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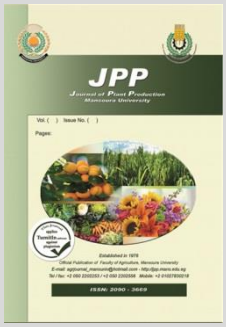
Influence of Garlic Foliar Treatment with some Plant Extracts on Purple Blotch Disease in Relation to Plant Growth, Yield and some Biochemical Responses

Osman, Y.M. M.[1*]; Maha H. Mohamed[2] and Hanaa R. M. Farag[3]

[1]Potato and Vegetatively Propagated Vegetables Res. Dept., Hort. Inst., Agric. Res. Centre, Giza, Egypt.
[2]Plant Pathology Dept., Fac. Agric., Ain Shams Univ., Cairo, Egypt.
[3]Agricultural Biochemistry Dept., Fac. Agric., Ain Shams Univ., Cairo, Egypt



ABSTRACT

The experiments were carried out during 2018/2019 and 2019/2020 seasons. Greenhouse experiments carried out at Department of Plant pathology, Faculty of Agriculture, Ain Shams University. Field experiments were carried out at Kaha Vegetable Research Farm, Kaliobia Governorate. Compared with untreated infected garlic plants Sids-40 cultivar, all foliar applications with aqueous extracts of each of henna (*Lawsonia inermis*), licorice (*Glycyrrhiza glabra*), rosemary (*Salvia rosmarinus*), mixtures of them at levels 5% and 10% for each extract or Ridomil plus (2.5g /L) showed significant decrease in purple blotch severity and resulted in significant increases in phenolic compounds, oxidative enzymes activity *i.e.*, CAT, POD and PPO during two growing seasons. However, significant decreases of proline content were resulted in the treated infected leaves during both seasons. Spraying with licorice and mixtures of extracts at5% and10% resulted the highest significant decreases of disease severity under both greenhouse and field conditions. Also, licorice at10% exhibited the highest values of plant length, fresh and dry weight of plants, values of bulb weight, total yield at harvest and after curing. Mycelial inhibition test proved that all extracts have an inhibitory effect on mycelial growth of *A. porri* and licorice extract exhibited the highest effect, even still lower than Ridomil Plus effect. It can be concluded that all aqueous extracts tested were able to reduce the disease severity as a result of either direct microbial growth inhibition or induction of garlic plant resistance against *A. porri*, thus as well was associated with improved plant growth parameters and garlic yield.

***Keywords*:** Purple blotch disease, Plant extracts, Total yield, Phenolic compounds, Antioxidant defensive enzymes, Mycelial growth inhibition.

INTRODUCTION

Garlic (*Allium sativum* L.) is the most important vegetable bulb and spice crop. It is considered as the second most widely used cultivated bulb crop after onion in Egypt. It is widely used as a spice in different vegetable dishes also has great important in the medical aspects. Purple blotch disease caused by *Alternaria porri* (Ellis) Cif. considered as one of the major foliage disease that affect the quantity and quality of garlic production (Bisht *et al*., 1993). The pathogen can infect other Allium crops like onion and shallot. Conditions that favour the development of the disease are high relative humidity about 80 to 90% and moderate temperature 25 to 30˚C (Dar *et al.*, 2020).

Chemical control considered the most practises to manage purple blotch disease but it can cause environmental pollution and development of resistant strains of pathogen against fungicides in addition to expensive cost (Dar *et al.*, 2020). So, several studies have turned to use safe alternative control strategies for disease management. Recently, trends to use extracts of many higher plants have an effective and safe role against plant fungal diseases (Draz *et al*., 2019; Ragupathi *et al.*, 2020).

Plant aqueous extracts of henna (*Lawsonia inermis*), acalypha (*Acalypha wilkesiana*), chinaberry (*Melia azedarach*), pomegranate (*Punica granatum*) and lantana (*Lantana camara*) acts as inducers of wheat resistance against leaf rust disease "*Puccinia triticina*" under field conditions and led to a significant increase in plant content of total phenolics, oxidative enzymes activities *i.e.*, peroxidise, polyphenol oxidase and yield during two growing seasons (Draz *et al*., 2019). Moreover, Ragupathi *et al*., (2020) reported that 18 aqueous plant extracts including *Lawsonia inermis* on tomato plant have an antifungal activity against *Alternaria solani*.

Aside from, several studies have indicated the effectiveness of use the plant extracts against purple blotch disease (Abdel-Hafez *et al*., 2014; Brahmane *et al*., 2015). Aqueous leaves extract of neem (*Azadirachta indica*) resulted significant reduction of disease severity of purple blotch under greenhouse conditions (Abdel-Hafez *et al.*, 2014). Also, Brahmane *et al.*, (2015) found that neem seed kernel extract gave the maximum decrease of purple blotch severity on onion followed by *Mentha arvensis*, *Allium sativum*, *Zingiber officinale* and *Vitex negundo.*

The secondary metabolites produced by several plants are considered as a source of bioactive substances against several plant diseases. Thakur *et al*., (2016) showed that the aqueous plant extract of licorice root attained the maximum free radical scavenging ability against reactive oxygen species (ROS) compared to methanolic and

* **Corresponding author.**
E-mail address: yasser.osman77@gmail.com
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ethanolic extracts. However, rosemary extract had significant anti-oxidative activity, which was mainly attributed of two phenolic diterpenes, namely, carnosol and carnosic acid (Richheimer *et al.*, 1996). Egyptian licorice roots are rich in many essential minerals, flavonoids and natural antioxidants (Morsi *et al*., 2008). In addition, Thakur *et al*., (2016) recorded the presence of glycyrrhizin, saponins, flavonoids, steroids in aqueous extract of licorice root, they found that glycyrrhizin was the major product in the aqueous extract. The chemical components of henna leaf aqueous extract were carbohydrates, proteins, flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones, and fatty acids. These chemical components were proven to have antibacterial and antifungal activities (Rao *et al.*, 2016). Aside from, several studies have shown the importance of medicinal plant extracts in improving the growth of plants. Foliar spraying with licorice extract has a favorable biological effect on vegetative growth of plant, flowering, total yield and fruits quality in several plants (Byan, 2014; Shafeek *et al*., 2015).

This study aimed to evaluate the efficacy of some plant extracts individually and in combinations as foliar application as well as Ridomil plus in controlling purple blotch disease under field conditions and study their role in enhancing growth, yield, improving bulb quality, storability, lipid peroxidation, proline content, phenolic compounds and antioxidant defensive enzymes.

MATERIALS AND METHODS

The experiments were carried out during 2018/2019 and 2019/2020 seasons. Greenhouse experiments carried out at Department of Plant pathology, Faculty of Agriculture, Ain Shams University. Field experiments were carried out at Kaha Vegetable Research Farm, Kaliobia Governorate.

1. Isolation and identification of the pathogen

The diseased garlic leaves that appeared typical symptoms of purple blotch disease were collected from Al Qalyubia Governorate fields. The infected leaf samples were cut into small pieces and surface sterilized with sodium hypochlorite solution 1% for 30 seconds. The surface sterilized pieces were washed with sterilized distilled water and dried between two filter papers, then placed on water agar medium in Petri dishes. The dishes were incubated at 25±2˚C for 48 h. For obtaining purified cultures of *A. porri*, its visible single colonies were sub-cultured on PDA and incubated as usual for six days. The obtained isolates were purified by single spore technique.

