Pathogenicity and Pectinase Activity of Some Facultative Mycoparasites Isolated from *Vicia faba* Diseased Leaves in Relation to Photosynthetic Pigments of Plant

**A Saleem**1,**2*, **AHM El-Said**1, **TA Maghraby**1 and **MA Hussein**1

1Botany Department, Faculty of Science, South Valley University, 83523 Qena, Egypt
2Biology Department, Faculty of Science, Tabbah University, 30002 Al-Madinah Al-Monawarah, Saudi Arabia

**Abstract**

Fourteen dematiaceous hyphomycetes fungi related to 9 genera isolated from diseased leaves of broad bean plant were investigated for their pathogenicity. Eight fungal species (represent 57.15% of total fungi tested) were positive and successfully able to infect broad bean leaves appearing leaf spot symptoms. Among these fungi *Alternaria alternata* was the most active virulent and produced leaf spots on more than 75% of infected leaves. Six fungal species (42.85%) had negative pathogenicity result and unable to infect the leaves of plant failing to produce any leaf spot symptoms. The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were significantly decreased as a result of the infection of phytopathogenic fungi. The concentrations of photosynthetic pigments were adversely affected by the degree of pathogenicity. Eight phytopathogenic fungi were screened for their abilities to produce pectinase enzyme using cup-plate method. All isolates tested were pectinase producers, but with variable degrees. Three fungal isolates (37.5% of total isolates) exhibited high pectinase activity and these were: *Alternaria citr., A. raphani* and *A. tenuissima*. Three other isolates (37.5%) were found to be moderate pectinase activity and these were: *Alternaria alternata*, *Curvularia lunata* and *Ulocladium botrytis*. *Cochliobolus spicifer* and *Stachybotrys atra var. microspora* (25%) were low producers of the enzyme. Maximum production of pectinase produced by *A. citr. and A. raphani* was recorded after 8 days at 30°C and pH 6 in the liquid medium supplemented with citrus pectin and ammonium sulphate as carbon and nitrogen sources respectively.

**Keywords:** Pathogenicity; Pectinase enzyme; Photosynthetic pigments; Broad bean

**Introduction**

Fungal plant pathogens are a group of microorganisms that show a very high versatility during their infection cycles [1]. This versatility allows them to infect a wide variety of crops [2]. They employ diverse strategies to infect and colonize the plants, and they also establish a complex interaction between fungus species and their hosts [3-5]. Common strategies of phytopathogenic fungi include forming specialized infection structures (haustoria and appressoria, etc.). In Japan, Rahman et al. [6] studied of the pathogenicity of *Alternaria tenuissima* isolates, collected from broad bean, on four broad bean cultivars. Two isolates of *Alternaria tenuissima* failed to produce lesions and two other isolates were less virulent. Gherawy [7] studied the pathogenicity of *Alternaria* species collected from several Egyptian crops on tomato fruits. 62% of isolates were pathogenic to wounded tomato fruit. Tuohy et al. [8] examined the pathogenic variation of *Drechslera teres* f. teres and *D. teres f. maculata*, the causal agent of net and spot blotch disease of barley. Significant differences were found in overall virulence of net form and spot form isolates. The spot form isolates were responsible for higher levels of disease than net form isolates. Kumar et al. [9] studied the pathogenicity of eleven *Alternaria solani* isolates, the causal agent of early blight of tomato. Six isolates were found to be virulent, causing severe disease in all tested varieties. The percent disease incidence of virulent isolates ranged between 73.90 and 83.35% while other isolates was categorized under less virulent and avirulent. Recently, Karani et al. [10] reported that, the pathogenicity of *Synchytrium poophorad* on attached and detached winged bean leaves gave positive disease symptoms appearing after 7 and 8 days of inoculations by Petri dish and moist chamber methods respectively. Abdel-Motaal [11] studied the pathogenicity of *Cladosporium herbam* on *Hyoscyamus muticus* plant. The disease symptoms first appeared after 2 weeks in the form of white spots, which enlarged and turned brown after 3 weeks. Plant-fungi interaction (Pathogenicity) was carried out in many investigations in several parts of the world and several plants [12-18].

