





# Penicillium AND Aspergillus SPECIES CHARACTERIZATION: ADAPTATION TO ENVIRONMENTAL FACTORS AND SENSITIVITY TO AQUEOUS MEDICINAL PLANTS EXTRACTS

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## ABSTRACT

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In this study, nine fungal species belonging to genus *Aspergillus* (6) and *Penicillium* (3), which were found on whiteflies cadavers adults collected from cucumber cultivation fields in Basra Iraq, are characterized regarding mycelial growth rate at different culture media, pH levels and temperature degrees, and their sensitivity under *in vitro* assay to aqueous extracts of *Ocimum sanctum*, *Mentha arvensis* and *Allium sativum* at different concentrations. *Aspergillus* and *Penicillium* species showed a wide range of tolerance to different culture media, pH levels and temperature degrees which decrease significantly their mycelial growth rate, although no of these parameters were able to inhibit them completely. This study has demonstrated further information on conditions favoring mycelial growth of *Aspergillus* and *Penicillium* species (at pH 6 and temperature between 25 and 30°C). In sensitivity assays, all tested species were susceptible to the aqueous extracts. The mycelial growth inhibition was significantly higher with the high concentrations of aqueous extract. The aqueous *M. arvensis* extracts at 150 mg/mL succeed to decrease the mycelial growth of *A. parasiticus* (82.5%) and *A. fumigatus* (81.67%). It appears that the growth rates of *A. parasiticus* (94.17%) and *A. fumigatus* (93.38%) was significantly inhibited by the aqueous *A. sativum* extracts at 150 mg/mL. *A. fumigatus* (80.83%) and *A. parasiticus* (76.67%) were high significantly inhibited by the aqueous extracts of *O. sanctum* at 150 mg/mL. The response of *Aspergillus* spp. and *Penicillium* spp. to natural situations and aqueous extracts is important to understand their behaviour and to predict fungal spoilage on crops fruits.

**Contribution/Originality:** This study documents the evaluation of the aqueous extracts efficacy of *Ocimum sanctum*, *Mentha arvensis* and *Allium sativum* against *Aspergillus* and *Penicillium* species and their sensitivity to culture media, pH and temperature under laboratory conditions.

## 1. INTRODUCTION

The *Aspergillus* and *Penicillium* genera belong to the order *Eurotiales* and contain a diverse number of species in terms of phylogenetic, morphological and physiological characters. *Aspergillus* spp. and *Penicillium* spp. possessed a worldwide distribution and over large a range of ecological habitats [1, 2]. These two genera are ubiquitous and can be colonize the soil, air, vegetation, insects, nematodes and indoor environments [3, 4].

*Penicillium* and *Aspergillus* species grow up in different environment habitats and necessitate various specific nutrition sources for their growth and reproduction. These fungi are easily influenced to nutritional and

physiological factors. In indeed, slight environmental factors variations may modify their morphological characters [2, 5]. In general, the necessities of nutritional for the fungi growth are not complex, but numerous fungal species require different physico-chemical and nutritional conditions [6-8]. Several researches evaluated the influence of different culture media components as well as the some physiological parameters on conditions favoring mycelial growth to genus *Aspergillus* and *Penicillium*. Consequently, the knowledge of their requirements for temperature, hydrogen ion concentration (pH), nutrients is important criteria for understanding the fungal ecology [5]. In ecological habitats of *Aspergillus* and *Penicillium* species, the nutrients availability at different pH levels and temperature degrees in numerous ecological conditions can influence growth, sporulation and activity of *Aspergillus* spp. and *Penicillium* spp [4, 9, 10]. Generally, these fungi may develop with a huge range of optimum temperatures from 20 to 30°C, but it is could be grow in the range 0-40°C under *in vitro* condition [4, 11]. In the same sense, the highest metabolic activities, cellular growth, conidial production and sporulation of *Aspergillus* spp. and *Penicillium* spp. were suitable for an optimum pH between 5 and 7 in liquid media [7, 10].

The post-harvest diseases, caused by *Aspergillus* spp. and *Penicillium* spp., are responsible in decreasing of the fruits quantity and quality in Iraq [12]. Biological control demonstrated high efficiency in controlling several species of *Aspergillus* and *Penicillium* globally, throughout using multiple strategies and tactics such as aqueous plants extracts, antagonistic fungi and bacteria, extracts algae, etc. to maintain these pathogens under the economic injury [13, 14]. Many aqueous plants extracts (*Ocimum sanctum*, *Mentha arvensis* and *Allium sativum*) presented high efficacy against these post-harvest diseases [15-17]. This eco-friendly approach present essentially no risk to human health and most studies show that they are relatively innocuous to natural antagonistics. Aqueous extracts are able to inhibit the mycelial growth and spore germination, block the appressorium formation and induce plant resistance [18, 19].

The study aims was to obtain new phenotypic information under *in vitro* conditions for these *Aspergillus* and *Penicillium* species by evaluating: their mycelial growth at different culture media, pH levels and temperature degrees; and their sensitivity to aqueous extracts of *O. sanctum*, *M. arvensis* and *A. sativum* at different concentrations.

## 2. MATERIAL AND METHODS

### 2.1. Fungal Material

Nine fungal species belonging to genus *Aspergillus*, i.e., *A. parasiticus*, *A. niger*, *A. carbonarius*, *A. flavus*, *A. nidulans* and *A. fumigatus*, and *Penicillium* i.e., *P. expansum*, *P. digitatum* and *P. italicum* were used in this study.

The fungal species used in the present research were obtained from the Laboratory of Plant Protection, College of Agriculture (Basra, Iraq), and they were isolated from whiteflies cadavers adults collected from experimental field cultivated by cucumber plants in Basra, Iraq during January-December 2017.

