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ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI FROM *BORAGO OFFICINALIS* L. AND EVALUATION OF THEIR BIOINSECTICIDAL EFFECT AGAINST MOSQUITO LARVAE *CULISETA LONGIAREOLATA* (MACQUART, 1838)

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Abstract

Description of the subject: Endophytic fungi and their products present a promising alternative to chemical control of mosquito larvae and adults.
Objective: This study aims to isolate and to identify the endophytic fungi from *Borago officinalis* L. (*B. officinalis* L.), and then to evaluate their bioinsecticidal power against fourth stage (L4) mosquito larvae of *Culiseta longiareolata* (Macquart, 1838) (*Cs. longiareolata*).
Methods: The endophytic fungi were isolated from the leaves of *B. officinalis* L. collected from the wilaya of Boumerdes, Algeria and then identified by macroscopic and microscopic examinations. The laboratory, bioassays were performed with six different concentrations (10^2, 10^3, 10^4, 10^5, 10^6, 10^7 spores/ml).
Results: The three endophytic fungi that were isolated and identified are *Fusarium sp.* (Bo1) ; *Penicillium sp.* (Bo2) and *Cladosporium sp*. (Bo3). The best larvicidal activity is attributed to *Fusarium sp*. (LC_{50}=6.45×10^5 spores/ml), followed by *Penicillium sp*. (LC_{50}=1.99×10^9 spores/ml) and finally *Cladosporium sp.* (LC_{50}=6.30×10^{10} spores/ml). The LT_{50} values ranged from 11 h to 7 days. (p<0.01)
Conclusion: These findings indicate that the leaves of *B. officinalis* L. can be a niche of several endophytic fungi with great efficiency against mosquito larvae, which suggests their use as an alternative to chemical control, more economical and less harmful for humans and environment.
Keywords: vector-borne diseases ; *B. officinalis* L. ; endophytic fungi ; *Cs. longiareolata* ; biological control.

ISOLEMENT ET IDENTIFICATION DES CHAMPIGNONS ENDOPHYTIQUES DE *BORAGO OFFICINALIS* L. ET ÉVALUATION DE LEUR EFFET BIOINSECTICIDE CONTRE LES LARVES DE MOUSTIQUE *CULISETA LONGIAREOLATA* (MACQUART, 1838)

Résumé

Description du sujet : Les champignons endophytes et leurs produits présentent une alternative prometteuse à la lutte chimique contre les larves et les adultes de moustiques.
Objectifs : Cette étude vise à isoler et à identifier les champignons endophytes de *Borago officinalis* L. (*B. officinalis* L.), et à évaluer leur pouvoir bioinsecticide contre les larves de moustique de quatrième stade (L4) de *Culiseta longiareolata* (Macquart, 1838) (*Cs. longiareolata*).
Méthodes : Les champignons endophytes ont été isolés à partir des feuilles de *B. officinalis* L. récoltées de la wilaya de Boumerdès, Algérie puis identifiés par des examens macroscopiques et microscopiques. Au laboratoire, les essais biologiques ont été réalisés avec six concentrations différentes (10^2, 10^3, 10^4, 10^5, 10^6, 10^7 spores/ml).
Résultats : Les trois champignons endophytes qui ont été isolés et identifiés sont *Fusarium sp.* (Bo1) ; *Penicillium sp.* (Bo2) ; *Cladosporium sp.* (Bo3). La meilleure activité larvicide est attribuée à *Fusarium sp.* (CL_{50}=6,45×10^5 spores/ml), suivi de *Penicillium sp.* (CL_{50}=1,99×10^9 spores/ml) et enfin *Cladosporium sp.* (CL_{50}=6,30×10^{10} spores/ml). Les valeurs TL_{50} varient de 11 h à 7 jours. (p<0,01)
Conclusion : Les résultats indiquent que les feuilles de *B. officinalis* L. peuvent être une niche de plusieurs champignons endophytes avec une grande efficacité contre les larves de moustiques, ce qui suggère leur utilisation comme alternative à la lutte chimique, plus économique et moins nocive pour l'homme et l'environnement.
Mots clés : maladies à transmission vectorielle ; *B. officinalis* L. ; champignons endophytes ; *Cs. longiareolata* ; lutte biologique.

