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Effect of some Plant Aqueous Extracts on Lettuce Growth, Chemical Constituents, Yield and Downy Mildew Disease.

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ABSTRACT

A plant bio-stimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance, disease resistance and/or crop quality traits, regardless of its nutrients content. Certain aqueous plant extracts of five plants i.e., Neem (*Azadirachta indica*), Moringa (*Moringa oleifera*), Garlic (*Allium sativum)*, Basil (*Ocimum basilicum*) and Eucalyptus (*Cinnamomum camphora)* extracts and fungicide (Dithane M-45) as well as tap water as control were evaluated as foliar spray under field conduction at Experimental Farm of Horticulture Department Faculty of Agriculture Menoufia University Shibin El-Kom, during fall seasons of 2018 and 2019 against Downy mildew disease caused by *(Bremia lactucae*), growth, yield, quality, water relation , photosynthetic pigments and some chemical analysis of lettuce plants cv. Balady (Local lettuce cultivar). The obtained results indicated that, application of plant extracts, and fungicide significantly decreased the disease severity, disease incidence and increased the growth characters and yield of lettuce plants.

Keywords: lettuce, biostimulants, growth, yield and downy mildew disease.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a popular, fresh vegetable and a typical cool-season crop worldwide with moderately high content of certain vitamins and minerals, principally vitamins A and C and calcium (Davis *et al*., 1997). There are many factors responsible for the low yield of lettuce crop production and among them fungal diseases play an important role. Powdery and Downy mildews as well as Early and Late blights are the most serious foliar diseases attacked vegetables grown in plastic houses (Abdel-Kader *et al*., 2012).

Downy mildew caused by the biotrophic oomycete *Bremia lactucae* is often considered to be one of the oldest, most frequent and most feared diseases affecting lettuce. The disease can cause considerable yield losses because lettuce at all growth stages can be attacked, the disease can spread exponentially under favorable, cool and humid climate conditions and the pathogen adapts rapidly to overcome the resistance in lettuce cultivars or to become resistant to frequently applied fungicides (Michelmore and Wong 2008).

As a result of the extensive using of fungicides for long time, pathogens acquired gradually resistance to those fungicides. Recently, attention has been paid towards exploitation of higher plant products as novel methods in plant protection. The popularity of botanical pesticides is increasing and some plant products are being used globally as green pesticides (Malkhan *et al*., 2012). Plant extracts are toxic to plant pathogens. Moreover, they are generally less phytotoxic, may be systemic, are easily biodegradable and can stimulate host metabolism (Stephanet *et al*., 2005).

Consumers are increasingly demanding natural, safe and healthy food produced sustainably. The consumption of fresh fruits and vegetablesare particularly encouraged, not only because of mineral nutrients and fibers but also due to their bioactive phytochemicals naturally rich in health benefiting properties. Plants have ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These compounds show highly antimicrobial effect and serves as plant defense mechanisms against pathogenic microorganisms (Cowan, 1999 and Das *et al*., 2010). Malkhan*et al*. (2012) showed that, neem tree and garlic plant have terpenoids and sulfoxide compound which are activity against fungi and bacteria. According to Sikandar *et al*. (2018) aqueous garlic extract inducing defense responses in pepper plants prior to *Phytopthora capsici* inoculation. In addition improved the growth by increased the plant height, number of leaves and root growth. Eucalyptus and basil plants extract have a great effect on reduced the growth of *Aspergillus candidus*, *Aspergillus flavus* and *Botrytis cinerea* (Satish *et al*. 2007 and Mermer-Doğu and Zobar, 2014).

One such strategy is the use of plant-derived biostimulants (PDBs) Posmyk and Szafrańska (2016). PDBs can assist the agricultural sector to target specific growth and reproduction issues to increase production more sustainably. The European Biostimulant Industry Councilde fined "plant biostimulants as containing substances and/or micro-organisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality".

Natural biostimulant compounds are classed on the basis of their source and content (humic substances, seaweed extracts, and amino acids containing products (Kauffman *et al*., 2007).

