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Pathological and physiological studies of Downy Mildew of Basil (Ocimum basilicum) Caused by Peronospora belbahrii in Egypt

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Abstract

Basil is the most commercially significant medicinal and aromatic plant, used both fresh and dried, as well as a source of essential oil for perfume and food flavor manufacture. Basil's main composition includes a significant amount of antioxidants and antimicrobial agents. The obligate, biotrophic oomycete pathogen Peronospora belbahrii Thines causes basil downy mildew (BDM). It became a very destructive disease that has caused severe damage and crop loss of sweet basil in Egypt and worldwide. A field survey for disease severity (DS) and disease incidence (DI) of BDM was done in basil cultivation areas in Egypt during two successive growing seasons 2019-2020. The highest percentage of (DS) and (DI) of BDM was (93% and 100%) in 2019 and (95% and 100%) in 2020 in Nassir city of Beni Suef Governorate. Laboratory studies indicated that the highest percentage of spores germination was 33% at temperatures 18^{o}C and the lowest was 3% at 12^{o}C, while no spores germinated at 10^{o}C., the relative humidity percentages 100% and 95% were the most suitable for the highest germination percentage (35% and 33%). The effect of light and dark hours interval (12 hours of light and 12 hours of darkness) was the most suitable for the highest percentage of spores germination (30%). Pathogenicity test by detached leaves method explained that the sporangiophores of P. belbahrii appeared after two days from infection and the severity increased until the 4th day when the whole leaf was infected. Pathogenicity test under greenhouse condition revealed that P. belbahrii severity and incidence reached (96.6 and 100%, respectively) 7 days post-inoculation in case of sowing basil (Baladi cv.) by seeds while in case of using transplants the disease severity and incidence reached (91.4 and 100%, respectively) at 10 days post-inoculation. The varietal reaction of some basil cultivars to BDM under greenhouse showed that Lemon Basil O. americanum var. citriodorum had the lowest disease severity and incidence (11.0% and 21.6%).

Keywords: Basil, Basil downy mildew, Peronospora belbahrii, Basil variety, Survey

1. Introduction

Basil (Ocimum basilicum L., Fam. Lamiaceae) is the most economically important medicinal and aromatic herb crop includes more than 50 species used for both fresh and dry consumption and as a source of essential oil and oleoresin for manufacturing perfumes, and food flavors [1]. The chemical composition of basil contains an important source of antioxidants [2], antimicrobial agents [3] with potential use in food preservation [4], insecticidal activities and it has been found to have in vivo anti-malarial activity [5].

Basil downy mildew was first reported in Uganda in1932 caused by Peronospora spp. causing defoliation and death of sweet basil [6-7]. The disease emerged in Switzerland in 2001 [6], Italy in 2003 [8], France and Belgium in 2004 [9-10], South Africa and Malta in 2005 [11], Iran in 2006, Cameroon 2007 [12] and the United States in 2007 [13-14-15], Argentina in 2008, Cuba and Taiwan in 2009, Hungary and U.K. in 2010 [16-17], Israel and Canada in 2011 [18-19]., and the Czech Republic in 2012 [20], Currently, BDM occurs in all parts of the world where sweet basil is grown. In Egypt basil downy mildew, incited by P. belbahrii Thines, was observed for the first time especially in Beni Suef governorate in 2013 [21] it has become a serious disease in sweet basil and the rapid spread of the pathogen P. belbahrii throughout various herb production regions causing complete crop losses [22].

Epidemics of basil downy mildew largely depend upon climate conditions. The major environmental factors are air humidity, temperature, light, and wind speed. Relative humidity seems to be the major factor for the development of P. belbahrii infection on basil. It became severe when foliage stays wet for extended periods (6 to 12 h) 95% humidity and 18°C [23-18].

The aim of this study is the survey of Basil downy mildew disease at the major production areas in the open field and aqua-ponique system. Characterize disease symptoms and carry up morphological, pathological, and physiological studies.

2. Materials and Methods

Survey of basil downy mildew

An intensive survey for disease severity and incidence was conducted in many regions in Egypt especially in Faiyum governorate in Yosef El Sediq, Beni Suef governorate (6 districts) Beni-Suef city, Nassir, Biba, Sumusta, Al-Fashn, and Ahnasya, in Asyut governorate in Abnob as the sweet basil plants, was cultivated there in large areas and on 6th of October and 10th of Ramadan cites under aqua-ponique system during two successive growing seasons 2019 and 2020.

Disease assessment

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

$$DI = \frac{\text{Number of plants infected}}{\text{Total number of examined plant}} \times 100$$

Scale from 0-3 in which 0=no visible sporulation; 1= scarce sporulation; 2 =moderate sporulation; and 3=heavy sporulation. According to [24-25] a modified

scale by Eslam M. Abdullah was made from (0–6) corresponding to scale as follow: 0= no visible symptoms; 1= from 1 to10 %; 2= from 11 to 25 %; 3= from 26 to50 %; 4= from 51 to75 %; 5= from 76 to 95 %; and 6= from 96 to 100%.

Disease severity was recorded according to the following equation:

$$DS\ \% = [\sum (n \times c)] / (N \times C) \times 100$$

Whereas: n = Number of infected leaves, c = scale number, N=Total number of examined leaves, and C= The highest category number of infections in the scale.

