Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2023 20(3):714-723. **OPEN ACCESS**

Snail wastes for chitinase production using entomopathogenic fungi

Ouidad Abdelaziz[1,2], Nora Bassa[2], Awatif Boumaza[3] , Asmaguemas[1],Chaima Djebbarl[1], Ilhem meriane[4], Ali Kemal Birgücü[5], İsmail Karaca[5],Saleh M. Al-maaqar[6,7], Majed A. Al-Shaeri[7,8], Naser A. Alkenani[7,8*]

[1]Laboratory of Applied Biochemistry, Faculty of Natural and life sciences, Constantine 1 University, Constantine. **Algeria**
[2]Laboratory of Biomolecules and Plant Breeding, Larbi Ben M'hidi University, 04000Oum El Bouaghi, **Algeria**
[3] Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Universite, Université 8 Mai 1945 , Guelma , **Algéria**
[4] Laboratory of Microbiological Engineering and Application, Faculty of Natural and life sciences, Constantine1 University, **Algeria**
[5]Faculty of Agriculture, Department of Plant Protection, Isparta University of Applied Sciences, 32260 Isparta, **Turkey**
[6] Faculty of Education, Department of Biology, Albaydha University, Al-Baydha, **Yemen**
[7]Environmental Protection & Sustainability Research Group, Faculty of Sciences, King Abdulaziz University, Jeddah, **Saudi Arabia**
[8] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, **Saudi Arabia**

Correspondence:nalkenani@kau.edu.sa*Received:11-05-2023, Revised: 25-07-2023, Accepted:29-07-2023 e-Published: 30-07-2023**

This research aims to reveal the ability of fungi to slow down the development of *Ectomyeloisceratoniae* (Zeller) larvae and to study chitinase production. The process is based on the isolation and identification of *entomopathogenic* fungi from *Ectomyeloisceratoniae* (Zeller) larvae. A total of nine strains of fungi from the *Aspergillus*, *Fusarium*, Alternaria, and Cladosporium genera were isolated and identified after purification. Among them, Fusarium oxysporum and Aspergillus tubingensis showed a substantial effect against *Ectomyeloisceratoniae* (Zeller) larvae with percentages of 77.78% and 55.83% respectively. The impact of temperature and pH on *Aspergillus tubingensis'* ability to produce chitinase using snail excrement was examined. Different pH levels (4, 7, and 10) and temperatures (10, 30, and 50 °C) were considered. These pH and temperature levels demonstrate that *Aspergillus tubingensis* exhibits the highest level of chitinolytic activity. Furthermore, the best enzymatic activity was observed in *Aspergillus tubingensis* at pH=7 with an activity of ($2.3x10^7$ U), followed by pH=4 of ($1.8x10^7$ U), and pH=10 of ($1.5x10^7$ U)., On the other hand, produced the best activity ($2x10^7$U)at 50°Cfollowed by ($1.90x10^7$U) at 30°C, then ($1.80x10^7$U) at 10°C.

Keywords: Entomopathogenic fungi, *Ectomyeloisceratoniae (Zeller), Chitnase, snail wast*.

INTRODUCTION

Biotechnology is a multidisciplinary science that utilizes different techniques and processes. It tends to be the most advanced emerging technology. Recently, biotechnology has been accelerated due to the great advances in the molecular industry. Therefore, biotechnology can obtain new synthetic organisms and proteins easier. Due to the increase in pollution, biotechnology is seen as a tool for pollution prevention, waste treatment, and new cleaner technologies. Biotechnology broadly uses microorganisms to produce bioactive molecules with significant biological activities. Moreover, entomo pathogenic fungi deserve attention and can be used as biological control (Lacey and Undeen, 1986). They play an important role in regulating insect populations (Roberts, 1992). The largest number of these pathogens is found in the Zygomycete class.

Enzymes are natural catalysts. they are specific and provide thermodynamic efficiency. Enzymes are the oldest forms of biotechnology. Enzymes also play a significant role in synthesizing chemical compounds, analytical and diagnostic methods, the treatment of disease, clean technologies, etc.

Biological catalysts, also called biocatalysts, such as enzymes, quicken biochemical reactions in living things. They can also be taken out of cells and used to catalyze various crucial commercial processes. In addition, chitinases are enzymes that break down chitin. They can be found in mammals, higher plants, insects, fungi, viruses, and bacteria.

Chitinolytic enzymes or chitinases have been studied in biological studies. They are grouped into families based on their amino acid sequence similarity. They have gained interest in various biotechnology applications because of

their ability to degrade chitin in fungi and insects' cell walls. Consequently, they have been used as antimicrobials or insecticides. They are implicated in plant resistance to fungal pathogens due to their inducible nature and anti-fungal activities in vitro. Also, chitinase usage tends to be in the respective bioconversion, transforming chitosan to Acetyl Glucosamine and chitooligo saccharides with pharmacological properties (Taira et al. 2002). This work aims to study the chitinase production by *Aspergillus tubingensis* strain on a medium based on snail waste at different temperatures (10, 30, and 50 ° C) and pH (4, 7, and 10).

MATERIALS AND METHODS

Sampling

Ectomyeloi*sceramic toniae* (Zeller) larvae were collected from stored dates. The dates were cut using a sterilized scalpel and examined for larvae or nymphs of moths by observation. Then, the larvae were collected in Petri dishes to isolate Entomopathogenic Fungi (EPF).

Fungi isolation

isolation

The larvae were disinfected with 1% sterile bleach for five minutes and rinsed with ethanol and sterile distilled water for five minutes. They were dried with sterile absorbent paper and placed in Petri dishes containing Dextrose Agar (PDA) potatoes supplemented by Clamoxyl (1g). The plates were incubated at 25°C in the dark and then incubated daily for three weeks for fungal growth (Zimmermann 1986). The colonies obtained were therefore purified and identified morphologically.

