Detection the influence of some endophytic Aspergillus strains on Galleria mellonella (Lepidoptera: Pyralidae)

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Abstract

In the present study, endophytes are important source for the discovery of bioactive compounds to control Galleria mellonella, the destructive pest to apiary. The isolates were identified as Aspergillus parasiticus speare 13939 and Aspergillus carbonarius van Tieghem 13943. The isolates were assessed for their crude extracts against the 4th instar larvae of G. mellonella. A. parasiticus exhibited the most potent larvicidal activity after 3, 5, 7 and 9 days post treatment, respectively. A. parasiticus filtrate caused the highest mortality after 7 days recording (50 %) at 8 % concentration. The mortality was strong by using 5 % of A. carbonarius filtrate (75 %) after 3 days of treatment. However, the mortality was weak with 9% of A. carbonarius (25 % at 9 days). In the same time, 1% of A. carbonarius showed weak effect on the percents of wax moth larval mortality (50 % at 9 days). As well as, the highest mortality percent of A. carbonarius was 62.5 % when the wax moth larvae were treated by 7 ml containing 34.6×10^{6} spore ml^{-1} after 11 days of incubation. The highest mortality percentage 75 % as 1ml from different volumes of A. parasiticus was obtained using 7 ml containing 11.13×10^{6} spore ml^{-1} on wax moth larvae after 7 days of incubation. The effective concentrations of spore suspensions could be arranged in descending manner according to the mortality of wax moth larvae. Finally, all results indicated that these endophytic fungi could be a potential ecofriendly source of bioactive metabolites with insecticidal activity and can be used in integrated management program.

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Introduction

The present study concerned with the detection of some fungal isolates' toxicity on the mortality levels of G. mellonella. Invertebrate models of infection that are less expensive and morally acceptable have been developed, such as those using G. mellonella larvae. G. mellonella can be used in addition to traditional animal models to explore a variety of aspects of host-fungal interactions. When human pathogenic bacteria infect wax moth larvae and mammals, they frequently use the same virulence and pathogenicity components as are used to infect invertebrates, which have immune systems that function similarly to those of mammals' innate immune systems. Additionally, wax moth larvae and mammals share many human illnesses' similar levels of

pathogenicity. Before using more expensive mammalian models, it is possible to quickly, cheaply, and accurately assess the toxicity and effectiveness of new antimicrobial drugs invivo by using the wax moth larva model. By eliminating substances with a poor likelihood of success and bolstering the need for more research in mammalian systems, this straightforward insect model can close the gap between invitro investigations and mammalian experiments. It is advised that wax moth larva models be used more frequently in anti-infective drug research and development programmed to decrease the usage of mammals in preclinical testing and lower the overall cost of drug development **(Desbois and Coote, 2012).**

Secondary metabolites produced by endophytes have been well documented to provide protection to the plant against insects **(Golla et al., 2018** and **Negi et al., 2018)**. The insecticidal activity of the endophytes could be mediated by the production of metabolites inhibiting the digestive enzymes of the insects. In insects, α-glycosidases are found in the alimentary canal, salivary secretions and hemolymph. Several insects are dependent on α-amylase to utilize starch for carbohydrate metabolism, making them good targets for biocontrol. A phenolic compound isolated from Cladosporium sp. has been reported to exhibit insecticidal activity due α-glucosidase inhibition (**Singh et al., 2015)**. Another phenolic compound chlorogenic acid has been isolated from endophytic fungus Cladosporium velox and characterized using mass spectroscopic analysis reported to suppress the activity of gut α-glycosidases (in vivo) of the polyphagous insect pest Spodoptera litura **(Singh et al., 2016)**.

Slater et al. (2011) showed that the pathogenicity of six knockout strains of Aspergillus fumigatus was comparable in murine and wax moth larva infection models, but that some discrepancies were observed between the murine and D. melanogaster models.