The identification of obtained isolates was applied on cultural characteristics and conidial specifications according to (Chethana *et al*., 2018). Cultural characteristics of different isolates were recorded on PDA medium after 5 days of single spore sub-culturing. Conidial characterizations were determined on slides of fungal colonies after 14 days old. Thirty conidia for each isolate were chosen randomly from different microscopic fields.

2. Pathogenicity test

Eight isolates obtained of genus *Alternaria* spp. were tested for their pathogenicity on garlic (Sids-40 cultivar) in greenhouse. Eleven days old PDA cultures of *Alternaria* isolates were used for preparation of inoculum. Conidial suspension of each isolate was adjusted up to 1 x 10^6/ml in distilled water by using haemocytometer. One clove of garlic was planted in earthen pots (20 cm in diameter) containing sterilized sandy loam soil (1:1) with 10 replicates for each isolate. Fifty days after planting, plant leaves were inoculated by spraying the inoculum suspensions on foliage, then plants were covered by polyethylene bags for 24 h. to retain high relative humidity and ensure the pathogen penetration to leaves, then bags were removed and plants were kept under greenhouse conditions. Disease severity was recorded 30 days after inoculations.

3. Plant materials and preparation of extracts

Plant extracts of roots of licorice (*Glycyrrhiza glabra*) and leaves of rosemary (*Salvia rosmarinus*) which were obtained from the local market, and leaves of henna (*Lowsonia inermis*) which were collected from Horticultural Research Institute, Agriculture Research Centre, Giza, Egypt. All plant materials were air dried and crushed in the electrical mill to prepare plant powder. Ten grams of plant powder of licorice and rosemary were soaked in 100 ml of distilled water, and then transferred to a shaker for 72 h at room temperature. Supernatant of each extract was separated from the residue by squeezing through double layers muslin cloth and then obtained aqueous extracts were filtrated through Whatman no. 1 filter paper (Satish *et al*., 2007). However, the henna extract was prepared according to the method of Kumar and Kathireswari (2016). The aqueous extract of henna was prepared by soaking 10 grams of henaa powder in 100 ml of distilled water and then boiled for 20 minutes. After cooling, extract was filtered as described above. Stock solution of each extract was kept in the fridge at - 4°C until use.

The supernatant was taken as standard plant extract solution (100%). Further, it was diluted with sterilized distilled water to get 5 and 10% concentrations of each extract. Mixtures of the three extracts were prepared by mixing each of levels of 5% or 10% of each extract.

4. Effect of aqueous plant extracts on purple blotch disease and parameters of garlic plant growth

Bulbs of garlic Sids-40 cultivar were obtained from Horticultural Research Institute, Dokki, Giza, Egypt. Cloves of garlic were soaked in tap water for 24 h. before planting for all experiments. The foliar treatments of plants extracts included; henna, licorice, rosemary, or mixtures of them at levels 5% and 10% for each extract, control plants were received tab water and Ridomil plus at 2.5 g /L were sprayed in separate treatments for comparison with plant extracts effect. Triton X-100 at the rate of 0.1% v/v was added for all sprayed solutions as surfactant. The spray solutions have completely covered the plant foliage. Foliar applications were applied four times at 45, 66, 87, 108 days after planting, during the growing season.

In greenhouse experiments, cloves of garlic were cultivated in earthen pots (20 cm in diameter, one clove/pot) containing sandy loam soil (1:1) with twelve replicates for each treatments. The isolate (IS4) of *A. porri* that appeared a highest degree of infection in pathogenicity test was used for artificial inoculation as usual. Plant extracts were applied as mentioned above.

The soil was clay loam in texture. Dates of planting were during October 3th and September 23th in 2018 and 2019 for the first and the second seasons, respectively. The

experiment included ten treatments which were arranged in a randomized complete blocks design (RCBD), with three replicates of each treatment. The experimental plot area was 10.50 m^2 which contained 3 rows, with 5 m length and 0.70 m width. Garlic cloves were planted on both sides of the rows at 10 cm apart. All agriculture practices for cultivation were performed as recommended by Ministry of Agriculture.

Disease assessment

Disease assessment was determined at 150 days after planting in both greenhouse and field experiments. The disease severity and disease incidence in greenhouse were recorded on all the plants. However, in field experiments the disease severity and disease incidence were recorded on thirty randomly selected plants per each treatment. Disease severity was determined according to Mayee and Datar, (1986) (Fig.1).

Fig. 1. The degree of purple blotch infection on garlic leaf by using modified scoring scale from 0 to 9 according to (Mayee and Datar, 1986); 0: no disease symptoms; 1: lesions covering 1% of leaf area; 2: lesions covering 2-5% of leaf area; 3: lesions covering 6-10% of leaf area; 4: lesions covering 11-15% of leaf area; 5: lesions covering 16-25% of leaf area; 6: lesions covering 26-40% of leaf area; 7: lesions covering 41-60% of leaf area; 8: lesions covering 61-75% of leaf area ; 9: >75% of leaf area covered with lesions in addition to dryness of most leaves.

2. Plant growth parameters

Random samples of three plants from each experimental plot were uprooted after 150 days from planting to determine plant length (cm.), number of leaves per-plant, neck and bulb diameter (cm.), bulbing ratio according to Mann (1952), fresh and dry weight of plant (gm.). Total yield at harvest and total yield after curing were determined for each experimental plot. The plants were placed for 15 days in an aerated area for curing. Five cured bulbs were randomly taken from each experimental plot to determine garlic bulbs quality including bulb weight (gm), neck and bulb diameters (cm.) and bulbing ratio.

3. Storability

After curing, two kilograms of bulbs without stems were randomly taken from each experimental plot in both seasons and placed in net bags and stored at room temperature at (25±3°C). Bulb weight loss was determined at 90 and 180 days of storage period as follows:

Weight loss (%) = [(initial weight of storage bulb-weight at sampling date) x100]/initial weight of storage bulb.

5. Biochemical analysis

Random leaf samples of three plants of garlic from each experimental plot were uprooted after 15 days of final treatment with all aqueous plants extracts to determine biochemical parameters *i.e.*, lipid peroxidation, proline content, phenolic compounds and antioxidant defensive enzymes activities.

1. Determination of Lipid peroxidation

The level of lipid peroxidation in leaves was determined using the method described by **Heath and Packer (1968)**. Lipid peroxidation was expressed as µM MDA g^{-1} Fresh Weight (FW).

2. Determination of proline concentration

Proline concentration was determined using a ninhydrin colorimetric according to the method of Troll and Lindsley (1955) as modified by Peters *et al*. (1997). The proline concentration was calculated from the standard curve of L-proline. Proline concentration was expressed as µg proline.g^{-1} FW.

3. Determination of total soluble phenols

Total soluble phenols in garlic leaves were performed using the method described by **Shahidi and Naczk** (**1995**). The concentration of total soluble phenols was calculated using the standard curve of gallic acid. Total soluble phenols concentration was expressed as µg of gallic acid. g^{-1} FW of the sample.

4. Enzymes assay

1. Enzyme extraction

Frozen tissues of leaf samples were ground using cold mortar and pestle and homogenized with cold sodium phosphate buffer (100 mM, pH=7) containing 1% (w/v) polyvinylpyrrolidone (PVP) and 0.1 mM EDTA. The extraction ratio was 4 ml extraction buffer for each one gram of plant tissues. The homogenate was centrifuged at 6000 rpm at 4º C for 15 min. The supernatant was used for measurement of peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO) and superoxide dismutase (SOD) activities.

1. Peroxidase activity

The activity of peroxidase (POD, EC1.11.1.7) was assayed by the method of Hammerschmidt *et al*. (1982). POD activity was expressed as unit.mg^{-1} protein.

