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Effect of Some Ecological Factors on Growth of *pestalotiopsis* spp. Isolated From Mastic Shrubs Leaves

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ABSTRACT:

In this study the effects of nutrient media, temperature, Relative Humidity, light and pH on mycelial growth of three species of *Pestalotiopsis* (*P. fici*,*P. guepinii* and *P. palmarum*) were evaluated for the better growth. PSA was most suitable for mycelial growth, whereas, the PLA appeared to be the best medium for conidiomata formation. The fungi grew from 10 to 40ºC, with optimum growth at 20-30ºC with no growth at 5ºC. The growth of the fungus was observed to increase with increase in relative humidity from 35% to 100%. Different light regimes had impact on mycelium growth Darkness was more suitable for the growth, while alternative light caused growth rings appearance in cultures. No visible growth at 4 pH. The fungus grew at pH 4.5 – 7. Optimum growth occurred at pH 7 with 94% for *P. palmarum* and 74% for *P. guepinii* after 5 days of incubation at 25ºC. A notable decline in growth occurred when pH increased from 7.5 to 9.

Keyword: *mastic shrub, fungi, Pestalotiopsis spp., linear growth, ecological factors*

INTRODUCTION

Mastic, *Pestacia lentiscus* L. (Anacardiaceae) is a small evergreen tree or shrub, up to 4 min height, and distributed in the Mediterranean region up to 700 m above sea level. It is an important medicinal plant grown in several regions geographical in Al-Jabal Al-Akhdar province at 45' - 59' 32° N and long. 44' - 30' 21° E in northeastern of Libya. Three different symptoms, leaf blight, death the tip of leaf and silvering gray leaf spots were observed on mastic leaves and their causes as plant pathogenic fungi, *Pestalotiopsis fici*, *P. guepinii* and *P. palmarum* respectively.were the first recorded by [1]. Environmental factors such as humidity and temperature plays an important role in dispersing fungi spores in air for short and long distances and when spores deposited a solid or liquid surface and if conditions of moisture and food are appropriate, they germinate [2,3]. Several studies were conducted on the effect of environmental factors on the growth of the pathogen [4,5,6]. Environmental factors such as relative humidity, temperature, light, pH and media were reported to have a profound influence on the infectiveness of a variety of fungi. Growth rate of fungi vary depending on different ecological factors [7,8,9,10]. Bearing in mind the economic importance of mastic and the disease complexes associated with the shrubs, this study which was aimed to give a better understanding of the organisms involved in the disease expression and serves as a pointer towards control mechanisms.

MATERIALS AND METHODS:

Fungi isolates

Pestalotiopsis fici, *P. guepinii* and *P. palmarum* were obtained from microbiology lab. in Department of Plant Protection, Faculty of Agriculture, Omer AlMuhktar University, El-Beida city, Libya

Environmental studies.

The effect of some cultural conditions such as nutrient media, incubation temperature, Relative Humidity (RH), light and pH on the linear growth of three fungi was carried out. Five mm diameter agar plugs were removed with a sterile cork borer from the edges of colonies and one such plug was placed in the center of each 90 mm Petri plate containing media. Plates were then wrapped with Parafilm. There were four replicate of each treatment. At each treatment the plates were arranged in a randomized complete block design. Colony diameter in each plate was measured.

Growth variation on solid media

Potato Sucrose Agar (PSA), Pestacia Leaf Agar (PLA) and Water Agar (WA) were used for the study of radial growth, mycelial pigments and conidiomata formation. Twenty ml of the media were poured after sterilization in 90 mm petri plates Petri Plates were inoculated with fungi. and incubated in darkness at 25°C for 5 days. After the end of incubation period, the linear growth (cm) was determined.

Growth variation at different temperature

PSA media Petri dishes were inoculated with *Pestalotiopsis fici*, *P. guepinii* and *P. palmarum* and incubated in darkness at 5, 10, 15, 20, 25, 30, 35 and 40°C for 5 days. After the end of incubation period, the linear growth (cm) was determined.

