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Research Article

Determining the Susceptibility of Different Basil Varieties to Downy Mildew Caused by *Peronospora belbahrii*

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ABSTRACT

Field experiments were carried out under natural infection of basil downy mildew in the Experimental Farm of Sids Agricultural Research Station, Agricultural Research Center, Beni-Sweif governorate in 2016 and 2017 for screening a number of local varieties of basil and French basil grown in Beni-Sweif governorate, Egypt for susceptibility to basil downy mildew. Generally, all varieties tested varied in their response to downy mildew infection. The five basil varieties tested were divided into five groups based on the resistance reaction screened under field condition into: resistant variety (Lv 1), moderately resistant (Lv 2), moderately susceptible (Lv 3), susceptible (French basil) and highly susceptible (Lv 4) and classified to three chemotypes due to their content of the dominant component as follows, methyl cinnamate chemotype (Lv 1 and Lv 3), linalool chemo type (Lv 4 and French basil cultivar) and methyl chavicol chemotype (Lv 2). However, phenols, peroxidase and polyphenol oxidase activities were found higher in resistant varieties as compared to susceptible varieties.

Keywords: Downy mildew; Resistance; Chemotypes; Methyl cinnamate; Linalool; Phenols; peroxidise; Polyphenol oxidase; Basil

INTRODUCTION

Genus *Ocimum* (Lamiaceae) includes around 30 species from tropical and subtropical regions of Asia, Africa, which are much differentiated in respect of morphological and chemical features [1,2]. Most members of this genus are annual or perennial, highly aromatic, branched herbs or shrubs [3,4]. These are accredited with various medicinal properties and used in folk medicine for the treatment of abdominal pains, colds, coughs, measles, insomnia, rheumatism, sunstroke, gonorrhea, inflammation, snake bite/insect bites, stomach and kidney malfunctions etc. [5,6]. Among the species of the genus, *Ocimum basilicum* L. (sweet basil) is the major essential oil crop around the world, cultivated in many countries which used as a spicy and medicinal herb.

The aromatic character of each type is determined by genotype and depends on the major chemical compounds of essential oil [2,7]. The essential oil constituents vary among sweet basil cultivars and the main ones are linalool, methyl chavicol, eugenol, 1,8-cineole, geranial, neral, methyl cinnamate [7,8].

The broad morphological variations in the wide variety of annual and perennial herbs and shrubs in the genus *Ocimum* [9] are primarily attributed to geographical distinctions, polyploidy, inter-specific hybridization, and general shifts in classification [10]. Basils differ in many aspects including vigor, form, plant height, branching, pubescence, leaf size, leaf shape, leaf texture, leaf color, leaf dimension, plant color, flower color, flowering period, taste and aroma [11-13].

The essential oil of *Ocimum* taxa showed huge inter and intra specific compositional variability due to existence of numerous genotype/ chemotype/ cultivars within taxa and because of endogenous variables, including variations in geographic range and climate and agricultural conditions etc. [6,14].

Composition of volatile oil constituents used to classify *Ocimum* species into chemotypes that are plants chemically distinct in the composition of secondary metabolites [15]. *O. basilicum* can be

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grouped into four chemotypes: linalool type, methyl chavicol type, methyl eugenol type and methyl cinnamate type according to plants that yield oil rich in these components [13,16,17] and numerous subtypes with mixed proportion of these constituents were identified in *O. basilicum*.

Earlier reports on the antimicrobial potential of *O. basilicum* revealed that basil oil and its specific constituents exhibited broad range of antagonistic activity against a wide range of microorganisms [7,16,18-21]. Methyl cinnamate (MC) is a methyl ester of cinnamate. It is one of the major components of essential oils extracted from herbal plants [22,23]. MC and essential oils containing MC possess antimicrobial, antifungal, insecticidal, and antioxidant effects [24-27].

Basil extract has antimicrobial, fungistatic and antioxidant activities due to its phenolic and aromatic compounds [28,29]. The main phenolics reported in basil are phenolic acids and flavonol-glycosides [30,31]. Rosmarinic acid is the most prevalent basil phenolic [31,32] but other caffeic acid derivatives, such as chicoric acid found in substantial concentrations [31]. However, changes in chemical composition of basil essential oil, based on dominance of major constituents was found to have a direct effect on activity potential of basil essential oil [33].