2. Catalase (CAT) activity

Catalase (CAT, EC 1.11.1.6) activity was determined according to the method of Chance and Maehly (1955) as modified by Cakmak *et al*. (1993). CAT activity was expressed as unit.mg^{-1} protein.

3. Polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO, EC 1.14.18.1) activity was measured according to Oktay *et al*. (1995). The enzyme activity was expressed as unit.mg^{-1} protein.

4. Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD, EC 1.15.1.1) assay was based on the method described by Beyer and Fridovich (1987). The enzyme activity was expressed as unit. mg^{-1}protein.

Protein concentration was quantified according to Bradford (1976) using bovine serum albumin for the standard curve.

6. Fungal growth inhibition assay

The inhibitory effect of tested plant extracts on the mycelial growth of *Alternaria porii* was carried out on PDA medium. Plant extracts of henna, licorice, rosemary and the mixture of them were sterilized before mixing with PDA by filtration through 0.22 µm sterile filters (Millipore). Sterilized plant extracts and fungicide Ridomil plus at level

of 2.5 g /L. were added to sterilized PDA just before solidification and the mix was poured into dishes (9 cm). Control dishes were not treated with plant extracts or fungicide. Five plates were used as replicates for each treatment. After solidification of the media, agar disks 5 mm of fungal growth culture were set on the center of each dish. All dishes were incubated at 25±2˚C until control dishes were filled with fungal growth. The percentage of fungal growth inhibition in different treatments was calculated.

7. Statistical analysis

The data were analyzed by analysis of variance (ANOVA) using CoStat. Least significant differences were calculated at p=0.05 and Duncan's multiple range test was applied to compare between means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Results

1. Isolation and identification of the pathogen

Eight isolates of *Alternaria* spp. (IS1 to IS8) were isolated from garlic leaves showed typical symptoms of purple plutch. Identification of the isolates ensured that all of them have identical specifications of *A. porri*. The isolates exhibited similar culture characteristics with little differences including the colony growth, colony diameter, colony colour in both upper and reverse surface, margin of growth, sectors, zonation (Fig. 2, Table 1). Isolate "IS8" showed the highest average of colony diameter as 79.0 mm, while the lowest value was 44.3 mm of isolate "IS3". As shown in (Fig. 3, Table 2), all isolates showed remarkable variations in conidial dimensions as isolate "IS4" showed the maximum length of both conidia (48.1µm) and conidial beak (32.2µm) as compared with other isolates. Conidia of all isolates showed pyriform shape with 3-5 transverse septa and 1-2 longitudinal septa. The beak length and width were varied from 18.7 to 32.2 µm and 3.0 to 3.9 µm, respectively.

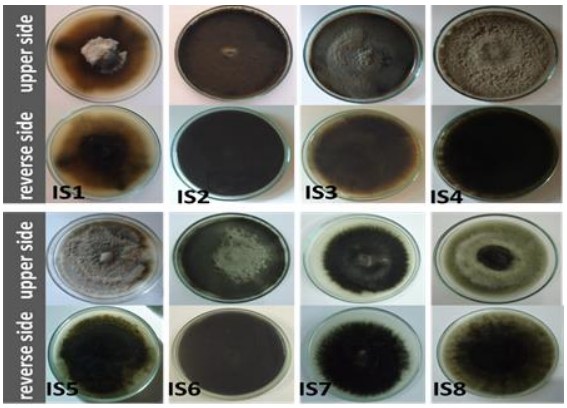

Fig. 2. Culture morphology of Alternaria isolates grown on PDA medium on the upper and reverse sides of dishes.

Table 1. Culture characteristics of Alternaria isolates grown on PDA medium.

isolates	Colony diameter (mm)	Colour on upper side	Colour on reverse side	Margin growth	Sectors	Zonation
IS1	64.3±2.1c	ashy black	brown	flat, smooth	present	absent
IS2	69.3±1.1b	ashy black	black	flat, smooth	absent	absent
IS3	44.3±4.0e	ashy black	brown	flat, thick	present	absent
IS4	57.7±2.5d	ashy	black	flat, rough, thick	absent	absent
IS5	62.3±2.1c	ashy	black	flat, thick	absent	absent
IS6	55.0±1.0d	ashy black	black	flat, thick	absent	absent
IS7	45.3±1.5e	ashy black	black	flat, smooth	absent	absent
IS8	79.0±0.0a	ashy	black	flat, thick	absent	present

- Colony diameter recorded after 5 days of subculture.

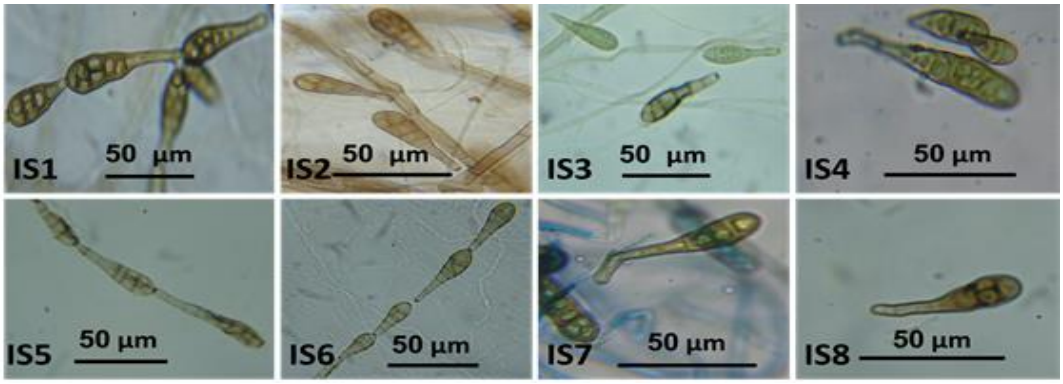

Fig. 3. Conidial morphology of Alternaria isolates in PDA medium.

Table 2. Conidial morphology of Alternaria isolates retired from infected garlic leaves.

Isolates	Conidia					Beak	
	Shape	Length (µm)	Width (µm)	No. of septa L	No. of septa T	Length (µm)	Width (µm)
IS1	pyriform	42.7±2.9b	12.8±0.5ab	2	3-5	22.9±1.6c	3.1±0.3d
IS2	pyriform	34.4±1.8d	12.9±0.4ab	1	4	21.9±0.6dc	3.7±0.1ab
IS3	pyriform	30.6±1.1e	11.9±0.4b	1	4	19.9±2.7de	3.9±0.3a
IS4	pyriform	48.1±1.6a	12.6±0.1ab	1	5	32.2±0.9a	3.5±0.1bc
IS5	pyriform	38.2±0.9c	10.6±0.9c	1	4	31.4±1.0a	3.3±0.1dc
IS6	pyriform	31.6±0.7de	9.6±0.3c	1	4	18.7±1.4e	3.2±0.1d
IS7	pyriform	32.8±1.4de	13.8±1.4a	1	3	31.1±0.6a	3.0±0.1d
IS8	pyriform	32.7±1.3de	12.8±0.8ab	1	3	25.6±0.8b	3.1±0.1d

- T: transverse, L: longitudinal.

2. Pathogenicity test

Data in Figs (4&5) illustrate that three isolates (IS4, IS5 & IS7) showed typical symptoms of purple blotch disease on inoculated garlic leaves. Isolate (IS4) showed the highest percentage of disease severity expressed as the percentage of covered leaf area with lesions.