Fluorescent microscopy characterized the dissemination and development of Aspergillus within larvae. By 6 h, fungal infection had spread to distal sites of the larvae and is marked by the formation of well- defined melanized nodules consisting of granulocytes infiltration and encapsulation of A. fumigatus germinating conidia **(Dubovskiy et al., 2016).** A. fumigatus remained viable and grew through melanized nodules 6 and 24 h post infection thus highlighting the importance of nodulation as an early response to an invading fungal inoculum (**Alekseeva et al., 2009)**. A. fumigatus toxins gliotoxin and fumagillin inhibit the microbicidal activity of human neutrophils and insect hemocytes by blocking the formation of F-ctin **(Fallon et al., 2010 and Renwick et al., 2007).** A. terrus infection in G. mellonella demonstrated unique histological findings consistent with those observed in disseminated aspergillosis in mammels **(Maurer et al., 2015).** There were virtually completed sequenced fungal genomes representing Aspergillus nidulans and Fusarium graminearum. A. nidulans has a single proteinase K subtilase (An 5581). Also, there is multiple intron loss in the phylogeny in F. graminearum to emphasize the difficulty of reconstructing the evolution of gene structure because of a labile nature intron presence or absence **(Krzywinski and Besansky, 2002)**. The availability of these genome sequences facilitates

the pathogenicity to insects. An association between the presence of dsRNA and hypo virulence has been documented for several Aspergillus species **(Elias and Cotty, 1996 and Van Diepeningen et al., 2008).**

Materials and methods

1- Isolation of fungal species: -

Each plant freshly healthy leaves and twigs were thoroughly washed in running water, cut into segments, and surface sterilized using a modified version of **Raviraja's (2005)** procedure that involved immersion in 70 % ethanol for 1 minute, followed by 2 minutes in a solution of 4% sodium hypochlorite, and then 1 minute of washing in sterile distilled water. After being placed on a sterile towel, sterilized samples were surface dried in a sterile environment. To avoid bacterial contamination, each individual piece was put in a petri dish with potato dextrose agar and chloramphenicol **(Raper and Fennel, 1965)**. Each petri dish had five or six segments plated in it, and the dishes were incubated at 27°C for a week to 15 days. It was determined whether the sterilizing process was effective.

To verify the sterility of the work space, control plate medium devoid of plant components was employed **(Schulz et al., 1998).** On PDA broth, endophytic fungi A. parasiticus speare 13939 (from leaves of Cacteceae) and A. carbonarius van Tieghem 13943 (from stems of Moringa) were cultivated using a modified version of **Pinkerton and Strobel's (1976)** technique. After 21 days, metabolites from each endophyte were harvested using triplicate inoculations. The filtrate is known as cell free culture filtrate and is used for pathogenicity testing on the fourth instar larvae of G. mellonella after their corresponding culture fluid was passed through four layers of cheese cloth to remove particles and filtrates were kept in separate vials.

2-Effect of different dilutions of Aspergillus parasiticus and Aspergillus carbonarius filtrates on wax moth

50 ml of YES broth were used to culture one ml of each isolate's spore suspension, which was then incubated at 30°C for 10 days. The cultures were run through Whatman No. 1 filter paper after the incubation period. To the whole volume of fungal filtrate, various amounts of sterilized distilled water were added individually to produce the following percentages in each tube: 1, 2, 3, 4, 5, 6, 7, and 9%. The surface of the diet was covered with one ml of the diluted filtrate, which was then allowed to dry. Three copies of each therapy were given. Using a sterilized fine brush, the G. mellonella larvae were applied to the prepared diet surface on petri plates. For the petri dishes, the incubator was adjusted to 28.2 °C and 65% R.H. A second set of petri dishes were prepared with the same diet and treated with water alone as a control. These plates were then allowed to dry with an equal number of the maintained larvae placed on their surface. To determine the best concentration of fungus filtrate, larval mortalities were measured after 1, 2, 3, 5, 7, 9 and 11 days.

3-Effect of different dilutions of spore suspensions of some fungi on wax moth

In comparison to G. mellonella larvae of the 4th instar, the effectiveness of several spore solutions of the most efficient fungus was calculated. Using pure water, serial successive concentrations of each suspension starting at 1% were made. The diet was dipped into each concentration for 30 seconds, with three replicates made for each concentration under test and a control (10 replicates). Mortality rates were reported 72 h following therapy. The mortality % corrected using **Abbott's** formula **(1925).**

Results

1- The efficacy of some endophytic fungal isolates extracts against the 4th instar G. mellonella larval mortality:

The fungal filtrates of A. parasiticus and A. carbonarius had satisfactory levels of mortality. The results were recorded at different incubation intervals (1, 3, 5, 7 and 9 days) and the obtained data were recorded. More than 8 % of fungal filtrate decreased larval mortality. From these data, it could be concluded that 6 % of fungal filtrates of both fungal strains produced the highest increase in larval mortality, as compared with other concentrations.