Basil downy mildew, caused by *Peronospora belbahrii* Thines [34-36], is a new disease of basil observed for the first time in Egypt especially in Beni-Sweif governorate in 2013 [37]. Since then the disease has been spread quickly and become epidemic in all basil growing areas, causing complete crop losses in some fields [38]. Basil downy mildew has been previously reported as a destructive disease in several countries and continents [39-44]. Although there is no research on how the pathogen arrived in Egypt. Frequently, infection is found immediately after seeding, suggesting that the pathogen can be spread through contaminated seed and/or infected plant materials are believed to facilitate the spread [45].

Epidemiology of basil downy mildew strongly depends upon weather conditions. Basil downy mildew develops during periods of moderate temperatures, high air humidity and prolonged leaf wetness [46,47]. The pathogen requires at least 6-12 h of leaf wetness (free leaf moisture) after inoculation for severe infections and, most important, in order to sporulate it requires the presence of moisture-saturated atmosphere (relative humidity ≥ 95%) in the dark which completed within about 11 hours at 18°C [47,48].

Chemical control is commonly used to protect many crops from downy mildew infection but this is difficult for medicinal and exportation crops like basil, due to the risk of the presence of residues at harvest. However, there are reports showed resistant isolates from the pathogen to the fungicide mefenoxam after one year from its appearance, thus making one of the most effective registered fungicides, ineffective [49,50].

Resistance to *P. belbahrii* in commercial sweet basil has not been reported in Egypt: apparently, all varieties are susceptible. Importantly, breeding program can be constructed to develop improved sweet basil varieties with tolerance to basil downy mildew. Therefore, the objective of the following study was to evaluate a number of local varieties of basil which handled as Balady variety and French basil which cultivated as a commercial crop and ornamental plants grown in Beni-Sweif governorate, Egypt for susceptibility to basil downy mildew and antifungal potential of the leaves extract and essential oil obtained from these varieties against the pathogen. Moreover, the essential oil percentage and composition and phenol content as well as peroxidase, polyphenol oxidase activities estimation.

MATERIALS AND METHODS

A two-year field experiment was conducted under natural infection in the Experimental Farm of Sids Agricultural Research Station, Agricultural Research Center, Beni-Sweif governorate during the seasons of 2016 and 2017 to study the response of five basil varieties grown in Beni-Sweif governorate, Egypt to *P. belbahrii* infection.

The climate at Beni-Sweif study area (Latitude 29° 4'28 N, Longitude 31° 5'52 E), during the basil growing seasons (2016 and 2017) was assessed using database obtained from the Central Laboratory for Agricultural Climate (CLAC), Giza, Egypt. The average minimum and maximum temperatures of 18-38°C and relative humidity of about 41-78% (Table 1).

Table 1: Some physical and chemical properties of experimental soil in the two seasons.

Season	Clay (%)	Silt (%)	Sand (%)	Organic Matter (%)	pH	EC DSm-1	Available nutrients (ppm)		
							N	P	K
2016	53.4	30.1	16.5	1.3	8.1	1.2	26.2	10.1	176
2017	53.6	30.2	16.2	1.4	8.0	1.3	26.7	10.3	179

In each season, all agriculture practices i.e., soil preparation, fertilization, propagation, irrigation and weed control were carried out according to the good agriculture practices for basil production recommended by the ministry of agriculture.

The experiment was designed in a randomized complete block design arrangement, with three replications. The experimental plot size was 3 × 3.5 m.

The uniform healthy basil varieties seedlings, 10-15 cm length were transplanted into the field on May, 1st in the two experimental seasons. The seedlings were obtained from Sids Agricultural Research Station, Agricultural Research Center, Beni-Sweif governorate (Figure 1). Monitoring and scouting the plants were carried out weekly for downy mildew, disease incidence and severity were estimated as follow:

Disease incidence

Percentage of disease incidence was recorded as the number of diseased plants relative to the number of growing plants, and then the average of disease incidence was calculated.