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As PDBs are natural products and naturally present in all higher plants (although in varying concentrations and combinations) a global trend in recent years shows an interest use in horticulture to maintain yield particularly under low input conditions in the horticultural sector (Kurepin *et al*., 2014). Garlic extract (Hayat *et al*., 2018), neem extract, Basil extract, Eucalyptus extract (Valvi *et al*., 2019) and most particularly extract obtained from moringa leaves Elzaawely *et al*. (2017) all have been evaluated comparing to fungicide application.

Therefore, the objective of this work aimed to study the effect of some plant aqueous extracts on lettuce growth, chemical constituents, yield and downy mildew disease.

MATERIALS AND METHODS

Preparation of plant extracts

Five locally available plants with defined biostimulants were used in the present study. The plant and their parts such as leaves of neem (*Azadirachta indica*), moringa (*Moringa oleifera*), basil (*Ocimum basilicum*) and eucalyptus (*Cinnamomum camphora)* and bulbs of garlic (*Allium sativum)* extracts were used individually and fungicide (Dithane M-45) was used for comparing with the previous extracts.

Aqueous plant extracts were obtained as per the method described by Bhatti (1988). A 100-gram sample of each plant was washed with distilled, sterile water. Then each sample was ground separately by using sterile pestle and mortal in 100 ml distilled sterile water. The extract of each sample thus obtained was filtered separately through a sterilized double layered muslin cloth to remove the bits of plant material. Then, this extract was again filtered through a filter paper (Whatman No.1). The filtered extract was centrifuged at 4000 rpm for 5 minutes to get homogenous aqueous solution. After centrifuging, the supernatant of each extract was collected. The extracts thus collected were passed separately through a Sintered glass filter to avoid bacterial contamination. This formed the standard plant extracts solution (100%).

Field experiment

This investigation was conducted during the 2018-2019 two growing fall seasons using c.v. Balady (Local lettuce cultivar) at the Experimental Farm of the Faculty of Agriculture Menoufia University Shibin El-Kom, Egypt. The experimental field was cleared, ploughed, harrowed and divided into plots. The soil texture at the experimental site was clay loam soil with approximately 1.7% organic matter, pH 7.5, EC 0.42 m.mhos. The 0-30 soil layers contained respectively 50.1 mg/100 g soil total nitrogen, 15.3 mg/100 g soil total phosphorous and 41.2 mg/100 g soil total potassium. Before sowing, Calcium superphosphate (15.5% P_2O_5) were applied at rate of 200 kg/fed. through soil preparation. Potassium sulphate (48% K_2O) was applied at rate of 50 kg/fed. in two equal split applications, i.e., 4 and 8 weeks from transplanting. Also, Nitrogen fertilizer in form of ammonium sulphate (20.6% N) was applied at rate of 100 kg/fed. in two equal portions at 21 and 45 days after transplanting.

Lettuce seeds were planted in the nursery on 15th and 18th of September 2018 and 2019 seasons, respectively. Then four weeks after emergence on the 20th and 25th October, respectively lettuce seedlings at the 5-6 leaf stage were planted out in a field at both sides of ridges. Spacing between plants within rows was 25 cm. Each plot contained five rows, 4 m in length and 0.70 m in width, occupying an area of 14 m^2. The experimental design was a randomized complete block and each treatment was replicated three times. Just three weeks after transplanting, biostimulants treatments as foliar application were started at the rates of 10% and repeated twice with an interval of two weeks between each of them.

All recommended cultural practices such as irrigation; eradication of weeds was adopted uniformly according to standard crop requirements. Harvesting was carried out at 88 and 90 days after transplanting in the first and the second seasons, respectively.

Data recorded

Growth Characters:

A random sample of ten lettuce plants was taken from the two outer rows of each plot, 12 weeks after transplanting to investigate the following growth parameters: shoot length (cm), leaf area (cm^2 /plant), leaves number/plant, fresh and dry weight of leaves (g) / plant, as well as diameter and length of the stem (cm).