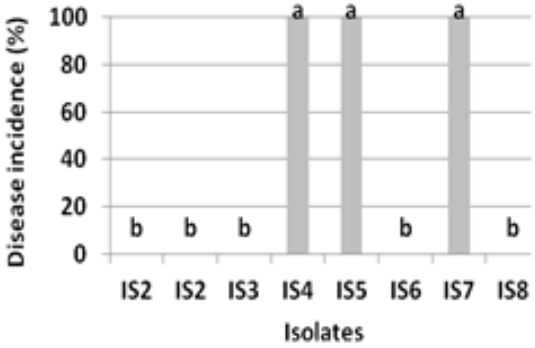

Fig. 4. Purple blotch incidence on garlic leaves by Alternaria isolates under greenhouse conditions.

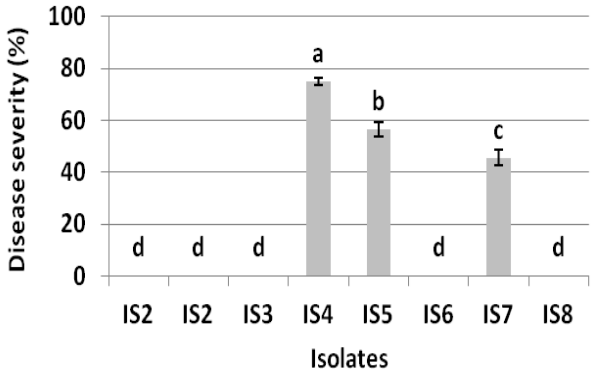

Fig. 5. Disease severity of Alternaria isolates on garlic leaves under greenhouse conditions.

3. Effect of aqueous plant extracts on purple blotch disease and parameters of garlic plant growth

The data of plant extracts or Ridomil Plus on the incidence of purple blotch under greenhouse conditions were illustrated in Figs. (6&7) and in field experiment were shown in Figs. (8, 9, 10 &11). Data in Fig. (6) clarify that Ridomil Plus, licorice extract at 10% and mix of the three extracts at 5% and 10% have significantly decreased the percentage of infected plants than control or than other treatments. Meanwhile, the mixed extracts at 10% recorded the highest reduction in disease incidence. Data in Fig. (7) showed that all treatments resulted in clear significant decrease in disease severity. Hereagain, mix (10%) and Ridomil Plus gave the highest values of reduction in disease severity, followed by mix (5%) and licorice (10%).

In addition, the results of both incidence and severity of purple blotch disease tended in similar trend in the two seasons of cultivation in the field experiment (Figs 8, 9, 10 &11). Application of mixed extracts (5% &10%) followed by licorice 10% resulted the highest decrease of purple blotch severity as compared with all other treatments during the two seasons of field tests. Interestingly, such three treatments of plant extracts have surpassed the effect of fungicide Ridomil Plus in disease discouragement.

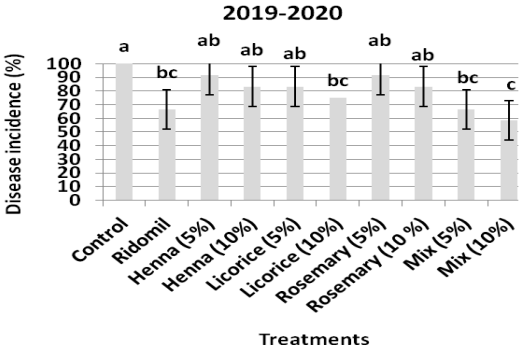

Fig. 6. Effect of some plant extracts as foliar application on the incidence of purple blotch disease on garlic under greenhouse conditions during 2019/2020.

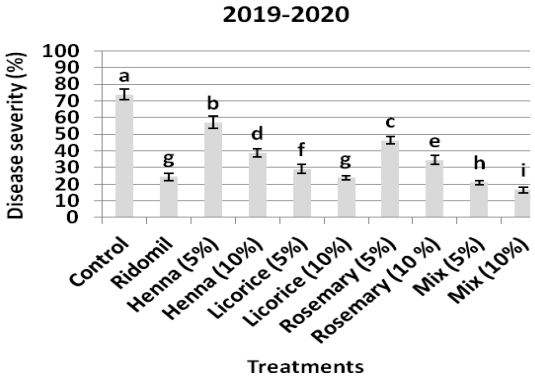

Fig. 7. Effect of some plant extracts as foliar application on the severity of purple blotch diseases on garlic under greenhouse conditions during 2019/2020.

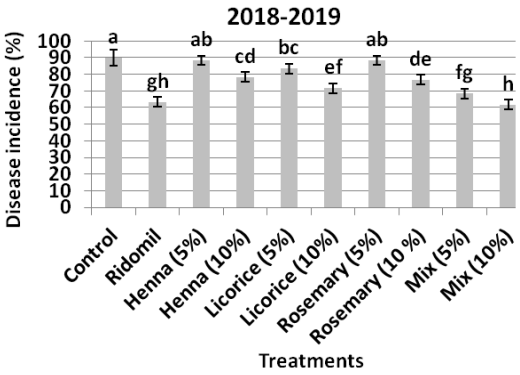

Fig. 8. Effect of some plant extracts as foliar application on the incidence of purple blotch disease on garlic under open field conditions during 2018/2019.

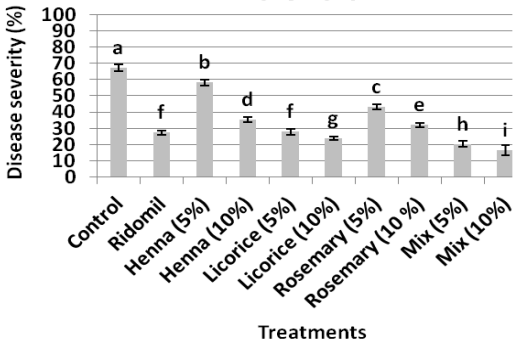

Fig. 9. Effect of some plant extracts as foliar application on the severity of purple blotch disease on garlic under open field conditions during 2018/2019.

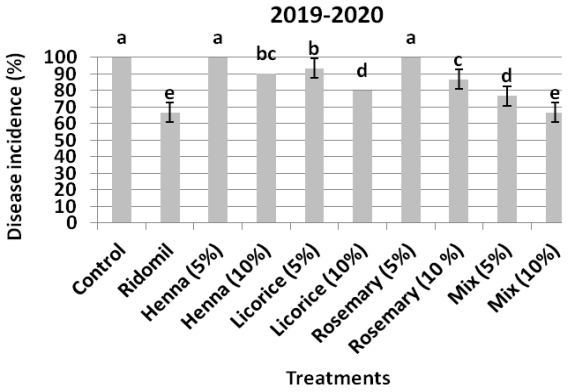

Fig. 10. Effect of some plant extracts as foliar application on the incidence of purple blotch disease on garlic under open field conditions during 2019/2020.

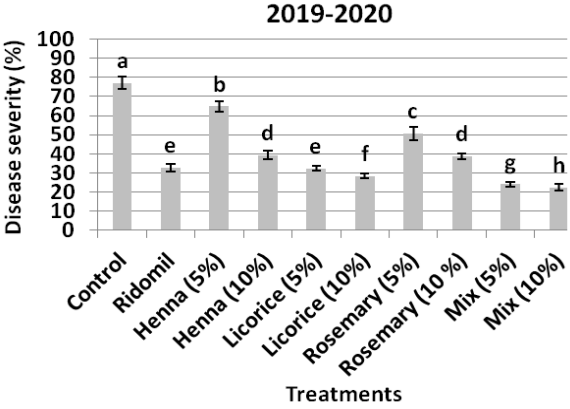

Fig. 11. Effect of some plant extracts as foliar application on the severity of purple blotch disease on garlic under open field conditions during 2019/2020.