Disease severity

Percentage of disease severity was recorded according to the following equation [51]:

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

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

Downy mildew severity ratings were recorded by examining 100 randomly selected leaf samples from 10 selected plants randomly distributed for each plot.

The varieties were categorized into following groups namely resistant (0-10%), moderately resistant (11-25%), moderately susceptible (26-50%), susceptible (51-75%) and highly susceptible (>75%) [52]. Three samples were harvested randomly at full bloom stage of plant growth in the second season for extraction of essential oils to determine the essential oil percentage and its composition, phenolic content and the antifungal activity of leaf extract and essential oils.

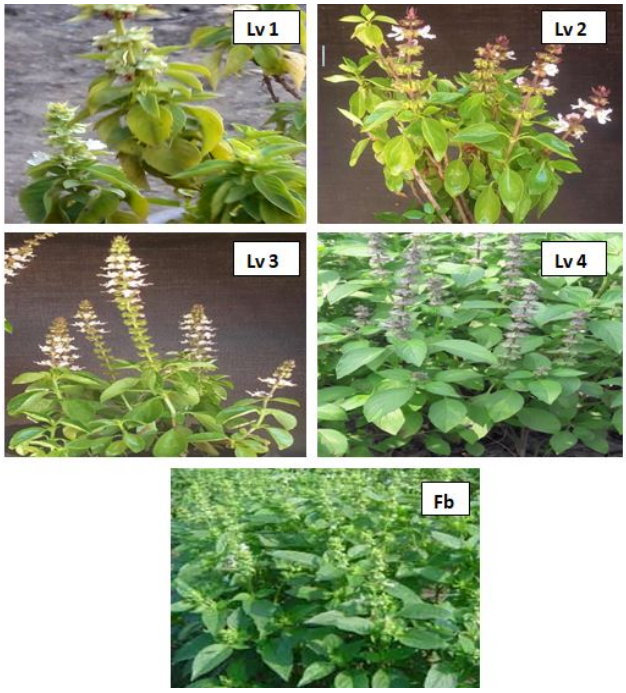

Figure 1: Pictures of basil varieties used in this study. Lv : Local variety; Fb: French basil.

Isolation of essential oils

Air dried herb of samples (10 g each) were hydro distilled in a Clevenger apparatus according to Guenther [53] in triplicate, Essential oil percentage was determined and expressed as (%), The essential oils of each variety were collected and dehydrated over anhydrous sodium sulphate and kept in a refrigerator until GC analysis.

Gas chromatography analysis (GC) of essential oils

The GC analysis of the volatile oil samples was carried out using gas chromatography instrument with the following specifications. DsChrom 6200 Gas Chromatograph equipped with a flame ionization detector, Column: BPX-5, 5% phenyl (equiv.) polysillphenylene-siloxane 30 m × 0.25 mm ID × 0.25 µm film., Sample size: 1il, Temperature program ramp increase with a rate of 10°C/min from 70°C to 200°C, Detector temperature (FID): 280°C, Carrier gas: nitrogen, Flow rate: N2 30 ml/min; H2 30 ml/min; air 300 ml/min.

Main compounds of the volatile oils were identified by matching their retention times with those of the authentic samples injected under the same conditions. The relative percentage of each compound was calculated from the area of the peak corresponding to each compound.

The isolation of essential oils and gas chromatography analysis were carried out at the Medicinal and Aromatic Plants Research Dept. Laboratory, Horticulture Research Institute, ARC.

Antifungal activity of basil leaves extract and essential oils

Basil leaves were collected randomly from the basil varieties tested. Ten leaves were dipped individually in beaker containing 10 ml sterilized distilled water for 48 hours. After specified dipping periods, the leaves were removed and the water containing leaf extract was collected in culture tubes and stored at 4°C for further use [54]. Drops of the extracts were placed on glass slides and sporangia of *P. belbahrii* were directly lifted with help of small paint brush from heavily infected basil leaves.

For antifungal activity of basil essential oils, the essential oils from different basil varieties were added individually with a few drops of the emulsifier Tween 20 (0.01%) for easy diffuse on glass slides. The slides were then placed in moist chambers prepared by placing two moist filter papers in the inner surfaces of a Petri plate. Three replications were made for each treatment. The slides were incubated at 18°C for 24 h and the percent of germination was calculated under a light microscope.