Yield and its Components:

At harvesting time, heads from the two inner rows of each plot were used in estimating lettuce yield and some of its components as follow:

- Average head fresh weight (g/plant).
- Average head dry weight (g/plant).
- Total head yield (kg/m^2)

Water relations:

Total water content (TWC, %) (Gosev, 1960 and Kreeb, 1990), leaf water deficit (LWD, %), relative water content (RWC, %) (Barrs and Weatherley, 1962), osmotic pressure (Gosev, 1960), transpiration rate (Kreeb, 1990).

Photosynthetic pigments:

The photosynthetic pigments were extracted from fresh leaf sample by 85% acetone and determined according to the method described by Wettestein's formula in A.O.A.C. (1995).

Chemical analysis:

Total carbohydrates were determinate using the phenol sulfuric acid method as described by A.O.A.C. (1995). Antioxidant enzymes activities as peroxidase and phynoloxidase were determined according to Fehrman and Dimond (1967) and Broesh (1954). Protein concentration was measured as described by A.O.A.C. (1995).

Disease severity and Disease incidence:

Disease severity of downy mildew was assessed with the score chart of 0 to 5 scale (0) No infection, (1) 0-10, (2) 10.1-15, (3) 15.1-25, (4) 25.1-50 and (5) More than 50 percent of leaf area being covered with mildew growth as described by Jamadar and Desai (1997).

$$\textbf{Disease Severity \%} = \Sigma (a \times b) / N \times K \times 100$$

Where:

a = Number of infected leaves in each category.
b = Numerical value of each category.
N = Total number of examined leaves.
K = The highest degree of infection category

$$\textbf{Disease incidence} = (\text{Number of diseased plants} / \text{number of total plants}) \times 100$$

Statistical Analysis:

The obtained data were subjected to analysis of variance (ANOVA) using Costat software, version 6.4 (2008). Duncan' s multiple range test (DMRT) at $p < 0.05$ level was used for means separation (Gomez and Gomez 1984)

RESULTS AND DISCUSSION

Results

Foliar application of biostimulant significantly promoted the plant growth in terms of leaf length, leaf width, number of leaves/plant, plant edible weight and total fresh weight over the control (Table1). Application of moringa leaf extract produced more growth parameters per plant and it was superior to other extracts or Diathene-M45 with a significant difference. Leaf length and leaf width was maximum (36.2 cm at 2018 and 36.9 cm at 2019) (36.6 cm at 2018 and 37.0 cm at 2019), respectively in the plants applied with the foliar spray of moringa leaf extract with statistical difference, followed by the treatment applied with garlic extract (34.6 cm at 2018 and 34.7 cm at 2019) (36.0 cm at 2018 and 36.8 cm at 2019), respectively. On the other hand, lowest leaf length and leaf width (27.2 cm at 2018 and 28.4 cm at 2019) (32.1 cm at 2018 and 33.1 cm at 2019), respectively was recorded for the control plants. The same trend was recorded in terms of No. of leaves/plant, plant edible weight and total fresh weight of leaves. The most effective treatments were obtained by spraying lettuce plants with moringa followed by garlic extract and eucalyptus extract compared to the unsprayed plants.

Table 1. Effect of aqueous plant extracts on some growth parameters of lettuce plants.