Purification

Fungal colonies were picked up and subcultured on the same medium used in the selective isolation, and they are incubated at 25°C for 6 days. Then they are examined by macroscopic and microscopic observations to confirm their purity. The colonies were maintained in slants for further studies.

Identification

The identification was based on fungal mycelium examinations:

Macroscopic examination: This aims to determine the color of the fungal colony during its development and to measure its diameter.

Microscopic examination: This is aimed at detecting the presence of thallus, the presence or absence of septum, the nature and characteristics of fructification, and the spores produced. (Samson and Haesks, 1988).

The Mycelium was fixed using a cotton blue lactophenol solution (Packer and Thomas, 1990).

Pathogenicity test

In order to identify different isolates, the direct dipping inoculation method was adopted (Butt &Goettel, 2000), which is recommended by FAO as a standard method for the detection and measurement of insect resistance to insecticides (FAO, 2004) and particularly for measuring the toxicity of fungi by contact with insect (Butt &Goettel, 2000)

Preparation of the entomopathogenic solution

Using a sterile spatula, small fragments of *Aspergillus tubingensis* and *Fusariumoxysporium*in fruiting stage; were removed by scraping and then inserted in a test tube containing 9ml distilled water and 0.05% Tween 80. The resulting "stock solution" solution was vortex mixed for 10 minutes for maximum spore release. The concentration of entomopathogenic fungi solution (10^8) was evaluated using hematimetric chamber: "Thoma cell counting chamber ".

Pathogenicity test

For the test, larvae of date moth (*Ectomyeloisceratoniae* (Zeller)) were selected. They indicate the dates damage stage. Larvae were soaked for 10 seconds in suspensions of *Aspergillus tubingensis*and Fusariumoxysporium prepared with 10^8 spores/ml. (Gannasi et al. 2006). Then, they were distributed on rectangular sections of dates, previously disinfected with bleach, rinsed with distilled water several times, and put in Petri dishes (5 larvae per Petri dish (ϕ = 8.5cm)). Six replicates were applied per treatment. The larvae used as control were soaked in distilled water and 5% Tween before being distributed in Petri dishes as described previously. The larvae mortality was monitored daily: from the first day of treatment until death (about 11 days). At each control, dead insects were eliminated before pathogens sporulation to avoid horizontal transmission of infection in the same plate.

Statistical analysis

The insecticidal properties of the two isolates were estimated by analyzing their mean lethal time and percent mortality by correction (Abbott, 1925).One-analysis analysis of variances using SPSS 23.0 statistical software (SPSS.Inc. Chicago. USA) was performed to determine the differences between the used treatments.

Chitinase screening

Isolated fungal strains were seeded on white agar with 20% chitin colloidal. (MCC),The plates were incubated at 28oC for seven days. Enzymatic activity was shown by the formation of a clear area around the colonies.

Fungi inoculum preparation

*Aspergillus tubingensis*was propagated onto an Dextrose-potato agar medium in plates at 28°C.Within 2 weeks, the plates were scraped with 10 ml of sterile water containing 0.05% Tween 80. The spore suspension was adjusted to

the desired concentration (107 conidia/ml) after being counted with Malassez cells.

Fermentation condition

Helix aspersa snail waste, regarded as a basic substrate provided by Oran Market, west of Algeria, was used as the medium for chitinase production. Snail waste was air-dried at room temperature before being ground. 50 ml of distilled water was used to soak two grams of snail powder. Erlenmeyer flasks containing 50 ml of culture medium were inoculated with a fungal suspension containing 10^7 conidia /1 ml.

Figure 1: Snail waste of *H.aspersa.*

Effect of pH

The mediums are incubated at 28°C for 7 days, and the pH range is tested between 4 and 10. The experience was multiplied by three and the mean values were calculated.

Effect of temperature

The medium is incubated for 7 days at three different temperatures (10, 30 and 50 °C). The experiments are carried out in triplicate.

Fermentation method

In an Erlenmeyer containing the broth (flour from snail was te), the chosen mold strain *Aspergillustubingens* was inoc ulated with 107 conidia/ml, and the pH of the medium was changed to 4, 7, and 1 Without shaking, the Erlenmeyer fl asks were incubated for 7 days at three different temperat ures (10 °C, 30 °C, and 50 °C). After fermentation, the culture broths were filtered through the Whatman paper n°1 dried, and weighed. The clear obtained filtrate represents the crude enzyme extract. It was frozen for chitinolytic assays. The parameters (pH and chitinolytic activity) are estimated at the end of the fermentations.

pH measurement

For each sample taken during the fermentations (erlens), the pH of the enzymatic extract was measured

Determination of chitinolytic activity

The reduction of sugars by DNS (3, 5-DiNitroSalicylic acid) served as the basis for determining the chitinolytic activity (Miller, 1959). 1 ml of crude enzyme extract and 1 ml of chitin in sodium phosphate buffer (50 mM, pH 5.0) make up the reaction mixture (0.2 g chitin in 2 ml buffer). For 30 minutes at 50 °C, the mixture is incubated in a water bath shaker. After 15 minutes of centrifugation at 5000 rpm, the reaction was stopped by combining 1 ml of the supernatant with 3 ml of DNS reagent. The mixture was brought to 100 °C in a water bath for five minutes, after which it was cooled in an ice bath. At 540 nm, the absorbance is measured by a spectrophotometer. Based on the standard reference curve of N-acetylglucosamine (GlcNAc), the amount of reducing sugars released is calculated (Toharisman et al. 2005). The amount of enzyme required to catalyze the release of 1 mol of N-acetyl glucosamine per ml in 1 min is referred to as an enzyme activity unit.