Total phenol was estimated using Folin ciocalteau reagent method described by Lafka et al. [55]. Peroxidase activity was determined using the method described in the Worthington enzyme manual [56]. Polyphenol oxidase activity was measured following the method described by Esterbaner et al. [57].

Data were statistically analyzed for computing L.S.D. test at 5% probability according to the procedure outlined by Snedecor and Cochran [58].

RESULTS

Evaluation of host resistance against downy mildew

Results of natural screening of the five tested basil varieties showed significant variation in their response against *P. belbahrii* (Table 2). Among them all plants of Lv 1 appeared symptomless until the end of experiment during the two growing seasons. Lv 2 had moderate disease incidence and progression until the end of crop growth where initial symptoms of downy mildew began to appear on some plants approximately 40 days after transplanting and recorded the minimum disease incidence 5.6% which reached maximum percent after five months from transplanting being, 56.4% followed by Lv 3.

The highest disease incidence was observed in Lv 4 plants followed by plants of French basil (Fb). The disease visible symptoms appear after 30 days from transplanting and reached its maximum percent after 3 months from transplanting, being 100.0% during the first growing season (2016). The same trend was observed in the second season (2017).

Table 2: Evaluation the disease incidence of *P. belbahrii* on different basil varieties during the two growing seasons 2016 and 2017 under field conditions.

Varieties	Incubation period/day	Disease incidence (%)					
		2016 growing season					
		1 month	2 month	3 month	4 month	5 month	Mean
Lv 1	0	0.0	0.0	0.0	0.0	0.0	0.0
Lv 2	40	0.0	5.6	19.2	33.0	56.4	22.8
Lv 3	33	0.0	30.4	62.0	100.0	100.0	58.5
Lv 4	30	44.8	82.6	100.0	100.0	100.0	85.5
Fb	30	33.0	76.8	100.0	100.0	100.0	82.0
L.S.D. at 0.05		2.0	5.7	4.9	6.7	6.7	~
		2017 growing season					
Lv 1	0	0.0	0.0	0.0	0.0	0.0	0.0
Lv 2	39	0.0	4.5	15.8	34.0	55.7	22.0
Lv 3	35	0.0	34.0	61.9	100.0	100.0	59.2
Lv 4	27	56.7	92.0	100.0	100.0	100.0	89.7
Fb	29	45.6	82.3	100.0	100.0	100.0	85.6
L.S.D. at 0.05		3.3	4.1	4.8	5.1	5.0	~

Lv : Local variety; Fb: French basil

Data in Table 3 showed significant differences among varieties for the disease severity. The basil varieties tested were categorized based on the resistance reaction screened under field condition into five groups. The Lv 1 was observed to be resistant to downy mildew pathogen with no infection until the end of crop growth during the two experimental years. Lv 2 showed moderately resistant with disease severity scored 13.7% in the 2016 growing season where in Lv 3 was found to be moderately susceptible (41.9%).

On the contrary, Fb was found susceptible to downy mildew infection with the average of disease severity, being 74.2% followed by Lv 4 which showed highly susceptible to downy mildew pathogen and had highest disease progression until the end of crop growth that reached to 97.2% at fourth month with the average, being 76.8%. The same trend was observed in the 2017 growing season.

Antifungal activity of basil leaves extract and essential oils

Leaves extract and essential oils of different basil varieties showed significant differences in germination of *P. belbahrii* sporangia (Table 4). In this regard, leaves extract of Lv 1 showed 0.7% germination percent while its essential oil completely inhibited sporangia germination. By contrast, leaf extract and essential oil of highly susceptible variety (Lv 4) caused the highest percent of sporangia germination which recorded 24.6% and 20.0%, respectively followed by French basil that showed 21.2% germination with leaf extract and 15.8% with essential oil. Leaves extract and essential oil of Lv 2 caused minimum sporangia germination, being 7.5 and 3.9%, respectively followed by Lv 3.

Table 3: Severity of *P. belbahrii* on different basil varieties during the two growing seasons 2016 and 2017 under field conditions.