Photosynthetic pigments are responsible for absorbing solar energy for the process of photosynthesis in the host plants. Any change in the pigment content would be reflected immediately on the photosynthetic efficacy and subsequently on growth and yield of plant. Plant productivity is quite sensitive to various stress factors of both biotic and abiotic nature [19]. The observed pronounced decrease in growth and pigments at the infected plants in several reports [20-25]. Faheed et al. [26] mentioned that, infected tomato seedlings with *Alternaria solani* showed highly significant decrease in the contents of chlorophyll a, chlorophyll b, carotenoids and consequently total pigments compared to the control. Elwakil et al. [27] showed significant reductions of photosynthetic pigments in faba bean seedlings infected with *Cephalosporium sp., Fusarium solani, F. oxysporum, F. verticillioides, Rhizoctonia solani and Verticillium dahliae* pathogens. Recently, Lobato et al. [28] evaluated the infection of *Colletotrichum lindemuthianum* pathogen on photosynthetic pigments. The total

*Corresponding author: A. Saleem, Botany Department, Faculty of Science, South Valley University, 83523 Qena, Egypt, E-mail: abdosaleem@yahoo.com
Received September 14, 2012; Accepted October 22, 2012; Published October 26, 2012
Copyright: © 2012 Saleem A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Pectins are high molecular weight polysaccharides found in higher plants. They form the primary cell wall and the main components of the middle lamella [29]. The hydrolysis of pectin backbone is obtained by the synergistic action of several enzymes [30]. Among the enzymes secreted, polygalacturonase and pectinase are responsible for cell maceration and death of plant tissue [31]. The involvement of pectic enzymes in the degradation of pectic constituents of cell walls and middle lamella of plant tissues has been reported for diverse types of diseases such as soft rot, dry rot, wilts, blights and leaf spots which are caused by pathogenic agents such as fungi, bacteria and nematodes [32]. Enzymes that attack pectic substances in the plant cell wall play a major role in pathogenicity [33]. The role of pectin degrading enzymes in causing cell wall degradation is so important that it determines the virulence of many pathogens [34]. Pectolytic enzymes have been reported to be induced by several substances [35,36]. Osman [37] reported to be induced by several substances [35,36]. Osman [37] reported that the optimal temperature for production of pectinase and pectin methylase by Aspergillus fumus being 35°C after 7 days of incubation. Yadav et al. [38] found that the optimum pH for pectinase production in culture filtrate of Aspergillus flavus, A. niger, A. phoenicis and A. wentii were 8.0, 7.0, 5.0 and 7.0, respectively. Pedrolly et al. [39] found that polygalacturonase produced by Aspergillus giganteus was maximal on orange bagasse followed by citrus pectin, at pH 6 and 30°C. Recently, Palaniyappan et al. [40] reported that the optimal pH of the medium for pectinase production by Aspergillus niger MTCC 281 was 5.5 with optimal temperature being 30°C. Banu et al. [41] recorded that the production of pectinase by Penicillium chrysogenum was higher at pH 6.5 at 35°C using sucrose and ammonium sulphate as carbon and nitrogen sources. The production of pectolytic enzymes was measured using different sources and physical parameters in several literatures [32,42-51]. This work was carried out to study the pathogenicity of several dematiaceous hyphomycetes on broad bean plant and the ability of these fungi to produce pectinase enzyme which is one of the most important cell wall degrading enzymes for pathogenicity, in addition to the correlation between pathogenicity and formation of photosynthetic pigments of plant.

Materials and Methods

Collection of Vicia faba plant samples

Fifty infected leaves samples of broad bean (Vicia faba L.) plant were collected from different cultivated regions in Qena governorate in Upper Egypt. Each sample was put in a sterile polyethylene bag and transferred to mycological laboratory in Botany Department, Faculty of Science at Qena, South Valley University. Samples were kept in a cool place (5°C) until fungal analysis.

Myological analysis of broad bean leaves

Determination of leaf surface fungi: Leaf surface fungi of broad bean plant were isolated from diseased leaves according to the methods described by El-Kholi et al. [52] on different types of media (Glucose-Czapek’s agar, Dichloran-chloramphenicol-malt extract agar, Dichloran-chloramphenicol-peptone agar) to be able to isolate various groups of facultative phytopathogenic fungi.

