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The anti-acetylcholinesterase, insecticidal and antifungal activities of the entophytic fungus *Trichoderma* sp. isolated from *Ricinus communis* L. against *Locusta migratoria* L. and *Botrytis cinerea* Pers.: Fr.

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Abstract: This study was conducted to evaluate anti-acetylcholinesterase and insecticidal and antifungal activities of the endophytic fungus *Trichoderma* sp, isolated from *Ricinus communis* L. leaves, against *Locusta migratoria* L. and *Botrytis cinerea* Pers.: Fr.. To evaluate the insecticidal and antifungal activities, different concentrations of the fungal extract were applied against *L. migratoria* (0.2, 0.3, 0.4 g/l) and against *B. cinerea* (1, 2, 3 g/l). It was found that the mortality of the targeted insects was positively proportional to fungal extract concentration and time after exposure (24, 48, 72 hours). The concentration 0.4 g/l appeared to be the most effective after 72 hours with mortality rate of 56.52%. Regarding antifungal activity, the concentration 3 g/l was the most effective against *B. cinerea* after 7 days, with an inhibition rate of 92.06% (excellent antifungal activity). Moreover, it was found that at 4 ug/ml the fungal extract had a maximum inhibitory capacity of Ache of 80% for acetylcholinesterase. Preliminary phytochemical analyses revealed the presence of alkaloids, flavonoids, phenols and saponins. In addition the colony of this endophytic fungus produced chitinases and proteases, which explained its important antifungal and insecticidal activities.

Keywords: insecticidal activity, antifungal activity, endophytic fungi, *Trichoderma sp*, *Locusta migratoria*, *Botrytis cinerea*

Introduction

Numerous insects and phytopathogenic agents are responsible for significant crop losses worldwide [1]. Among them the migratory locust, *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae), which is a pestilential insect with a polyphagous habit [2], exhibits extensive ecological plasticity and a large geographic range. Its gregarious phase is influenced by population density [3] which leads to the formation of spectacular swarms that cause considerable damage to crops [4, 5]. *Botrytis cinerea* (Pers, 1794) is an airborne plant pathogen with a necrotrophic lifestyle, attacking over 200 crop hosts worldwide [6]. It causes a disease known as “grey mould” which is responsible for economic losses that are difficult to calculate because of its wide range of hosts [7]. The control methods for *L. migratoria* and *B. cinerea* are dominated by chemical methods involving the application of considerable amounts of chemical pesticides [7, 8]. These chemicals are effective, but their extensive application has created problems such as resistance, environmental pollution, and adverse effects on human health [9]. Therefore, finding effective alternatives for crop protection against pests and pathogens is now more than a necessity [10]. Among the alternatives, endophytic fungi are considered to be one of the most promising biological groups for plant protection against a large number of insects and pathogens [11, 12]. Endophytes are microorganisms that reside in plant tissues without causing any apparent symptoms. They are an important source of bioactive compounds and are considered a promising tool in agronomy [13].

Trichoderma species are recognized as mycoparasites and are widely used in agriculture as bio fungicides and bioremediation agents [14]. Their biological activities are caused by various secondary metabolites [15].

Thus, the aim of this study was evaluating the insecticidal, antifungal, and anti-acetylcholinesterase activities of the endophytic fungus *Trichoderma sp*, isolated from the leaves of the castor, *Ricinus communis* L. against *L. migratoria* and *B. cinerea*.

Material and Methods

Biological material

R. communis leaves were collected with a scalpel from five healthy plants in the Beni Bechir region, Skikda, Algeria in March 2019. *L. migratoria* is a potential pest of many crops in Algeria and this insect was collected in August 2019 from the same region as was *R. communis*. *B. cinerea* was isolated from infected strawberries.

Methods

1. Insects breeding

The objective of the first step was to obtain a large number of *L. migratoria* with which to conduct our experiment (Locust treatment and mortality rate calculation). Locusts were bred in the laboratory according to a previously described method [16], with slight modifications. Wooden cages of 50 cm³ covered with tulle to ensure aeration and avoid condensation of moisture (50-60% maximum), were used, they were equipped with a sliding door to allow different manipulations and a heating system maintained a constant temperature of approximately 30±1°C, which allowed the insects to thermoregulation and attain their optimal temperature for molting and egg laying. A photoperiod with 12 hours of continuous light per day was provided with a 60-watt incandescent bulb.