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INTRODUCTION

Belonging to the Culicidae family *Cs. longiareolata* (Macquart, 1838) is one of the medically and veterinary important mosquitoes that transmit a wide range of vector-borne diseases around the world [1-3]. During a blood meal, females ingest pathogenic microorganisms (virus, parasite, bacteria) present in an infected host (human or animal), to reinject them into a new host after the pathogen has reproduced. Often, once a vector becomes infectious, it is able to transmit the pathogen for the rest of its life cycle with each subsequent blood meal or bite [4 and 5]. Females of *Cs. longiareolata* (Macquart) rarely bite humans, however, these small insects can be found in human dwellings where they become an important vector of brucellosis, avian influenza and West Nile encephalitis as a secondary vector. *Cs. longiareolata* (Macquart) can also be an intermediate vector of avian Plasmodium causing Malta fever [1, 3, 6 and 7]. This species is mainly of veterinary interest through the transmission of a wide range of pathogenic parasites and arboviruses such as malaria in birds (*Plasmodium spp.*) [4 and 5]. This multivoltine, thermophilic, and ornithophilic species is widely distributed around the word mainely the Mediterranean Sea, Africa, Europe and Asia [8 and 9]. The use of chemical insecticides such as organochlorines, organophosphates, carbamates and synthetic perythrenoids seem to be the most effective method to reduce mosquito population [10 and 11]. However, their intensive use has created a number of problems such as human health, the development of insect resistant, ecological imbalance and damage to non-target organisms due to their high toxicity [11]. Comparatively to chemical pesticides, endophytic fungi are currently considered to be one of the most promising biological groups in vector control which lead to the commercialization of a large number of biopesticide products from fungi [12]. In fact, these endophytic fungi can act on the cuticle of insects without negative effect on humans and environment [13-15]. They also have the advantage of being inexpensive, easy for application with high efficiency [16 and 17]. Despite the wide distribution of *Cs. longiareolata* (Macquart) in Algeria especially in arid areas [18], limited or no works were interested in testing the sensitivity of these insects using endophytic fungi. In this perspective, the aim of this study is to isolate and to identify for the first time in Algeria, the endophytic fungi of a Boraginaceae (*B. officinalis* L.) and to evaluate their effects on *Cs. longiareolata* (Macquart, 1838) larvae.

MATERIEL AND METHODS

1. Plant material

The leaves of *B. officinalis* L. were collected with a sterile scissor from the wilaya of Boumerdes, Algeria (36° 46′ 0″ North, 3° 28′ 0″ East). It was harvested away from air pollution and appearing to have no pathological symptoms or superficial damage. The fresh part of the plant was sent to the laboratory in sterile glass jars within a period not exceeding 24 h [19].

2. Animal material

The fourth instar larvae (L4) of *Cs. longiareolata* (Macquart, 1838) come from an untreated site at the University of Boumerdes by using a metal ladle.

3. Isolation and purification of endophytic fungi

In order to get rid of the hyphae and spores of epiphytic fungi, the leaves of *B. officinalis* L. underwent a series of surface disinfection in soapy water for 3 min, in sterile distilled water for 10 min, then an immersion in ethanol 70% for 1min. This was followed by sodium hypochlorite NAOCL 3% sterilization for 4min, then a second immersion in ethanol 70% (30sec), and finally rinsing with sterile distilled water twice for 1min [20 and 21]. To check the effectiveness of the surface disinfection, the PDA medium (Potato Dextrose Agar) was inoculated with a drop of distilled water obtained from the last rinse. The leaves thus obtained from the first step are cut into small pieces ($2mm^2$) using a sterile scalpel and then placed aseptically in Petri dishes containing a PDA culture medium supplemented aseptically with 18ml of Tetracycline to inhibit bacterial growth. The Petri dishes were then incubated for 7 days at 25°C. The purification was carried out by a successive subculture on a new sterile culture medium PDA. Endophytic fungi were recorded with a two-letter code followed by a number. The first and second letters correspond successively to the genus and species of the plant studied. The number indicates the order of appearance.

4. Endophytic fungi identification

The identification is mainly based on macroscopic (color, diameter, speed of growth and appearance) and microscopic criteria (nature of the mycelium and types of conidia).

5. Bioinsecticidal activity

-*Preparation of the entomopathogenic solution*: Using a sterile spatula, small fragments of each fruiting fungus were scraped from the surface of the dish to be introduced into a test tube containing 9ml of distilled water and 2-3 drops of Tween 80. The solution thus obtained was subjected to the action of a magnetic stirrer (Ibx instruments, Germany) for 10 min. In total, six concentrations were prepared from the stock solution C1, C2, C3, C4, C5, and C6 corresponding to 10^7, 10^6, 10^5, 10^4, 10^3, 10^2 spores/ml for each fungus.