Growth variation at different Relative Humidity levels

Seven levels of RH were maintained by mixtures of appropriate combinations of concentrated sulphoric acid and distilled water (Table 1) as described by Ayyasamy and Baskaran [11]. Mixtures were taken in the desiccators for each level of RH.

Table 1. Preparation of solutions for maintenance of different Relative Humidity (RH) levels

Treatment No.	Distilled water (mL)	Sulphoric acid (mL)	RH (%)
1	100.0	0.0	100
2	88.5	11.5	95
3	80.0	20.0	90
4	70.0	30.0	75
5	62.0	38.0	65
6	56.0	44.0	50
7	49.0	51.0	35

PSA media Petri dishes were inoculated with tested fungi, kept in desiccators and covered with lids and sealed off with parafelem tape. Desiccators were incubated in darkness at 25°C for 5 days. After the end of incubation period, the linear growth (cm) was determined.

Growth variation at photo-periods:

The effect of light on mycelial growth was evaluated on PSA. Flourescent lamp and black carbon paper were used to maintain different photo-period *viz.*, continuous light, continuous dark, 12 hrs light and 12 hrs dark There were four replicate plates for each medium under each light regime. Observations were recorded as mentioned above.

Growth variation at different pH levels:

Eleven different pH levels study from 4.0 to 8.0 with a 0.5 difference were adjusted to the PSA medium were used to evaluate their effect on the mycelial growth. This was done before autoclaving with the help of HCL (0.1 N) and NaOH (0.1 N) by using the digital pH meter. The pH adjusted medium were poured into Petri plates, inoculated and incubated as aforementioned for the studies. Variations in radial growth was recorded

RESULTS AND DISCUSSION:

Effect of culture media: The radial mycelial growth rates of *Pestalotiopsis* spp. were affected by culture media (Fig. 1). In general, PSA was most favorable for fast radial growth of mycelium than PLA and WA. Colony diameter was observed significantly superior on PSA medium (87- 91,5 mm) followed by PLA medium (68.9- 78 mm) after 5 days of inoculation. WA medium (28.3 30.3 mm) recorded comparatively less growth of tested fungi. Formation of pigments and conidiomata on different media were studied and results are presented in Fig. 2 a.

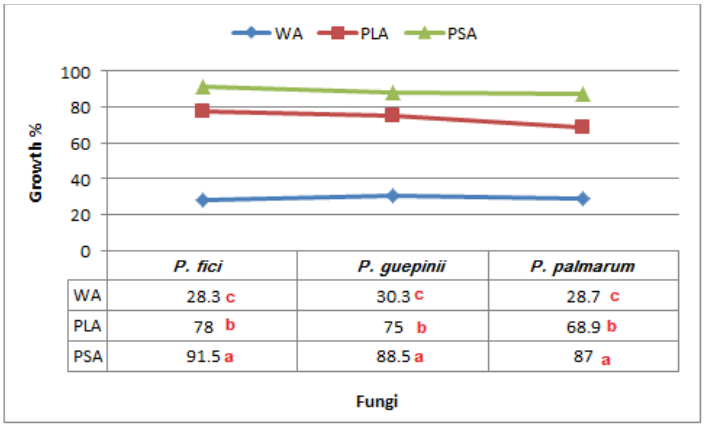

Figure 1. Effect of different growth media on the linear growth (%) of tested fungi

At the beginning color of the mycelia was white and became darker with the age. The reverse side of colonies were olive brown and brown to black on PLA medium. The highest number of conidimata per plate was formed on PLA, whereas; PSA and WA either produced least number of conidiomata or not at all respectively (Fig. 2 b). Type of culture media and their chemical compositions significantly affected the mycelia growth rate and conidial production of *Phoma exigua* [12]. Several study was used PSA culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi [13,14,15]. Osman et al. [16] observed that growth rate of *Alternaria alternata* and *Fusarium oxysporum* was faster on sucrose containing media as the carbon source. Most fungi thrive on PDA, but this can be too rich in nutrients, thus encouraging the mycelial growth with ultimate loss of sporulation [17]. In the present study, *Pestalotiopsis* spp. showed heavy conidiomata formation in PLA. This result may be attributed to its low glucose/sucrose content suppresses the overgrowth of fast growing species and induces sporulation. Qureshi and Meah [18] recorded highest number of pycnidia on mango leaf extract followed by PDA. .