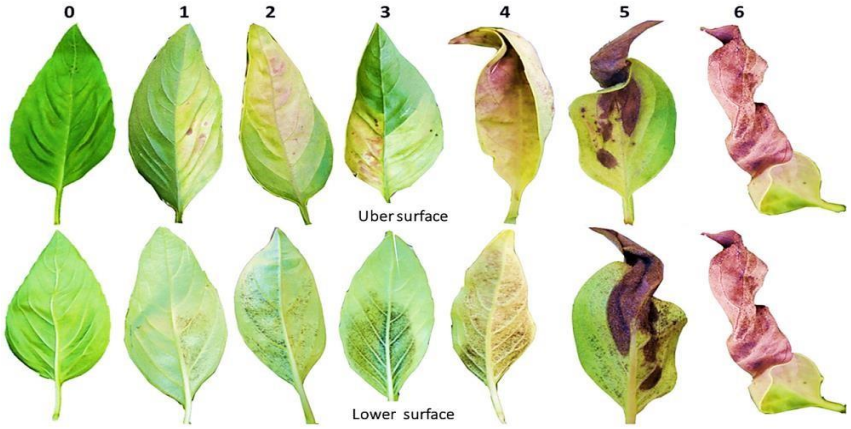

Fig. (1) Symptoms severity of BDM on upper leaf surfaces and sporulation on lower leaf surfaces arranged according to the modified scale from 0 to 6.

Identification of basil downy mildew

Sporangiophores and sporangia were scraped from basil leaves and transferred to microscope slides and mounted in lactophenol for microscopic evaluation. Morphological characteristics of the samples were scrutinized as described by Thines [26] using a compound light microscope (A. KRUSS OPTROIC, Germany, camera software analysis scope image 9.0 H9D). The micrographs were prepared from two samples and two-electron micrographs were taken by scanning using the electron microscope (Quanta FEG 250 model Lowvaccum, FEIUSA). The samples were fixed by immersion (2.5%v/v glutaraldehyde in phosphate buffer, 0.1M, pH7) for 24 h using the modified protocol of [27].

Pathogenicity tests

Preparation of inoculum

The artificial inoculum of P. belbahrii was prepared by washing off fresh sporangia collected from the upper surface of the infected basil leaves from the infected field into cold distilled water containing a few drops of tween 20 and transferred to the laboratory. The obtained sporangial suspension was adjusted with the aid of a hemocytometer to ($1x10^5$ sporangia/ml) [24].

Detached Leaf Assay (DLA)

Fourteen basil leaves from healthy basil plants at age of 40 days were cut and placed into a 12mm Petri dish containing moist Whatman filter paper No.1. All the Petri dishes were sprayed with adjusted sporangia suspension and arrangement with control and 3 replicates replications, wrapped in plastic bags, packed in the incubator, and maintained in the dark overnight. Sporangia were gently washed off from each leaf and counted using a hemocytometer under a microscope [28].

Pathogenicity test under greenhouse

This experiment was conducted in a greenhouse at the Nanophytopathlogy lab - Desert Research Center (DRC). The uniform healthy basil seedlings (Balady variety), and incubated at growth chamber, 10-15 cm length were transplanted individually into 25cm-diameter pots, filled with 3 kg clay-sandy soil (four seedlings per pot), and five replicates for each treatment with one replicate as control. Healthy basil plants were inoculated by spraying with adjusted sporangia suspension on both leaves surfaces until runoff. Inoculated plants were placed on the benches and covered with a transparent polyethylene sheet immediately after artificial inoculation and maintained until the last assessment to obtain the high relative humidity conditions. [29]. Monitoring and scouting the plants for downy mildew and disease incidence and severity were estimated at intervals of 7 days.

Effect of physiological Factors that affect sporulation and germination Effect of Temperature

Several temperatures degrees were tested on the germination of BDM sporangia at the laboratory. Sporangia were harvested with a small paintbrush from the lower surface of basil leaves in a petri dish containing cold sterilized distilled water.1ml of P. belbahrii $1x10^5$ sporangia suspension was put in cavity slides on 9 mm plastic plates. Plates were immediately placed into incubation chambers maintained at 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28°C in darkness. Incubation chamber temperatures were confirmed using analog thermometers. Three cavity slides were used for each temperature treatment. from 24h after incubation, sporangial germination, slides were examined with a 10X light microscope. The germination status of 100 sporangia from each slide was determined. Sporangia were deemed germinated with sub-terminal or lateral

germ tubes only the sporangia were estimated germinated. **[30]**. These experiments were repeated twice.

Effect of Relative Humidity (RH)

The relationship between sporangia germination and relative humidity (R.H) was studied in a growth chamber using different degrees of relative humidity which were maintained in desiccators by proportions of H_2SO_4 mixtures with distilled water that was varied to give vapor pressures equivalent to 75, 80, 85, 90, 95 and 100 percent [31]. R.H. Sporangia were collected by a small paintbrush over clean glass cavity slides. The cavity slides containing sporangia were placed in desiccators. The desiccators were then incubated in an incubator at 18 oC for 24 hours. The percent of germination was calculated by taking the number of germinated sporangia present in microscopic fields [32].

Effect of Light and darkness

To determine the effect of light and dark interval on the production of sporangia and sporangiophores of P. belbahrii, detached basil leaves on a light period and placed on moist filter paper inside 14 cm Petri dishes, and sprayed with$1x10^5$ sporangia suspension. The dishes were covered with transparent poly-ethylene bags to ensure a moist atmosphere (100% R.H.) and placed in illuminated growth chambers, light was supplied by 40W (CW) fluorescent tubes. Aluminum foil was used to cover dark control trays, which were then put in the same cabinet. Different period of light was used in this experiment to 4 four different light (L) /dark (D) periods; as follow 24h L /0h D, 12h L /12h D, 8h L/16h D, and 0h L /24h D for 5 days at 18˚C. Sporulation intensity on individual leaves was visually assessed using a scale was mentioned previously [33]. Another experiment was conducted to determine the impact of different light regimes on sporangia germination. Sporangia which were collected previously were put in cavity slides and incubated at 18˚C. and the light periods was adjusted to 24h L /0h D, 12h L /12h D, 8h L/16h D, and 0h L /24h D for 24 h, then the slide was examined under a light microscope to calculate the number of germinated sporangia.