-*Experiment method*: The bio-tests were carried out in cups containing 49 ml of distilled water, 1 ml of each entomopathogenic solution and 20 mosquitoes larvae of fourth stage. The same number of larvae was placed in a control cup containing 50 ml of the breeding water. According to Abbott [22], the mortality rate and corrected mortality were calculated every 24h, 48h, 72h, 96h of treatement using the following formulas:

Mortality rate% = (Death number) / (Total number of individuals) ×100.

Corrected mortality% = [Mortality in treated group - Mortality in the control group /100-Mortality in the control group] ×100

6. Statistical analysis

Results were expressed as mean ± SEM of three repetitions. The statistical comparison between groups was performed with one-way ANOVA analysis using Statistica version 6.

RESULTS

1. Isolation of endophytic fungi

After seven days of incubation at 25°C, the endophytic fungi begin to grow from the leaf segments of *B. officinalis* L. The efficiency of the disinfection was checked and showed no microbial growth indicating that the epiphytes were completely removed according to the disinfection protocol. A total of three fungal isolates were obtained from *B. officinalis* L leaves (Bo1, Bo2 and Bo3). The colonization rate (CR) and isolation frequency (IF) of a taxon were calculated. All the results are mentioned in the following table (Table 1):

Table 1. Colonization rate and frequency of isolation

Fungal isolates	Total number of inoculated segments	Number of segments with endophytes	CR (%)	Number of taxon isolation	IF (%)
Bo1	07	05	71.43	2	28.57
Bo2				1	14.28
Bo3				2	28.57

CR : colonization rate; FI : isolation frequency

2. Macroscopic and microscopic identification

The macroscopic and microscopic identification were carried out. The microscopic identification was performed by the study in the fresh state and with the methylene blue technique (tape method). Totally, the three endophytic fungi which were isolated from the leaves segments of *B. officinalis* L. are *Fusarium sp*. (Bo1); *Penicillium sp*. (Bo2) and *Cladosporium sp*. (Bo3). (Table 2)

3. Bioinsecticidal results

In the conducted experiment, the mortality kinetics of *Cs. longiareolata* (Macquart) larvae show significant sensitivity to all entomopathogenic. A proportional variation in time, doses and endophytic fungus species was recorded (Fig. 1). The value of P is highly significant (p <0.01).

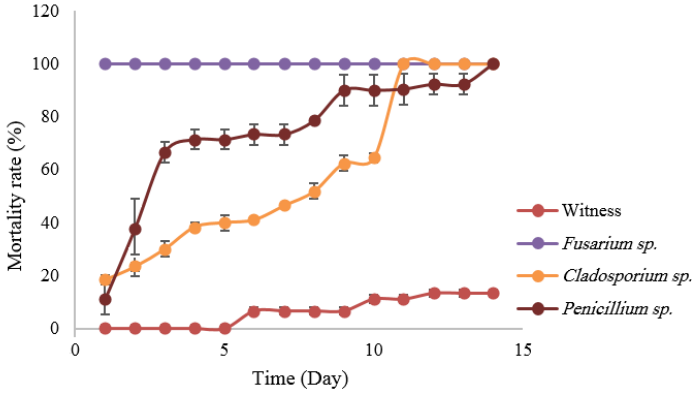
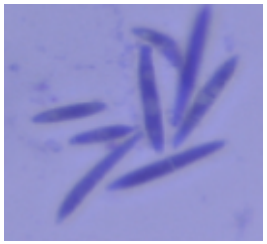
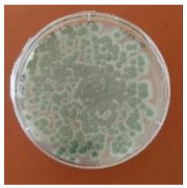
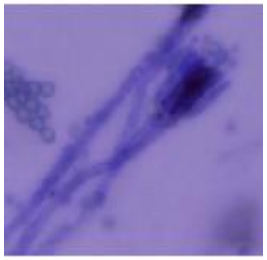
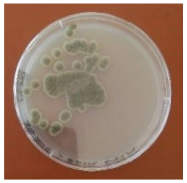
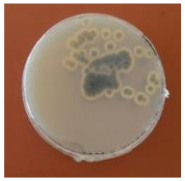
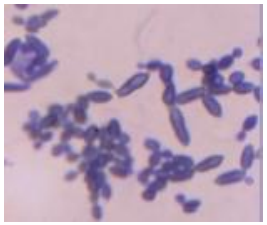