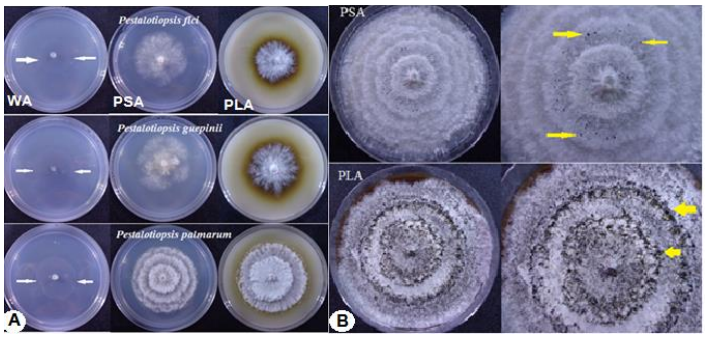

Figure 2. Effect of different media on formation of pigment (A) and conidiomata (B). after 5 and 20 days of incubation.

Effect of temperature:

The results of the effect of temperature on the growth of *Pestalotiopsis* species is showed in Fig. 3. No colony growth was noted at 5°C, whereas all the temperature regimes tested supported the growth fungi. However, it was observed that at 20°C and 25°C the fungi attained maximum growth of 80% to 94% after 5 days of incubation. The growth fungi was drastically reduced below 10°C and started to decline above 35°C. Slight changes in colony morphology were observed at 10 and 40ºC, being slower at 10ºC with fluffy aerial mycelium. At 30- 40ºC colony sometimes was not completely circular, but with thin mycelium. Temperature plays an important role in influencing the growth of fungi [19]. Normally, the growth temperature for the majority of fungi is between 25˚C to 30˚C and above 40˚C the growth is poor [20]. The various optimal temperatures for mycelial growth, conidiomata production and germination of *Pestalotiopsis* spp. obtained in the laboratory coincide favorably with the commonly observed field temperatures. This may therefore not only account for the survival of the pathogen in the field (soil), but also enhances disease incitement and development.

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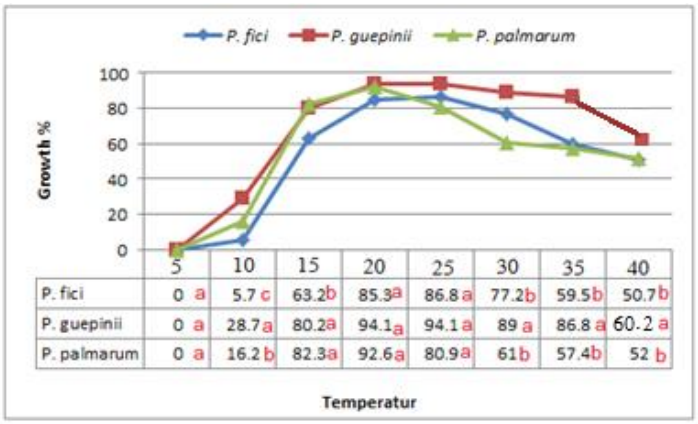
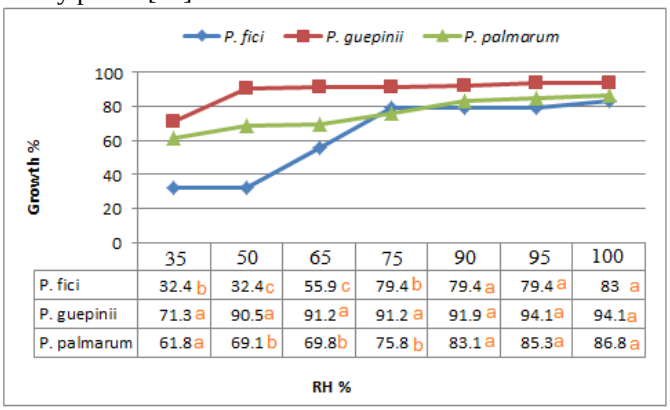