RESULTS

Isolation, purification, and identification of entomopathogenic fungi

Nine fungal strains are isolated, they belong to 4 genera: *Aspergillus*, *Fusarium*, *Cladosporium,* and *Alternaria*. The major genus is *Aspergillus*(70%) represented by 5 species: *Aspergillusparasiticus*, *Aspergillus tubingensis*, *Aspergillus niger* (2 species), *Aspergillus flavus* and *Aspergillus fumigatus*, followed by *Fusariumoxysporium*, *Cladosporiumcladosporoides* and *Aletrnariaalternata* with a percentage of 10% (fig.2)

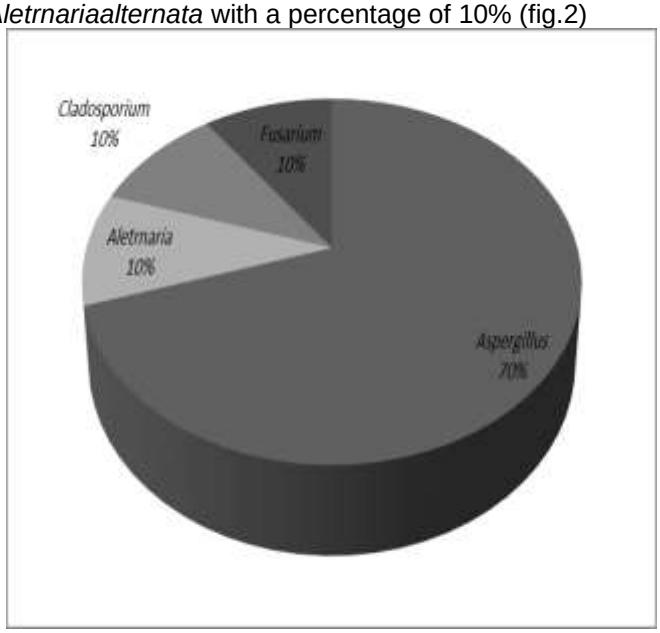


Figure 2: Isolates frequency

Pathogenicity test

With percentages of 77.78% and 55.83%, respectively , the results demonstrated that both Fusarium oxysporium and Aspergillus tubingensis have a significant impact agai nst Ectomyelois ceratoniae (Zeller).Ectomyelois ceratonia e was more successfully combatted by Drechslera sp. tha n Aspergillus tubingensis. After the first, third, fifth,

seventh, ninth, and eleventh days of treatment, respectively, the measured cell densities for Fusariumoxysporium were 4.66, 4.16, 3.33, 2.33, 2.33, 1.00, and 0.83, and for Aspergillus tubingensis were 4.00, 3.83, 3.16, 2.33, 1.86, and 1.86. Additionally, after the fifth day, the statistical analysis showed a highly significant difference. After the first, third, fifth, seventh, ninth, and eleventh days of treatment, the results were [F3DAA= (3.5,p0.07)], [F5DAA= (10.83,p0.053)], [F7DAA= (34.09,p0.000)], [F9DAA= (29.11,p0.00)], and [F11 DAA=24,39 (p<0.000)] respectively (Table 1).

App: Application; DAA1: 1st day after application; DAA3: 3rd day after application; DAA5: 5th day after application; DAA7: 7th day after application; DAA9: 9th day after application; DAA11: 11th day after application df: Degrees of freedom; F: *Fisher test;* Sig: Signification

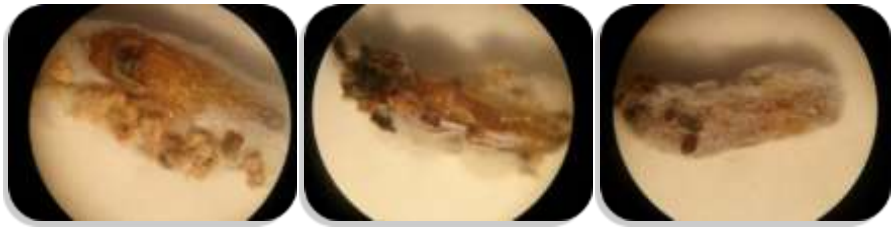
A total mortality of *Ectomyeloisceratoniae* larvae was observed after being dipped in *Fusariumoxysporium* sporal suspension. We noticed a very high mortality rate of the larvae when using *Asperfillustubingensis*.In addition, the test highlighted several modes of action of fungal strains on *Ectomyeloisceratoniae* larvae:*Fusariumoxyporium* had wrapped up with its whitish mycelium all over the surface of the larvae leading to their disintegration. However, *Aspergillus tubingensis.*acted by penetration of its hyphae into the body of the larva giving an appearance of needle bites; causing the body destruction of the larvae (crumbling). The pathogenicity test results of entomopathogenic fungi on *Ectomyeloisceratoniae*larvae (Zeller) are shown in Table 2.

Table 1: Univariate analysis of variance

		Sum of Squares	Df	MeanSquare	F	Sig.
DAA1	Between Groups	3,111	2	1,556	7,000	,007
	Within Groups	3,333	15	,222		
	Total	6,444	17			
DAA3	Between Groups	3,111	2	1,556	3,590	,053
	Within Groups	6,500	15	,433		
	Total	9,611	17			
DAA5	Between Groups	10,111	2	5,056	10,833	,001
	Within Groups	7,000	15	,467		
	Total	17,111	17			
DAA7	Between Groups	25,000	2	12,500	34,091	,000
	Within Groups	5,500	15	,367		
	Total	30,500	17			
DAA9	Between Groups	40,111	2	20,056	29,113	,000
	Within Groups	10,333	15	,689		
	Total	50,444	17			
DAA11	Between Groups	31,444	2	15,722	24,397	,000
	Within Groups	9,667	15	,644		
	Total	41,111	17			

Table 2: **The pathogenicity test results**

	The appearance of date moth larvae after treatment
Control	

Aspergillustubingensis	
Fusariumoxysporium	

Chitinase activity

For chitinase activity, *Aspergillus tubingensis* showed the highest zone around the colony with ϕ = 2cm, followed by *Fusariumoxysporium* with ϕ = 1.4 cm (Fig.3), On the other hand, the other species showed mycelia growth on the colloidal chitin medium.