1. Plant growth parameters

It is clearly shown in Table (3) that foliar applications with different plant extracts resulted significant increases in plant length and bulbing ratio in the first season, fresh and dry weight of plant in the first and second seasons. However, the obtained increases in treated garlic plant growth parameters *i.e.*, plant length and bulbing ratio in second season, diameter of both neck and bulb in the first and second seasons were not significant. Foliar spraying with licorice at 10% exhibited the highest value of plant length compared with control plants in first season. As well as licorice at 10% gave the highest values of plant fresh and dry weights in both seasons. Meantime, the spray with Ridomil Plus gave no significant effect on different parameters of garlic plant through the two seasons. However, henna at 10% scored the lowest value of plant dry weight in second season.

2. Yield and storability

Data in Table (4) indicated that all plant extracts as foliar application have significantly increased the total yield at harvest as well as after curing, except with treated of mix extracts at 10% at both seasons and with rosemary at 5% in the second season. As for the results of application of plant extracts on garlic yield components after curing *i.e*, neck diameter and bulbing ratio, results showed insignificant variations at both seasons. However, bulb weight significantly increased in both seasons whereas bulb diameter resulted significant increases with all treatments in the first season. The highest values of bulb weight, total yield at harvest and total yield after cured in both seasons and bulb diameter in the first season were obtained from foliar spray with licorice at 10% compared with untreated control plants.

Table 3. Effect of foliar spray treatments with plant extracts on plant length, leaf number, neck and bulb diameter, bulbing ratio, fresh and dry weigh of garlic plant after 150 days from planting in both seasons 2018/2019 and 2019/2020

Treatments	Plant length (cm)	Leaf number/ plant	Neck diameter (cm)	Bulb diameter (cm)	Bulbing ratio	Plant F.W (g.)	Plant D.W (g.)
			2018/2019 season				
Control	59.83 f	10.00 abc	1.40 bc	5.10 a	0.28 bc	100.40 d	19.35 g
Ridomil	60.33 f	10.17 ab	1.25 c	5.23 a	0.24 c	101.45 d	19.79 g
Henna (5%)	65.00 e	10.50 a	1.48 abc	4.98 a	0.30 ab	109.68 c	21.98 ef
Henna (10%)	67.00 de	9.67 bc	1.50 abc	5.05 a	0.30 ab	111.37 c	23.22 cd
Licorice (5%)	71.67 bc	10.00 abc	1.65 ab	5.12 a	0.32 a	127.24 a	22.31 e
Licorice (10%)	75.00 a	10.00 abc	1.58 ab	5.30 a	0.30 ab	128.69 a	24.44 a
Rosemary (5%)	70.00 c	9.67 bc	1.73 a	5.25 a	0.33 a	126.02 a	24.14 ab
Rosemary (10%)	72.33 abc	9.33 c	1.58 ab	5.17 a	0.31 ab	117.76 b	22.65 de
Mix (5%)	69.50 cd	10.00 abc	1.68 a	5.18 a	0.32 a	125.74 a	23.61 bc
Mix (10%)	73.67 ab	9.50 bc	1.53 ab	5.02 a	0.31 ab	113.60 bc	21.25 f
			2019/2020 season				
Control	81.75 ab	8.67 b	1.78 ab	4.04 b	0.44 a	97.69 e	19.11 bc
Ridomil	84.33 ab	9.00 ab	1.93 a	4.15 ab	0.47 a	101.66 cde	19.74 ab
Henna (5%)	86.13 a	9.00 ab	1.90 a	4.30 ab	0.44 a	108.07 bc	20.82 a
Henna (10%)	84.67 ab	9.50 ab	1.70 ab	4.04 b	0.42 a	100.57 de	18.51 c
Licorice (5%)	86.17 a	9.67 a	1.52 b	4.18 ab	0.38 a	110.94 ab	20.70 a
Licorice (10%)	86.25 a	9.17 ab	1.82 ab	4.46 a	0.41 a	115.92 a	20.83 a
Rosemary (5%)	83.08 ab	9.17 ab	1.77 ab	4.17 ab	0.42 a	102.73 cde	18.81 bc
Rosemary (10%)	84.78 a	9.33 ab	1.65 ab	4.21 ab	0.39 a	104.40 bcd	19.17 bc
Mix (5%)	79.75 b	8.83 ab	1.67 ab	4.13 ab	0.41 a	102.87 cde	19.90 ab
Mix (10%)	85.70 a	8.83 ab	1.78 ab	4.11 ab	0.43 a	103.99 cde	20.59 a

Means followed by the same letters were not significantly differed according to Duncan's multiple range test.

Table 4. Effect of foliar spray treatments on total yield and its components and percentage of bulbs weight loss after 90 and 180 days in storage of garlic plant in both seasons 2018/2019 and 2019/2020

Treatments	Total yield at harvest (ton/fed.)	Total yield after cured (ton/fed.)	Bulb weight (gm.)	Neck diameter (cm)	Bulb diameter (cm)	Bulbing ratio	bulb weight loss% after 90 days in storage	bulb weight loss% after 180 days in storage
2018/2019 season								
Control	5.371 g	4.501 e	51.89 ef	0.96 ab	5.70 c	0.17 a	6.01 ab	13.25 a
Ridomil	5.976 f	4.736 e	50.75 f	0.88 bc	5.73 c	0.15 ab	4.79 b	11.84 a
Henna (5%)	6.797 de	5.486 cd	55.73 de	0.86 bc	5.88 c	0.15 bc	6.22 ab	12.27 a
Henna (10%)	7.765 b	6.188 b	56.72 cd	0.89 abc	6.30 ab	0.14 bc	6.85 a	13.23 a
Licorice (5%)	6.788 de	5.427 cd	62.20 b	0.84 c	6.23 b	0.13 c	6.26 ab	14.30 a
Licorice (10%)	9.066 a	6.960 a	67.14 a	0.95 ab	6.47 a	0.15 bc	7.15 a	13.72 a
Rosemary (5%)	7.518 bc	5.940 bc	61.06 bc	0.93 abc	6.20 b	0.15 abc	7.56 a	14.10 a
Rosemary (10%)	8.939 a	6.868 a	63.35 ab	0.94 abc	6.32 ab	0.15 bc	7.57 a	13.34 a
Mix (5%)	7.068 cd	5.720 bcd	63.68 ab	0.96 ab	6.32 ab	0.15 ab	6.90 a	13.05 a
Mix (10%)	6.446 ef	5.105 de	65.21 ab	0.99 a	6.35 ab	0.16 ab	6.69 ab	13.99 a
2019/2020 season								
Control	6.579 e	4.646 d	49.60 f	0.98 ab	5.12 a	0.19 a	7.18 bcd	14.71 bcd
Ridomil	6.866 d	4.703 cd	53.21 cd	0.86 abc	5.09 a	0.17 ab	5.10 e	11.43 f
Henna (5%)	7.911 b	5.458 b	53.06 cde	0.98 a	5.23 a	0.19 ab	5.27 e	12.15 ef
Henna (10%)	7.937 b	5.495 b	56.71 ab	1.00 a	5.16 a	0.19 a	6.60 cde	13.25 cdef
Licorice (5%)	8.760 a	6.149 a	54.40 bc	0.84 bc	5.32 a	0.16 ab	8.06 abc	15.13 abc
Licorice (10%)	8.773 a	6.167 a	58.22 a	0.98 ab	5.31 a	0.18 ab	9.58 a	16.90 a
Rosemary (5%)	7.331 c	5.041 bcd	55.65 abc	0.90 abc	5.28 a	0.17 ab	8.88 ab	16.26 ab
Rosemary (10%)	7.970 b	5.511 b	56.45 ab	0.83 c	5.33 a	0.16 b	6.72 cde	14.02 cde
Mix (5%)	7.243 c	5.208 bc	50.06 ef	0.88 abc	5.13 a	0.17 ab	5.88 de	13.11 def
Mix (10%)	6.958 d	4.767 cd	51.22 def	0.97 abc	5.07 a	0.19 a	5.79 de	12.40 ef

Means followed by the same letters were not significantly differed according to Duncan's multiple range test.