Figure 1: Corrected mortality kinetics (± ESM) of *Cs. longiareolata* (Macquart) larvae treated with the 10^7 spores/ml concentration of the three entomopathogenic after 15 days of treatment

Table 2: Macroscopic and microscopic identification of the three fungal isolates

Fungal isolates	Macroscopic identification						Microscopic identification		
	Color		Diameter (cm)		Growth rate	Aspect	Microscopic observation	Characteristics	Genus
	Front	Back	5 days	10 days					
Bo1	Whitish 	light orange 	7.6	8.8	3 days	Cottony, white filament, irregular outline, absence of water droplets (exudate)		* Septate and colorless mycelium; * Three types of conidia: sickle-shaped macroconidia with several transverse septa; scalariform microconidia; blastoconidia with 0 to 3 septa.	*Fusarium sp.*
Bo2	Belu green 	Yellow 	2.95	Pigmented colonies	8 days	Powdery, irregular pigmented, presence of water droplets (exudate)		A brush-like organization in the form of a septate and hyaline mycelial filament, of simple conidiospores which end in a penicil. The phialides are arranged in a biverticillea at the tips of the conidiospores.	*Penicillium sp.*
Bo3	Dark green with white outline 	Green surrounded by white 	1.65	Pigmented colonies	7 days	Powdery, hard, rounded relief		Pigmented septate hyphae; conidiospores of variable length; acropetal chain blastopores	*Cladosporium sp.*

For the other concentrations (10^6; 10^5; 10^4; 10^3 and 10^2 spores/ml), this mortality rate was only obtained after the 9th, 12th, 13th, 13th and 14th days respectively).

It emerges that the best bioinsecticidal activity is attributed to the endophytic fungus *Fusarium sp.* (Bo1) which recorded 100% mortality from the first day of treatment. The effect of *Cladosporium sp.* (Bo2) on the larval population started from the first day but did not reach its maximum until after the 11th day ($C_1=10^7$ spores/ml) ($P < 0.01$). For *Penicillium sp.* (Bo3), the larvicidal effect was a bit prolonged in time (14th day of treatement) compared to the other entopathogenic solutions. The larvae showing sensitivity to entomopathogenic solutions underwent regression in size, total degeneration and change in color in the body of the insect (observed only with *Fusarium sp*. (Bo1)) (Fig. 2).

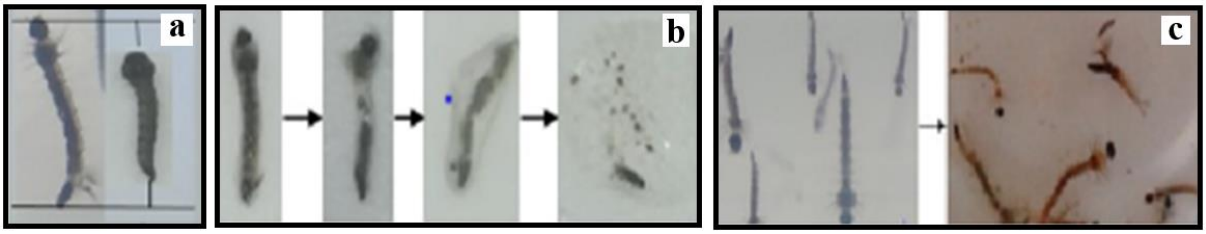


Figure 2: The effect of the three endophytic fungi (*Fusarium sp.; Penicillium sp.; Cladosporium sp*.) on the morphology of *Cs. longiareolata* (Macquart) larvae

a: a decrease in size; b: degeneration; c: a change in color in the insect's body

Values of LC_{50} and LT_{50} were obtained by plotting the Probits=f (log concentrations) and Probits=f (log time) regression lines (Table 3). The lowest concentration which causes half of larval mortality was obtained from the endophytic fungus Fusarium sp. (6.45×105 spores / ml) with the shortest LT_{50} value (11h). For the other solutions these values are much more prolonged in time, three days for Penicillium sp. and seven days for Cladosporium sp. with respective values of LC_{50} equal to 1.99×109 and 6.30×1010 spores/ml.