Varieties	Incubation period/day	Disease severity %					
		2016 growing season					
		1 month	2 month	3 month	4 month	5 month	Mean
Lv 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lv 2	40	0.0	2.1	12.6	32.6	21.2	13.7
Lv 3	33	0.0	24.8	47.8	71.5	65.3	41.9
Lv 4	30	36.6	67.0	93.7	97.2	89.5	76.8
Fb	30	32.7	66.6	89.4	94.9	87.3	74.2
L.S.D. at 0.05		2.0	4.3	5.6	4.1	4.1	~
		2017 growing season					
Lv 1	0	0.0	0.0	0.0	0.0	0.0	0.0
Lv 2	39	0.0	1.2	13.8	25.2	18.0	11.6
Lv 3	35	0.0	23.5	49.1	76.3	65.2	42.8
Lv 4	27	39.0	70.9	91.7	98.5	88.0	77.6
Fb	29	36.3	65.8	86.2	94.6	85.7	73.7
L.S.D. at 0.05		2.6	3.1	4.1	4.2	4.1	~

Lv : Local variety; Fb: French basil

Table 4: Effect of leaves extract and essential oils of tested basil varieties on sporangial germination of *P. belbahrii*

Varieties	Spore germination (%)	
	leaves extract	Essential oils
Lv 1	0.7	0.0
Lv 2	7.5	3.9
Lv 3	14.0	11.2
Lv 4	24.6	20.0
Fb	21.2	15.8
L.S.D. at 0.05	1.5	1.6

Lv : Local varity; Fb: French basil

Essential oil content

The essential oil content obtained from aerial parts of selected basil local varieties (1, 2, 3, 4) and French cultivar are shown in Table 5. The presented data indicate that the content of essential oil were (0.31, 0.68, 1.38, 0.73 and 1.12, respectively) which average amount was 0.84%.

Table 5: Essential oil content (%).

Varieties	Lv 1	Lv 2	Lv 3	Lv 4	Fb	Mean
Oil%	0.31	0.68	1.38	0.73	1.12	0.84

Lv : Local varity; Fb: French basil

Gas chromatography analysis (GC) of essential oils

Data in Table 6 and Figures 2-6, showed the gas chromatography analysis (GC) of essential oils obtained from basil local varieties (1, 2, 3, 4) and French cultivar. Considering five oils together, a total of eighteen different compounds were identified: 18 for local varieties (1, 2, 3, 4) (98.40, 96.18, 97.18, and 96.05% of the total oil, respectively), 16 for the French cultivar (96.52% of the oil).

The main constituents found in the oil of varieties under study were 1,8-cineole (5.97, 5.71, 8.14, 5.24 and 13.50%, respectively), linalool (12.21, 11.97, 12.63, 24.85 and 28.76%, respectively), methyl chavicol (8.30, 21.87, 6.27, 12.61 and 5.01%, respectively), Isoestragole (3.21, 8.73, 5.66, 1.13 and 1.49%, respectively), Bornyl acetate (4.60, 4.53, 3.31, 2.33 and 3.55%, respectively), eugenol (8.13, 4.04, 5.76, 5.34 and 10.69%, respectively) and methyl cinnamate (34.16, 15.41, 31.65, 15.24 and 7.39%, respectively).

Table 6: Gas chromatography analysis (GC) of essential oils.