	5	10	15	20	25	30	35	40
P. fici	0 a	5.7 c	63.2b	85.3a	86.8 a	77.2b	59.5b	50.7b
P. guepinii	0 a	28.7a	80.2a	94.1a	94.1 a	89 a	86.8 a	60.2 a
P. palmarum	0 a	16.2 b	82.3a	92.6a	80.9a	61b	57.4b	52 b

Figure 3. Effect of incubation temperature on the linear growth (%) of tested fungi

Effect of relative humidity:

This study also revealed that 65% to 100% relative humidity were most suitable for the growth of all fungi (Fig. 4). Results of the present study partly agree with the report of Al-Garni, et al. [21] and Nawar [22] found that the growth of *A. niger* increased regularly with increasing RH up to 100%, and with Ibrahim, et al. [5] who showed maximum growth of *Heminthsporium fulvum* at 92.5 and 100% relative humidity. Likewise Sharma and Sharma [23] reported that relative humidity of 95% supported maximum growth of *Trichophyton mentagrophytes*. Also Knight [24] investigated the effect of relative humidity on the growth *Trichophyton mentagrophytes* and found that relative humidity of 97% was the best for the growth of the fungus. High atmospheric humidity has been reported to favour the initiation of the diseases of many plants [25].



	35	50	65	75	90	95	100
P. fici	32.4 b	32.4c	55.9 c	79.4 b	79.4 a	79.4 a	83 a
P. guepinii	71.3 a	90.5a	91.2a	91.2 a	91.9 a	94.1a	94.1a
P. palmarum	61.8a	69.1 b	69.8b	75.8 b	83.1 a	85.3a	86.8 a

Figure 4. Effect of Relative Humidity on the linear growth (%) of tested fungi

Effect of light:

When the tested fungi were grown on PSA medium solidified under continuous light in a Petri dish, the vegetative growth was reduced. The best growth of fungi was shown under the dark continuous. Alternating light 12 hours light plus 12 hours darkness produced alternative rings between fluffy and abundant in cultures growth (Figuers 5 and 6). Changed growth rate or yield, spore production and pigment formation are among the responses of many fungi to light [26, 27]. Light inhibits growth, glucose uptake and phosphorylation but does not inhibit the uptake of lysine. A low molecular weight substance produced or accumulating in the light inhibits the phosphorylation of glucose. It is suggested that the inhibition of glucose uptake and phosphorylation precedes conidiation and that conidiation may be the result of starvation caused by this light-induced inhibition [27, 28].

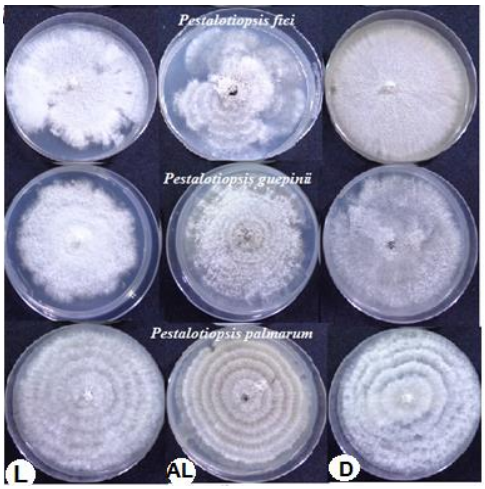
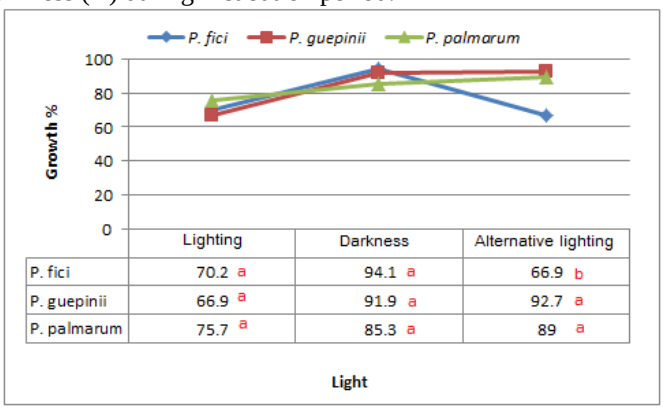


Figure 5. Growth rings formation on cultures of tested fungi after expose to alternative lighting (AL) compared with lighting (L) and darkness (D) during incubation period.