Different Ages response to downy mildew

This experiment was prepared to study the response of different ages of basil plants to infection by P. belbahrii.

Experiment design: Plants of different ages were selected from cotyledon. (2,4,6,8,10 and 12 of real leaves), then sprayed with P. belbahrii Spore suspension adjusted (1 x 10^5 sporangia/ml) that was prepared before by collecting Fresh spores from sporulating plants into cold distilled water. Control plants were similarly treated with tap water. The control and inoculated plants were placed overnight in a dew chamber at (18 ◦C and 95% RH) in the dark to ensure infection. Care was taken to avoid any contact between the plants [34].

Disease Assessment: Disease symptoms and sporulation were visually estimated daily post inoculation depending on plant age at the time of inoculation [24].

The varietal reaction of some basil cultivars to basil downy mildew

Six cultivars of sweet basil which were brought from BUSTAN AQUAPONICS Co. i.e., Balady, Grand-Vert, Italian, Thai., lemon, and Cinnamon were chosen to test their reaction for infection with P. belbahrii. Seedlings were four plants in each pot, one experiment included 5 replicates. At the four-leaf stage, the plants were inoculated with the sporangial suspensions ($1x10^5$ sporangia/ml) as mentioned before in the pathogenicity test. For each variety with control and five replicates. Disease incidence and severity on the growing varieties were estimated as mentioned before [25].

Fig. (2) Morphological characterization of six basil cultivars (a) Cinnamon, (b) lemon, (c) Italian, (d) Thai. basil, (e) Balady, and (f) Grand-Vert.

Statistical analysis

The obtained results were exposed to statistical analysis of either simple regression & correlation or analysis of variance (ANOVA) [35].

3. Results and Discussion

Survey of basil downy mildew

Data presented in Table(1), showed a survey of basil downy mildew which took place in Faiyum, Beni-Suef, Asyut governorates in open fields, and on the 10th of Ramadan and 6th of October under the aqua-ponique system in two successful seasons during years 2019 and 2020. The highest percentage of Disease Severity (DS) and Disease Incidence (DI) value was recorded in the Beni-Suef governorate in all cities. However, at Nassir city was recorded (93% and 100% of DS and DI) respectively, while in the second season recorded (95% and100% of DS and DI) followed by Sumusta and Ahnasya. The lowest value for DS and DI was recorded in Abnob city Asyut Governorate in the first season (25% and 41% for DS and DI) respectively, while in the second season was recorded (23% and 39% of DS and DI). The other places showed moderate disease incidence and disease severity.

Table (1) Values of basil downy mildew incidence and severity under field condition survey in different cultivation areas in two successful seasons during 2019 and 2020 years

Cities	Disease severity %		Disease incidence %	
	2019	**2020**	**2019**	**2020**
10th of Ramadan	54	50	56	61
6th of October	53	46	64	62
Yosef El Sediq	49	44	52	48
Beni Suef city	91	92	98	95
Sumusta	93	91	100	100
Biba	90	95	98	96
Nassir	93	95	100	100
Al-Fashn	92	95	94	96
Ahnasya	92	91	100	100
Abnob	25	23	41	39
LSD 1%	3.30	2.66	2.41	2.27
Governorates	**2019**	**2020**	**2019**	**2020**
Sharkia	54	50	56	61
Giza	53	46	64	62
Faiyum	64	44	52	48
Beni Suef	92	93	98	98
Asyut	25	23	41	39
		LSD 1%		
Any two governorates but Beni Suef	1.81	1.46	1.32	1.24
Any governorate vs Beni Suef	2.52	2.03	1.84	1.73

***10th Ramadan and 6th October under the aqua-ponique system.**

Survey of basil plantations in either Faiyum, Beni Suef, and Asyut governorates in open fields or in 6th of October and 10th of Ramadan cites under the aqua-ponique system was resulted to confirm that downy mildew is the most destructive disease among the foliar diseases attacking basil plantations. The disease was more virulence in Beni Suef Heavy on the 6th of October and 10th of Ramadan and moderate in Asyut, this could be attributed to the arid cold climate prevailing in Beni-Suef, providing the low temperature and high relative humidity which is favored for downy mildew overall, While under the aqua-ponique system, which depends on growing plants over fish ponds, it may be attributed to the availability of adequate moisture for the pathogen to cause the infection In addition to the absorption of nitrogenous substances from the ponds by basil from fish products, makes the leaves larger and juicier, which leads to easy infection of plants and speed spread of disease.

Disease symptoms and characterization:

Plants of basil Baladi variety were susceptible to downy mildew infection at all almost stages, however, disease symptoms were observed after 20 days of sowing. Leaf yellowing is often the first symptom of basil downy mildew. Yellowed areas are usually bordered by leaf veins. When spores are produced, a characteristic fuzzy, dark gray to purple growth on the underside of the leaves. Morphological identification of P. belbahrii sporangia from infected plants was smooth, ovoid to subglobose range between 73.0 Long x 53.1 Width µm and 46.4 Long x 36.0 Width µm, light brown, presented in Table (2). Sporangiophore arising from stromata, erect, firstly cylindrical then branching dichotomously three to five times, and hyaline. the terminal ends are claw-shaped, each branch carried one sporangium, this description was belonging to Peronospora's genius. The least length of Sporangiophore was (240 µm x 3 µm width) in Abnob samples while the longest length (680 µm x 7 µm width) was provided by samples of 10th of Ramadan and 6th of October. Other wide sporangiophores characterized were nearby.