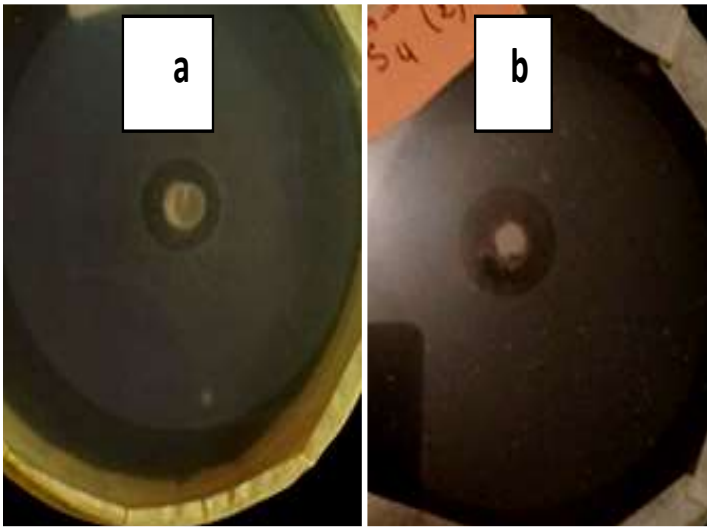


Figure 3: Screening of fungi chitinase enzyme production, a.*Aspergillustubingensis*, b.*Fusariumoxysporium*

Effect of pH

Measurement of pH

Fig 4 Shows a significant pH increase in the liquid cultures.By the end of the cultures. The pH reaches 7,72 at pH 4 and 7,77 at pH=7. On the other hand, the culture based on snail waste at pH 10 shows a significant decrease 7,9.

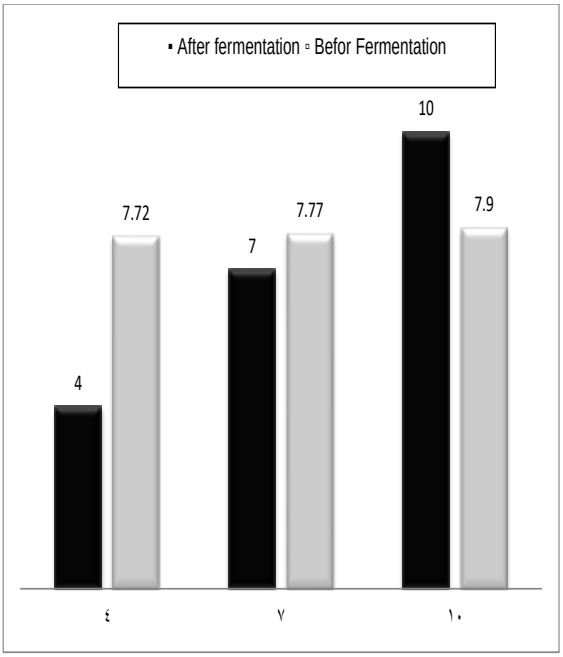


Figure 4: pH variation after and before fermentation of *Aspergillustubingensis*.

Chitinolytic Activity

Fig.5 shows the best enzymatic activity that was observed in *Aspergillus tubingensis* at pH=7 with an activity of ($2.3x10^7$ U), followed by pH=4 of ($1.8x10^7$ U) and finally pH=10 with ($1.5x10^7$ U).

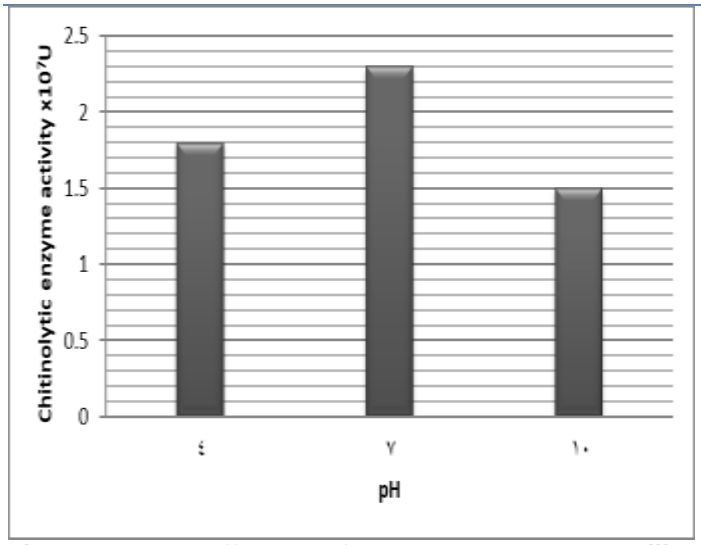


Figure 5: Effect of pH on *Aspergillus stubingensis*'schitinolytic activity

Effect of temperature

pH measurement

The results show a significant increase in pH in the liquid cultures. This could be explained by the fact that the snail has an excellent buffering capacity. This is the case for most agro-industrial residues. Additionally, Aspergillus tubingensis showed the highest pH at 30°C (7,96), followed by 10°C (7,78), and then 50°C (7.68). (fig 6).

