيات في مصر وجميع أنحاء العالم. أجريت هذه الدراسة لإيجاد طرق بديلة آمنة للسيطرة على مرض البياض الزغبي. حيث أظهرت النتائج أن المعاملة بالمستخلصات النباتية والزيوت الأساسية ومنظمات النمو والتسميد الورقي بالنيتروجين والبوتاسيوم والفوسفور تقلل من الإصابة بالمرض وتؤدي إلى زيادة معنوية في المحصول مقارنة بالنباتات الغير معاملة. تمت زيادة خصائص نمو النباتات مثل ارتفاع النبات، مساحة الورقة، طول الجذر، طول الساق، الوزن الرطب للنبات والوزن الجاف للنبات بشكل إيجابي نتيجة لتطبيق المعاملات مقارنة بالنباتات الغير معاملة. كما زاد المحتوى المائي الكلي (TWC) والمحتوى المائي النسبي (RWC) ومعدل النتح في نباتات الكوسة بشكل ملحوظ استجابةً لمعاملات كل من الجبريلين، وزيت الكمون الأسود، وزيت القرنفل (١٠٪)، وزيت النعناع (٥٪)، ومستخلص العفص. مع أو بدون استخدام المبيد الفطري المتخصص بالمقارنة مع النباتات الغير معاملة. أدى تطبيق طرق مكافحة المرض على نباتات الكوسة إلى زيادة الكلوروفيل ومحتوى السكريات الكلية وتركيز البرولين مقارنة بالنباتات الغير معاملة.