2. Isolation, purification and identification of endophytic *Trichoderma* sp.

The isolation of the endophytic fungi was performed 48 hours after the collection of *R. communis* leaves according to a previously described protocol [17], with a slight modifications. The samples were carefully washed under running water to remove soil particles and cut into 5 x 5 mm segments with a flame-sterilized razor blade. The resulting segments were placed in 75% ethanol for 1 minute, washed three times with sterile distilled water for a few minutes each to remove excess ethanol, and dried on sterile filter paper under aseptic conditions. Next the samples were uniformly and aseptically transferred into Petri dishes containing modified PDA medium with streptomycin (200 mg/l) to inhibit bacterial growth. These Petri dishes were sealed using diagonal parafilm and incubated at room temperature (25-30°C) for 21 days to ensure the growth of endophytic fungi. The emerging fungi from the ends of segments (plant tissue) were immediately isolated and transplanted into a new PDA medium without antibiotics. Next, the fungal strains were incubated at 25 °C for 3 to 6 days. Microscopic observations were made using an optical microscope.

3. Submerged fermentation and production of the fungal extract.

The next step was conducted according to a previous method [18], with slight modifications. Agar discs with associated mycelium of the fungal endophyte, *Trichoderma* sp, were placed in 250 ml flasks containing 50 ml of PDB medium and closed to prevent contamination. The cultures were incubated for 21 days at 25±2°C and shaken back and forth each hour on mechanical shaker. After 21 days, mycelia were separated from the medium by filtration through Whatman No. 1 filter paper. The filtrate was mixed for 15 minutes with an equal volume of ethyl acetate and allowed to stand for 10 minutes in order to separate it into the two immiscible organic and aqueous layers. Then the ethyl acetate was evaporated using a rotary evaporator. Each 1 mg of the fungal extract was dissolved in 1 ml of distilled water and stored at 4°C for later use.

4. Test of chitinolytic and proteolytic activities of the endophytic fungi *Trichoderma sp.*

The chitinolytic and proteolytic activity of the endophytic fungus *Trichoderma sp.* was determined according to a previous method [19]. The culture medium revealing the proteolytic activity was essentially composed of: 3 g NaCl, 3 g K₂HPO₄, 3 g MgSO₄·7H₂O, 10 g Agar, 5 g casein, and 1 l distilled water. The culture medium revealing the chitinolytic activity was essentially composed of: 3 g NaCl, 3 g K₂HPO₄, 3 g MgSO₄·7H₂O, 10 g agar, 5 g colloidal chitin, and 1 l distilled water.

Fragments (5x5 mm) of the endophytic fungus *Trichoderma sp.* were inoculated in the center of Petri dishes which is previously containing the solid medium revealing the chitinolytic and/or proteolytic activity. The resulting strains were incubated in an oven at 25°C. After 24 to 48 hours of inoculation the presence of a translucent halo indicated the presence of chitinolytic and/or proteolytic activities.

5. Preliminary phytochemical analyses of the fungal extract.

Detection of the major classic of metabolites was conducted using previously described techniques described [20]. Extracts were dissolved individually in dilute Hydrochloric acid and filtered then treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow colored precipitate indicated the presence of alkaloids. For the detection of flavonoids the extract was treated with few drops of sodium hydroxide solution. The formation of intense yellow color, which became colorless upon the addition of dilute acid, indicated the presence of flavonoids. For the detection of phenols the fungal extract was dissolved in 5 ml of distilled water and a few drops of 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds. For the saponins the fungal extract was vigorously shaken with distilled water and was then allowed to stand for 10 minutes, formation of a fairly stable emulsion indicated the presence of saponins. For detection of tannins, the crude fungal extract was treated with an alcoholic FeCl₃ reagent, and a bluish black color that disappeared upon the addition of a little dilute H₂SO₄ followed by the formation of a yellowish brown precipitate indicated the presence of tannins.