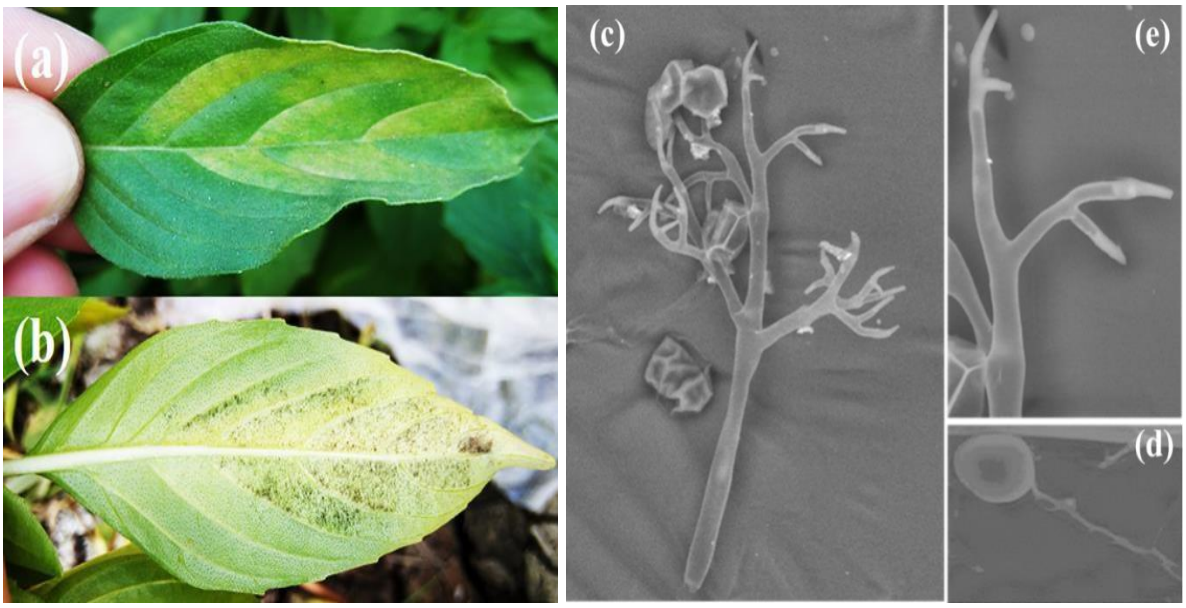

Fig (3) Disease symptoms of downy mildew on the upper and lower surface of basil leaves (a) yellowish on the upper surface (b) sporangia and sporangiophore on the lower surface at the left side and scanning electron microscope image showing; (c) sporangiophore tree-like structure, (d) sporangia (e) sporangiophore branches end in two division, the terminal ends are claw shaped, each branch carried one sporangium, this description was belonging to Peronospora genius at the right side.

Table (2) Morphological measurements of P. belbahrii sporangia collected from different cultivation areas using light microscope camera with software analysis under 40X microscope lens.

Governorates	Cites	Sporangia	
		Long (µm)	Wide (µm)
Sharkia	**10th of Ramadan**	73	53.1
Giza	**6th of October**	72.1	54.4
Faiyum	**Yosef El Sediq**	57.2	51.2
Beni Suef	**Sumusta**	69.7	61.6
	Biba	65.3	52,6
	Ahnasya	64.6	55.1
	Nassir	67.2	58.5
	Al-Fashn	64.2	56.8
	Beni Suef city	62.8	55.5
Asyut	**Abnob**	46.4	36.0

Pathogenicity tests Detached leave assay (DLA)

Inoculation of detached basil leaves with a sporangial suspension P. belbahrii the causal agent of downy mildew, resulted in well-defined necrosis Fig. (4) that quickly propagated throughout the leaves. All leaves completely infected with P. belbahrii in a few days, which had previously been demonstrated to be harmful in whole plant inoculations, developed necrosis. The basil leaves were sprayed with P. belbahrii spore suspension and incubated at 18 ^{o}C, 95 % (RH) in light and dark intervals. The sporangiophores were paired on the second day of incubation, and the infection was enhanced by the day until it was full on the fourth day. When compared to non-infected leaves (control).

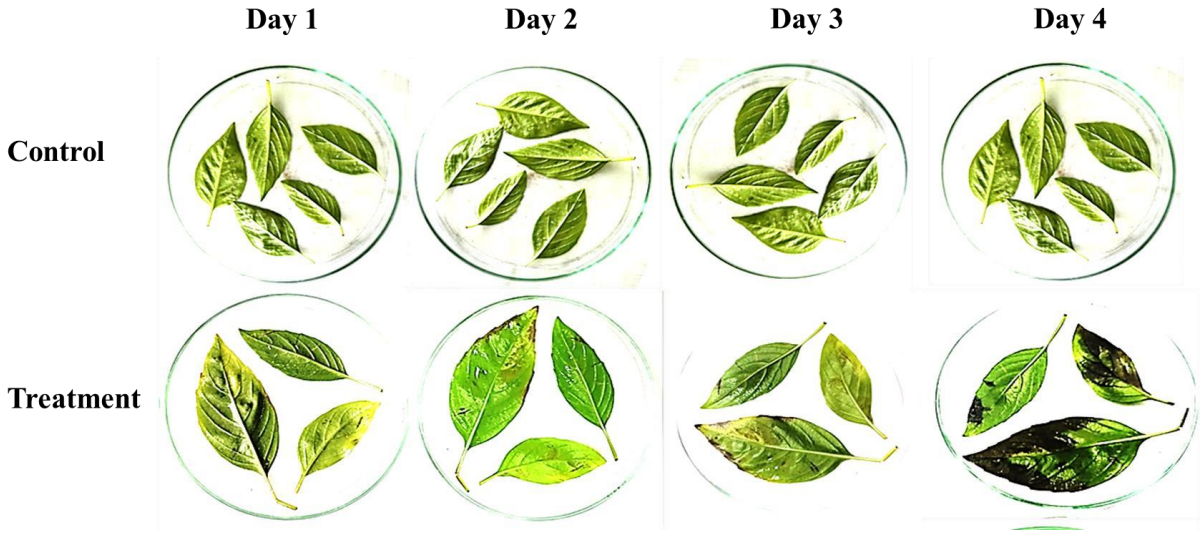

Fig. (4) Results of Detached Leaf Assays (DLA) of basil leaves, non-infected (control) and infected with Downy mildew pathogen P. belbahrii respectively under laboratory condition during 4 consecutive days.