### 2.2. Effect of Different Culture Media on Mycelial Growth of *Aspergillus* and *Penicillium* Species

Three culture media (PDA, Komada medium, Carrot Agar (CA)) were used to determine the most appropriate for the mycelial development of *Penicillium* and *Aspergillus* species. One disc plug (8 mm diameter) of each fungal species (10-days-old culture) was placed in the center of the each medium.

The mycelial growth rate (MGR as cm per day (cm/day)) was measured when it reached at least two thirds of the Petri dish (almost at 7 days of colony growth) by evaluating the perpendicular diameters average of each colony. Three replicates (five plates / replicate) for each individual treatment were conducted and the plates were incubated at 25°C. The optimum culture media (O<sub>CM</sub>) for mycelial growth rate of each *Penicillium* and *Aspergillus* species was plotted against culture media and a curve was fitted by a cubic polynomial regression ( $y = a + bx + cx^2 + dx^3$ ).

### 2.3. Effect of pH on Mycelial Growth of *Aspergillus* and *Penicillium* Species

The pH effect was determined using cultures grown on PDA to assess the most appropriate for the mycelial development of *Penicillium* and *Aspergillus* species. One disc plug (8 mm diameter) of each fungal species (10-days-old culture) was placed in the center of PDA medium which were adjusted to pH 4, 6 and 8. The MGR was measured when it reached at least two thirds of the Petri dish (almost at 7 days of colony growth) by evaluating the perpendicular diameters average of each colony. Three replicates (five plates / replicate) for each individual treatment were conducted and the plates were incubated at 25°C. The optimum pH for mycelial growth rate of each *Penicillium* and *Aspergillus* species was plotted against pH and a curve was fitted by a cubic polynomial regression ( $y = a + bx + cx^2 + dx^3$ ).

### 2.4. Effect of Temperature on Mycelial Growth of *Aspergillus* and *Penicillium* Species

The temperature effect was determined using cultures grown on PDA to assess the most appropriate for the mycelial development of *Penicillium* and *Aspergillus* species. One disc plug (8 mm diameter) of each fungal species (10-days-old culture) was placed in the center of PDA medium. The MGR was measured when it reached at least two thirds of the Petri dish (almost at 7 days of colony growth) by evaluating the perpendicular diameters average of each colony. The plates were incubated at 20, 25, 30 and 40°C. Three replicates (five plates / replicate) for each individual treatment were conducted. The optimum temperature for mycelial growth rate of each *Penicillium* and *Aspergillus* species was plotted against temperature and a curve was fitted by a cubic polynomial regression ( $y = a + bx + cx^2 + dx^3$ ).

### 2.5. Preparation of Aqueous Plant Extracts

*Ocimum sanctum* (Lamiaceae), *Mentha arvensis* (Lamiaceae) and *Allium sativum* (Liliaceae) were thoroughly washed in tap water, and surface-sterilized with sodium hypochlorite (NaOCl) 3% for 10 min and then rinsed in three changes of sterile distilled water. Fresh vegetables materials were air dried and used for fresh extract preparation. In case of leaves (*O. sanctum* and *M. arvensis*) and bulbs (*A. sativum*), extracts were prepared by crushing known weight of fresh materials with distilled water at ratio of 1:1 (w/v). The pulverized mass of leaves plant was squeezed through four folds of the fine cloth and the extracts were centrifuged at 3000 rpm for 20 min. The supernatants were filtered through Whatman filter paper and the filtrate was collected in Erlenmeyer flasks (250 mL) [3].

### 2.6. In Vitro Evaluation of Aqueous Plant Extracts

Relative efficacy of aqueous plant extracts on mycelial growth inhibition was studied *in vitro*, using poisoned food technique. In this experiment, three aqueous plant extracts were used for their efficacy. However, the requisite amount of the filtrate of each plant extract was incorporated to PDA medium to get different concentrations (75, 100 and 150 mg/mL) under aseptic conditions [3]. 15 ml of poisoned medium was poured in each sterilized petriplates and suitable checks were maintained. One disc plug (0.5 cm diameter) of each fungal species (4 days-old culture) was placed in the center of the poisoned medium. A plug of pathogen was used as control treatment (without treatment). Three replicates (five plates / replicate) for each individual treatment were conducted and the plates were incubated at 25±2°C for 7 days. The percent of radial mycelial growth inhibition (I) was evaluated according to the formula of Rhouma, et al. [20].  $I (\%) = (1 - C_n/C_0) \times 100$ ; where:  $C_n$  is the radial growth diameter of the tested fungal in the presence of the treatment.  $C_0$  is the growth diameter of the tested fungal in the control treatment. The degree of mycelial inhibition were estimated on the basis of a rating scale described by Anith, et al. [13] (with +: Poor inhibition (pathogen mycelium couldn't overgrow the antagonist colony); ++: Moderate inhibition (pathogen mycelium could not reach the antagonist colony); +++: High inhibition (zone of inhibition >2 mm but <5 mm); ++++: Very high inhibition (>5 mm)) [21].

### 2.7. Statistical Analysis

The data were analyzed by ANOVA using SPSS version 20.0 statistical software (SPSS, SAS Institute, USA), to evaluate parameter values differences. Differences between treatments were determined by Duncan multiple range test at 5% of significance level.