As for weight loss percentage of garlic bulbs during storage, results in Table (4) showed that there were no significant differences between foliar treatments on bulbs weight loss% after the 90 and 180 days in storage in the first season. On the contrary, Ridomil plus at 2.5 g/L and henna at 5% recorded significant decreases in bulbs weight loss as compared to untreated control, during the period of storage after 90 and 180 days in the second seasons. While, licorice at 10% recorded significant increases in bulbs weight loss as compared to untreated control after the 90 and 180 days of storage in the second season. Aside from, the effect of other treatments did not show significant differences except mix of all extracts at 10% after 180 days in storage (at the end of storage period) in the second season.

4. Biochemical parameters

Results in Fig. (12) showed that control plants either treated with Ridomil Plus or untreated plants led to significant increases in MDA content in garlic leaves in both seasons compared to foliar treatments of all aqueous plant extracts. However, there was no significant differences between treatment of plant extracts in both seasons.

The level of phenolic compounds was significantly increased in all foliar treatments with plant extracts as well as sprayed plant with Ridomil Plus compared with untreated plants, in both seasons (Fig 13). In the first season, foliar application with licorice and henna at 10% was involved in the highest phenolic compounds 1811 and 1792 µg/g FW, respectively compared to untreated plants 1079 µg/g FW. Also, in the second season, the maximum level of phenols was detected in application with licorice at 10 % as it obtained 1863 µg/g FW compared to untreated plants 954 µg/g FW. Data illustrated in Fig. (14) indicated that proline content in leaves of infected garlic treated with either plant extracts or Ridomil was significantly decreased compared to untreated plants. Among all treatments, foliar application with aqueous extract of licorice at 10% caused the maximum value in both seasons (196.8 and 82.5 µg/g FW, respectively).

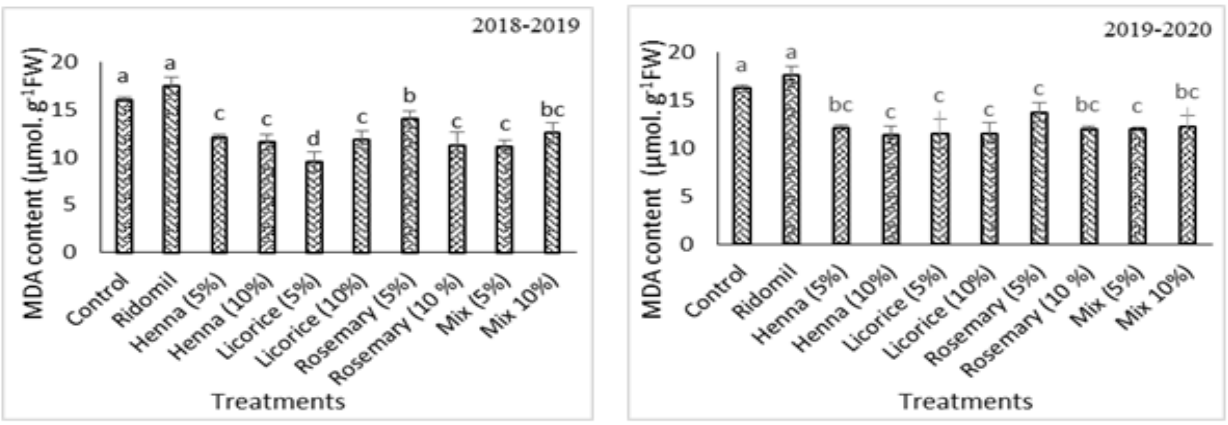

Fig 12. Effect of plants aqueous extracts as a foliar application on MDA content in infected garlic with purple blotch during two seasons 2018/2019 and 2019/2020.

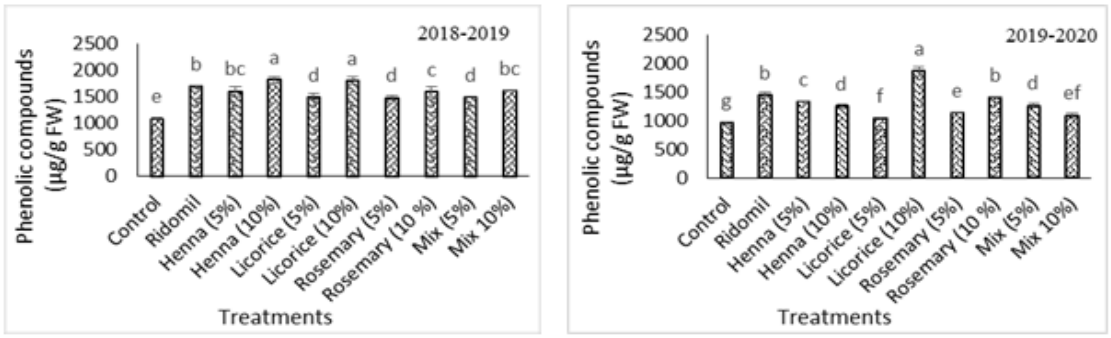

Fig 13. Effect of plants aqueous extracts as a foliar application on phenolic compounds in infected garlic with purple blotch during two seasons 2018/2019 and 2019/2020.

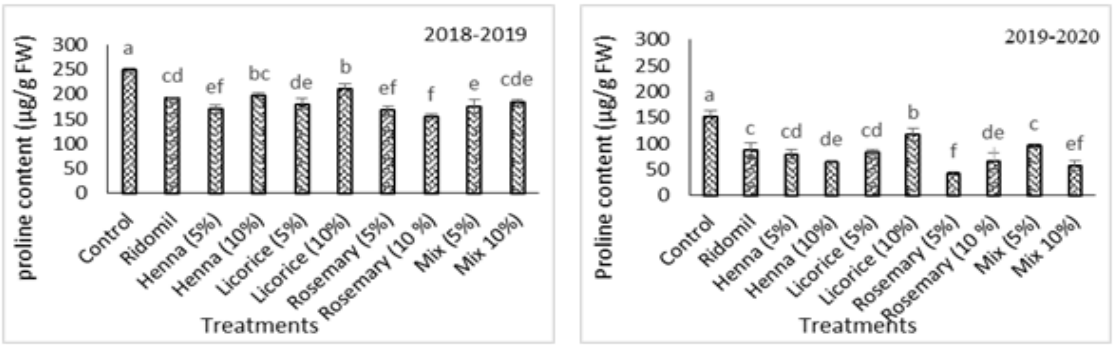

Fig. 14. Effect of plants aqueous extracts as a foliar application on proline content in infected garlic with purple blotch during two seasons 2018/2019 and 2019/2020.