Greenhouse experiment

Data present in Table (3) showed that all tested P. belbahrii isolates proved to be pathogenic to basil plants, causing symptoms of downy mildew, compared with the control. In this regard, Baldy cv. significantly recorded the highest disease severity and incidence (96.6 and 100%, DS and DI respectively). Sowing Baladi cv. of basil by seeds recorded higher downy mildew severity and incidence than transplants recorded (91.4 and 100% DS and DI respectively). It's worthy to mention that the incubation period of basil downy mildew was shorter in sowing basil as seeds than transplants which recorded 7 and10 days respectively.

Table (3) Pathogenicity tests for P. belbahrii causal agent of downy mildew on basil plants (cv. Baladi), under greenhouse condition.

Source	Days post-inoculation	Disease severity %	DS % per day of infection inducement	Disease incidence%	DI % per day of infection inducement
Seeds	7	96.6	13.80	100	14.29
Transplants	10	91.4	9.14	100	10.00
LSD 1 %		1.24	0.65	-	-

In this study, the detected symptoms on artificially inoculated basil plants under greenhouses or on detached leaves in were similar to those observed in the field, with leaves of infected plants initially slightly chlorotic, especially near the center vein, within 2-3 days, a characteristic grey, furry growth appeared on the lower surface of infected leaves, and these symptoms occasionally occurred on the top side of leaves, although the distribution of the disease was generally uniform. however, these symptoms were like those described by [36-37-38]. P. belbahrii sporulation is known to occur on the abaxial side of affected leaves, with dark purplish-brown sporangia formed during suitable weather circumstances, similar to other downy mildew infections [39].

Effect of physiological Factors that affect sporulation and germination Effect of Temperature

Temperature is an important environmental factor that influences the germination of sporangia. The sporangia of P. belbahrii were incubated at 10, 12, 14, 16, 18, 20, 22, 24, and 28 ºC for 24 hours in sterile distilled water. Data in Table (4) and Fig. (5) shown that the sporangia did not germinate when they were incubated at a temperature of 10 ºC and 28 ºC, while germination started from the degree of 12 ºC (3%), The maximum germination of sporangia were obtained at 18 ºC (33%), moreover, the germination rate of sporangia was not significantly different between 18 and 20 °C, While there was a clear significant difference between these two degrees and the rest of the different temperatures degrees. The effect of temperature on sporangia germination was determined, the higher the temperature, the higher the rate of sporangia germination until it reached the maximal germination rate between 18 and 20, then the germination rate began to decline when the temperature was increased.

Table (4) Effect of different temperature degrees on sporangia germination of P. belbahrii.

Temperature °C	Sporangia Germination %
10	0
12	3
14	10
16	20
18	33
20	31
22	21
24	12
26	7
28	0
LSD 1 %	1.59

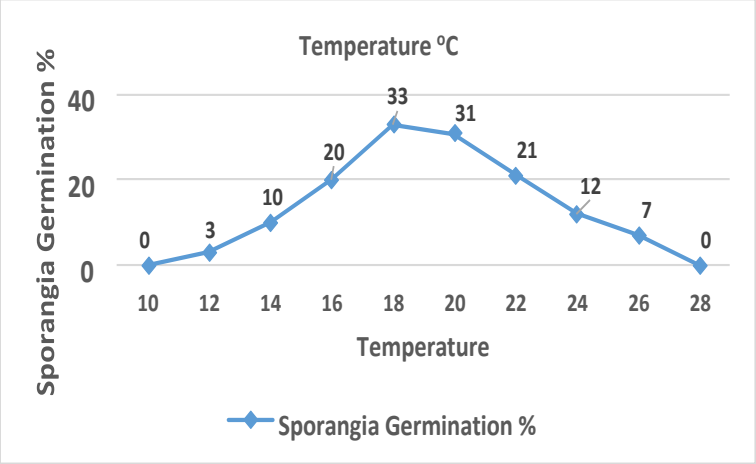

Fig. (5) Effect of various degrees of temperature on P. belbahrii sporangia germination.

Temperature levels play a major role in the germination of sporangia, infection, and basil downy mildew disease development [23]and other downy mildew [40-41]. Therefore, we focused our work to study the impacts of temperature on sporangia germination in vitro under a wide range of temperatures, between (10 ◦C and 28 ◦C). The germination of sporangia started at 12 °C and maximum germination was observed under 18°C (33%). As the temperature increased, the reduction of sporangial germination was observed. This result agreed with [42] who reported that temperature is an important environmental factor that affects the germination of sporangia, and the results are consistent with [43] who found that sporangia of P. belbahrii required incubation at 15–20°C to germinate.

Effect of Relative Humidity (RH)

The data provided in Table (5) and Fig. (6) illustrate that there is a significant increase in the percentage of P. belbahrii sporangia germination as the relative humidity was increased. Moreover, we found a positive correlation between a percentage of humidity and sporangia germination rate, with the sporangia attaining the highest percent of germination as the surrounding RH increased. At RH 100 %, the percentage of germination was (35 %), while at RH 95 %, the percentage of germination was (33 %), but the germination significantly decreased at RH 90 % with a germination rate (27 %) and almost nullified at RH 75 percent (0 %).