This experiment aimed to study the effect of different dilutions of fungal filtrates on wax moth larvae mortality by fungal isolates. (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9 %) of A. parasiticus and A. carbonarius were separately added as 1 ml of fungal filtrate to wax moth diet. One ml of solution added without fungal filtrate in a separate pettri dish served as control. Results revealed that the tested fungal extracts caused low initial mortalities at the first days of treatment by the initial concentrations while their highest mortality percents recorded from the third till 9 days post treatment. A. parasiticus caused the highest mortality after 9 days recording (50 %).

After incubation at 30°C, each sample was tested for its mortality concentrations. However, the mortality was weak with 9 % of A. carbonarius (25 % at 9 days). In the same time, 1% of A. carbonarius showed weak effect on the percents of wax moth larval mortality (50 % at 9 days). However, the percents of mortality were greatly affected at 5% after 72h of treatment by A. carbonarius recording (75 %) as shown in Table 1. The results of this experiment indicated that fungal filtrate was effective in wax moth larvae mortality by the two isolates A. parasiticus and A. carbonarius and the effect was dose- dependant.

Table 1: The efficacy of some fungal filtrates (A. parasiticus speare 13939 and A. carbonarius van Tieghem 13943) on G. mellonella larvae after 3, 5, 7 and 9 days at 28± 2ºC and 65± 5%RH.

Conc (%)	Mortality percentages (%) during different incubation periods (days)									
	A. parasiticus					A. carbonarius				
	1	3	5	7	9	1	3	5	7	9
1%	0	25	25	25	25	25	25	50	50	50
2%	0	25	25	25	25	25	50	62.5	62.5	75
3%	0	0	0	0	25	25	62.5	62.5	62.5	62.5
4%	0	0	0	0	25	50	50	62.5	62.5	62.5
5%	0	0	0	0	25	0	75	75	75	75
6%	0	25	25	25	37.5	0	62.5	62.5	62.5	62.5
7%	0	0	25	25	37.5	0	37.5	37.5	37.5	37.5
8%	0	25	25	50	50	12.5	50	50	50	50
9%	0	0	0	0	50	0	25	25	25	25
Control	0	0	0	0	0	0	0	0	0	0

2- The efficacy of most potent endophytic fungal spore suspensions against the 4th instar G. mellonella larval mortality:

The previous data clearly indicated that A. parasiticus and A. carbonarius had the maximum mortality percent. As well as, the same isolates had the highest insecticidal action against wax moth. Therefore, the previous isolates were selected for the further experimental studies. Two experiments were carried out to study the wax moth larval mortality by spore suspension of 2 fungi A. parasiticus and A. carbonarius under the influence of incubation time. To select the optimum incubation time for larval mortality by the 2 fungal strains, A. parasiticus (318 \10 µL saline) were suspended in 100 ml of sterilized saline solution incubated for different time intervals, then taken different volumes (1, 2, 3, 5, 7, 9 and 11 days) at 30ºC. As well, A. carbonarius (990 \10 µL saline). At the end of the incubation period, all samples were recorded and the mortality percentages were determined, as previously mentioned. To select the proper lethal concentration which supported successful larval mortality, 9 different concentrations of spore suspension of fungal strains A.

parasiticus and A. carbonarius were tested. Analysis of the various concentration throughout the experimental periods revealed that variability in wax moth larval mortality was obtained using different concentrations. The mortality percents of each concentration were measured and the results were presented. The highest mortality percentage 75 % as 1ml from different volumes of A. parasiticus was obtained using 7 ml containing 11.13×10^6 spore ml^{-1} on wax moth larvae after 7 days of incubation. The effective concentrations of spore suspensions could be arranged descendingly according to the mortality of wax moth larvae. As well as, the highest mortality percent of A. carbonarius was 62.5 % when the wax moth larvae were treated by 7 ml containing 34.6×10^6 spore ml^{-1} after 11 days of incubation. According to the results of this experiment, the larvae have been treated in 7 ml of spore suspensions of A. parasiticus and A. carbonarius throughout the subsequent experiments.