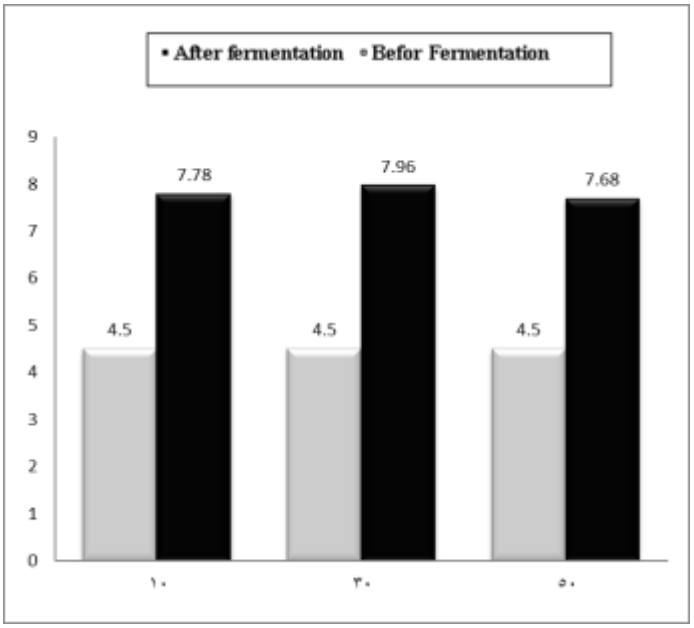


Figure 6: Effect of temperature on pH variation of chitinolytic enzyme activity

Chitnolytic activity

Based on the obtained results, the comparison of chitinolytic activities in *Aspergillus tubingensis* indicates a significant production of chitinase 10°C, 30°C and 50°C but with different amounts: (1.80x10^7U), (1.90x10^7U)and (2x10^7U) respectively (fig.7).

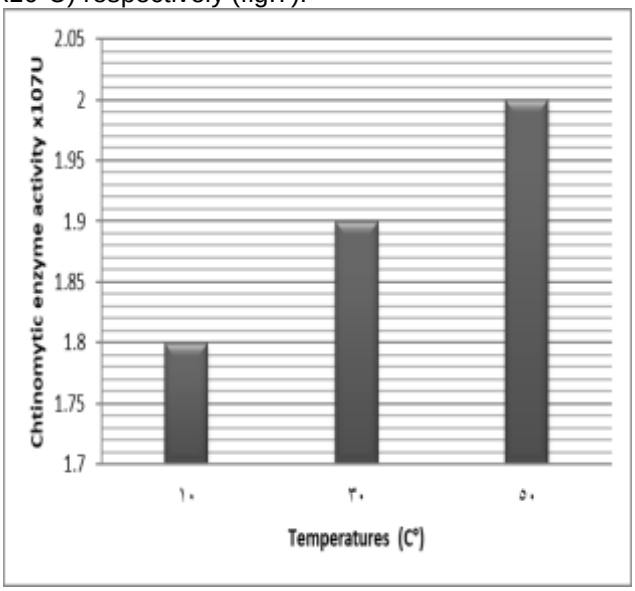


Figure 7: Effect of temperature on the chitinolytic activity of *Aspergillus tubingensis*(*H.aspersa*).

DISCUSSION

The 17 million palm trees in Algeria produce an annual average of 600,000 tons of dates (D. S. A., 2013). One of the most widely used cultivars worldwide is DegletNour.Date export is an unavoidable economic problem, and ensuring its sustainability is aninvestment in the social stability of arid regions. However, dates are vulnerable to numerous diseases and pests that reduce their yield and degrade their quality, especially those that are destined for export. The primary insect pest of dates is the date moths. It is listed on List A of the pests that require mandatory control (Executive Decree No 95-387 of 28 November 1995). Every year, in all the phoenician cultural zones of the various ecological entities, the Algerian Ministry of Agriculture and Rural Development takes care of treating nearly 3900,000 palm trees against Boufaroua disease for all cultivars and Myeloid disease only for DegletNour cultivar. (I. N. P.V., 1984). The Algerian Ministry has allocated 85.7 million Algerian Dinars to the Southern Wilayas growing dates in order to combat Boufaroua and Myelois diseases (I. N. P.V., 1984).The only method for managing date moths is chemical control. Unfortunately, this means is still only limited because larvae feed and grow inside the fruit, which harms biodiversity. Date infestations in fields and storage facilities significantly reduce the commercial quality of dates and pose a threat to exports, especially of the DegletNour variety (Bensalah and Ouakid, 2015). Numerous studies have been conducted in Algeria and other nations on the development of larvae infestation at various date fruit developmental stages. Researchers

have discovered varying infestation rates. One of the most dangerous palm pests in the world is Ectomyeloisceratoniae Zeller (Lepidoptera: Pyralidae). It harms many valuable fruit trees, palms, and crops like carob, pomegranate, and dates worldwide. In North Africa, Wertheimer (1958) and Warner (1988) reported a damage rate of more than 10% and up to 30%. According to Munier (1973), the pest infested 8% to 10% to as many as 80% of the dates. Pomegranate cultivation in a Tunisian oasis was vanishing as a result of moth attacks, which could cause up to 80% of damage (Khoualdia et al. 1995). Doumandji-Mitiche 1983 reported a percentage of 42.5% of date attacks in collecting areas under the date palms and an increase of up to 64.7% at storage areas as well as an average loss rate of 2 to 10% (Nay and Perring, 2005), as well as up to 57% under specific circumstances (Idder et al. 2009).

In terms of methods and strategies, controlling Ectomyeloisceratoniae (Zeller) is problematic; it actually presents new difficulties. A successful defense against pest attacks might be biological control. Microorganisms could be used as an alternative to parasitoids, which are currently used to control the date moth. This is especially true given the high efficacy of microbial treatment against other crop pests. Entomopathogens (fungi and bacteria) are used for this control (Saiah et al. 2011). Most insects are susceptible to microorganisms like fungi, bacteria, and viruses (Scholte et al. 2004). In aquatic, terrestrial, and underground habitats, about 750 species of fungi have been identified as obligate or facultative pathogens on one or more stages of insect development (McCoy et al. 1988).

Insect biology traits like survival, development, fecundity, and food intake are all impacted by entomopathogenic fungi and their metabolites. Ascomycetes, which are members of the order Hypocreales, are the most researched fungi in biological aphid control (Beauveria, Metarhizium, Nomura, Verticillium, and Peacilomyces). Regarding the high mortality rate against insects (epizootics) that was recorded, Beauveriabassiana and Metarhiziumanisopliae were thought to be the most effective (Burges, 1981; McCoy et al. 1988).