6. Acetylcholinesterase inhibitory activity of the fungal extract.

Acetylcholinesterase (Ache) inhibitory activity was detected according to a previously described method [21]. The protocol was as follows: 150 µl of 100 mM sodium phosphate buffer (Ph=8) was added to 10 µl of different concentrations of the dissolved extract (in methanol) and 20 µl of Ache. This mixture was incubated at 25°C for 15 minutes and 10 µl of DNTB (0.5 mM) and 10 µl of acetyl choline iodide (0.71 mM) was added to it. Readings at 412 nm at 0 and 15 minutes were performed using a plate reader. The percentage of inhibition of the Ache enzyme was calculated by the following formula:

$$\text{Inhibition of the enzyme Ache (\%)} = \frac{E - S}{E} \times 100$$

where E = the activity of the enzyme without extract, S = the activity of the enzyme with the extract.

7. Insecticidal activity of the endophytic fungal extract.

An assay was conducted by directly spraying of adult insects inside a plastic boxes (five insects per box) with three concentrations of the fungal extract were applied (0.1 g/l, 0.2 g/l, and 0.3 g/l). There were five repetitions for each concentration and five repetitions treated with distilled water were considered controls. The mortality rate (in percentage) was calculated for each treatment after 24 h, 48 h and 72 h.

8. Antifungal activity of the endophytic fungal extract.

Three concentrations (1 g/l, 2 g/l, and 3 g/l) of the fungal extract were mixed with the medium culture (agar-agar) and then inoculated with 5 x 5 mm segments of *B. cinerea*. The strains were incubated at 25°C for 7 days. Based on previous protocol [22] the antifungal activity was calculated by using the following formula:

$$P.I.C.G = \frac{(D_t - D_e)}{D_t} \times 100$$

where P.I.C.G (%) = percentage of inhibition of colony growth;

D_t = Average diameter of the colony unexposed to the extract (control);

D_e = Average diameter of the colony exposed to the extract.

The antifungal activity of the fungal extract was evaluated according to the percentage of the inhibition of the colony growth: from 30% to 40% - low activity; from 50% to 60% - moderate activity; from 60% to 70% - good activity; > 70% - excellent activity.

Statistical data analysis.

An analysis of variance (ANOVA) and Tukey's (HSD) test (with a confidence interval of 95%) were performed in order to compare recorded corrected mortality, Ache inhibition, and antifungal activity means, and rank these results into homogeneous groups. All statistical data analyzes were performed using the XLSTAT 2019 software (Addinsoft, 2019. XLSTAT Statistical Data Analysis Solution, Paris, France).

Results and Discussion

Evaluation of the insecticidal activity of the fungal extract.

From the results (Figures 1 and 2) it was determined that the fungal extract had a variable insecticidal activity against *L. migratoria*. The effect of the extract changed according to the concentration and time after exposure. The concentration 0.4 g/l was the most effective concentration against the insects after 72 hours with a mortality rate of 56.52%. For the Analysis of variance (Anova) and the Tukey (HSD) test (with a 95% confidence interval). The capital letters A, B, C, and D indicates a significant difference among insecticidal activity of different concentrations of the extract on different days (at 0.05 significance level). The lowercase letters a, b, c, and d indicates a significant differences among insecticidal activity at different concentrations of extract at the same day (at 0.05 significance level).

Figure 1. Mortality rate (%) of insects according to different concentrations of the fungal extract

Figure 2. *L. migratoria* 72 h after the application of the fungal extract

Evaluation of the antifungal activity of the fungal extract against *B. cinerea*.

It was determined that the fungal extract had a variable antifungal activity against *B. cinerea* (Figures 3 and 4). The effect of the extract changed according to the concentration and time after exposure. The concentration 3g/l was the most effective concentration against *B. cinerea* after 7 days with a maximum inhibition rate of 92.06% (excellent activity antifungal). For the Analysis of variance (Anova) and the Tukey

(HSD) test (with a 95% confidence interval). The capital letters A, B, C, and D indicates a significant difference among antifungal activity at the same concentration of extract on different days (at the 0.05 significance level). The lowercase letters a, b, c, and d indicates a significant difference among antifungal activity on the same day (at 0.05 significance level).

Figure 3. Inhibition percentage of *B. cinerea* colony growth by different concentrations of the fungal extract

Figure 4. Inhibition of *B. cinerea* colony growth based on the concentration (3g/l) of the fungal extract

Anti-cholinesterase activity of the fungal extract.