Table 3: Toxicity parameters after two days of treatment

Endophytic fungus	Equation	R^2	LC_{50} (spores/ml)	Equation	R^2	LT_{50}
Fusarium sp.	Y=0.7x+0.935	0.61	$6.45x10^5$	y=6.0381x – 1.0782	0.74	11h
Penicillium sp.	Y=0.72x-1.693	0.91	$1.99x10^9$	y=2.7138x + 0.0224	0.79	3d
Cladosporium sp.	Y=0.24x+2.147	0.94	$6.30x10^{10}$	y=1.2582x + 2.2074	0.92	7d

R^2 : coefficient of determination; LC_{50}: lethal concentration ; LT_{50}: lethal time 50; h : hour ; d : days

DISCUSSION

Several studies confirm the presence of these fungi in various medicinal plants. In fact, isolates belonging to the genus *Fusarium* (Bo1) were isolated as an endophyte from the roots, leaves and stems of *Hyoscyamus muticus* L. (Egyptian Henbane) [23]; and from samples of fresh bark of *Acacia catechu* willd [24].

Ramesha et *al*. [25], isolate *Fusarium sp*., *Fusarium semitectum* from the stems and flowers of *Nerium oleander* L. (Oleander), from the old and fresh leaves of the same plant [26] and from roots of plants growing in salty environments [27].

According to Abdel-Motaal et *al*. [23], the genus *Penicillium* (Bo2) was isolated as an endophyte from the roots, leaves and stems of the Egyptian Henbane *Hyoscyamus muticus* L. From Oleander flowers *Nerium oleander* L. [25]; roots of *Triticum durum* (wheat) [28]; leaves of *Olea europaea* L. (European olive tree) [29] and from the leaves of *Peganum harmala* (Harmel) [30].

Fungi of the genus *Cladosporium* (Bo3) has also been isolated as an endophyte from several medicinal plants. Huang et *al*. [31], isolate it from the stems of *Nerium oleander* L. Others have isolated it from the leaves, the stems and roots [23, 24, 28, 30 and 32].

The study of Halouane et *al.* [33], show that Deuteromycetes or imperfect fungi (including *Fusarium*, *Penicillium* and *Cladosporium* genus) are the most widely used in biological control to control insect populations. Indeed, Singh and Prakash [34], have shown that culture filtrates when combined, in the 1: 1: 1 ratios of *Fusarium oxysporum, Lagenidium giganteum, Trichophyton ajelloi* exhibit considerable mortality to control *Culex quinquefasciatus* adults. Furthermore, Govindarajan et *al*. [35], showed that the mortality rate of *Fusarium vasinfectum* culture filtrates isolated from the soil on 3rd instar of *Culex quinquefasciatus* larvae is observed after 24 h with a LC_{50} equal to 50.03 mg/ml.

According to Riba et *al.* [36], *Penicillium citrinum* has shown a very aggressive effect against *Culex pipiens, Aedes aegypti* and *Anopheles stephensi*. *Penicillium citrinum* mosquito larvae without multiplying in the host. In addition, Russell et *al.* [37], concluded that *Penicillium citrinum* helped to reduce the viability of *Aedes aegypti* eggs. Lara da Costa et *al*. [38], carried out *in vitro* biological tests in order to evaluate the pathogenicity of 13 strains of *Penicillium* in 2nd stage larvae *Aedes aegypti*, *Aedes fluviatiles*, *Anopheles aquasalis* and *Culex quinquefasciatus*. Death rates started within the first 24 h, ranging from 0 to 100%.

Ragavendran et *al.* [39], aimed to verify the larvicidal potential of fungal mycelia (with an ethyl acetate-based solvent) extracted from *Penicillium daleae* against larvae of stages 1-5 of *Culex quinquefasciatus* and *Aedes aegypti* at the highest concentration (1000 mg/ml) of extract, mortality begins at 18 h of exposure and reaches 100% mortality after 48 h of exposure.

The studies carried out by Laib [40], also isolated this fungus (*Cladosporium sp.*) from the leaves of Oleander *Nerium oleander* L. (Apocynaceae, Gentianales) and which showed that the filtrates of this endophytic fungus are endowed with good insecticidal activity against *Acanthoscelides obtectus* with a mortality rate of 84% recorded after 48 h of spraying the preparation at 100%.

CONCLUSION

The macroscopic and microscopic examinations allowed to identify the endophytic fungi from the leaves of *B. officinalis* L. namely, *Fusarium sp*. (Bo1) ; *Penicillium sp.* (Bo2); and *Cladosporium sp*. (Bo3).