## 3. RESULTS AND DISCUSSION

### 3.1. Effect of Different Culture Media on Mycelial Growth of *Aspergillus* and *Penicillium* Species

The different culture media exerted high significant differences on mycelia growth rate of *Penicillium* and *Aspergillus* species ( $P < 0.01$ ). All *Penicillium* spp. and *Aspergillus* spp. grew at all culture media (PDA, CA and KOMADA) with varying MGR. Statistical analyses indicated that the most favorable medium was PDA with a MGR ranged from 0.9 (*P. digitatum*) to 1.09 cm/day (*P. italicum*), followed by the CA (with MGR varied from 0.27 (*P. digitatum*) to 0.46 cm/day (*A. niger*)) and KOMADA (with MGR varied from 0.17 (*A. nidulans*) to 0.36 (*A. niger*)) media (Figure 1). The cubic polynomial regression ( $y = a + bx + cx^2 + dx^3$ ) selected to describe the mycelial growth rate at different culture media, adjusted MGR data with  $R^2 > 0.99$  for all *Penicillium* spp. and *Aspergillus* spp. (Figure 1).

### 3.2. Effect of pH on Mycelial Growth of *Aspergillus* and *Penicillium* Species

The results of the pH effect on the mycelial growth rate of *Penicillium* and *Aspergillus* species are presented in Figure 2. High significant differences ( $P < 0.01$ ) were noted on MGR of the tested species. It appears that pH 6 exhibited the highest MGR for all tested species with a rate varied between 0.79 (*P. expansum*) and 1.09 cm/day (*A. niger*). However, the radial growth decreased progressively when the PDA medium was adjusted to pH 4, with a value ranged from 0.31 (*P. italicum*) to 0.48 cm/day (*A. nidulans*) (Figure 2). The cubic polynomial regression ( $y = a + bx + cx^2 + dx^3$ ) selected to describe the mycelial growth rate at different pH, adjusted MGR data with  $R^2 > 0.99$  for all *Penicillium* spp. and *Aspergillus* spp. (Figure 2).

### 3.3. Effect of Temperature on Mycelial Growth of *Aspergillus* and *Penicillium* Species

The temperature effect on mycelial growth rate is shown in Figure 3. All species were able to grow on PDA over a range of temperatures from 20 to 40°C with varying MGR. Statistical analysis revealed a high significant difference of MGR of *Penicillium* and *Aspergillus* species incubated at different temperature ( $P < 0.01$ ). Some exception was noted at 30°C; in fact the differences of the growth rates of tested species were almost negligible at this temperature ( $P \geq 0.05$ ). Optimum mycelial growth temperatures for all species ranged between 25 (with MGR ranged from 0.94 for *A. nidulans* to 1.12 cm/day for *P. italicum*) and 30°C (with MGR varied from 0.8 (*A. nidulans*) to 0.86 cm/day (*A. parasiticus* and *A. niger*)) (Figure 3). The cubic polynomial regression ( $y = a + bx + cx^2 + dx^3$ ) selected to describe the mycelial growth rate at different temperature, adjusted MGR data with  $R^2 > 0.762$  for all *Penicillium* spp. and *Aspergillus* spp. (Figure 3).

The main objective of this research was to obtain biological information about *Aspergillus* (6) and *Penicillium* (3) species, regarding mycelial growth at different culture media, pH and temperature levels. Our results reveal great variability of the MGR to culture media, pH and temperature of the *Aspergillus* and *Penicillium* species tested. This is the first detailed study of the culture media, pH and temperature effects on MGR of *Aspergillus* and *Penicillium* species in Iraq. This study has demonstrated further information on conditions favoring mycelial growth of *Aspergillus* spp. and *Penicillium* spp. (culture media = PDA; pH = 6; T = 25-30°C). It has been revealed that temperature and pH are central criteria for understanding the fungal ecology Ahmed and Naresh [5]. Rosfarizan, et al. [22] demonstrated that the highest metabolic activities and cellular growth of filamentous fungi (*Aspergillus* spp. and *Penicillium* spp.) were suitable for an optimum pH between 5 and 6 (acidic pH). Deshmukh, et al. [6] noted that *Aspergillus* spp. and *Penicillium* spp. grow on PDA over a range of pH from 3 to 8 with maximum production of dry

mycelial weight and sporulation at pH 5.5 and 6.5, respectively. David, et al. [10] reported that the mycelial growth of *Aspergillus* spp. isolated from grapes was influenced better at pH 4 and 7 than at pH 2.6, whatever water activity level. Ahmed and Naresh [5] noted that the mycelial growth of *Aspergillus* spp. could be affected by pH in development medium; directly (action on the surfaces of cell) or indirectly (effect on the nutrients availability). Abubakar, et al. [7] observed that the highest spores formation number and mycelial growth of *A. parasiticus* were obtained at pH 5 and the lowest at pH 10. These studies indicate that higher alkaline medium is not suitable for *A. parasiticus* development. Elizabeth and Trinci [23] studied the effect of pH on mycelial growth of *Penicillium* spp. These authors noted that the maximum hyphal growths were observed at pH 6. In general, *Aspergillus* spp. are more tolerant to alkaline pH while *Penicillium* spp. are more tolerant to acidic pH Wheeler, et al. [1]. Cao, et al. [11] revealed that all isolates tested of *Penicillium* spp. grew at optimally temperatures ranged from 17 to 28°C. The optimum temperature on germination and mycelial growth of *P. digitatum* and *P. italicum* was occurred at 25°C, but it is could be grow in the range 6-37°C [12]. These differences could be due to the culture medium used and the isolate studied. In another study, *P. italicum* can be germinated at lower temperatures than *P. digitatum*, and even at 0°C. This is supported by work done by Wyatt and Parish [2] on orange juice serum agar. In the same sense, *P. digitatum* and *P. italicum* grow optimally at 25°C [2]. Recently, Pang, et al. [4] suggested that the optimum mycelial growth temperatures of *Aspergillus* spp. were ranged from 25 to 30°C. The mycelial growth of *A. niger* may grow at temperatures ranging from 10 to 37°C, with an optimum varied from 30 to 37°C [9]. Furthermore, these optimum temperatures were observed in the field close to harvest time as previously reported by Bellí, et al. [9]. Our temperature experiments indicate that *Aspergillus* and *Penicillium* species are mesophilic fungal. Similar results were documented by Cao, et al. [11] and Boughalleb-M'Hamdi, et al. [24]. The ecological requirements knowledge of *Aspergillus* spp. and *Penicillium* spp. is important to understand their behaviour in natural situations and to predict fungal spoilage on crops fruits.