Table (5) Effect of relative humidity on sporangia germination of P. belbahrii.

Relative Humidity (RH)	75	80	85	90	95	100	**LSD 1 %**
Sporangia Germination %	0.0	7.0	15.0	27.0	33.0	35.0	1.43

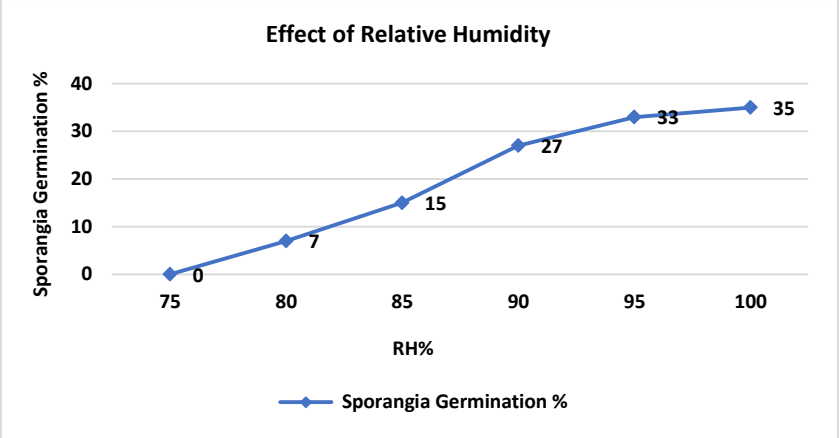

Fig. (6) Effect of relative humidity on P. belbahrii sporangia germination

Relative humidity is also an important environmental factor that influences sporangia germination. Germination and sporulation of the oomycete foliar plant pathogen P. belbahrii are strongly dependent on the availability of free moisture and high humidity. In this study, we found that germination of P. belbahrii requires high relative humidity, similar to other foliar downy mildew diseases. The absolute necessity of free leaf moisture for infection was reported for other foliar downy mildew agents [44-45]. In the present investigation, the gradual increase in sporangia germination was noticed with the increase in percent relative humidity. The maximum sporangial germination was observed at 100 % RH (35%). These results agreed with [43] established those microscopic examinations of sporulation in a dew chamber at 18 ^{o}C in the dark showed the following: at around 3 h, white sporangiophores start emerging from the stomata openings on the lower leaf surface; at 4 to 5 h, the sporangiophores branched once or twice at 6 h, the sporangiophores branched thrice at 7.5 to 8 h.

Effect of Light and Darkness intervals

The effect of light on sporulation of P. belbahrii was tested on sporangia grown on the basil leaves in the laboratory under a growth chamber. The sporangia were incubated at 18 ^{o}C using four different light and darkness. The best sporangia germination was at (12/12h) followed by (8 in light/16 in darkness). Sporangia did not germinate continuously in the light or dark. Light and darkness on detached leaves of basil there was a clear influence of light regime on sporulation of basil downy mildew on basil leaves for disease severity, Sporulation on the lower leaf surface of detached leaves were strongly suppressed by CW light Inoculum spore concentration had no effect on downy mildew severity in continuous light, sporulation was significantly inhibited. while in (12h light /12h dark) produced dichotomously branched sporangiophores with many spores. In (8h light /16h dark) conditions produced branched sporangiophores with few sporangia. In (0h light / 24h dark) conditions produced branched sporangiophores with no sporangia.

Table (6) Effect of light and darkness on P. belbahrii sporangia germination and sporangia formation on leaves

Hour light	Darkness	Sporangia Germination %	Disease severity%	Sporangiophore and sporangia on basil leaves
24	0	0	0	Not produce any sporangia and sporangiophore
12	12	30	76	Best production of sporangia and sporangiophore
8	16	18	15.2	Produce few no. of sporangiophore and sporangia
0	24	11	3	Produce few no. of sporangiophore no sporangia
LSD 1 %		1.18	1.67	

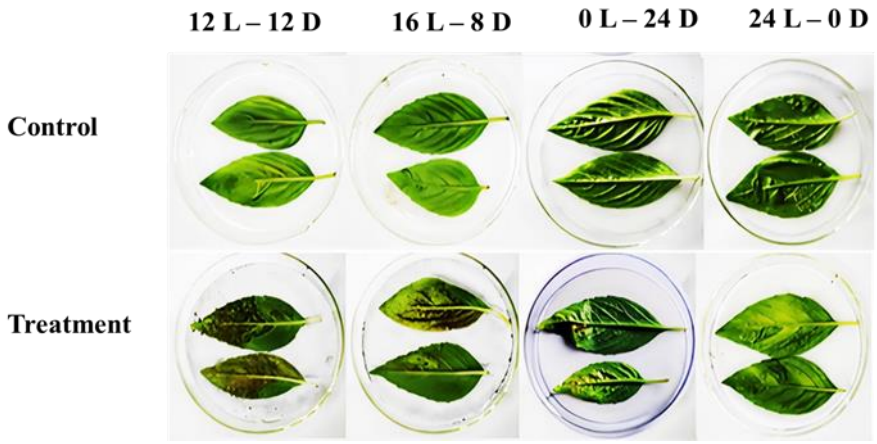

Fig (7) P. belbahrii sporangia formation on leaves under various hours of light and darknes5s; 12h in dark with 12 h in light, 16 h in light with 8 h in dark, 24 h in dark, and 24 h in light.