Furthermore, the bioinsecticidal effect of these three endophytic fungi at different concentrations appears to be effective in controlling the larval population of *Cs. longiareolata* (Macquart). However, the best bioinsecticidal activity is attributed to *Fusarium sp.* (Bo1). It emerges from this study that *B. officinalis* L. is a niche of several endophytic fungi that may have a major biotechnological importance for controlling disease-carrying mosquitoes with minimal effect on the environment.

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REFERENCES

[1]. Malsov, A.V. (1989). Blood-sucking mosquitoes of the subtribe Culisetina (Diptera, Culicidae) in world fauna (*Smithsonian Institution Libraries and The National*, Vol. 93. Washington, D.C.

[2]. Paksa, A., Sedaghat, M.M., Vatandoost, H., Yaghoobi-Ershadi, M. R, Moosa-Kazemi, S. H., Hazratian, T, Sanei-Dehkordi, A. & Oshaghi, M.A. (2019). Biodiversity of mos-quitoes (Diptera: Culicidae) with empha-sis on potential arbovirus vectors in East Azerbaijan Province northwestern. *Journal of Arthropod-Borne Diseases*, 13(1): 62–75.

[3]. Ghahvechi Khaligh, F., Vahedi, M., & Chavshin, A.R. (2020). Identification of symbiotic bacteria in the midgut of the medically important mosquito, *Culiseta longiareolata* (Diptera: Culicidae). *BMC Research Notes*, 13(1): 1–7. https://doi.org/https://doi.org/10.1186/s13104-020-05220-0.

[4]. Ghahvechi Khaligh, F., Naghian, A., Soltanbeiglou, S., & Gholizadeh, S. (2020). Autogeny in *Culiseta longiareolata* (Culicidae: Diptera) mosquitoes in laboratory conditions in Iran. *BMC Research Notes*, 13(1): 1–5. https://doi.org/doi: 10.1186/s13104-020-04942-5

[5]. Schoener, E., Susanne Uebleis, S., Butter, J., Nawratil, M., Cuk, C., Flechl, E., Kothmayer M., Obwaller A.G., Zechmeister T., Rubel, F. (2017). Avian plasmodium in Eastern Austrian mosquitoes. *Malaria Journal*, 16(1): 1–12. https://doi.org/https://doi.org/10.1186/s12936-017-2035-1

[6]. Bisanzio, D., Giacobini, M., Bertolotti, L., Mosca, A., Balbo, L., Kitron, U., & Vazquez-Prokopec, G. (2011). Spatio-temporal patterns of distribution of West Nile virus vectors in eastern Piedmont Region. *Parasites & Vectors*, 4(1): 1–11. https://doi.org/https://doi.org/10.1186/1756-3305-4-230

[7]. Hazratian, T., Paksa, A., Sedaghat, M. M., Vatandoost, H., Moosa-Kazemi, S. H., Sanei-Dehkordi, A., Salim-Abadi Y., Pirmohammadi M., Yousefi S., Masoumeh A. & Oshaghi M.A. (2019). Baseline susceptibility of *Culiseta longiareolata* (Diptera: Culicidae) to different imagicides, in eastern Azerbaijan, Iran. *Journal of Arthropod-Borne Diseases*, *13*(4): 407–415.

[8]. Cranston, P.S., Ramsdale, C.D., Snow, K.R., & White, G.B. (1987). Adults, Larvae, and Pupae of British Mosquitoes (Culicidae) A Key (*Cumbria: Freshwater Biological Association*, Vol. 48.

[9]. Kampen, H., Kronefeld , M., Zielke , D., & Werner, D. (2013). Three rarely encountered and one new *Culiseta* species (Diptera: Culicidae) in Germany. *Journal of the European Mosquito Control Association*, 31: 36–39.

[10]. Hamaidia, K., & Soltani, N. (2014). Laboratory evaluation of a biorational insecticide, kinoprene, against Culex pipiens larvae: Effects on growth and development. *Annual Research & Review in Biology*, 4(14): 2263–2273. https://doi.org/DOI: 10.9734/ARRB/2014/9729

[11]. Dris, D., Tine-Djebbar, F., & Soltani, N. (2017). *Lavandula dentata* essential oils : chemical composition and larvicidal activity against *Culiseta longiareolata* and *Culex pipiens* (Diptera : Culicidae). *African Entomology*, 25(2): 387–394. https://doi.org/https://doi.org/10.4001/003.025.0387

[12]. De Faria, M. R., & Wraight, S. P. (2007). Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control*, 43(3): 237–256. https://doi.org/https://doi.org/10.1016/j.biocontrol.2007.08.001

[13]. Inglis, G.D., Goettel, M.S., Butt, T.M., & Strasser, H (2001). Use of hyphomycetous fungi for managing insect pests. *Fungi as Biocontrol Agents*, 23–69.