Broad bean-fungi interaction (Pathogenicity test)

Fifteen fungal species of dematiaceous hyphomycetes, collected from diseased leaves of broad bean plant, were evaluated for their pathogenicity on broad bean seedlings according to the method described by Berner et al. [52] and Abdel-Motaal [11]. Broad bean plants were grown in a glasshouse throughout the experiment. Pathogenicity test was carried out by using autoclaved sandy clay soil 1:2 (w/v). Three kg of sterilized soil was put in 20 cm diameter pots. Seed surface were sterilized by 0.1% mercuric chloride for 2 minutes, rinsed several times with sterilized water. Three seeds were sowed in each pot and daily irrigated with water until appearance of seedlings. Spore suspensions of selected fungal species were used for inoculation of plants. These fungi were cultivated on potato dextrose agar medium. The pathogenicity of selected species was assayed on 20 days broad bean plants by spraying of leaves, which were grown in a glasshouse for 15 days. Three replicates were used for each tested isolate and control plant was sprayed by sterile distilled water. After 6-10 days of inoculation, spots appeared on the leaf surface of plants.

Photosynthetic pigments of broad bean plant

Extraction of photosynthetic pigments: The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined using the spectrophotometric method recommended by Metzner. The photosynthetic pigments were extracted from a known weight of fresh leaves in 85% aqueous acetone. The extract was centrifuged at 7000 rpm for 15 minutes and the supernatant was decanted and completed to a definite volume by using 85% aqueous acetone to become suitable for spectrophotometric measurements.

Determination of photosynthetic pigments: The pigments extract was measured against a blank of pure 85% acetone at wavelengths of 452.5, 644 and 663 nm. Taking into consideration the dilution factor. The concentration of pigment fractions (Chlorophyll a, Chlorophyll b and carotenoids) was determined (μg/ml) using the following equations. Finally pigment fractions were calculated as mg/g fresh matter.

Chlorophyll a=10.3 E 663 — 0.918 E 644 Chlorophyll b=19.7 E 664 — 3.87 E 644
Carotenoids=4.2 E 653 — (0.0264 Chl. a + 0.4260 Chl. b)

Pectinolytic activity

Screening of fungi for pectinase production: Seven fungal species representing 3 genera were screened for their abilities to produce extracellular pectinase as described by Osman [37]. Fungi were cultured on Czapek’s agar medium and incubated at 28°C for 5 days. Using a sterile cork borer (10 mm diameter) discs were cut to inoculate 50 ml sterile liquid medium (in 250 ml Erlenmeyier conical flasks) of Hankin et al. [53]. After 7 days of incubation at 28°C, the cultures were filtered and the filtrates were used to detect the activity of pectinase enzyme according to the method described by Ammar et al. [54]. Aliquots of 0.1 ml of culture filtrate were pipetted into 10 mm cavities which were made in plates containing solid medium. After 24 hours of incubation at 28°C, plates were flooded with iodine solution. Clear zone around the cavities indicated the activity of pectinase enzyme. The average diameter of clear zones (in mm) of triplicates for each isolates was recorded.

Factors affecting pectinase production: Alternaria citri and A. raphani were found to be the most active pectinase producers. So they employed to study the effect of different ecological and nutritional factors on pectinase production.

Effect of incubation periods: Flasks containing 50 ml of Hankin et al. [53] medium with pH 7 were inoculated with A. citri and A. raphani and incubated at 28°C for 14 days and harvested at 48 hours intervals.
Three replicates were used for each treatment. Filtrates were centrifuged at 4°C for 15 minutes at 15000 rpm and the clear supernatants were assayed for pectinase activity using the method described by Sherwood [55].

**Effect of temperature:** 50 ml of liquid medium was inoculated separately with *A. citri* and *A. raphani* (in 250 ml conical flasks) and incubated at 15, 20, 25, 30, 35 and 40°C for 8 days. Three replicates were made for each treatment. At the end of the incubation period, cultures were filtered, centrifuged at 4°C for 15 minutes at 15000 rpm and the clear supernatants were assayed for pectinase activity.