### 3.4. In Vitro Evaluation of Aqueous Plant Extracts

Data presented in Tables 1, 2 and 3 indicated clearly that the three aqueous plants extracts exerted high significant reduction (<0.01) on radial mycelial growth of fungal species at different concentrations after 7 days of incubation using poison food technique. All concentrations of aqueous extract exhibited better inhibition than the control. Concentration of the aqueous plant extracts affected mycelial growth, which, more the concentration is important more the percent of radial mycelial growth inhibition increased under *in vitro* conditions. In fact, the aqueous plants extracts at 150 mg/mL showed a good ability to limit the mycelial growth of all tested fungal species. As shown in Table 1, the aqueous *M. arvensis* extracts with a concentration of 150 mg/mL succeed to decrease the mycelial growth of *A. parasiticus* (82.5%), *A. fumigatus* (81.67%) and *A. carbonarius* (72.92%). However, *P. expansum* and *P. digitatum* showed a good resistance against the three concentrations of aqueous extract with inhibition rate below 40%. The results from the effect of aqueous *A. sativum* extracts on mycelial growth inhibition of *Aspergillus* and *Penicillium* species are shown in Table 2. It appears that the growth rates of *A. parasiticus* (94.17%), *A. fumigatus* (93.38%) and *A. niger* (89.58%) was significantly inhibited by the aqueous extracts at 150 mg/mL compared to the other tested concentrations. The results of the aqueous *O. sanctum* extracts efficacy on mycelial growth inhibition under *in vitro* condition are presented in Table 3. *A. fumigatus* (80.83%) and *A. parasiticus* (76.67%) were high significantly inhibited by the aqueous extracts of *O. sanctum* at 150 mg/mL. At 100 mg/mL, the extract also showed a significant inhibitory effect against these two fungi with a value of 67.08 and 62.92%, respectively. The obtained data showed that *A. sativum*, *O. sanctum* and *M. arvensis* extract strongly inhibited *Aspergillus* species mycelium growth than of *Penicillium* species. The scale of potency of the three extracts in inhibiting the *Aspergillus* and *Penicillium* species mycelial growth is as follows: *A. sativum* > *O. sanctum* > *M. arvensis*. Abnormal hyphal swelling, curling, short branching, and accumulation of protoplasm in mycelia were observed on the mycelia of *A.*

*parasiticus*, *A. fumigatus*, *A. carponrius* and *A. niger* exposed to 150 mg/mL of all aqueous plant extract, control mycelia grew profusely and normally with uniform thickness.

Present results are in analogy with many reports. [Gibriel, et al. \[25\]](#) showed that *Mentha* sp. extracts inhibits the *A. flavus* mycelial growth. The extracts are also effective on *A. ochraceus* mycelial growth inhibition and ochratoxin production. [Manoorkar and Gachande \[26\]](#) reported that the leaf extract of *M. arvensis* and *O. sanctum* at 30% concentration were the most effective against *A. niger*, *A. flavus*, *A. terreus*, *A. fumigatus* and *P. citrinum* under *in vitro* condition. [Koka, et al. \[16\]](#) noted that the solvent extracted of *M. arvensis* revealed a good antifungal activity against *P. expansum*, *P. chrysogenum* and *A. niger*. The leaf extract of *Ocimum* sp. showed the highest mycelial growth inhibition (82.8-87.7%) against *A. flavus* [Iram, et al. \[14\]](#). [Saranya, et al. \[17\]](#) demonstrated that *O. sanctum* leaves extract had significant inhibitory effect against both *Penicillium* sp. and *A. niger*, but slightly higher rate of inhibition was recorded in *Penicillium* sp. It was observed by [Rizwana \[19\]](#) that aqueous *O. sanctum* extract at 100% concentration was more effective in *A. niger* growth inhibition. Furthermore, [Irkin and Korukluoglu \[18\]](#) showed that the plant extracts of *Allium* spp. greatly (325 mg/mL) decreased the mycelial growth of *A. niger*, with a reduction of the colony diameter up to 50%. [Akinmusire, et al. \[15\]](#) determined the interaction between aqueous *A. sativum* extract at 200 mg/mL concentration and *A. niger* (93.03%), *A. ustus* (100%) and *Penicillium* spp. (92.97%) involving increased the radial mycelial growth inhibition, which supports our arguments. Earlier, [Pai and Platt \[27\]](#) evaluated the efficacy of some botanicals plants against *A. nidulans* and *P. niger*. They observed that extracts of *A. sativum* bulbs effectively reduced the mycelial growth. The appressorium is an important fungal structure during the penetration process; therefore, the aqueous plant extracts shows a spore germination inhibition and appressorium formation blocking [Iram, et al. \[14\]](#). [Chohan, et al. \[28\]](#) revealed that the aqueous plant extracts are rich in secondary metabolites (flavonoids, saponins, terpenoids, steroids, tannins, coumarins, alkaloids, phenols, etc.), which could be responsible for their higher antifungal activity against *Penicillium* and *Aspergillus* species. Previous study noted that coumarins and their derivatives showed good antimicrobial activities against fungal [\[28\]](#).

**Table-1.** Effect of aqueous leaf extracts of *M. arvensis* at different concentrations (75, 100 and 150 mg/mL) on mycelial growth inhibition of *Aspergillus* and *Penicillium* species after 7 days of incubation at 25±2°C.