[14]. Sayed, S.M., Esmat, F.A., & Saqer , S.A.-O. (2019). Efficacy of indigenous entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, isolates against the rose aphid, *Macrosiphum rosae* L.(Hemiptera: Aphididae) in rose production. *Egyptian Journal of Biological Pest Control*, 29(1): 1–7. https://doi.org/https://doi.org/10.1186/s41938-019-0123-y

[15]. Fernández-Grandon, G.M., Harte, S.J., Ewany, J., Bray, D. & Stevenson, P. C. (2020). Additive effect of botanical insecticide and entomopathogenic fungi on pest mortality and the behavioral response of its natural enemy. *Plants*, 9(2): 173-. https://doi.org/https://doi.org/10.3390/plants9020173

[16]. Lacey, L.A., Frutos, R., Kaya, H.K., & Vail, P. (2001). Insect pathogens as biological control agents: do they have a future? *Biological Control*, 21(3): 230–248. https://doi.org/https://doi.org/10.1006/bcon.2001.0938

[17]. Eidy, M., Rafiee-Dastjerdi, H., Zargarzadeh, F., Golizadeh, A., & Mahdavi, V. (2016). Pathogenicity of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) and *Verticillium lecanii* (Zimmerman) against aphid *Macrosiphum rosae*, Linnaeus (Hemiptera: Aphididae) under laboratory conditions. *Jordan Journal of Biological Sciences*, 9(1): 25–28.

[18]. Bouaziz, A., Boudjelida, H., & Soltani, N. (2011). Toxicity and perturbation of the metabolite contents by a chitin synthesis inhibitor in the mosquito larvae of *Culiseta longiareolata*. *Annals of Biological Research*, 2(3): 134–142.

[19]. Toubal, S., Bouchenak, O., Elhaddad, D., Yahiaoui, K., Boumaza, S., & Arab, K. (2018). MALDI-TOF MS detection of endophytic bacteria associated with great nettle (*Urtica dioica* L.), grown in Algeria. *Polish Journal of Microbiology*, 67(1): 67–72. https://doi.org/ DOI: 10.5604/01.3001.0011.6145

[20]. Petrini, O, Sieber, T.N., Toti, L., & Viret, O. (1993). Ecology, metabolite production, and substrate utilization in endophytic fungi. *Natural Toxins*, 1(3): 185–196. https://doi.org/https://doi.org/10.1002/nt.2620010306

[21]. Liu, A.R., Wu, X.P., & Xu, T. (2007). Research advances in endophytic fungi of mangrove. *The Journal of Applied Ecology*, 18(4): 912–918.

[22]. Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18(2): 265–267. https://doi.org/https://doi.org/10.1093/jee/18.2.265a

[23]. Abdel-Motaal, F.F., Nassar, M.S., El-Zayat, S.A., El-Sayed , M.A., & Ito, S.I. (2010). Antifungal activity of endophytic fungi isolated from Egyptian henbane (*Hyoscyamus muticus* L.). *Pakistan Journal of Botany*, 42(4): 2883–2894.

[24]. Nagaraja, T.G., & Devkar, P.G. (2010). Seasonal occurrence of endophytic mycoflora of inner bark of medicinal plant *Acacia catechu* Willd. *The Bioscan*, 5(2): 243–245.

[25]. Ramesha, A., Sunitha, V.H., & Srinivas, C. (2013). Antimicrobial activity of secondary metabolites from endophytic fungi isolated from *Nerium oleander* L. *International Journal of Pharma and Bio Sciences*, 4(1) :683–693.

[26]. Laib, D., Lombarkia, N., & Bensaci, O.A. (2015). Utilisation des filtrats du champignon endophyte *Fusarium sp.* de *Nerium oleander* L. dans la lutte biologique contre *Sitophilus zeamais* en Algérie. *Revue Agriculture*, 6(2) :38–43.