5. Antioxidant defensive enzymes

The enzyme activity of catalase was significantly increased in all treatments compared to untreated control in both seasons as shown in Table (5). Plants treated with mixtures of aqueous extracts at levels of 5% and 10% exhibited the highest CAT activity in infected leaves (2.40 and 2.19 unit.mg^{-1} protein respectively), while the effect of henna (5 and 10%), licorice (5 and 10%) and rosemary (5 and 10%) aqueous extracts was lower than mixtures (1.85, 1.69, 1.58, 1.82, 1.40 & 1.52 unit.mg^{-1} protein, respectively) in the first season. On the other hand, licorice 5% aqueous extract achieved the highest activity of CAT in infected garlic (2.78 unit.mg^{-1} protein) compared with control (0.70 unit. mg^{-1} protein) in the second season. The enzyme activities of POD were significantly increased in all treatments compared to untreated control as shown in Table (5) in the two successive seasons. Moreover, in the first season, the maximum increase in POD activity of foliar treatment with mixture of all extracts at 10% was 90.44 unit.mg^{-1} protein compared to untreated control (53.01 unit.mg^{-1} protein). On the other hand, in the second season, the maximum increase in POD activity of foliar treatment with licorice (10%) was 101.09 unit.mg^{-1} protein compared to untreated control (38.22 unit.mg^{-1}protein). Data presented in Table (5) showed also that all foliar treatments with aqueous plants extracts and Ridomil in infected leave garlic led to significant increase in PPO activity compared with untreated plants in both seasons. Aqueous extracts of rosemary (5%) and mixture (10%) attained the highest PPO activity in garlic in the first season. Furthermore, application with aqueous extracts of licorice and rosemary at 5 & 10% led to the highest PPO activity compared with untreated control in the second season. SOD activity was significantly affected by foliar treatments with Ridomil, henna, licorice and rosemary at 5 & 10% comparing with the control in both seasons. Otherwise, there were no significant differences in SOD activities between control and mixture of all extracts in the first season and between control and 10 % mixture in the second season.

Table 5. Effect of foliar applications of aqueous plant extracts on antioxidant defense enzymes in garlic leaves under purple blotch disease pressure

Treatments	Antioxidant defensive enzymes							
	CAT (unit.mg^{-1}protein)		POD (unit.mg^{-1}protein)		PPO (unit.mg^{-1}protein)		SOD (unit.mg^{-1} protein)	
	2018-2019	2019-2020	2018-2019	2019-2020	2018-2019	2019-2020	2018-2019	2019-2020
Control	0.7^{d}±0.11	0.7^{g}±0.06	53.0^{f}±1.25	38.2^{f}±4.26	1600.8^{d}±167.4	787.30^{e}±92.40	129.2^{d}±15.1	134.44^{f}±7.8
Ridomil	0.9^{d}±0.08	1.7^{c}±0.12	83.3^{b}±0.75	79.6^{c}±6.49	2206.6^{c}±161.5	1309.1^{c}±101.3	205.2^{bc}±21.5	255.3^{ab}±11.1
Henna (5%)	1.9^{bc}±0.25	1.3^{de}±0.02	71.1^{d}±3.29	92.6^{b}±5.97	2644.7^{b}±40.30	1595.0^{b}±137.4	231.8^{b}±19.8	251.4^{abc}±17.1
Henna (10%)	1.7^{c}±0.35	1.4^{d}±0.10	83.6^{b}±3.96	78.7^{c}±5.5	2251.0^{c}±11.70	903.10^{d}±67.90	292.9^{a}±20.5	263.3^{a}±10.9
Licorice (5%)	1.6^{c}±0.50	2.8^{a}±0.11	78.9^{bc}±0.58	59.7^{e}±2.26	2813.2^{b}±107.3	1840.2^{a}±117.9	197.7^{c}±6.7	219.9^{bcd}±30.8
Licorice (10%)	1.8^{bc}±0.31	2.3^{b}±0.10	61.1^{e}±1.53	101.1^{a}±2.7	2287.0^{c}±147.6	1936.6^{a}±154.5	215.7^{bc}±7.0	214.5^{cd}±19.6
Rosemary (5%)	1.4^{c}±0.16	2.3^{b}±0.10	60.6^{e}±1.41	69.8^{d}±4.09	3039.1^{a}±20.10	1789.8^{a}±80.30	220.2^{bc}±16.2	282.6^{a}±7.7
Rosemary (10%)	1.5^{c}±0.19	0.9^{f}±0.10	76.0^{cd}±4.33	89.9^{b}±0.83	2379.6^{c}±107.0	1936.5^{a}±24.30	222.7^{bc}±17.2	182.8^{de}±35.8
Mix (5%)	2.4^{a}±0.17	1.8^{c}±0.11	76.5^{c}±1.15	69.8^{d}±1.27	2322.5^{c}±102.5	908.50^{d}±30.80	136.6^{d}±12.1	182.6^{de}±30.9
Mix (10%)	2.2^{ab}±0.20	1.2^{e}±0.14	90.4^{a}±6.87	73.2^{cd}±4.8	3213.7^{a}±115.6	1335.9^{c}±51.60	140.7^{d}±15.5	162.9^{ef}±30.1

Data represent the means ± standard deviation of three replicates. Different letters refer to significant differences at $p \leq 0.05$.

6. Effect of plant extracts on the mycelial growth inhibition of *A. porri*

It was revealed from the results (Fig. 15, Table 6) that all concentrations of plant extracts caused significant inhibitions in the mycelial linear growth of *A. porri*. Among all plant extracts used, licorice at 10 % was the most effective against *A. porri* as compared with the other extract and caused highest inhibition in the mycelial linear growth (57.88 %). However, Ridomil Plus inhibited the growth of *A. porri* on media as compared with all other treatments, followed by licorice at 5% (51.16 %), and mix 10% (50.38 %).

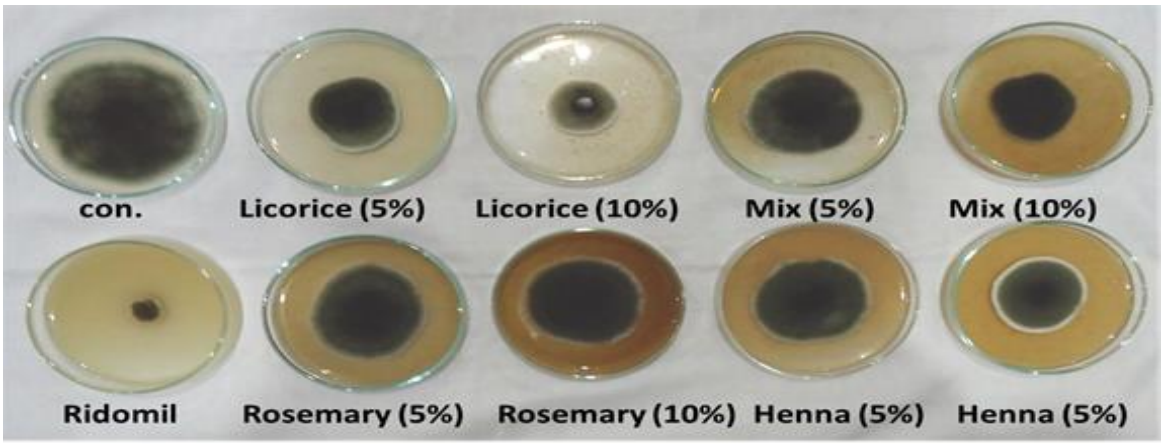

Fig. 15. Effect of aqueous plant extracts on the inhibition of linear growth of *Alternaria porri* after 7 days of incubation at 25±2°C.