Treatments	Percent of radial mycelial growth inhibition				Degree of mycelial inhibition		
	75 mg/mL	100 mg/mL	150 mg/mL	P-value	75 mg/mL	100 mg/mL	150 mg/mL
<i>Aspergillus parasiticus</i>	57.5 <sup>aC(a)</sup>	68.75 <sup>aB</sup>	82.5 <sup>aA</sup>	<0.01	++++	++++	++++
<i>A. niger</i>	45 <sup>a</sup>	56.25 <sup>a</sup>	70 <sup>a</sup>	≥0.05	+++	++++	++++
<i>A. carponrius</i> □	47.92 <sup>a</sup>	59.17 <sup>a</sup>	72.92 <sup>a</sup>	≥0.05	+++	++++	++++
<i>A. flavus</i>	23.33 <sup>bcC</sup>	34.58 <sup>bcB</sup>	48.33 <sup>bcA</sup>	<0.01	++	+++	+++
<i>A. nidulans</i>	46.67 <sup>a</sup>	57.92 <sup>a</sup>	71.67 <sup>a</sup>	≥0.05	+++	++++	++++
<i>A. fumigatus</i>	56.67 <sup>aB</sup>	67.92 <sup>aAB</sup>	81.67 <sup>aA</sup>	<0.05	++++	++++	++++
<i>Penicillium expansum</i>	15.42 <sup>c</sup>	26.67 <sup>c</sup>	40.42 <sup>c</sup>	≥0.05	++	+++	+++
<i>P. digitatum</i>	17.08 <sup>cC</sup>	28.33 <sup>cb</sup>	42.08 <sup>ca</sup>	<0.01	++	+++	+++
<i>P. italicum</i>	38.75 <sup>abC</sup>	50 <sup>abB</sup>	63.75 <sup>ba</sup>	<0.01	+++	++++	++++
P-value <sup>(b)</sup>	<0.01	<0.01	<0.01	Nd	Nd	Nd	Nd
Interactions	T	<0.01			Nd	Nd	Nd
	C	<0.01			Nd	Nd	Nd
	T x C	≥0.05			Nd	Nd	Nd

Note:

<sup>(a)</sup> Duncan's Multiple Range Test, values followed by different superscripts are significantly different at P≤0.05.

<sup>(b)</sup> Probabilities associated with individual F tests.

Capital letters are for means comparison in the same row.

Small letters are for comparison of means in the same column.

Data are the average of 5 Petri dishes per replicate (with 3 replicates).

Mycelial growth inhibition percentage (I %) = (1 - Cn/Co) x 100; where: Cn is the radial growth diameter of the pathogen in the presence of the treatment. Co is the growth diameter of the pathogen in the control treatment.

+: Poor inhibition (pathogen mycelium couldn't overgrow the antagonist colony); ++: Moderate inhibition (pathogen mycelium could not reach the antagonist colony); +++: High inhibition (zone of inhibition >2 mm but <5 mm); ++++: Very high inhibition (>5 mm).

Nd: not determined.

T: Treatments.

C: Concentrations.

The higher concentration (150 mg/mL) of *A. sativum*, *O. sanctum* and *M. arvensis* extracts exerted maximum inhibitory effects on mycelial growth of *Penicillium* and *Aspergillus* species. Similar results were documented by Iram, et al. [14]. Chohan, et al. [28] and Saranya, et al. [17] demonstrated that the mycelial growth inhibition was significantly higher with the high concentrations of extract of *A. sativum* and *O. basilicum*.

**Table-2.** Effect of aqueous bulb extracts of *A. sativum* at different concentrations (75, 100 and 150 mg/mL) on mycelial growth inhibition of *Aspergillus* and *Penicillium* species after 7 days of incubation at 25±2°C.

Treatments	Percent of radial mycelial growth inhibition				Degree of mycelial inhibition		
	75 mg/mL	100 mg/mL	150 mg/mL	P-value	75 mg/mL	100 mg/mL	150 mg/mL
<i>Aspergillus parasiticus</i>	69.17 <sup>aC(a)</sup>	80.42 <sup>aB</sup>	94.17 <sup>aA</sup>	<0.01	++++	++++	++++
<i>A. niger</i>	64.58 <sup>abB</sup>	75.83 <sup>aAB</sup>	89.58 <sup>aA</sup>	<0.05	++++	++++	++++
<i>A. carbonarius</i>	57 <sup>ab</sup>	68.25 <sup>ab</sup>	82 <sup>ab</sup>	≥0.05	++++	++++	++++
<i>A. flavus</i>	35.83 <sup>cdC</sup>	47.08 <sup>cdB</sup>	60.83 <sup>cdA</sup>	<0.01	+++	+++	++++
<i>A. nidulans</i>	59.17 <sup>ab</sup>	70.42 <sup>ab</sup>	84.17 <sup>a</sup>	≥0.05	++++	++++	++++
<i>A. fumigatus</i>	68.38 <sup>aB</sup>	79.63 <sup>aB</sup>	93.38 <sup>aA</sup>	<0.01	+++	+++	++++
<i>Penicillium expansum</i>	36.25 <sup>cdC</sup>	47.5 <sup>cdB</sup>	61.25 <sup>cdA</sup>	<0.01	+++	+++	++++
<i>P. digitatum</i>	33.75 <sup>d</sup>	45 <sup>d</sup>	58.75 <sup>d</sup>	≥0.05	++	++	++++
<i>P. italicum</i>	51.25 <sup>bcC</sup>	62.5 <sup>bcB</sup>	76.25 <sup>bcA</sup>	<0.01	++++	++++	++++
P-value <sup>(b)</sup>	<0.01	<0.01	<0.01	Nd	Nd	Nd	Nd
Interactions	T	<0.01			Nd	Nd	Nd
	C	<0.01			Nd	Nd	Nd
	T x C	≥0.05			Nd	Nd	Nd

Note:

(a) Duncan's Multiple Range Test, values followed by different superscripts are significantly different at P≤0.05.