Nine isolates from the four genera of Aspergillus, Fusarium, Cladosporium, and Alternaria were able to be listed in the current study thanks to the successful isolation of fungal strains. The findings indicate that Aspergillus has a 70% impact on insects, which is consistent with the findings of Won et al. (2015). Environmental, industrial, and agricultural lifestyles were all represented by different Aspergillus species. Several of them have the potential to be opportunistic pathogens of a variety of organisms, including agricultural pests (Gibbons and Rokas, 2012). It has been discovered through toxicity testing that Aspergillus species may be effective aphid pest control agents. However, these saprophytic fungi not only target insects but also weak immune systems in humans, mammals, and birds (Tell, 2005).

Biological control research is currently being done on A. flavus strains that don't produce any aflatoxins and aren't toxicogenic (Ehrlich, 2014). The recorded mortality rate for Fusarium (10%) was different from that reported by Guesmi-Jouani et al. (2010), who found a mortality rate of more than 90%. Fusarium had a higher efficacy than what was specifically reported for Glover gossypii aphids (Ganassi et al. 2001). Susceptibility and the fungi's vulnerability (Glare et al. 2012). On the other hand, after the seventh day, Cladosporium and Fusarium exerted a significant effect of (10%). (Saranya et al.2008) demonstrated that C. oxysporum conidial suspension, when used at a concentration of 108 conidia/ml incorporated in a Teepol solution, could cause mortality in the order of 77.5% in individuals of Aphis craccivora (Koch). According to Bensaci et al. (2015), C. oxysporum's maximum mortality rate against A. fabea was 67.96%. Selection of entomopathogenic fungi for biological control of insects remains It was discovered that Aspergillus tubingensis degraded chitin. *Lecanicilliumlecanii*, *Peacilomycesvariotii*, Beauveriabassiana (Dhawan and Joshi, 2017; Suresh and Chandrasekaran, 1998), and Beauveria feline all have chitinases (Patidar et al. 2005). The comparison of the amounts of enzymes produced by the selected strain: *Aspergillus tubingensis* is carried out by fermentation on medium-based snail pieces in Erlenmeyer flasks with an inoculums rate (10^7 conidia/ml).

The outcomes demonstrate that snail fragments are an advantageous substrate for chitinase production. It also demonstrates the richness of the chitin. Furthermore, the outcomes concur with those reported by (Kaya et al. 2015). In grasshoppers, chitin content ranges from 5.3% to 8.9%. Chitinases are being investigated for their potential as biological pest control agents for insects. They are regarded as some of the crucial enzymes for breaking down insect cuticle polymeric chitin (Stoykov et al. 2015). The results that were obtained are comparable to those that Suresh and Chandrasekaran (1998) reported for the production of chitinase by Beauveriabassiana on shrimp waste medium with a ratio of 5/1 (shrimp waste/seawater for 96 hours).

However, according to Dhawane and Joshi (2017), B.bassiana MTCC 4495 had the highest average chitinase activity (0.51 U/ml), followed by B.bassiana MTCC 2028 (0.49 U/ml). The findings are consistent with those made public by Lee et al. (2009), and they also demonstrated that Penicillium sp. LYG 0704 exhibited its highest chitinase activity on the third day of culture. Furthermore, Binod et al. (2005) pointed out that 72 hours is the ideal incubation period for producing chitinase.

Including enzymatic processes, metabolic processes have an impact on the pH of the medium. Because of this, changes in pH are signs of changes in metabolic activity (Sandhya et al. 2005). After the culture, the pH rises to a value of 7.97. According to Botton et al. (1990), this

increase in pH is caused by the medium's release of amino acids and ammonium ions (NH4+) after a period of intense proteolysis, as well as the medium's depletion of nutritive substrates and the onset of mycelial autolysis.

For most fungal chitinases, including those of the species Lecanicillum, the ideal pH was observed to be 4-6. (Tikhonov et al. 2002). Gliocladumcatenulatum (Ma et al. 2012), M. anisopliae (Kang et al. 1999), Talaromyces flavus (Duo-Chuan et al. 2005), and Aspergillus fumigatus (Xia et al. 2009). According to earlier studies, the ideal pH range for fungal chitinases is between 4 and 8. (Sashai and Manocha, 1993).

CONCLUSION

At the plantation level as well as on all stock levels, careful and permanent control of storage conditions, as well as the use of biochemical and biological control methods, must be applied to prevent insect attacks on our agricultural and food products.

Our work is carried out to manage the date moth *Ectomyeloisceratoniae* (zeller) which represents the potentially devastating pest in the palm grove because it occurs at the beginning and the end of maturity stages of date crops and causes considerable damage due to the date contamination as well as the climatic factors. Storing dates in poorly conditioned areas favors the development of *Ectomyeloisceratoniae (Zeller).*

Our results confirm the potential damage caused by *Ectomyeloisceratoniae (zeller)* which is widely recognized as a date moth and demonstrates the insecticidal activity of entomopathogenic fungi against this major pest of stored dates. Thus, they could be used as an alternative to the chemical treatments

Chitinase has an interest because of its potential use in many industrial sectors. This enzyme has great importance in biotechnology and it could be found in many fields such as agric-food and medical fields. The production of this enzyme has been successful by some bacteria, insects, higher plants, mammals, and fungi. The isolation of fungal strains allowed the listing of 9 isolates representing 4 genera: *Aspergillus*, *Fusarium, Cladosporium, and Alternaria*. The major genus is *Aspergillus* with a frequency of 70% grouping 5 species: *Aspergillus parasiticus, Aspergillus tubingensis, Aspergillus niger (2 species), Aspergillus flavus* and *Aspergillus Fumigatus*, followed by the genus *Fusariumoxysporium , Cladosporiumcladosporoides* and *Aletrnaria alternate* with a percentage of 10%. Among them, *Fusariumoxysporium* and *Aspergillustubingensis* that showed a considerable effect against *Ectomyeloisceratoniae (Zeller)* with a percentage of 77.78% and 55.83% respectively.