It was found that the fungal extract had a variable Ache inhibitory activity (Figure 5) a maximum Ache inhibition rate of 80% was recorded for the concentration of 4 μ g/ml of the fungal extract. The capital letters A, B, C, and D indicates a significant difference among Ache inhibitory activity at different concentrations of extract at the 0.05 significance level. The lowercase letters a, b, c, and d indicates a

significant difference among ache inhibitory values at the same concentration of extract (at the 0.05 significance level).

Figure 5. Acetylcholinesterase inhibition based on different concentrations of the fungal extract

Preliminary test of chitinolytic and proteolytic activities of the endophytic fungi *Trichoderma sp.*

It was determined that the endophytic fungus *Trichoderma sp.* had chitinolytic and proteolytic activities as evidenced by a translucent halo that formed around the fungal colony (Figure 6).

Figure 6. A translucent halo surrounding the fungal colony: A. Fungal colony in chitinolytic activity medium, B. Fungal colony in proteolytic activity medium

Preliminary phytochemical analyses of endophytic *Trichoderma* sp. extract.

Preliminary phytochemical analyses revealed the presence of alkaloids, flavonoids, phenols and saponins.

Discussion

Endophytic microorganisms are those that occur internally in plant organs, especially in leaves, branches and stems, while apparently not harming the hosts [23]. The capability of colonizing internal host tissues has made endophytes a valuable tool to improve crop performance in agriculture [24]. Fungal endophytes provide protection against herbivorous insects [25, 26] and phytopathogenic fungi [27] by producing numerous insecticidal, insect repellent, and antifungal secondary metabolites during their active growth phase which are either similar to, or have a higher activity than those of their respective hosts [28, 29]. They have been reported to have numerous applications in agro-chemistry [30]. Among these insecticidal and antifungal secondary metabolites the chitinases [31], proteases [19] and acetylcholinesterase inhibiting molecules such as alkaloids and flavonoids [32], which are considered responsible for the insecticidal and the antifungal activities against *L. migratoria* and *B. cinerea*. Many fungal species secrete proteases when they are grown in a medium containing casein as a source of nitrogen [19]. Chitinases can be induced in the presence of colloidal chitin as source of carbon in the medium [33] and possess a hydrolytic activity against chitin, which is decomposed by the hydrolysis of the β -1,4-glycosidic bonds between the N-acetylglucosamine molecules [34] into low molecular weight molecules such as chitotetraose, chitotriose and chitobiose [35]. The halo corresponds to the zone of degradation of colloidal chitin by the chitinases secreted by these fungi [36]. The results of casein degradation by fungal strains indicate that they have proteolytic activity [19, 37]. *Trichoderma* spp. are well-known producers of degradative enzymes such as chitinases and proteases [38]. Chitinases and proteases are virulence factors that play an important role during insect infection [39] and a resistance mechanism provided by endophytic fungi to their host plants against phytopathogenic fungi [40]. Their production is a common feature of most entomopathogenic fungi [41] including *Trichoderma* spp. [36]. These fungi act by different mechanisms including wounding the peritrophic membrane of the insect's gut, which results in the inhibition of food intake and consequently leading to death [35]. Alternatively they break down the cuticle of insects [31] as well as inhibit phytopathogenic fungi by destroying their cell wall integrity [42]. The fungi produced a wide range of secondary metabolites that can contribute positively in an agricultural context against several pests [43]. Among them the acetylcholinesterase inhibitory molecules could be applied in agriculture to control insects and other arthropod pests [44], by acting against their nervous systems [45] and increasing their acetylcholine level which induces a repetitive activity of the neurons leading to the death of the insect [46]. In comparison with our study, a 70% mortality rate of *Helicoverpa armigera* Hub. larvae was recorded after a topical application of *Trichoderma*