Table 2: The efficacy of some fungal spore suspensions (A. parasiticus speare 13939 and A. carbonarius van Tieghem 13943) on G. mellonella larvae after 1, 2, 3, 5, 7, 9 and 11 days at 28± 2ºC and 65± 5%RH.

a)- A. parasiticus

Concentrations of A. parasiticus (Spore ml^{-1})	Mortality percentages (%)						
	24 h	48 h	72 h	5 days	7days	9 days	11days
1 (1.59×10^6)	0	0	0	0	0	25	25
2 (3.18×10^6)	0	0	0	0	25	25	37.5
3 (4.77×10^6)	0	0	0	0	0	25	37.5
4 (6.36×10^6)	0	50	50	50	50	50	62.5
5 (7.95×10^6)	0	0	0	0	0	37.5	62.5
6 (9.54×10^6)	0	25	25	25	50	50	62.5
7 (11.13×10^6)	0	25	25	50	75	75	75
8 (12.72×10^6)	25	25	25	25	50	50	50
9 (14.3×10^6)	0	0	0	0	0	0	25
Control	0	0	0	0	0	0	0

318 in 10 µl (total volume 100 ml)

b)- **A. carbonarius**

Concentrations of A. carbonarius (Spore ml^{-1})	Mortality percentages (%)						
	24 h	48 h	72 h	5 days	7days	9 days	11days
1 (4.95×10^6)	990 in 10 µl (total volume 100 ml)	0	0	0	0	0	25	25
2 (9.9×10^6)		0	0	0	0	0	25	50
3 (14.85×10^6)		0	0	0	0	0	25	50
4 (19.8×10^6)		0	25	25	25	25	25	50
5 (24.75×10^6)		0	0	25	25	25	37.5	50
6 (29.7×10^6)		0	25	25	25	25	37.5	50
7 (34.6×10^6)		0	0	0	0	0	25	62.5
8 (39.6×10^6)		0	0	0	0	25	25	62.5
9 (44.55×10^6)		0	0	0	0	0	25	37.5
Control		0	0	0	0	0	0	0

Discussion

In this investigation, A. parasiticus and A. carbonarius produced the maximum mortality of G. mellonella. According to **Raper and Fennell (1965)** and **Moubasher (1993)**, the two isolates were recognized using the traditional methods of identification (macroscopic and microscopic criteria). A. parasiticus speare 13939, A carbonarius van Tieghem 13943 were the isolates' designations as a consequence. On the 21st day, the most powerful isolates (A. parasiticus and A. carbonarius) were collected from their broth cultures for the purpose of extracting secondary metabolites.

A. parasiticus induced the production of a novel compound present inside mycelium 2-(-4-bromophenyl)-2-oxoethyl benzoate, that is considered food repellent in Coleoptera (T. castaneum) as well as having an antivirulence strategy **(Cartagena et al., 2014).** Virulence of A. parasiticus may also depend on the media on which it is being cultured. Hence, strain of A. parsiticus was suitable as biological control agent against C. heimi **(Aihetasham et al., 2015).** A. carbonarius is

the main fungal species producing a range of secondary metabolite. The function role of the transcription factor AcOTAbZIP gene in culture filtrate of A. carbonarius is involved in metabolite synthesis (polyketide biosynthesic pathway). This gene is involved in 1,8 dihydroxynaphtalene melanin biosynthesis **(Gerin et al., 2021).** Hexamerin is a storage protein which is endocytosed by fat body cells has ferritin subunits by which the larvae can limit the growth of the fungus within the hemocoel. The balance of iron availability play role in the immune system- fungal iron sequestration. The synthesis of ferritin as means of sequestering iron from Aspergillus siderophores may play role in protecting the host against the higher fungal inoculum by depleting the fungus of its iron requirement **(Vierstraete et al., 2004 and Schrettl et al., 2004).** Calcineurin is a calcium calmodulin- dependent phosphatase conserved widely across eukaryotes, including pathogenic fungi. The role of calcineurin varies depending on the fungal species, for example calcineurin in A. fumigatus, calcineurin mutants exhibit delayed germination, hyphal growth with irregular branching and abnormal septa **(Juvvadi et al., 2014),** where the calcineurin inhibitor FK506 forces Mucor to grow only as yeast **(Lee et al., 2013).**

The findings demonstrated that the investigated extracts had greater larvicidal action than the spore solution of the endophytic fungus and may retard the growth of G. mellonella larvae. Numerous studies have shown the importance of endophytes in preventing insect growth, killing insects, and repelling insects **(Clay, 1988; Singh et al., 2016 and Azevedo et al., 2000).** According to the results of this experiment, the larvae have been treated in 7 ml of spore suspensions of A. parasiticus and A. carbonarius throughout the subsequent experiments.