No.	Component	Basil varieties				
		Lv 1	Lv 2	Lv 3	Lv 4	Fb
1	α -pinene	1.39	1.38	1.59	0.73	1.04
2	sabinene	1.10	0.90	1.13	2.46	2.84
3	β -pinene	0.55	1.29	2.17	1.58	1.23
4	Myrcene	0.94	0.42	2.94	0.86	1.13
5	Limonene	1.10	3.66	2.26	2.94	3.06
6	1,8-cineol	5.97	5.71	8.14	5.24	13.50
7	α -terpinene	1.79	2.46	2.44	2.14	3.20
8	Linalool oxide	4.77	1.89	1.58	3.67	4.78
9	Linalool	12.21	11.97	12.63	24.85	28.76
10	Camphor	0.37	2.52	1.90	5.26	4.85
11	Methyl chavicol	8.30	21.87	6.27	12.61	5.01
12	Isoestragole	3.21	8.73	5.66	1.13	1.49
13	Chavicol	3.80	3.52	3.13	2.96	4.02
14	Bornyl acetate	4.60	4.53	3.31	2.33	3.55
15	Eugenol	8.13	4.04	5.76	5.34	10.69
16	Methyl cinnamate	34.16	15.41	31.65	15.24	7.39
17	β - caryophyllene	1.69	2.54	1.36	3.28	0.00
18	Cadinol	4.31	3.31	3.24	3.43	0.00
	Total identified	98.4	96.18	97.18	96.05	96.52
*	Total unidentified	1.60	3.82	2.82	3.95	3.48
	Total	100.00	100.00	100.00	100.00	100.00

Lv : Local varity; Fb: French basil

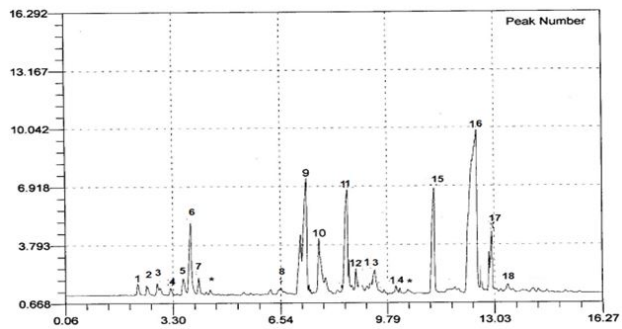

Figure 2: GC analysis for local varity (1).

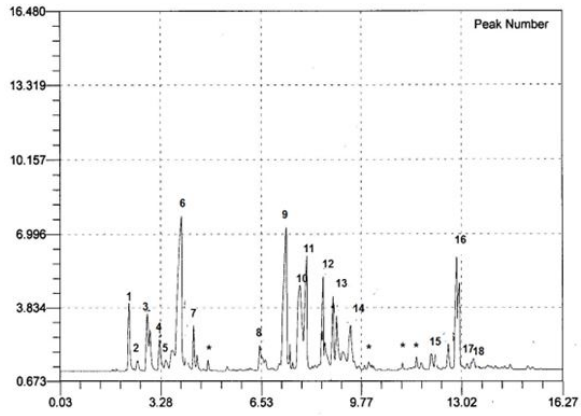

Figure 3: GC analysis for local variety (2).

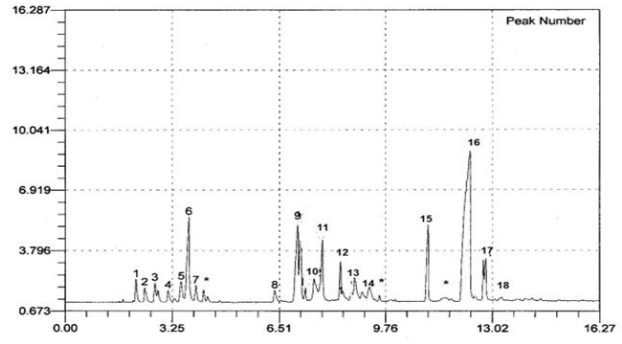

Figure 4: GC analysis for local variety (3).

In regard to these component, the varieties investigated under this study could be classified to three chemotypes due to their content of the dominant component as follows, methyl cinnamate chemotype which present in the two local varieties (Lv 1 and Lv 3) that recorded the highest amount of methyl cinnamate (34.16 and 31.65%, respectively), linalool chemotype which present in the two varieties (Lv 4 and French basil cultivar) that recorded the highest amount of linalool (24.85 and 28.76%, respectively), the local variety (Lv 2) characterized by the highest amount of methyl chavicol (21.87%) which could belong to methyl chavicol chemotype.