(b) Probabilities associated with individual F tests.

Capital letters are for means comparison in the same row.

Small letters are for comparison of means in the same column.

Data are the average of 5 Petri dishes per replicate (with 3 replicates).

Mycelial growth inhibition percentage (I %) = (1 - Cn/C0) x 100; where: Cn is the radial growth diameter of the pathogen in the presence of the treatment. C0 is the growth diameter of the pathogen in the control treatment.

+: Poor inhibition (pathogen mycelium couldn't overgrow the antagonist colony); ++: Moderate inhibition (pathogen mycelium could not reach the antagonist colony); +++: High inhibition (zone of inhibition >2 mm but <5 mm); ++++: Very high inhibition (>5 mm).

Nd: not determined.

T: Treatments.

C: Concentrations.

**Table-3.** Effect of aqueous leaf extracts of *O. sanctum* at different concentrations (75, 100 and 150 mg/mL) on mycelial growth inhibition of *Aspergillus* and *Penicillium* species after 7 days of incubation at 25±2°C.

Treatments	Percent of radial mycelial growth inhibition				Degree of mycelial inhibition		
	75 mg/mL	100 mg/mL	150 mg/mL	P-value	75 mg/mL	100 mg/mL	150 mg/mL
<i>Aspergillus parasiticus</i>	51.67 <sup>a(a)</sup>	62.92 <sup>a</sup>	76.67 <sup>a</sup>	≥0.05	++++	++++	++++
<i>A. niger</i>	42.92 <sup>a</sup>	54.17 <sup>a</sup>	67.92 <sup>a</sup>	≥0.05	+++	++++	++++
<i>A. carbonarius</i>	46.67 <sup>a</sup>	57.92 <sup>a</sup>	71.67 <sup>a</sup>	≥0.05	+++	++++	++++
<i>A. flavus</i>	21.25 <sup>bB</sup>	32.5 <sup>bB</sup>	46.25 <sup>bA</sup>	<0.01	++	+++	+++
<i>A. nidulans</i>	44.17 <sup>a</sup>	55.42 <sup>a</sup>	69.17 <sup>a</sup>	≥0.05	+++	++++	++++
<i>A. fumigatus</i>	55.83 <sup>aB</sup>	67.08 <sup>aB</sup>	80.83 <sup>aA</sup>	<0.01	++++	++++	++++
<i>Penicillium expansum</i>	13.33 <sup>c</sup>	24.58 <sup>c</sup>	38.33 <sup>c</sup>	≥0.05	++	++	+++
<i>P. digitatum</i>	14.17 <sup>cC</sup>	25.42 <sup>cB</sup>	39.17 <sup>cA</sup>	<0.01	++	++	+++
<i>P. italicum</i>	37.08 <sup>abC</sup>	48.33 <sup>abB</sup>	62.08 <sup>aA</sup>	<0.01	+++	+++	++++
P-value <sup>(b)</sup>	<0.01	<0.01	<0.01	Nd	Nd	Nd	Nd
Interactions	T	<0.01			Nd	Nd	Nd
	C	<0.01			Nd	Nd	Nd
	T x C	≥0.05			Nd	Nd	Nd

Note:

<sup>a</sup> Duncan's Multiple Range Test, values followed by different superscripts are significantly different at P≤0.05.

<sup>b</sup> Probabilities associated with individual F tests.

Capital letters are for means comparison in the same row.

Small letters are for comparison of means in the same column.

Data are the average of 5 Petri dishes per replicate (with 3 replicates).

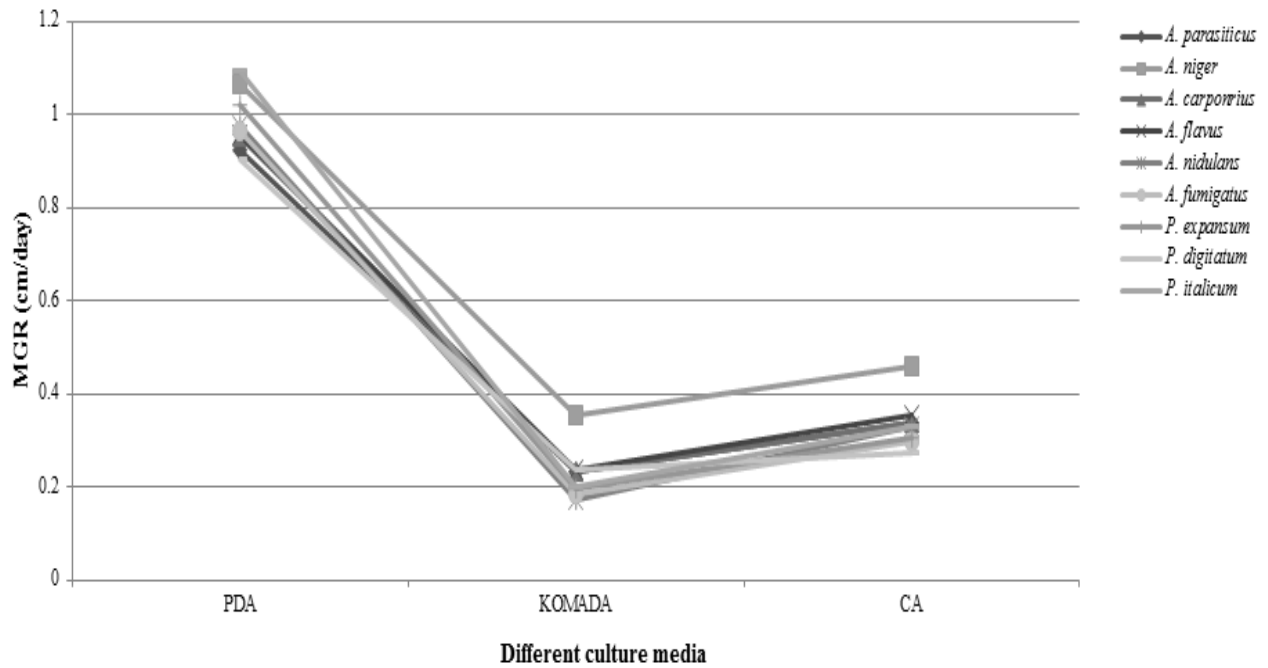
Mycelial growth inhibition percentage (I %) = (1 - Cn/C0) x 100; where: Cn is the radial growth diameter of the pathogen in the presence of the treatment. C0 is the growth diameter of the pathogen in the control treatment.