In this study, light and darkness regimes were tested on the sporangia of P. belbahrii the causal agent of downy mildew disease in vitro. Where the effect of periods of light and dark on sporangia was studied to measure the germination rate of sporangia and sporulation of P. belbahrii on detached basil leaves. Light microscopy revealed that while dichotomously branched sporangiophores with abundant spores were formed in the dark, abnormally branched sporangiophores, with no spores, were formed under light conditions where a degree of 12h /12h was recorded as the best light and dark hours, due to the fact that germination of sporangia needs darkness, while sporangiophores need light periods. This result agreed with [18-33].

Different Ages Response to Downy mildew

The results showed a negative correlation was found between plant age and disease infection almost all plants infected at the cotyledon stage became after 7 days post-inoculation (dpi). A gradual decrease in the proportion of infected plants occurred in older plants. Thus, inoculation at the 2, 4, 6, 8, 10, and 12-leaf stages produced about 95, 87, 70, 63, 32, and 25% infected plants, respectively Table (7). Neither disease symptoms, nor sporulation was seen on the control plants treated with water.

Table (7) The relationship between plant age and the appearance of infection of P. belbahrii in basil plants.

Growth stage	Age(days)	Tested plants	Days for infection appearance	Infected Plants (%)	Infection inducement per day
Cotyledons	6	100	7	96	13.71
2 leaves	10	100	7	95	13.57
4 leaves	13	100	7	87	12.43
6 leaves	16	100	10	70	7.00
8 leaves	19	100	10	63	6.30
10 leaves	21	100	14	32	2.29
12 leaves	24	100	14	25	1.79

***dpi =days post-inoculation**

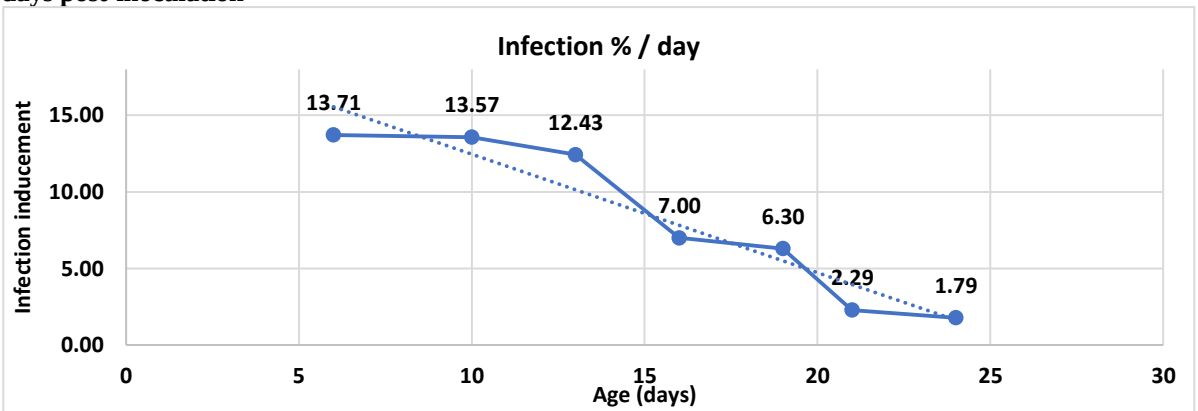

Fig (8) Infection inducement % / day in response to plant age at the time of artificial infection inducement.

Fig (9) Shows the relation between various ages of basil plants and the infection of P. belbahrii, from the cotyledons stage to the 12-leaf stage. The inoculated plants were incubated in a growth chamber to allow the infection.

The major purposes of the present study were to examine the ability of downy mildew pathogen P belbahrii to infect basil plants at any age and the most affected stages for the infection. Here, we show that infection can be readily produced in artificially inoculated basil plants under growth chamber conditions. When P. belbahrii can infect the basil plants stages from cotyledon stage to 12-leaf stage the inoculated plants became artificially infected within 7 and 14 days after inoculation, respectively. Where the older plants showed more resistance to infection by downy mildew due to structural resistance The thickness of the cuticle increases as the plants age increases the thickening of the cell walls, which make it difficult to penetrate through the germ tubes of the pathogen. In contrast to plants in the younger stages, which were more susceptible to infection [34-46].

The varietal reaction of some basil cultivars to basil downy mildew

Data presented in Table (8) show the response of 6 basil cultivars Baladi, Grand-Vert, Italian, Thai., lemon, and Cinnamon grown under greenhouse conditions from April to May 2020 to the infection with P. belbahrii (BDM, basil downy mildew). Leaves showed various morphological changes like (fold up, fold down, flat, flat-down, or flat-up) as shown in Fig. (10) Basil plants of CVs. Baladi and Italian were more susceptible to P. belbahrii infection than cv. Grand-Vert, Thai. While Lemon was highly resistant. Concerning Italian cv. had the shorter incubation period (7 days), followed by Baladi cv. plants which had (8 days), and the greater severity and incidence was (95.4% and 100 %) as compared to basil cv. Grand-Vert, Thai. and lemon that expressed (10 days) incubation period. (100 % DI and 95 % DS for Italian), (97.8 % DI and 94 % DS for Grand-Vert), (96 % DI and 95% DS for Thai Basil), and (21.6% DI and 11 % DS for Lemon Basil).

Table (8) Response of six basil cultivars to BDM infection under greenhouse conditions.