**Effect of pH values:** Flasks containing 50 ml medium were adjusted to different pH levels ranging from 2 to 12 using 0.1 N HCl or 0.1 NaOH. Cultures were inoculated with *A. citri* and *A. raphani*. The inoculated flasks were incubated at 30°C for 8 days. Three flasks for each pH value were prepared. At the end of the incubation period, cultures were filtered, centrifuged at 4°C for 15 minutes at 15000 rpm and the clear supernatants were assayed for pectinase activity.

**Effect of different carbon sources:** The medium was supplemented with 0.5% of one of the following carbon sources: citrus pectin, CMC, cellulose powder, starch, glucose, fructose, maltose, lactose and sucrose. Cultures were inoculated with *A. citri* and *A. raphani*. The inoculated flasks were incubated at 30°C for 8 days and cultures were filtered. After centrifugation the clear filtrate was used to detect the pectinase activity.

**Effect of various nitrogen sources:** *A. citri* and *A. raphani* were cultured on liquid medium free of ammonium sulphate and adjusted to the best pH for pectinase production. The following nitrogen sources were incorporated separately in the basal medium at a concentration of 0.2% w/v: Ammonium nitrate, potassium nitrate, sodium nitrate, sodium citrate, calcium nitrate, magnesium nitrate and peptone in addition to ammonium sulphate as a control. After 8 days of incubation at 30°C, cultures were filtered, centrifuged at 4°C for 15 minutes at 15000 rpm and the pectinase activity was determined.

### Results and Discussion

#### Pathogenicity test

The pathogenicity of 14 dematiaceous hyphomycetes fungi attributed to 9 genera was tested. Eight fungal species (represent 57.15% of total fungi) were positive and successfully able to infect *Vicia faba* leaves appearing infection symptoms of leaf spot, but the degree of infection was varied. *Alternaria alternata* was highly virulent (+++) and produced leaf spots on more than 75% of infected leaves. *Stachybotrys atrata var. microspora* had moderate ability (+) to infect leaves of plant and its lesion cover 50 to 75% of infected leaves. The other 6 species (represent 42.85% of total fungi) including *Alternaria citri*, *A. raphani*, *A. tenuissima*, *Cochliobolus spicifer*, *Curvularia lunata* and *Ulocladium botrytis* were less virulent (+). Its lesions were observed on less than 50% of infected leaves. On the other hand, the remaining 6 fungal species (represent 42.85% of total fungi) had negative pathogenicity result and unable to infect the leaves of broad bean plant failing to produce any leaf spot symptoms on inoculated leaves. These species were *Cladosporium cladosporioides*, *C. sphaerospereum*, *Drechslera papendorfii*, *Mycosphaerella tassiana*, *Stachybotrys parvispora* and *Setosphaeria rostrata* (Table 1, Figure 1). Many workers demonstrated that *Alternaria* species infected many plants including broad beans [6], *Minneola* [13], *tomato* [26,56] and *Trifolium repens* [20,22]. Rahman et al. [6] reported that, among several *Alternaria tenuissima* isolates collected from broad bean, eighty percent were highly virulent, and 7% were in moderate virulent category. Two isolates of *Alternaria tenuissima* failed to produce lesions on four cultivars of broad bean and two other isolates were less virulent. Elena [13] recorded that, the symptoms of the brown spot disease caused by *Alternaria species* were reproduced only on fruits of Minneola hybrids and not appeared on fruits of mandarin and clementine when inoculated by *Alternaria isolates*. Kumar et al. [9] found that, six isolates from eleven *Alternaria solani isolates*, the causal agent of early blight of tomato, were found to be virulent, causing severe disease in all tested tomato varieties. Other isolates were rated as less virulent and avirulent. The percent disease incidence of virulent isolates ranged between 73.90 and 83.35%. Karami et al. [10] reported that, the pathogenicity of *Synchytrium psophocarpici* on attached and detached winged bean plant leaves gave positive disease symptoms appearing 8 days after inoculations by moist chamber method and 7 days by Petri dish method. The appearance of orange gall was observed on 70 and 90% of leaves inoculated in the moist chamber and Petri dish respectively. Recently, Abdel-Motaal [11] mentioned that the disease symptoms caused by *Cladosporium herbarum* on *Hyoscyamus muticus* plant, first appeared after 2 weeks as white spots, which enlarged and turned brown after 3 weeks.
Photosynthetic pigments