[27]. Kouadria, R., Bouzouina, M., & Lotmani, B. (2018). Isolation and identification of endophytic fungi on plants growing in salt environments, using ITS and 18S molecular methods. *Plant Archives*, 18(2) : 1835–1840. https://doi.org/DOI: 10.4236/ajps.2015.619313

[28]. Sadrati, N., Daoud, H., Zerroug, A., Dahamna, S., & Bouharati, S. (2013). Screening of antimicrobial and antioxidant secondary metabolites from endophytic fungi isolated from wheat (*Triticum durum*). *Journal of Plant Protection Research*, 53(2) : 129–136.

[29]. Malhadas, C., Malheiro, R., Pereira, J.A., de Pinho, P.G., & Baptista, P. (2017). Antimicrobial activity of endophytic fungi from olive tree leaves. *World Journal of Microbiology and Biotechnology*, 33(3) :46-. https://doi.org/10.1007/s11274-017-2216-7

[30]. Ouzid, Y., Smail-Saadoun, N., & Houali, K. (2018). Comparative study of in vitro antioxidant activity of foliar endophytic fungi and leaves extracts of *Peganum harmala* of Dayate aiat (Laghouat, Algeria). *Journal of Fundamental and Applied Sciences*, 10(1) : 147–157. https://doi.org/DOI: 10.4314/jfas.v10i1.10

[31]. Huang, W.Y., Cai, Y.Z., Hyde, K.D., Corke, H., & Sun, M. (2007). Endophytic fungi from *Nerium oleander* L. (Apocynaceae): main constituents and antioxidant activity. *World Journal of Microbiology and Biotechnology*, 23(9) : 1253–1263. https://doi.org/DOI https://doi.org/10.1007/s11274-007-9357-z

[32]. Khan, R., Shahzad, S., Choudhary, M.I., & Khan, S.A. (2010). Communities of endophytic fungi in medicinal plant *Withania somnifera*. *Pakistan Journal of Botany*, 42(2) : 1281–1287.

[33]. Halouane, F., Bissaad, F.Z., Doumandji-Mitiche, B., Benzina, F., Chahbar, N., & Hamid, S. (2013). Study of the effect of *Beauveria bassiana* (Vuil,) on the biochemistry and structure of the cuticle of *Schistocerca gregaria* (Forskal). *Annals of Biological Research*, 4(12) :68–74.

[34]. Singh, G., & Prakash, S. (2011). Studies on fungal cultural filtrates against adult *Culex quinquefasciatus* (Diptera: Culicidae) a vector of filariasis. *Journal of Parasitology Research*, 1–5. https://doi.org/https://doi.org/10.1155/2011/147373

[35]. Govindarajan, M., Jebanesan, A., & Reetha, D. (2005). Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say. *Tropical Biomedicine*, 22(1) : 1–3.

[36]. Riba, G., KEITA, A., & Vincent, J. J.(1984). Sensibilité des larves de moustiques à différentes espèces d'Hyphomycètes entomopathogènes. *Cah. ORSTOM Ser. Entomol. Med. Parasitol*, 22 : 271–276.

[37]. Russell, B. M., Kay, B.H., & Shipton, W. (2001). Survival of Aedes aegypti (Diptera: Culicidae) eggs in surface and subterranean breeding sites during the northern Queensland dry season. *Journal of Medical Entomology*, 38(3) :441–445. https://doi.org/https://doi.org/10.1603/0022-2585-38.3.441

[38]. Lara da Costa, G., Lage de Moraes, A.M., & Cunha de Oliveira, P. (1998). Pathogenic action of *Penicillium* species on mosquito vectors of human tropical diseases. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms*, 38 : 337–341. https://doi.org/https://doi.org/10.1002/(SICI)1521-4028(199811)38:5/6<337::AID-JOBM337>3.0.CO;2-N

[39]. Ragavendran, C., Mariappan, T., & Natarajan, D. (2017). Larvicidal, histopathological efficacy of *Penicillium daleae* against larvae of *Culex quinquefasciatus* and *Aedes aegypti* plus biotoxicity on *Artemia nauplii* a non-target aquatic organism. *Frontiers in Pharmacology*, 8 : 1–14. https://doi.org/https://doi.org/10.3389/fphar.2017.00773.

[40]. Laib, D. (2014). Etude de l'activité insecticide du champignon endophyte *Cladosporium sp*. isolé du Laurier rose *Nerium oleander* L.(Apocynaceae, Gentianales) sur la bruche des haricots *Acanthoscelides obtectus* Say (Coleoptera, Bruchidae). *Nature & Technology*, (10) : 39–44.