Cultivars	Days post-inoculation	DS %	DS % / day	DI %	DI % / day
Baladi **O. basilicum**	**10**	**95.4**	**9.54**	**100**	**10.00**
Grand-Vert **O. basilicum var. basil.**	**10**	**94.0**	**9.40**	**97.8**	**9.78**
Italian **O. basilicum**	**8**	**95.0**	**11.88**	**100**	**12.50**
Thai Basil **O. basilicum var. thyrsiflorum**	**10**	**95.2**	**9.52**	**96.0**	**9.60**
Lemon Basil **O. americanum var. citriodorum**	**10**	**11.0**	**1.10**	**21.6**	**2.16**
Cinnamon **O. basilicum var. cinnamomum**	**14**	**95.0**	**6.79**	**93.2**	**6.66**
LSD 1 %		**1.97**	**0.29**	**2.01**	**0.28**

Disease Severity (DS) is shown as a percentage of infested leaf area, Disease incidence (DI) is shown as a percentage of total infected plants. 14 days after inoculating selected basil cultivars at the 4-leaf stage with P. belbahrii isolate (1 × 10^5 sporangia suspension).

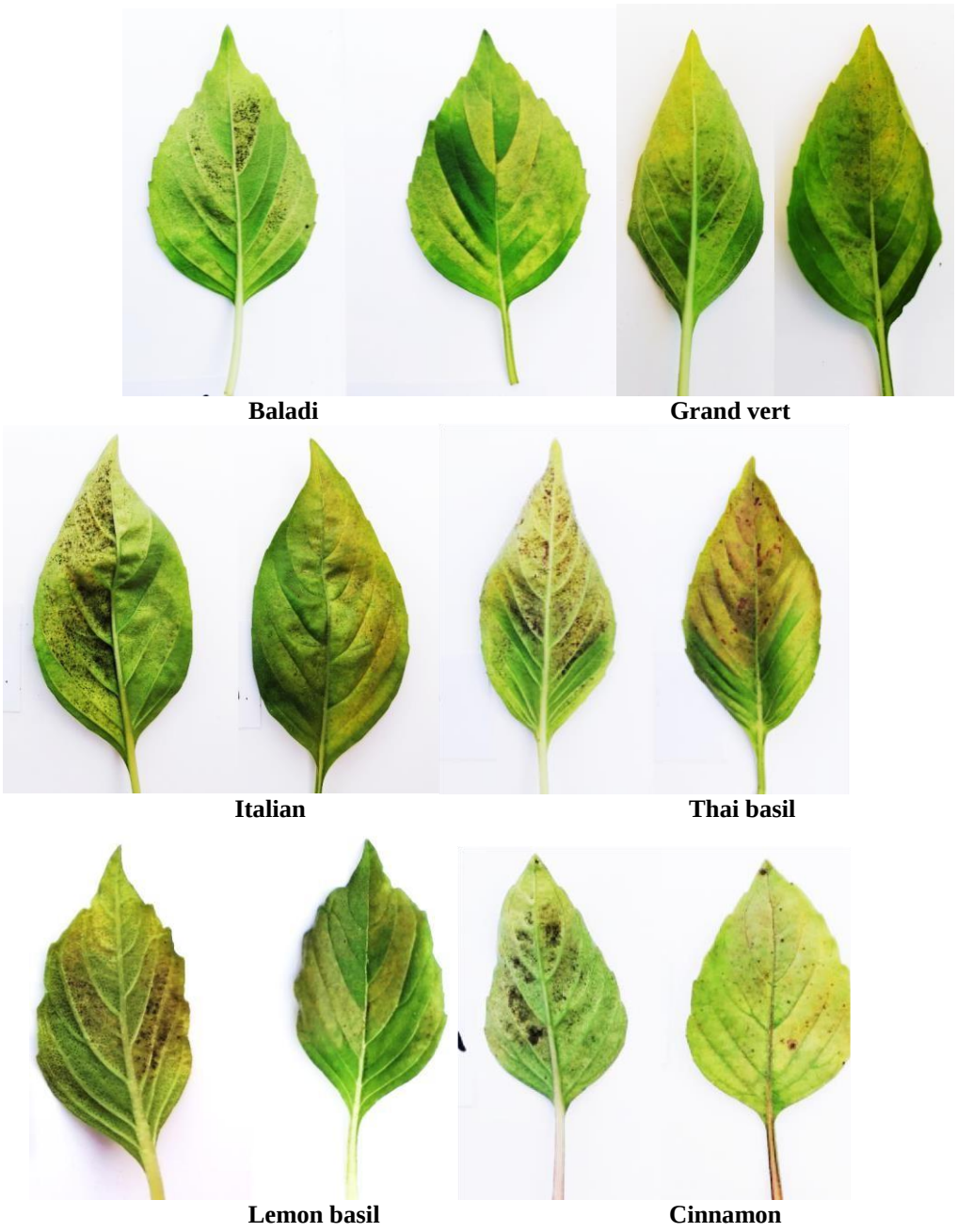

Fig. (10) Downy mildew disease symptoms on different species of basil.

The reaction of six sweet basil cultivars to downy mildew disease was studied. The results indicated that Balady Italian Grand vert, Thai Basil, and Cinnamon were highly significantly susceptible to downy mildew disease, while Lemon Basil expressed high resistance for the infection only very light sporulation was visible on the margins of older, and chlorotic leaves. These results were those reported by several researchers who concluded that plant cultivars are variance in their reactions to downy mildew infection this agreed with [24-25-47] which showed tolerance as well as with basils originating from O. citriodorum (Lemon Basil) and O. americanum displayed no signs or symptoms of basil downy mildew, but the leaf morphology, habit, and aroma of this cultivar differ significantly from that of other O. basilicum species.

4. Conclusion

According to the survey that was conducted in basil cultivation areas, Beni Suef Governorate recorded the highest infection rate of (DS) and (DI) caused by P. belbahrii. Under laboratory conditions, the favorable conditions to germinating P. belbahrii sporangia were 18^{o}C at 100% and 95% (RH), and a period of (12 hours of light and 12 hours of darkness). The P. belbahrii isolate was able to infect all basil variety that was tested with the high rate of DS and DI with Balady var. and the lowest rate with Lemon var.

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