Treatment	Plant edible weight (gm)		Total fresh weight (gm)		Number of leaf / plant		Leaf width (cm)		Leaf length (cm)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Neem	321.0^{d}	329.7^{b}	26b^{c}	10.0^{bc}	10.2^{c}	28^{bc}	35.0.^{d}	36.0.^{c}	32.4^{c}	33.3^{d}
Moringa	329.0^{a}	331.8^{a}	29^{a}	11.5^{a}	11.2^{a}	32^{a}	36.6.^{a}	37.0.^{a}	36.2^{a}	36.9^{a}
Garlic	325.4^{b}	330.0^{b}	27^{b}	10.6^{ab}	10.7^{b}	28^{bc}	36.0^{b}	36.8.^{b}	34.6^{b}	34.7^{c}
Basil	310.0^{e}	320.0^{d}	24d^{e}	9.9b^{c}	10.0^{cd}	26^{cd}	34.7^{e}	34.6.^{e}	30.8^{d}	32.1^{e}
Eucyalupts	323.4^{c}	327.4^{c}	25^{cd}	9.7b^{c}	10.1^{c}	24de	34.2.^{f}	34.1.^{f}	29.8^{d}	30.3^{f}
Diathene-M45	307.7^{f}	319.7^{d}	30^{a}	11.4^{a}	10.9^{b}	29.^{b}	35.6.^{c}	35.5.^{d}	35.5^{ab}	36.4^{b}
control	280.0^{g}	301.7^{e}	23^{e}	9.0^{c}	9.9^{d}	23^{e}	32.0.^{g}	3.31.^{g}	27.2^{e}	28.4^{g}

Duncan's multiple range test was used-values followed by the same letters are not significantly differed ($p \leq 0.05$).

Various biostimulants management practices had significant effect on the total head yield during both seasons among the treatments received Dithane M-45 or control one received tab water produced more total head yield as illustrated in Fig (1). Foliar application of moringa leaf extract resulted in an enhancement in total head yield kg/m^2 (7.81 at 2018 and 7.94 at 2019), respectively and was significantly superior over garlic extract(6.55 at 2018 and 6.70 at 2019) or neem extract (5.86 at 2018 and 5.95 at 2019), respectively. The lowest total head yield per m^{2}was found in the control extract (4.05 at 2018 and 4.07 at 2019), respectively.

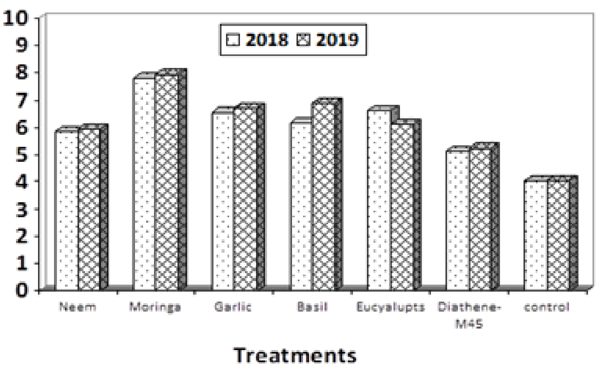

Fig.1. Effect of aqueous plant extracts on total yield of lettuce plants.

The results presented in Table (2) showed that, there was a remarkable increase in total water content, relative water content and transpiration rate in lettuce leaves which spread with the plant extracts as neem, moringa, eucalypts and fungicide. Mean while there are a reduction in the leaf water deficit in lettuce leaves at the same treatments, compared with the control plants. In addition, there was a negative effect on lettuce leaf water relations when treated with the garlic and basil plant extracts. The all treatments gave the same results in the second season.

The recorded data in Table (3) cleared that, the photosynthetic pigments, total carbohydrates and total protein recorded a higher values in lettuce leaves which treated with the extracts of neem, moringa, eucalypts and pesticide. Meanwhile, at the same treatments there was a decrease in the enzyme activates. The garlic and basil plant extracts caused a decrease in photosynthetic pigments, total carbohydrates and total protein in lettuce leaves which treated with garlic and basil plant extracts, compared with untreated plants. All results in the second season at the same of the first one.

Table 2. Effect of aqueous plant extracts on water relation of lettuce plants.

Treatments	T. Water content (%)	Leaf water def. (%)	Rel.water content (%)	Trans.rate mg/gfw.h	T. Water content (%)	Leaf water def. (%)	Rel.water content (%)	Trans.rate mg/gfw.h
	Season 2018				Season 2019			
Neem	95.88	15.09	84.91	4.85	95.72	14.27	85.73	3.82
Moringa	97.14	12.07	87.93	7.81	96.12	13.04	86.96	7.02
Garlic	94.03	17.97	82.03	3.24	93.94	15.70	84.30	2.57
Basil	94.66	17.45	82.55	3.78	94.36	14.68	85.32	2.62
Eucyalupts	95.70	15.14	84.86	4.81	95.66	14.34	85.66	3.71
Diathene-M45	96.80	12.87	87.13	4.96	96.06	13.46	86.54	3.85
Control	94.92	16.32	83.68	3.81	95.25	14.35	85.65	3.37
LSD 5%	0.359	0.263	0.382	0.001	0.213	0.141	1.177	0.003

Table 3. Effect of aqueous plant extracts on chemicals constituents of leaves of lettuce plants.