Table 6. Effect of aqueous plant extracts on the inhibition of linear growth of *Alternaria porri* after 7 days of incubation at 25±2°C

Treatments	% Reduction in growth of *Alternaria porri*
Control	00.00
Ridomil plus	89.66
Henna (5%)	29.97
Henna (10%)	41.08
Licorice (5%)	51.16
Licorice (10%)	57.88
Rosemary (5%)	21.18
Rosemary (10%)	26.09
Mix (5%)	31.00
Mix (10%)	50.38

Discussion

Foliar applications with aqueous extracts of henna, licorice, rosemary or mixtures of them at levels 5% and 10% significantly decreased garlic purple blotch disease index under green house as well as field conditions during two successive seasons. Such results are consistent with works of several researchers (Abdel-Hafez *et al*., 2014; Brahmane *et al.*, 2015). The effect of such plant aqueous extracts on purple blotch disease seemed to be due to induction of plant resistance (Draz *et al*., 2019) or their acts as antifungal activity (Benaissa and Belhamra, 2017).

Malondialdehyde (MDA) is a secondary product of lipid peroxidation, it can be used as a biomarker for oxidative damage accompanied by abiotic and biotic stresses in plant cells (Farmer and Mueller 2013). All foliar applications with aqueous plant extracts caused significant decreases in MDA content of garlic leaves in both seasons compared to untreated infected plants which can be attributed to induction of antioxidant defense mechanisms. However, untreated infected garlic plants showed significant increases in MDA content due to the higher production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), and hydroxyl radical (OH^-). The activity of ROS frequently causes cellular membrane damage through peroxidation of fatty acids (Su *et al.*, 2019).

Proline plays three major beneficial roles in plants during different stress conditions, as a metal chelator, a signaling molecule and an antioxidative defense molecule **(Dar *et al*., 2016)**. The observed accumulation of proline in untreated infected plants was correlated to a high level of lipid peroxidation induced by pathogen. So, the proline accumulation could be accepted as a metabolic indicator of purple blotch induced oxidative stress in garlic plants. In addition, the clear reduction in proline content concomitant with a decrease in disease index in extracts treated garlic plants leads to the elicitation that such treatments have alleviated the ROS activity of the pathogen that led to proline decline as a reaction of disease control on plants.

All tested extracts led to activation of phenol biosynthesis in garlic leaves as well as antioxidant defensive enzymes (POD, PPO, CAT and SOD). Phenolic compounds act as ROS scavengers directly or as substrates for antioxidant enzymes like peroxidases (Bendary *et al*., 2013). Also, phenols are the principal components of lignin which increase plant cell wall resistance against degrading enzymes, limiting the penetration by the invading pathogen (Bhuiyan *et al.*, 2009). In addition, the enhanced POD activity was reported to be associated with the induced systemic resistance in plant against pathogen during induced several plant defense mechanisms, such as oxidative cross-linking and lignin biosynthesis in plant cell walls (Bestwick *et al*., 1998). Polyphenol oxidase (PPO) is involved in the oxidation of polyphenols into quinones (antimicrobial compounds), lignification of plant cells during microbial invasion, and participates in induction of plant resistance against fungi (Mayer, 2006). Our results is in agreement with Draz *et al*. (2019) who showed that the aqueous extract of henna led to effective protection against the leaf rust disease caused by *P. triticina* by increasing plant contents of total phenolics, oxidative enzymes activities POD and PPO.

Aside from, all tested aqueous plant extracts have increased the plant growth parameters and total yield and the highest effect was clear when licorice used, these results are similar with (Hussian and Al-Rakabi, 2006; Kamal and Ghanem, 2012; Byan, 2014; Shafeek *et al.*, 2015) they showed that foliar spraying with licorice extract has a favorable effect on vegetative growth of plant, flowering, total yield and fruits quality in several plants.

All tested aqueous plant extracts relatively inhibited mycelial growth of *A. porri in vitro* but was remarkably lower than Ridomil effect. Licorice extract showed the best effect compared with other extracts. Several reports stated that extracts of different medicinal plants have an inhibitory effect against phytopathogenic fungi (Abbas *et al.*, 2015; Benaissa and Belhamra, 2017). However, the extract of *Glycyrrhiza glabra* roots exhibited significant antimicrobial activity against different fungal and bacterial strains (Abbas *et al.*, 2015). Otherwise, aqueous extract of *Lawsonia inermis* has an antifungal activity affect against *Rhizoctonia* sp., *Phtophtora* sp, *Pytium* sp. caused damping off disease and against *A. solani* the cause of leaf blight disease in tomato plants (Benaissa and Belhamra, 2017). The antimicrobial activity of licorice aqueous extract may be due to the presence of various phytochemical groups such as saponins, flavonoids, alkaloids, tannins, steroids, and anthraquinones in extract, which are considered antimicrobial compounds (Field and Lettinga, 1992).

It can be concluded from these results that henna, licorice, and rosemary aqueous extracts were able to reduce the disease severity of purple blotch disease. Such disease decline may be due to both mycelial growth inhibition and induced resistance in garlic against *Alternaria porri* through reduction of lipid peroxidation during a mechanism involved activation of antioxidant defensive enzymes (CAT, POD and PPO) as well as phenol biosynthesis, and decrease of proline content compared with untreated infected plants during both seasons. This was associated with the improvement of plant growth parameters, and total garlic yield.

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تأثير معاملة الثوم ببعض المستخلصات النباتية على مرض اللطعة الأرجوانية وعلاقتها بصفات نمو النبات والمحصول وبعض الاستجابات البيوكيميائية

جميع التجارب تم اجرائها خلال الموسمين الزراعين ٢٠١٨/٢٠١٩ و ٢٠١٩/ ٢٠٢٠ . تم اجراء تجارب الصوب بقسم أمراض النبات، كلية الزراعة، جامعة عين شمس. وتم اجراء التجارب الحقلية بمزرعة بحوث الخضر بقها بمحافظة القليوبية.أدت جميع معاملات الرش الورقى بالمستخلصات المائية لكل من الحناء (*Lawsonia inermis*) ، والعرق سوس (*Glycyrrhiza glabra*) ، وإكليل الجبل (*Salvia rosmarinus*) بتركيز ٥ و١٠% لكل منهم، وخليط منهم بمستويات ٥٪ و ١٠٪ أو مبيد ريدوميل (٢,٥ جم / لتر) إلى احداث خفضاً معنوياً في شدة الاصابة بمرض اللطعة الأرجوانية ، كما وأدى إلى احداث زيادات معنوية في المركبات الفينولية ونشاط الإنزيمات المؤكسدة مثل CAT و POD و PPO خلال موسمي النمو (٢٠١٨/٢٠١٩ و ٢٠١٩/٢٠٢٠) مقارنة مع نباتات الثوم المصابة غير المعاملة. ومع ذلك ، فقد أدت المعاملات إلى حدوث انخفاض معنوي في محتوى البرولين في الأوراق المصابة المعاملة خلال الموسمين. أظهر الرش بالعرق سوس ومزيج من المستخلصات بنسبة ٥٪ و ١٠٪ أفضل تأثير معنوي في خفض شدة المرض تحت ظروف الصوبة الزراعية وظروف الحقل. كما أظهر العرق سوس ١٠٪ أعلى طول للنبات والوزن الطازج والجاف ووزن البصلة والمحصول الكلي عند الحصاد وبعد العلاج التجفيفى.أثبت اختبار التثبيط الفطري أن جميع المستخلصات النباتية لها تأثير مثبط على نمو فطر *A. porri* وأظهر مستخلص عرق السوس أعلى تأثير مثبط بعد تأثير المبيد الفطري Ridomil Plus . يمكن الاستنتاج أن جميع المستخلصات المائية المختبرة كانت قادرة على تقليل شدة المرض نتيجة إما لتثبيط النمو الميكروبي المباشر أو حث مقاومة نبات الثوم على مقاومة فطر *A. porri* ، وقد ارتبطت المعاملات أيضًا بتحسين صفات نمو النبات وإنتاجية الثوم.