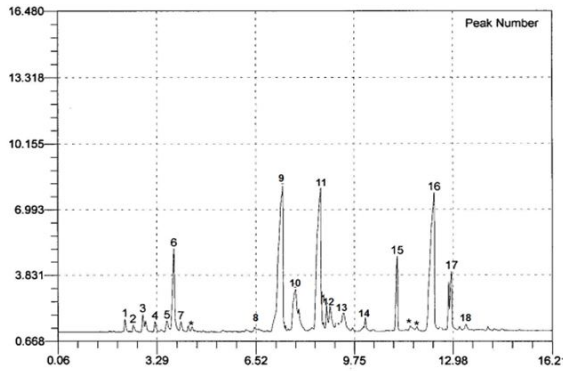

Figure 5: GC analysis for local variety (4).

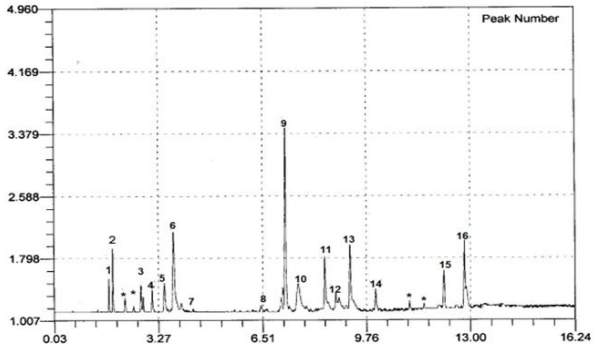

Figure 6: GC analysis for French basil.

Regarding, the total content of phenylpropanoids (Methylchavicol, Isoestragole, Chavicol and Eugenol) and esters (Bornyl acetate and Methyl cinnamate) which recorded (62.21, 58.10, 55.78, 39.61 and 32.15% of the total oil) for the investigated varieties (Lv 1, Lv 2, Lv 3, Lv 4 and Fb, respectively) which could explain the tolerance of these varieties against *P. belbahrii* infection.

Total phenols and defense-related enzymes

Phenols, peroxidase and polyphenol oxidase activities were found higher in resistant varieties as compared to susceptible varieties (Table 7). In this regard, resistant cultivar (Lv 1) showed high phenols (39.56 mg/l)), peroxidase (0.62) and polyphenol oxidase activities (0.23) followed by the moderately resistant cultivar (Lv 2) which recorded 37.98, 0.21 and 0.14, respectively. Lv 3 showed moderate content of phenols and enzymatic activities. In comparison to resistant cultivars, highly susceptible cultivar (Lv 4) showed lower phenols and enzyme activities, being 36.54 mg/l, 0.04 and 0.03, respectively followed by the susceptible variety (Fb) which recorded 36.83, 0.06 and 0.06, respectively.

Table 7: Total phenols and enzyme activities in basil varieties tested.

Varieties	Phenols		Enzymatic activities	
	Total phenol (mg/l)	Conjugate (mg/l)	Peroxidase	Polyphenol oxidase
Lv 1	39.56	38.10	0.62	0.23
Lv 2	37.98	37.63	0.21	0.14
Lv 3	37.45	37.00	0.09	0.10
Lv 4	36.54	35.78	0.04	0.03
Fb	36.83	36.33	0.06	0.06
Mean	37.67	36.97	0.21	0.11

DISCUSSION

The recent spread of basil downy mildew in Egypt [37] has caused enormous economic losses to growers where it responsible for considerable losses in quantity and quality of herb. This study was constructed to provide and improved sweet basil varieties with tolerance to basil downy mildew. Percentages of downy mildew incidence and severity varied significantly among basil varieties. There was clear separation between the resistant and the most susceptible one. Response to downy mildew among varieties evaluated in this study is consistent with a previous report [59-62]. The five basil varieties tested were divided into five groups based on the resistance reaction screened under field condition into: resistant variety (Lv 1), moderately resistant (Lv 2), moderately susceptible (Lv 3), susceptible (French basil) and highly susceptible (Lv 4). This suggests that genes for resistance reside mainly in Lv 1.