Based on the obtained results, the comparison of chitinolytic activities in *Aspergillus tubingensis* indicates a significant production of chitinase 10°C, 30°C and 50°C but with different amounts: ($1.80x10^7$U), ($1.90x10^7$U)and ($2x10^7$U) respectively, the best enzymatic activity that was observed in *Aspergillus tubingensis* at pH=7 with an activity of ($2.3x10^7$ U), followed by pH=4 of ($1.8x10^7$ U) and finally pH=10 with ($1.5x10^7$ U). *Aspergillus* is no longer considered a pathogenic strain, and it could be used in biological control (entomopathogenic), using food waste (animal, plant) as a basic substrate for enzyme production.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

ACKNOWLEDGEMENT

The authors thank Faculty of Natural and life sciences, Constantine 1 University, Constantine. Algeria , Larbi Ben M'hidi University, 04000Oum El Bouaghi, Algeria , Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et de l'Univers , Université 8 Mai 1945 , Guelma , Algérie , Faculty of Agriculture, Department of Plant Protection, Isparta University of Applied Sciences, 32260 Isparta, Turkey., Faculty of Education, Department of Biology, Albaydha University, Al-Baydha, Yemen , Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia; for their kind and great help.

AUTHOR CONTRIBUTIONS

OA, NB, AB, AG, CD and I M collaborated in the creation of the manuscript. Ik and ALB participated in the pathogenicity test. In addition to that, OA, SMA , MAA elaborated in the identification of the fungi and enzyme's test. Finally, OA and NAA produced chitinase enzyme. All authors read and approved the final manuscript.

REFERENCES

Abbott, W.S. 1925. A method for computing the effectiveness of an insecticide. Journal of EconomicEntomology 18:265-267.

Bensaci, O. A., H.Daoud, N. Lombarkia and Rouabah K.(2015). Formulation of the endophytic fungus Cladosporiumoxysporum Berk.& MA Curtis, isolated from Euphorbiabupleuroidessubsp. luteola, as a new biocontrol too against the black beanaphid (AphisfabaeScop.). Journal of plant protection research. 55(1): 80-87.

BensalahM.K.andOuakid M.L. (2015). Essai de luttebiologiquecontre la pyrale des dattes.Courrier du Savoir – N°20, Octobre 2015. Université Mohamed Khider – Biskra, Algérie, 2015

Botton, B., A.Breton, M.Fèvre, P. Guy, J.P. Larpentand Veau P. (1990). Moisissuresutilesetnuisibles. Importance industrielle. Paris

Binod, P., T.Pusztahelyi,V. Nagy, C.Sandhya , G.Szakács, L. Pócsi, and PandeyA. (2005). Production and purification of extra cellularchitinases from Penicillium aculeatum NRRL 2129 under solid-state fermentation .Enzyme and Microbial Technology.36(7): 880-887.

Butt, T.M. and Goettel, M.S. (2000) Bioassays of Entomogenous Fungi. In: Navon, A. and Ascher, K.R.S., Eds., Bioassays of Entomopathogenic Microbes and Nematodes, CAB International, Wallingford, UK, 141-195. http://dx.doi.org/10.1079/9780851994222.0141

Burges, H. D. (1981). Safety, safetytesting and quality control of microbial pesticides. In: Burges HD, editor. Microbial control of pests and plant diseases 1970-1980. London: Academic Press, pp. 737-767.

Dhawan, M., & Joshi, N. (2017). Enzymatic comparison and mortality of Beauveriabassiana against cabbage caterpillar Pierisbrassicae LINN. brazilian journal of microbiology, 48, 522-529.

DoumandjiMitiche, B. (1983). Contribution à l'étude bio-écologique des parasites et des prédateurs de la Pyrale des caroubes, Ectomyeloisceratoniae Zeller,(Lepidoptera, Pyralidae), en Algérie en vue d'une éventuelle lutte biologique contre ce ravageur (Doctoral dissertation).

D.S.A. (2013). Direction des services agricole (DSA) Biskra. Rapport : situation de l'agriculture de la wilaya de Biskra

Ehrlich, K. C. (2014). Non-aflatoxigenic Aspergillus flavus to preventaflatoxin contamination in crops: advantages and limitations. Front Microbiol. 5:50.

Ganassi, S., A. Moretti, C. Stornelli, B. Fratello, A.M. Pagliai and Logrieco A.(2001). Effect of Fusarium, Paecilomyces and Trichoderma formulations against aphidSchizaphisgraminum. Mycopathologia. 151: 131–138.

Glare, T., J. Caradus, W. Gelernter, T. Jackson, N. Keyhani, J. Köhl, P. Marrone, L. Morin and Stewart A. (2012) . Have biopesticides come of age. Trends Biotechnol. 30 :250–258.

Guesmi-Jouini, J., N. Boughalleb-M´hamdiand .Ben Halima-Kamel M. (2010). Etudes préliminairessur les champignons entomopathogènes des pucerons de l'artichaut en Tunisie.Entomologie faunistique – Faunistic Entomology. 63 (3) : 171-181.

Idder, M.A., H. Idder-Ighili, H. Saggou and Pinturea B. (2009). Infestation reteandmorphology of the carobmoth Ectomyloiscertoniae (Zeller) on differentvarieties of the palm date,phoenixdactylifera(L). Chierd'Agriculture. 18(1):63-71.

INPV.(1984). Institut National de Protection des Végétaux.Ravageurs du palmierdattier. Bull. Phytosan.N°34, 03 juin 1984.INPV.MAP. 03p.

Khoualdia , O., A. Rhouma, J.P. Mrro and Brun J. (1995).Un trichogramme, nouveau parasite d'E.certoniaezeller (lepidoptera, pyralidae).En Tunisie .Ann. Ed INRA.Tunisie : 145-151.

Kang, S. C., Park, S and Lee D.G . (1999). Purification and characterization of a novel chitinase from the entomopathogenic fungus, Metarhiziumanisopliae. Journal of invertebrate pathology. 73(3):276-281.