Photosynthetic pigments (Chlorophyll a, Chlorophyll b and Carotenoids) of broad bean leaves were significantly decreased due to the infection of pathogenic fungi. Alternaria alternata which was highly virulent exhibited the highest significantly inhibitive effect on the formation of photosynthetic pigments of plant compared with the control sample. The inhibitive effect was 66.89, 60.72, 67.42 and 65.07% for chlorophyll a, chlorophyll b, carotenoids and total photosynthetic pigments, respectively. The photosynthetic pigments of broad bean leaves were also decreased by the infection of A. citri, A. raphani and A. tenuissima, Cochliobolus spicifer, Curvularia lunata, Stachybotrys parvispora var. microspora, Mycosphaerella tassiana, Setosphaeria rostrata and Ulocladium botrytis pathogens. On the other hand, there is no significant difference in the contents of photosynthetic pigments in case of nonpathogenic isolates (Cladosporium cladosporioides, C. sphaeromperm, Drechlera papendorfii, Mycosphaerella tassiana, Setosphaeria rostrata and Stachybotrys parvispora) (Table 2). Faheed et al. [26] mentioned that infected tomato seedlings with Alternaria solani showed highly significant decrease in the contents of chlorophyll a, chlorophyll b, carotenoids and consequently total pigments as compared to the control. Li et al. [20] showed that the infection of Trifolium repens leaf tissue with Alternaria azukiae decrease its pigment content. Recently, Elwakil et al. [27] showed significant reductions of photosynthetic pigments in faba bean plants infected with Cephalosporium sp., Fusarium solani, F. oxysorium, F. verticillioides, Rhizoctonia solani and Verticillium dahliae.

Table 2: Effect of fungal infection on photosynthetic pigments of broad bean leaves.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Photosynthetic pigments (mg/g fresh leaves)</th>
<th>Chl. a</th>
<th>Chl. b</th>
<th>Carot.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.23</td>
<td>1.88</td>
<td>100</td>
<td>6.14</td>
</tr>
<tr>
<td>Alternaria alternata</td>
<td></td>
<td>2.16**</td>
<td>66.89</td>
<td>1.14**</td>
<td>60.72</td>
</tr>
<tr>
<td>A. raphani</td>
<td></td>
<td>2.66**</td>
<td>82.36</td>
<td>1.55**</td>
<td>67.42</td>
</tr>
<tr>
<td>A. tenuissima</td>
<td></td>
<td>2.77**</td>
<td>85.70</td>
<td>1.50**</td>
<td>67.98</td>
</tr>
<tr>
<td>A. tenuissima</td>
<td></td>
<td>2.51**</td>
<td>77.57</td>
<td>1.40**</td>
<td>74.31</td>
</tr>
<tr>
<td>Cladosporium cladosporioides</td>
<td></td>
<td>3.16</td>
<td>97.89</td>
<td>1.88</td>
<td>99.79</td>
</tr>
<tr>
<td>C. sphaeromperm</td>
<td></td>
<td>3.01</td>
<td>93.28</td>
<td>1.76</td>
<td>93.68</td>
</tr>
<tr>
<td>Cochliobolus spicifer</td>
<td></td>
<td>2.69**</td>
<td>83.41</td>
<td>1.71</td>
<td>90.68</td>
</tr>
<tr>
<td>Curvularia lunata</td>
<td></td>
<td>2.83**</td>
<td>81.28</td>
<td>1.70</td>
<td>90.34</td>
</tr>
<tr>
<td>Drechlera papendorfii</td>
<td></td>
<td>3.15</td>
<td>97.34</td>
<td>1.78</td>
<td>94.74</td>
</tr>
<tr>
<td>Mycosphaerella tassiana</td>
<td></td>
<td>3.16</td>
<td>97.80</td>
<td>1.79</td>
<td>95.26</td>
</tr>
<tr>
<td>Setosphaeria rostrata</td>
<td></td>
<td>3.09</td>
<td>95.88</td>
<td>1.76</td>
<td>93.42</td>
</tr>
<tr>
<td>Stachybotrys atra var. microspora</td>
<td></td>
<td>2.26**</td>
<td>69.99</td>
<td>1.27**</td>
<td>67.30</td>
</tr>
<tr>
<td>S. parvispora</td>
<td></td>
<td>3.04</td>
<td>94.09</td>
<td>1.71</td>
<td>92.09</td>
</tr>
<tr>
<td>Ulocladium botrytis</td>
<td></td>
<td>2.66**</td>
<td>82.18</td>
<td>1.47**</td>
<td>78.24</td>
</tr>
</tbody>
</table>