harzianum Pers. culture filtrate (chitinase concentration of 2000 U ml/l) [35]. The endophytic fungus *Alternaria* sp. Cas 1 (JX177676) isolated from *R. communis* had a maximum acetylcholinesterase inhibitory activity rate of 78% [44]. Some studies have shown that different strains of *Trichoderma* spp. have a significant antifungal activity against *B. cinerea* [47]. Trichodermin (**137**) was obtained from the endophytic fungus *T. brevicompactum* 0248 isolated from *Allium sativum* L. which had a potent inhibitory activity against *Rhizoctonia solani* (EC50 of 0.25 mg/ml) and against *B. cinerea* (EC50 of 2.02 mg/ml) [48]. The endophytic fungus *T. koningiopsis* YIM PH30002 harbored in *P. notoginseng* Bur. was the source of koningin O (**131**), koningin Q (**132**) and 7-O-methylkoninginin D (**133**), and koningiopisin C (**134**). These compounds exhibited different antifungal activities against *F. oxysporum* woll and *P. cucumerina* [50]. Two compounds dichlorodiaportinolide (**135**) and dichlorodiaportin (**136**) obtained from the endophytic fungus *Trichoderma* sp. 09 isolated from the root of *M. bontiodides* Sie & Zuc. showed different antifungal activities with MIC values ranging from 6.25 to 150 mg/ml against *C. musae* Arx and *R. solani* Kuh [50]. *T. koningiopsis* YIM PH30002 produced two koninginins R and S (**138–139**) which showed certain antifungal activities against *F. flocciferum* Cor. and *F. oxysporum* Sch. [51]. Ethyl acetate extracts of *Trichoderma* spp. isolates exhibited antimicrobial activity against *Vibrio* sp. Pac, *S. marcescens* Biz, *M. smegmatis* Trevi and *B. cereus* [52]. The harzianic acid (HA) and 6-pentyl-a-pyrone (6PP) showed an antifungal activity against the powdery mildew agent *U. necator* Schw [53]. *T. velutinum* ACR-P1 had a good antifungal activity against *F. oxysporum* Sch., *V. dahliae* Kleb, *A. alternata* Keis and *C. capsici* But & Bus [54].

Conclusion

In this study, we were able to highlight the antifungal; insecticidal and anti-acetylcholinesterase activities of the endophytic fungus *Trichoderma* sp. isolated from *R. communis* against *B. cinerea* and *L. migratoria*.

Three concentrations of the fungal extract (0.2, 0.3, and 0.4 g/l) were applied to test the insecticidal activity against *L. migratoria*, and it was found that the mortality of the targeted insects was positively proportional with concentration and time (24, 48, and 72hours). The concentration of 0.4 g/l was the most effective against these insects after 72 hours with a mortality rate of 56.52%.

Three other concentrations of the fungal extract (1, 2, and 3 g/l) were applied to test the antifungal activity against *B. cinerea*. The concentration of 3 g/l was the most effective against *B. cinerea* after 7 days with an inhibition rate of 92.06% (excellent antifungal activity). Moreover it was found that the endophytic fungus *Trichoderma* sp. extract had variable Ache inhibitory activity. The concentration 4 ug/ml was the most endowed with a maximum inhibition of Ache of 80%.

Preliminary phytochemical analyses revealed the presence of alkaloids, flavonoids, phenols and saponins and it was found that the colony of these endophytic fungi is a producer of chitinases and proteases which explains the action of its antifungal and insecticidal activities. These results demonstrate that the endophytic fungi *Trichoderma sp.* extract can be applied as a contact bioinsecticide and biofungicide against *L. migratoria* and *B. cinerea*.