Kaya, M and Baran T. (2015). Description of a new surface morphology for chitin extracted from wings of cockroach (Periplaneta americana). International journal of biologicalmacromolecules. 75: 7-12.

Lacey, L. A and Undeen A.H. (1986) .Microbial control of black flies and mosquitoes. AnnualReview of Entomology. 31(1): 265-296.

Lee, C. G., D. Hartl, G.R. Lee, B. Koller, B., Matsuura, H., Da Silva, C. A., ...and Humbles A .(2009) . Role of breastregressionprotein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13–induced tissue responses and apoptosis. Journal of ExperimentalMedicine. 206(5): 1149-1166.

Ma, G. Z., H.L.Gao, Y.H.Zhang, S.D. Li, B.Y. Xie AND Wu S.J. (2012). Purification and characterization of chitinase from Gliocladiumcatenulatum strain HL-1-1. African Journal of Microbiology Research. 6(20): 4377-4383.

McCoy, C.W., R. A. Samson and Boucias D.G. (1988). Entomogenous Fungi. In: Handbook of Natural Pesticides: Microorganisms. (Eds.: Ignoffo et.al.). CRC Press, pp. 260.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical chemistry. 31(3), 426-428.

Munier, P. (1973).Le palmierdattier. Techniques agricoleset productions tropicales. Maison Neuve et Larousse. Paris, France. 217

Nay J.E andPerring T.M. (2005) .Impact of antpredationantheat on carobmoth (Lepidoptera: Pyralidae) mortality in california date gardens. Journal of Economic Entomology .98(3) :725-731.

Packer, H. L and Thomas C.R .(1990). Morphological measurements on filamentous microorganisms by fully automatic image analysis. Biotechnology and bioengineering. 35(9): 870-881.

Patidar, P., D. Agrawal, T.Banerjee and Patil S.(2005) .Optimisation of process parameters for chitinase production by soilisolates of Penicillium chrysogenum under solid substrate fermentation. ProcessBiochemistry.40(9) : 2962-2967.

Roberts, D. Wand .HajekA.E .(1992). Entomopathogenic fungi as bio-insecticides.In Frontiers in industrial. Mycology (pp. 144-159).Springer, Boston, MA.

Saiah, F., A.Berkani, B.S. Bendahmane, M.Y. Benkadda ,W.Lakhdari and Kolai N.(2011). Isolement de champignons entomopathogènes à partir de

PhyllocnistiscitrellaStainton (Lepidoptera: Gracillariidae). Entomologie faunistique Faunistic Entomology. 2011;63(3):199–202

Sandhya, C., K.M.Nampoothiri and Pandey A. (2005) .Microbial proteases. In Microbial enzymes and biotrans formations (pp. 165-179). Humana Press.

Saranya, S., R. Ushakumari, S. Jacob and Babu Philip M. (2008). Efficacy of different entomopathogenic fungi against cowpeaaphid, Aphiscraccivora (Koch). J. Biopest. 3 (1 Special Issue) : 138 - 142.

Sashai, A. S and Manocha M.S.(1993). FEMS Microbiol.Rev. 11: 317-338 .

Scholte, EJ., B.G.J.Knols, R.A.Samson and W.Takken .2004.Entomopathogenic fungi for mosquito control: a review. J. InsectSci. 4(19) : 1-24. (insectscience.org/4.19).

Stoykov, Y. M., A.I.Pavlov and Krastanov A.I.(2015). Chitinase biotechnology: production, purification, and application. Engineering in Life Sciences. 15:30-38

Suresh, PV and Chandrasekaran M.(1998). Utilization of prawnwaste for chitinase production by the marine fungus Beauveriabassiana by solid state fermentation. World J. Mircobiol. Biotechnol. 14:655-660.

Tell, L. A. (2005). Aspergillosis in mammals and birds: impact on veterinary medicine. Medical Mycology. 43(Supplement_1): S71-S73.

Toharisman, A., M.T.Suhartono, M.Spindler-Barth, J.K.Hwang and Pyun Y.R.(2005).Purification and characterization of a thermo stable chitinase from Bacillus licheniformis Mb-2. World Journal of Microbiology and Biotechnology.21(5): 733-738.

Taira, T., Ohnuma, T., Yamagami, T., Aso, Y., Ishiguro, M., &Ishihara, M. (2002). Antifungal activity of rye (Secalecereale) seed chitinases: the different binding manner of class I and class II chitinases to the fungal cell walls. Bioscience, biotechnology, and biochemistry, 66(5), 970-977.

Tikhonov VE, Lopez-Llorca LV, Salinas J, JanssonHB .(2002) . and characterization of chitinases from the nematophagous fungi Verticilliumchlamydosporium and V. suchlasporium .Fungal Genet Biol. 35:67–78.

Warner, R.L. (1988) Contribution to the biology and themanagement of the carobmoth, Ectomyeloisceratoniae(Zeller) in 'DegletNoor' date gardens in the CoachellaValley of California.- Ph.D. Dissertation, University of California, Riverside, USA.

Wertheimer, M. (1958) Un des principaux parasites du palmierdattieralgerien: le myloisdecolore. Fruits.13(8) : 109-123 .

Won, W. L., Y. S. Tae, M. B. Sung and Soo D. W. (2015). Screening and evaluation of entomopathogenic fungi against the green peachaphid, Myzuspersicae, using multiple tools.J.Asia-Pac. Entomol. 18: 607–615.

Xia, J. L., J.Xiong, T. Xu, C.G.Zhang, R.Y.Zhang, Q.Zhang, Q ….andQiu G.Z.(2009). Purification and characterization of extracellular chitinase from a novelstrain Aspergillus fumigatus CS-01. Journal of Central South University of Technology.16(4): 552-557.

Zimmermann, G. (1986). The Galleriabait method ’for detection of entomo pathogenic fungi in soil. Journal of applied Entomology. 102(1-5):213-215.