+: Poor inhibition (pathogen mycelium couldn't overgrow the antagonist colony); ++: Moderate inhibition (pathogen mycelium could not reach the antagonist colony); +++: High inhibition (zone of inhibition >2 mm but <5 mm); ++++: Very high inhibition (>5 mm).

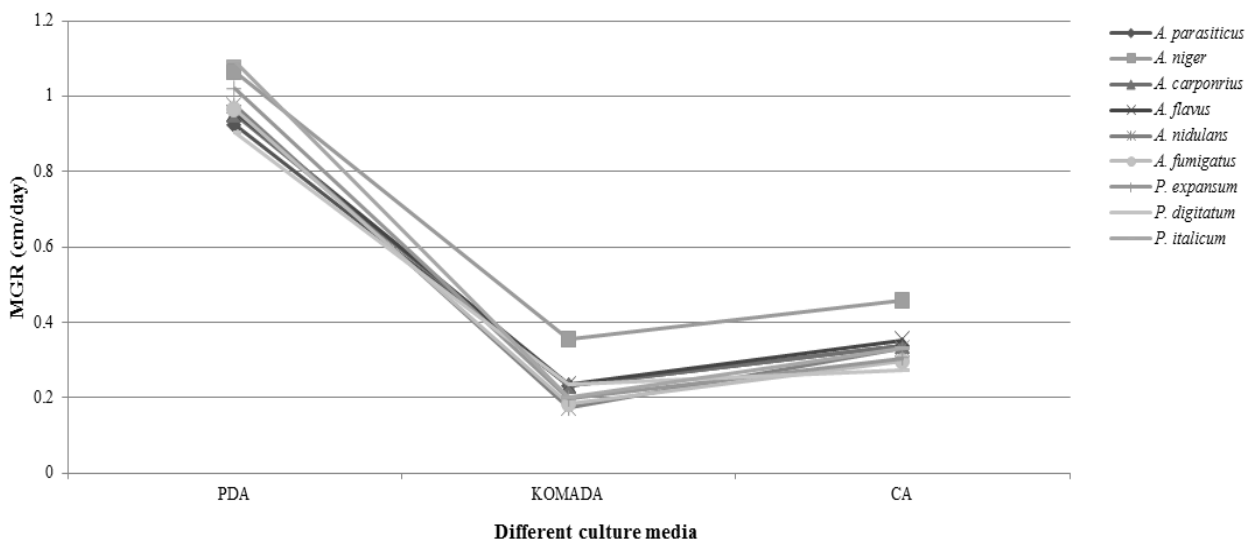
Nd: not determined.

T: Treatments.

C: Concentrations.