Pectinolytic activity

Screening of fungi for pectinase production: The ability of eight fungal species representing 5 genera (positively infected broad bean plant) was screened for their abilities to produce pectinase enzyme using cup-plate method. All fungal isolates were pectinase producers, but with variable degrees. Three fungal isolates (37.5% of total isolates) exhibited high pectinase activity and these were: Alternaria citri, A. raphani and A. tenuissima (Figure 2). Three other isolates (37.5% of total isolates) were found to be moderate pectinase activity and these were: Alternaria alternata, Curvularia lunata and Ulocladium botrytis. The remaining two isolates (25% of total isolates) which were Cochliobolus spicifer and Stachybotrys atra var. microspora were low producers of the enzyme (Table 3). Bocca et al. [57] found that, out of 248 fungal isolates recovered from coffee plants and soil samples, screened for their capability to produce pectinase, 119 isolates able to produce pectolytic enzyme with 13 high producers. All of these were related to either Aspergillus or Penicillium. Dartora et al. [58] found

Table 3: Screening of fungi for their abilities to produce pectinase enzyme.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Pectinase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria alternata</td>
<td>21 M</td>
</tr>
<tr>
<td>A. citri</td>
<td>25 H</td>
</tr>
<tr>
<td>A. raphani</td>
<td>24 H</td>
</tr>
<tr>
<td>A. tenuissima</td>
<td>23 H</td>
</tr>
<tr>
<td>Cochliobolus spicifer</td>
<td>18 W</td>
</tr>
<tr>
<td>Curvularia lunata</td>
<td>22 M</td>
</tr>
<tr>
<td>Stachybotrys atra var. microspora</td>
<td>20 W</td>
</tr>
<tr>
<td>Ulocladium botrytis</td>
<td>21 M</td>
</tr>
</tbody>
</table>

Degree of pectinase activity: High activity, H: from 23-25 mm; Moderate activity, M= 21-22 mm; Weak activity, W< less than 21 mm.
that out of 5 fungal strains tested for their capacity of producing endopolygalacturonase, strains of Aspergillus niger and A. oryzae were the best producers. Recently, Gouda [59] found that among 39 fungal isolates tested for the ability of producing pectinase enzymes on solid medium, one isolate only related to Emericella nidulans produced pectinase enzymes.

**Effect of different environmental and nutritional factors on pectinase production and fungal growth**

*Alternaria citri* and *A. raphani* were chosen for more study, since these fungi were the most active fungi for pectinase production. Pectinase production has been found to be affected by variable environmental and nutritional conditions.

**Effect of incubation periods:** Pectinase enzyme produced by *A. citri* and *A. raphani* fungi was increased by the increasing of the incubation period showing its maximum after 8 days. After 10, 12 and 14 days of incubation, the enzyme activity was decreased. However, maximum mycelial growth of these fungi was recorded after 6 days of incubation (Figure 3 and 4). In this respect, Yadav et al. [38] reported that maximum yield of pectinase by *A. raphani* was obtained after 6 days of incubation (Figure 3 and 4). In this respect, Yadav et al. [38] reported that, the optimum pH for pectinase production in culture filtrate of *Aspergillus flavus*, *A. niger*, *A. phoenicis* and *A. wentii* was 8.0, 7.0, 5.0 and 7.0, respectively. Also, pH 6.5 was the most suitable for pectinase production by *Penicillium chrysogenum* [41].

**Effect of different environmental and nutritional factors on pectinase production and fungal growth**

*Alternaria citri* and *A. raphani* were chosen for more study, since these fungi were the most active fungi for pectinase production. Pectinase production has been found to be affected by variable environmental and nutritional conditions.