Treatments	Chl. a (mg /g dwt)	Chl. b (mg /g dwt)	Carot. (mg /g dwt)	T. carbohydrates (Mg/g d. wt)	Per-oxidase O.D./g Fwt. after 2 min.	Phenol-oxidase O.D./g Fwt after 45 min.	T. protein %
				Season 2018			
Neem	2.68	1.52	2.32	151.28	0.27	0.52	15.86
Moringa	2.73	1.64	2.48	195.61	0.26	0.39	19.21
Garlic	1.85	0.96	1.03	182.26	0.48	0.76	11.38
Basil	2.18	1.13	1.49	139.80	0.33	0.69	12.76
Eucyalupts	2.61	1.40	2.20	145.31	0.30	0.63	14.91
Diathene-M45	2.71	1.59	2.40	115.67	0.27	0.36	17.44
control	2.50	1.33	1.77	130.64	0.31	0.69	14.56
LSD 5%	2.09	1.18	1.50	2.88	0.025	0.019	0.348
				Season 2019			
Neem	2.60	1.70	2.36	128.65	0.27	0.50	16.50
Moringa	2.79	1.89	2.57	145.55	0.27	0.31	19.58
Garlic	1.71	1.01	0.95	76.26	0.39	0.71	10.84
Basil	2.08	1.23	1.03	86.53	0.33	0.69	11.31
Eucyalupts	2.48	1.50	1.74	113.21	0.29	0.60	13.48
Diathene-M45	2.63	1.72	2.39	136.42	0.29	0.36	17.58
control	2.22	1.33	1.53	104.20	0.32	0.63	13.18
LSD 5%	2.30	1.32	1.79	3.67	0.017	0.018	0.317

Data presented in Table (4) showed that application of aqueous extracts and fungicide (Dithane M-45) significantly reduced the disease severity and disease incidence in the two seasons 2018-2019 compared to the control. The fungicide was superior in reducing disease severity and disease incidence (68.45 and 60.0 at 2018) and (67.94 and 61.29 at 2019), respectively. Among all aqueous plant extracts, the garlic was the most effective on reduced the disease severity with 65.91 and 68.02 at 2018 and 2019, respectively and reduced the disease incidence with 53.3 and 54.38 at 2018 and 2019respectively. While basil extract was the lowest effective on the reduction of disease severity (37.18 and 38.63) at 2018 and 2019, respectively and reduction of disease incidence 43.3 and 38.70 at 2018 and 2019, respectively.

Table 4. Effect of aqueous plant extracts on disease severity and disease incidence on lettuce plants.

Treatment	Disease severity	Reduction (%)	Disease incidence (%)	Reduction (%)	Disease severity	Reduction (%)	Disease incidence (%)	Reduction (%)
	2018				2019			
Neem	1.67^{d}	52.95	37.5^{bcd}	50.0	1.85^{d}	49.31	45.0^{d}	41.93
Moringa	1.31^{e}	63.09	35.0^{cd}	53.3	1.34^{e}	63.28	37.5^{e}	51.61
Garlic	1.21^{f}	65.91	35.0^{bc}	53.3	1.24^{f}	66.02	35.0^{f}	54.38
Basil	2.23^{b}	37.18	42.5^{b}	43.3	2.24^{b}	38.63	47.5^{c}	38.70
Eucyalupts	2.02^{c}	43.09	45.0^{b}	40.0	2.09^{c}	42.73	50.0^{b}	35.48
Dithane M-45	1.12^{g}	68.45	30.0^{d}	60.0	1.17^{g}	67.94	30.0^{g}	61.29
Control	3.55^{a}	-	75.0^{a}	-	3.65^{a}	-	77.5^{a}	-

Duncan's multiple range test was used-values followed by the same letters are not significantly differed($p \leq 0.05$).