References

- [1].Rahman, S.F.S.A; Singh, E; Pieterse, C.M.J; Schenk, P.M, Emerging microbial biocontrol strategies for plant pathogens, *Plant Science*, **2018**, 267, 102-111.
- [2].Popov, G.B.; Launois-Luong, M.H.; Van der veel, J.J, Les oothèques des criquets du Sahel. Ed. Cirad/Prifas, 'Collection Acridologie Opérationnelle 7ème édition, Montpellier, **1990**, p. 153.
- [3].Lecoq M, Les criquets du sahel. Ed. Cirad/Prifas, 'Collection Acridologie Opérationnelle 1ère édition, Montpellier, **1988**, p. 125.
- [4].Latchininsky, A.V, Locusts and remote sensing: a review. *Journal of Applied Remote Sensing*, **2013**, 7, 075-099.
- [5].Sangbaramou, R.; Camara, I.; Xin-zheng, H.; Shen, J.; Tan, S.; Shi, W, Behavioral thermoregulation in *Locusta migratoria manilensis* (Orthoptera: Acrididae) in response to the entomopathogenic fungus *Beauveria bassiana*. *Plos One*, **2018**, 13 (11), 1-19.
- [6].Williamson B.; Tudzynski B.; Tudzynski P.; Van kan A. A. L, *Botrytis cinerea*: the cause of grey mould disease. *Molecular plant pathology*, **2007**, 8 (5), 561-580.
- [7].Bolivar-Anillo, H.J; Garrido, C.; Collado, I.G, Endophytic microorganisms for biocontrol of the phytopathogenic fungus *Botrytis cinerea*, *Phytochemistry Reviews*, **2019**, 1-20.
- [8].Zhang, L.; Hunter, D, Management of locusts and grasshoppers in China. *Journal of Orthoptera Research*, **2017**, 26, 155.
- [9].Ali, A.; Ahmad, F.; Biondi, A.; Wang, Y.; Desneux, N, Potential for using *Datura alba* leaf extracts against two major stored grain pests, the khapra beetle *Trogoderma granarium* and the rice weevil *Sitophilus oryzae*, *Journal of Pest Science*, **2012**, 85, 359-366.
- [10].Pavela, R, Possibilities of botanical insecticide exploitation in plant protection, *Pest Technology*, **2007**, 1, 47-52.
- [11].Vega, F.E.; Goettel, M.S.; Blackwell, M.; Chandler, D.; Jackson, M.A.; Keller, S.; Koike, M.; Maniania, N.K.; Monzon, A.; Ownley, B.H.; Pell, J.K.; Rangel, D.E.N.; Roy, H.E, Fungal entomopathogens: new insights on their ecology, *Fungal Ecology*, **2009**, 2, 149-159.
- [12].Yan, D.H.; Song, X.; Li, H.; Luo, T.; Dou, G.; Strobel, G, Antifungal Activities of Volatile Secondary Metabolites of Four *Diaporthe* Strains Isolated from *Catharanthus roseus*. *Journal of Fungi*, **2018**, 4, 65.

- [13].Aly, A.H.; Debbab, A.; Proksch, P, Fungal endophytes: unique plant inhabitants with great promises. *Applied Microbiology and Biotechnology*, **2011**, 90 (6), 1829–1845.
- [14].Ghorbanpour, M.; Omidvari, M.; Abbaszadeh-Dahaji, P.; Omidvar, R.; Kariman, K, Mechanisms underlying the protective effects of beneficial fungi against plant diseases. *Biological Control*, **2018**, 117, 147-157.
- [15]. Harwoko, H.; Daletos, G.; Stuhldreier, F.; Lee, J, Wesselborg, S, Feldbrügge, M.; Müller, W.E.G; Kalscheuer, R; Ancheeva, E; Proksch, P, Dithiodiketopiperazine derivatives from endophytic fungi *Trichoderma harzianum* and *Epicoccum nigrum*. *Natural product research*, **2019**, 18, 1-9.
- [16].Ouedraogo, R.M.; Kamp, A.; Goettel, M.S.; Brodeur, J.; Bidochka, M.J, Attenuation of fungal infection in thermoregulating *Locusta migratoria* is accompanied by changes in hemolymph proteins. *Journal of Invertebrate Pathology*, **2002**, 81, 19-24.
- [17].Li, Y.L.; Xin, X.M.; Chang, Z.Y.; Shi, R.J.; Miao Z.M.; Ding J.; Hao G.P., The endophytic fungi of *Salvia miltiorrhiza* BGE. f. alba are a potential source of natural antioxidants. *Bot. Studies*, **2015**, 56(1), 5.
- [18].Dolatabad, H.K.; Nikkhah, M.J.; Shier, W.T, Evaluation of antifungal, phosphate solubilisation, and siderophore and chitinase release activities of endophytic fungi from *Pistacia vera*. *Mycology Progress*, **2017**, 16(8), 777-790.
- [19].Lopez-Illorca, L.V.; Carbonell, T.; Vidal, S.G., Degradation of insect cuticle by *Paecilomyces farinosus* proteases. *Mycological Progress*, **2002**, 1(3), 249-256.
- [20].Harborne, J.B., Phytochemical Methods. Chapman Hall, London, **1998**, 60-66.
- [21].Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherston, R.M., A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **1961**, 7, 88-95.
- [22].Abdellatif, S.; Abdelrahman, S.M.; Deraz S.F., Promising antifungal effect of some folkloric medicinal plants collected from el-hammam habitat, Egypt against dangerous pathogenic and toxicogenic fungi. *ARPV Journal of Agricultural and Biological Science*, **2011**, 6 (9), 26-32.
- [23].Porras Alfaro, A., Bayman, P., Hidden fungi, emergent proprieties: endophytes and microbiomes. *Annual Review of Phytopathology*, **2011**, 49, 291-315.
- [24].Zhang, Z.B.; Zeng, Q.G.; Yan, R.M.; Wang, Y.; Zou, Z.R.; Zhu, D., Endophytic fungus *Cladosporium cladosporioides* LF70 from *Huperzia serrata* produces Huperzine A. *World Journal of Microbiology Biotechnology*, **2011**, 27, 479-486.
- [25].Breen, J.P, Enhanced resistance to fall armyworm (Lepidoptera: Noctuidae) in *Acremonium* endophyteinfected turfgrasses. *Journal of Economic Entomology*, **1993**, 86, 621-629.
- [26].Clement, S. L.; Elberson, L. R.; Bosque-Perez, N. A.; Schotzko, D. J., Detrimental and neutral effects of wild barley – *Neotyphodium* fungal endophyte associations on insect survival. *Entomologia Experimentalis et*