**Effect of incubation periods:** Pectinase enzyme produced by *A. citri* and *A. raphani* fungi was increased by the increasing of the incubation period showing its maximum after 8 days. After 10, 12 and 14 days of incubation, the enzyme activity was decreased. However, maximum mycelial growth of these fungi was recorded after 6 days of incubation (Figure 3 and 4). In this respect, Yadav et al. [38] reported that, the optimum pH for pectinase production in culture filtrate of *Aspergillus flavus*, *A. niger*, *A. phoenicis* and *A. wentii* was 8.0, 7.0, 5.0 and 7.0, respectively. Also, pH 6.5 was the most suitable for pectinase production by *Penicillium chrysogenum* [41].

**Effect of different environmental and nutritional factors on pectinase production and fungal growth**

*Alternaria citri* and *A. raphani* were chosen for more study, since these fungi were the most active fungi for pectinase production. Pectinase production has been found to be affected by variable environmental and nutritional conditions.

**Effect of incubation periods:** Pectinase enzyme produced by *A. citri* and *A. raphani* fungi was increased by the increasing of the incubation period showing its maximum after 8 days. After 10, 12 and 14 days of incubation, the enzyme activity was decreased. However, maximum mycelial growth of these fungi was recorded after 6 days of incubation (Figure 3 and 4). In this respect, Yadav et al. [38] reported that, the optimum pH for pectinase production in culture filtrate of *Aspergillus flavus*, *A. niger*, *A. phoenicis* and *A. wentii* was 8.0, 7.0, 5.0 and 7.0, respectively. Also, pH 6.5 was the most suitable for pectinase production by *Penicillium chrysogenum* [41].

**Effect of different carbon sources:** Among nine carbon sources incorporated separately in the culture medium for enzyme production, citrus pectin yielded the maximum pectinase production by *A. citri* and *A. raphani*. Considerable amounts of pectinase were also achieved in the presence of fructose and starch as carbon sources. On the other hand, glucose and sucrose were the least inducible carbon sources for pectinase production by the two fungal species. The highest yields of mycelial growth of the two fungal isolates were obtained with the presence of lactose as carbon source followed by starch. The remaining carbon sources were less inducers for mycelial growth of the two fungal isolates (Figure 3 and 4). Pedrolli et al. [39] reported that, the highest production of polygalacturonase was maximal on orange bagasse followed by citrus pectin as carbon source. However, sucrose was the most favourable carbon source for pectinase production by *Penicillium chrysogenum* [41]. Mojsov [60] observed that the maximal production of endo-polygalacturonase by *Aspergillus niger* was achieved with apple pulp followed by apple pectin as carbon source. However, Glucose yielded maximum dry biomass of *Neurospora crassa* [61] and *Aspergillus niger* [60].

**Effect of various nitrogen sources:** The highest yields of pectinase produced by *A. citri* and *A. raphani* were achieved in the presence of ammonium sulphate as nitrogen source followed by peptone. Considerable amounts of pectinase enzyme were also synthesized in the presence of sodium nitrate as nitrogen source. On the other hand, the remaining nitrogen sources were less favourable for the enzyme production. Maximum mycelial growth of *A. citri* and *A. raphani* were obtained when calcium nitrate incorporated into the culture medium followed with peptone and ammonium sulphate. Sodium nitrite was the least inducible nitrogen source for mycelial growth of the two fungal species (Figure 3 and 4). Bai et al. [62] reported that, high pectinase activity and mycelial growth were obtained with ammonium sulphate, yeast extract powder, soya peptone and soybean powder. Recently, Banu et al. [41] reported that pectinase production by *P. chrysogenum* was higher using ammonium per sulphate followed by ammonium sulphate as nitrogen source.

**Conclusion**

*Alternaria alternata* which exhibited the highest pathogenicity level on broad bean plant induced the highest significant inhibitive effect on secretion of photosynthetic pigments of *Vicia faba* and had
moderate ability to produce pectinase enzyme although generally genus *Alternaria* were the most active genera for production of pectinase enzyme.

Screening of phytopathogenic fungi and their abilities to infect plant crops would contribute in the detection of the range of plant diseases distribution and protection of plants against fungal pathogens. Moreover, study of pectinase production and its environmental relationship plays an important role for the ability of fungal pathogens in biological degradation of host plant and its control.

References