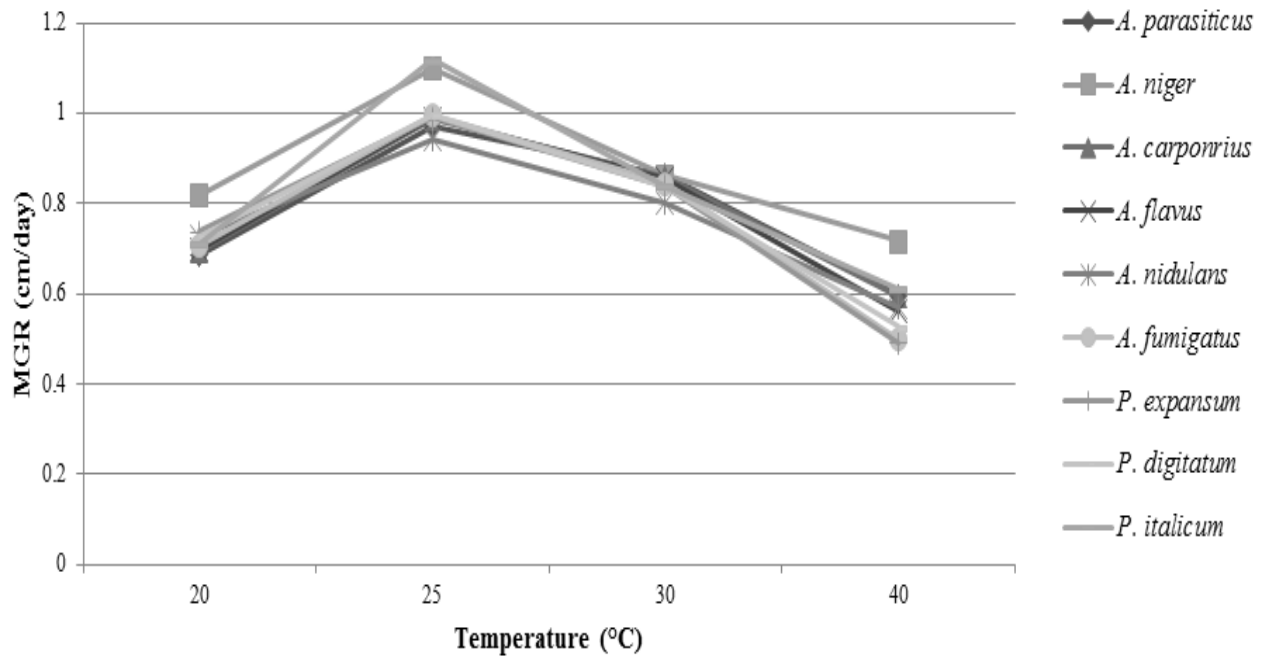


**Figure-1.** Effect of culture media (Potato dextrose agar (PDA), Komada medium, Carrot Agar (CA)) on the mycelial growth rate (MGR) of *Aspergillus* and *Penicillium* species. Regression equation, coefficient of determination ( $R^2$ ) and optimal culture media ( $O_{CM}$ ) for mycelial growth of *Aspergillus* and *Penicillium* species.  $y$  = adjusted with the values of the MGR at three culture media.  $O_{CM}$  = optimal culture media for mycelial growth of *Aspergillus* and *Penicillium* species calculated from the regression equation: *A. parasiticus* ( $y = 0.3979x^2 - 1.885x + 2.4121$ ;  $R^2 = 0.99$ ); *A. niger* ( $y = 0.4054x^2 - 1.9236x + 2.582$ ;  $R^2 = 0.99$ ); *A. carponrius* ( $y = 0.4138x^2 - 1.9619x + 2.5016$ ;  $R^2 = 0.99$ ); *A. flavus* ( $y = 0.4194x^2 - 1.9799x + 2.5184$ ;  $R^2 = 0.99$ ); *A. nidulans* ( $y = 0.4793x^2 - 2.2398x + 2.7368$ ;  $R^2 = 0.99$ ); *A. fumigatus* ( $y = 0.4471x^2 - 2.123x + 2.642$ ;  $R^2 = 0.99$ ); *P. expansum* ( $y = 0.4663x^2 - 2.2232x + 2.7785$ ;  $R^2 = 0.99$ ); *P. digitatum* ( $y = 0.3519x^2 - 1.7224x + 2.2742$ ;  $R^2 = 0.99$ ); *P. italicum* ( $y = 0.5104x^2 - 2.423x + 3.0064$ ;  $R^2 = 0.99$ ).



**Figure-2.** Effect of pH (4, 6 and 8) on the mycelial growth rate (MGR) of *Aspergillus* and *Penicillium* species. Regression equation, coefficient of determination ( $R^2$ ) and optimal pH ( $O_{pH}$ ) for mycelial growth of *Aspergillus* and *Penicillium* species.  $y$  = adjusted with the values of the MGR at different pH.  $O_{pH}$  = optimal culture media for mycelial growth of *Aspergillus* and *Penicillium* species calculated from the regression equation: *A. parasiticus* ( $y = -0.5404x^2 + 2.2811x - 1.4118$ ;  $R^2 = 0.99$ ); *A. niger* ( $y = -0.5192x^2 + 2.1888x - 1.2143$ ;  $R^2 = 0.99$ ); *A. carponrius* ( $y = -0.4767x^2 + 2.0174x - 1.1898$ ;  $R^2 = 0.99$ ); *A. flavus* ( $y = -0.4704x^2 + 1.9903x - 1.1695$ ;  $R^2 = 0.99$ ); *A. nidulans* ( $y = -0.3011x^2 + 1.2508x - 0.4705$ ;  $R^2 = 0.99$ ); *A. fumigatus* ( $y = -0.2952x^2 + 1.2514x - 0.5199$ ;  $R^2 = 0.99$ ); *P. expansum* ( $y = -0.3306x^2 + 1.4356x - 0.7583$ ;  $R^2 = 0.99$ ); *P. digitatum* ( $y = -0.4791x^2 + 2.032x - 1.1915$ ;  $R^2 = 0.99$ ); *P. italicum* ( $y = -0.4915x^2 + 2.1041x - 1.2994$ ;  $R^2 = 0.99$ ).





**Figure-3.** Effect of temperature (20, 25, 30 and 40) on the mycelial growth rate (MGR) of *Aspergillus* and *Penicillium* species. Regression equation, coefficient of determination ( $R^2$ ) and optimal temperature ( $O_T$ ) for mycelial growth of *Aspergillus* and *Penicillium* species.  $y$  = adjusted with the values of the MGR at different temperature.  $O_T$  = optimal culture media for mycelial growth of *Aspergillus* and *Penicillium* species calculated from the regression equation: *A. parasiticus* ( $y = -0.138x^2 + 0.6529x + 0.1823$ ;  $R^2 = 0.968$ ); *A. niger* ( $y = -0.1067x^2 + 0.4795x + 0.4763$ ;  $R^2 = 0.762$ ); *A. carbonarius* ( $y = -0.1394x^2 + 0.6545x + 0.1929$ ;  $R^2 = 0.954$ ); *A. flavus* ( $y = -0.1462x^2 + 0.6767x + 0.181$ ;  $R^2 = 0.965$ ); *A. nidulans* ( $y = -0.1112x^2 + 0.4945x + 0.3574$ ;  $R^2 = 0.950$ ); *A. fumigatus* ( $y = -0.1588x^2 + 0.7167x + 0.1616$ ;  $R^2 = 0.978$ ); *P. expansum* ( $y = -0.1494x^2 + 0.658x + 0.2401$ ;  $R^2 = 0.983$ ); *P. digitatum* ( $y = -0.1449x^2 + 0.6488x + 0.2369$ ;  $R^2 = 0.967$ ); *P. italicum* ( $y = -0.1579x^2 + 0.7319x + 0.1746$ ;  $R^2 = 0.809$ ).

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