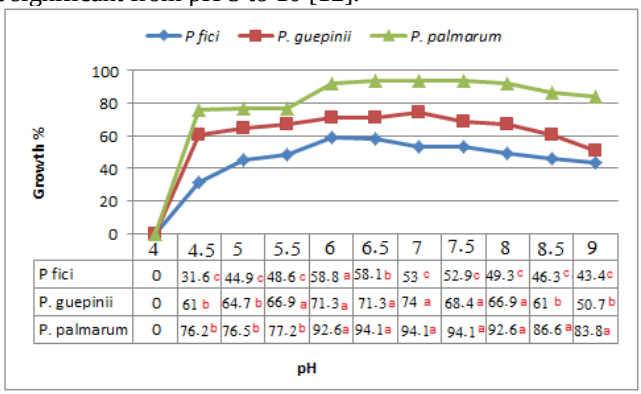


	Lighting	Darkness	Alternative lighting
P. fici	70.2 a	94.1 a	66.9 b
P. guepinii	66.9 a	91.9 a	92.7 a
P. palmarum	75.7 a	85.3 a	89 a

Figure 6. Effect of variation at photo-periods on the linear growth (%) of tested fungi

Effect of pH concentration:

Mycelia growth of of *P. fici* , *P. guepinii* and *P. palmarum* were studied at 11 pH level 4, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8, 8.5 and 9. Result in this study indicated that, all the three fungi grow on a wide range of pH. All tested fungi followed similar tends in response to changes in pH (Fig. 7). No visible growth at 4 pH. The fungus grew at pH 4.5 – 7. Optimum growth occurred at pH 7 with 94% for *P. palmarum* and 74% for *P. guepinii* after 5 days of incubation at 25ºC. A notable decline in growth occurred when pH increased from 7.5 to 9. The optimum pH for mycelial growth of most fungi is 5 - 6.5 [29]. Narasimha and Rajagopalan [30] reported that *A. helianthi* can grow at pH from 4.5- 10. However, the effect of pH on mycelial growth and conidial germination was not significant from pH 5 to 10 [12].



	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9
P fici	0	31.6 c	44.9 c	48.6 c	58.8 a	58.1 b	53 c	52.9c	49.3 c	46.3 c	43.4c
P. guepinii	0	61 b	64.7 b	66.9 a	71.3a	71.3a	74 a	68.4 a	66.9 a	61 b	50.7 b
P. palmarum	0	76.2 b	76.5 b	77.2 b	92.6a	94.1a	94.1a	94.1 a	92.6 a	86.6 a	83.8a

Figure 7. Effect of different concentration of pH on the linear growth (%) of tested fungi

CONCLUSION:

This study confirms that growth and sporulation of a pathogen is directly proportional to its aggressiveness on its host. Thus the study emphasizes the importance of the substrate which can influence the inoculum potential and aggressiveness of an organism and in turn influence the host – parasite relationship. The present study will help to maintain the fungus in the laboratory condition for preparation of inocula for different studies concerning control of the plant disease. The study also concluded that the culture media are essential growing factor for controlling the growth and sporulation of phytopathogenic fungi and effect of different temperature, relative humidity, lighting and pH studies provide the information about a optimum environmental condition necessary for excellent growth and sporulation of fungi. The results may be correlated with climatological data such as Rainfall, sunshine and relative humidity coupled temperature this determines whether there are adequate period of wetness for rot infection and subsequent disease establishment.

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