The study of peroxidase, polyphenol oxidase activities and phenolic content in relation to the downy mildew incidence of five basil varieties revealed that peroxidase and polyphenol oxidase activities as well as total phenol content were linearly related to degree of resistance. This shows that unlike the resistant varieties, susceptible varieties failed to show early reaction against the pathogen attack and this may be the reason for establishment of pathogen in host and cause of disease. Our results are in line with previous reports Dai et al. [63] and Satish et al. [64] who reported that the species of genus Vitis (*V. rotundifolia*) that contain high polyphenols content are more resistant to infection caused by *Uncinula necator* and when infected they produce larger amounts of polyphenols than varieties that are more susceptible. The resistant variety contained more gallic acid derivatives and catech in tannins as compared to the susceptible variety. The appearance of flavonoids during the early stage may play an important role in resistance.

Higher peroxidase activity in resistant cultivars can be correlated with higher lignin deposition. Thus, peroxidase plays a key role in scavenging reactive oxygen species as well as participates in physiological process such as lignin formation. Similarly, studies describing correlations of high polyphenol oxidase levels in cultivars or lines with high pathogen resistance continue to provide support for a pathogen defense role of polyphenol oxidase [65]. Li and Steffens [66] suggest several possibilities for the potential anti-pathogen effects of polyphenol oxidase mechanism, including (1) general toxicity of polyphenol oxidase-generated quinones to pathogens and plant cells, accelerating cell death, (2) alkylation and reduced bioavailability of cellular proteins to the pathogen, (3) cross-linking of quinones with protein or other phenolics, forming a physical barrier to pathogens in the cell wall, and (4) quinone redox cycling leading to H_2O_2 and other reactive oxygen species [67].

The essential oil content varied among the tested basil varieties ranged from 0.31-1.38%. The oil content of basil in this study was similar to several literature reports. Suchorska and Osińska [68] studied five forms of sweet basils from Germany, Romains, Hungary and Egypt and reported that the oil content varied from 0.1 to 0.55%. A study by Marotti et al. [11] showed that the content of essential oil in herb of 10 Italian basil cultivars ranged from 0.3 to 0.8%. Galambosi and Szebeni [69] reported oil contents in basil herb from 0.38 to 1.29%, while Seidler-Łoży kowska and Król from 0.23 to 1.67% [70]. In a large study on 270 sweet basil accession in Germany, oil content varied from traces to 2.65% [71]. Such variations in the essential oil content of basil across countries might be attributed to the varied agro climatic conditions of the regions.

On the other hand the five basil varieties classified to three chemotypes due to their content of the dominent component as follows, methyl cinnamate chemotype (Lv 1 and Lv 3), linalool chemotype (Lv 4 and French basil cultivar) and methyl chavicol chemotype (Lv 2). Due to the high content of linalool, methyl chavicol and methyl cinnamte, the studied varieties may become applied in food and perfume industries. However, the total content of phenylpropanoids (Methylchavicol, Isoestragole, Chavicol and Eugenol) and esters (Bornyl acetate and Methyl cinnamate) were recorded higher in the Lv 1, Lv 2 which could explain the tolerance of these varieties against *P. belbahrii* infection. Our results were confirmed by *in vitro* studies by testing leaves extracts and essential oil from different basil varieties on sporangial germination of *P. belbahrii*. Germination of *P. belbahrii* sporangia completely inhibited by leaf extract and essential oil of Lv 1 followed by Lv 2. Similar results obtained by Kocić-Tanackov et al. [72]; El-Shiekh et al. [73] and Elsherbiny et al. [74] investigated the antifungal potential of the basil extract (estragol, trans-α-bergamotene, eucalyptol, trans-ocimene, linalool, methyl-eugenol and methyl cinnamate) against various plant pathogens. Elsherbiny et al. [74] conclude that the antifungal activity of basil extract is apparently related to their higher percentage of aromatic oxygenated monoterpenes (methyl cinnamate) in the extract which increase the permeability of the plasma membrane and inhibit the respiration on mitochondrial membrane of fungi [75,76], lead to lipid peroxidation in fungi and increase ergosterol biosynthesis [77].

CONCLUSION

The resistant variety identified in the present study can be used as a source for downy mildew resistance in the basil-breeding program. The varieties of basil investigated under this study related to methyl cinnamate, linalool and methyl chavicol chemotypes. The phenolic compounds content in basil varieties are responsible for tolerance to fungal infection. The high content of methyl cinnamte, methyl chavicol and linalool in the studied varieties may become applied in food and perfume industries.

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