Applicata, **2005**, *114*, 119-125.

- [27].Reinhold-Hurek, B., Hurek T., Living inside plants:bacterial endophytes. *Current Opinion in Plant Biology*, **2011**, *14*, 435-44.
- [28].Strobel, G.A., Endophytes as source of bioactive products. *Microbes Infection*, **2003**, *5*, 535-544.
- [29].Mantzoukas, S.; Lagogiannis, I., Endophytic Colonization of Pepper (*Capsicum annum*) Controls Aphids (*Myzus persicae* Sulzer). *Applied Sciences*, **2019**, *9*, 2239, 1-12.
- [30].Nisa, H.; Kamili, AN.; Nawchoo, I.A.; Shafi, S.; Shameem, N.; Bandh S.A, Fungal endophytes as prolific source of phytochemicals and other bioactive natural products: a review. *Microb.Pathogen.*, **2015**, *82*, 50-59.
- [31].Pérez, L.D.S.; Florido, J.E.B.; Navarro, S.R.; Mayagoitia, J.F.C.; López, M.A.R., Enzymes of Entomopathogenic Fungi, Advances and Insights. *Advances in Enzyme Research*, **2014**, *2*, 65-76.
- [32].Tiwari, P.; Kumar, B.; Kaur, M.; Kaur, G.; Kaur, H., Phytochemical screening and Extraction: A Review. *Internationale pharmaceutica sciencia*, **2011**,*1*, 98-106.
- [33].Coutiño, L.R.; Marquez, J.E.; Peter, M.G.; Shirai, K., The effect of pH on the production of chitinolytic enzymes of *Verticillium fungicola* in submerged cultures. *Bioresource Technology*, **2010**, *101*, 9236-9240.
- [34].Kitamura, E.; Kamei, Y., Molecular cloning, sequencing and expression of the gene encoding a novel chitinase A from marine bacterium, *Pseudomonas sp.* PE2, and its domain structure. *Applied Microbiology and Biotechnology*, **2003**, *61*, 140-149.
- [35].Binod, P.; Sukumaran, R.K.; Shirke, S.V.; Rajput, J.C.; Pandey, A., Evaluation of fungal culture filtrate containing chitinase as a biocontrol agent against *Helicoverpa armigera*. *Journal of Applied Microbiology*, **2007**, *103*, 1845-1852.
- [36].Agrawal, T.; Kotasthane, A.S., Chitinolytic assay of indigenous *Trichoderma* isolates collected from different geographical locations of Chhattisgarh in Central India. *Springer Plus*, **2012**, *1*, 73.
- [37].Sapna Bai, N.; Remadevi, O.K.; Sasidharan, T.O.; Balachander, M.; Dharmarajan, P., cuticle degrading enzyme production by some isolates of the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.). *Journal of Biosciences*, **2012**, *20*, 25-32.
- [38].Di Pietro, A.; Lorito, M.; Hayes, C.K.; Broadway, R.M.; Harman, G.E., Endochitinases from *Gliocladium virens*: isolation, characterization and synergistic antifungal activity in combination with gliotoxin. *Phytopathology*, **1993**, *83*, 308-313.
- [39].Seidl, V., Chitinases of filamentous fungi: a large group of diverse proteins with multiple physiological functions. *Fungal Biology Reviews*, **2008**, *22*, 36-42.
- [40].Tan, R.X; Zou, W.X., Endophytes: a rich source of functional metabolites. *Natural Product Reports*, **2001**, *18*, 448-459.