Discussion

The antimicrobial substance allicin, which is a volatile phytoanticipin produced in garlic plants is active against a broad range of phytopathogenic organisms in vitro and in plant (Curtis *et al*. 2004) Allicin appears to have a multi-site mode of action so, it will be difficult for pathogens to mutate a resistance against it (Slusarenko *et al*. 2008).

Moringa extract provides a rich and rare combination of zeatin, quercetin, □-sitsterol, caffeoylquinic acid and kaempferol which have antifungal and antibacterial activities (Ashfaq *et al*., 2012). The antifungal compounds contained in basil with phenolic compounds such as linalool, methyl-cavicol (estragol), camphor, and eugenol. These compounds have antifungal activity (Vieira *et al*., 2014). According to Sadre *et al.* (1983) and Hanaa, Farag *et al.* (2011) application of neem extract to tomato plants significantly reduced fusarium wilt disease. The reduction may be due to the presence of gedunin i.e. tetranortriterpenoid and azadirachtin which possess antifungal properties.

The observed data showed concurring results of biostimulans on the growth and physiology of pepper and eggplants seedlings (Hayat *et al*., 2018). Application of neem, moringa leaf extract garlic, basil and eucalyptus extract also influenced the growth and development of the treated plants with statistical difference. These findings matched previous results on the bioactivity of water Garlic Extract in the growth of subject plants (Gao, 2006 and Otunola, 2010).Based on current results, plant growth parameters such as plant edible weight, total fresh weight, number of leaf /plant, leaf width and leaf length, as well as total head yield, were significantly altered. The growth improvement in the plant can be established by the fact that garlic extracts contain various growth-promoting compounds such as starch and vitamins, and organo sulfur compounds such as allicin and diallyl disulfide, etc. (Martins, 2016).Growth-promoting effects of aqueous garlic extracts have been reported previously intomatoes (Hayat *et al*., 2018). Plant derived biostimulants application may also lead to up-regulation of photosynthesis and improved nitrogen and carbon metabolism (Yasmeen *et al*., 2014 and Yasmeen *et al*., 2013). In other cases, bioactive peptides triggering signaling pathways involving phytohormone biosynthesis may explain the positive effects of plant derived bio stimulants on plant growth and yield (Elzaawely *et al*., 2017).Improved growth has been also attributed to the presence of sugars in plant derived bio stimulants, which acts as a source of energy and stimulate nitrogen assimilation (Kumar *et al*. 2019) and Lucini*et al*. 2015).However, in current findings, there seems to be strong correlation between the morphological indices and the developmental aspects such as chlorophyll or carotenoid contents. These findings are in corroboration with previous results. Yasmeen *et al.* (2014) found that, Plant derived biostimulants application has positive effect on photosynthesis and improved nitrogen and carbon metabolism.

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تأثير المستخلص المائى لبعض النباتات على النمو والانتاج ومرض البياض الزغبى للخس

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فى تجربة حقلية اجريت بمزرعة كلية الزراعة جامعة المنوفية عامى 2018 – 2019 تم دراسة تأثير المستخلص المائى لخمس نباتات وهم النيم – الثوم – المورينجا – الكافور – الريحان بالإضافة لمبيد دياثين م-45 على مرض البياض الزغبى وبعض صفات النمو والمحصول بالإضافة لدراسة العلاقات المائية وبعض المكونات الكيميائية لنباتات الخس. وقد أوضحت النتائج أن إضافة المستخلصات المائية قد خفضت بصورة معنوية نسبة وشدة الإصابة المرضية وحدوث زيادة فى نمو وإنتاجية المحصول بالإضافة لحدوث تغيرات فى الصفات الفسيولوجية محل الدراسة.