- [41].Yang, J.; Tian, B.; Liang, L.; Zhang, K.Q., Extracellular enzymes and the pathogenesis of nematophagous fungi. *Applied Microbiology and Biotechnology*, **2007**, *75*(1), 21-31.
- [42].Howell, C.R, Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Disease*, **2003**, *87*, 4-10.
- [43].Johnson, R.D.; Akagi Y.; Fleetwood, D.J; Gardiner, D.M.; Kodama, M.; Young, C.; Voisey, C.R., Fungal Toxins of Agricultural Importance. In: Kempken, F. (Ed). *Agricultural Applications*. **2013**, 75-99.
- [44].Singh, B.; Thakur, A.; Kaur, S.; Chadha, B.S.; Kaur, A., Acetylcholinesterase Inhibitory Potential and Insecticidal Activity of an Endophytic *Alternaria* sp. from *Ricinus communis*. *Applied Biochemistry and Biotechnology*, **2012**, *168*, 991-1002.
- [45].Singh, B.; Bhagat, J.; Chadha, B.S.; Kaur, A., Cholinesterase inhibitory potential of different *Alternaria* spp. and their phylogenetic relationships. *Biologia*, **2014**, *69*(1), 10-14.
- [46].Champ P., Mode d'action et utilisation des organophosphorés. Mode d'action et utilisation des insecticides: Insectes, insecticides, santé. Paris, **1985**, 207-223.
- [47].Demirci, E.; Dane, E.; Eken, C., *In vitro* antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*. *Turkish Journal of Biology*, **2011**, *35*, 457-462.
- [48].Shentu, X.; Zhan, X.; Ma, Z.; Yu, X.; Zhang, C., Antifungal activity of metabolites of the endophytic fungus *Trichoderma brevicompactum* from garlic. *Brazilian Journal of Microbiology*, **2014**, *45*, 248-254.
- [49].Liu, K.; Yang, Y.; Miao, C.P.; Zheng, Y.K.; Chen, J.L.; Chen, Y.W.; Xu, L.H.; Guang, H.L.; Ding, Z.T.; Zhao, L.X., Koningiopsisins A-H, polyketides with synergistic antifungal activities from the endophytic fungus *Trichoderma koningiopsis*. *Planta Medica*, **2016**, *82*, 371-376.
- [50].Li, W.; Xu, J.; Li, F.; Xu, L.; Li, C., A New antifungal isocoumarin from the endophytic fungus *Trichoderma* sp. 09 of *Myoporum bontioides* A. Gray. *Pharmacognocny Magazine*, **2016**, *12*, 259-261.
- [51].Hu, M.; Li, Q.L.; Yang, Y.B.; Liu, K.; Miao, C.P.; Zhao, L.X.; Ding, Z.T., Koninginins R-S from the endophytic fungus *Trichoderma koningiopsis*. *Natural Products Research*, **2017**, *31*, 835-839.
- [52].Karaoglu, S.A.; Bozdeveci, A.; Pehlivan, N., Characterization of Local *Trichoderma* spp. as Potential Bio-Control Agents, Screening of *in vitro* Antagonistic Activities and Fungicide Tolerance. *Hacettepe Journal of Biology & Chemistry*, **2018**, *46* (2), 247-261.
- [53].Pascale, A.; Vinale, F.; Manganiello, G.; Nigro, M.; Lanzuise, S.; Ruocco, M.; Marra, R.; Lombardi, N.; Woo, S.L.; Lorito, M., *Trichoderma* and its secondary metabolites improve yield and quality of grapes. *Crop Protection*, **2017**, *92*, 176-181.
- [54].Sharma, R.; Magotra, A.; Manhasa, R.S.; Chaubey, A., Antagonistic potential of a psychrotrophic fungus: *Trichoderma velutinum* ACR-P1, *Biological Control*, **2017**, *115*, 12-17.