This revealed the secondary metabolites in these extracts may have insecticidal properties. According to **Jeyasankar et al. (2014),** high larval mortality often suggests possible insecticidal efficacy of fungal secondary metabolite extract. This conclusion is in keeping with their findings. Insecticide properties of secondary metabolite substances include poisoning or the formation of harmful chemicals after intake. Larval death may be attributable to direct insecticidal action, feeding inhibition, gustatory repellency, or a reduction in the ability to assimilate food. In this regard, **Desire et al. (2014)** verified that these endophytes will increase the pool of possible medicines and pesticides.

According to **Andres et al. (2017),** screening naturally occurring compounds in microorganisms is one method of looking for such environmentally friendly insecticide products. It could be concluded that 6 % of fungal filtrates of both fungal strains produced the highest increase in larval mortality, as compared with other concentrations.

Serotonin (5- hydroxyl tryptamine) has been examined in the central nervous system of many insect species **(Coleman and Neckameyer, 2005).** Serotonin plays a key role in regulating and modulating physiological and behavioral processes in insects **(Yuan et al., 2006).** The entomopathogenic C. coronatus produces 2- β carboline alkaloids which influence the development of G. mellonella by affecting serotonin- regulating enzymes **(Harding et al., 2013).**

The larval mortality using some fungal strains has been also studied, in a trial for controlling the infection of the wax moth.

Endophytes can be exploited as one of the biological control agents in sustainable agricultural production, according to **Saad et al. (2019).**

Larval mortality after consuming a meal containing a fungus extract may be caused by a disruption in larval bioassays (**Gobbi et al., 1998).** Furthermore, some larvae were unable to reach the following advanced instar **(Khedr, 2002)**. The severely slowed rate of growth of the treated larvae as a result of the extracts' post-harmful effects may also be connected to the extracts' capability for repelling insects, and the insect's capacity to convert food into biomass.

The delayed development and the lowered adult emergence were detected in larvae given a diet supplemented with a fungus extract. The findings of the phytochemical analysis revealed that there were several phenolics, terpenoids, and proteins in the fungal extract. The results of the biosafety research indicated that the extract is safe. It was shown that the genotoxic and cytotoxic properties of S. litura and the associated capacity of S. commune to kill insects **(Kaur et al., 2018).**

Little is yet understood about the effectiveness of endophytic fungal extracts as biological controlling agents and their various modes of action against various insects. The majority of research on natural product regulating agents focused on the effects and mechanisms of action of plant extracts on insects, whereas endophytic fungal extracts and their metabolites received less attention.

<u>References:</u>

1. **Aihetasham, A.; Qayyum, F.; Xaaceph, M. (2015):** Pathogenicity of A. parsiticus against Coptotermes heimi (Wasmann). Punjab Univ. J. Zool., 30(2): 51-55.
2. **Alekseeva, L.; Huet, D.; Femenia, F.; Mouyna, I.; Abdelouahab, M. and Cagna, A. (2009):** Inducible expression of beta defensins by human respiratory epithelial cells exposed to A. fumigatus organisms. BMC Microbiol, 9-33.
3. **Andres, M.F.; Diaz, C.E.; Gimenez, C.; Cabrera, R. and Gonzalez- Coloma, A. (2017):** Endophytic fungi as novel sources of biopesticides: the Macaronesian Laurel forest, a case study. Phytochemistry review, 16(5): 1009- 1022.
4. **Azevedo, J.L.; Maccheroni, W.; Pereira, J.O. and Araujo, W.L. (2000)**: Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electron J. Biotechnol., 3: 40-65.
5. **Cartagena, E.; Marcinkevicius, K.; Luciardi, C.; Rodŕiguez, G.; Bardon, A. and Arena, M. (2014):** Activity of a novel compound produced by A. parasiticus in the presence of red flour beetle Tribolium castaneum against Pseudomonas aeruginosa and coleopteran insects. J pest science, 87: 521-530.

6. **Clay, K. (1988):** Fungal endophytes of grasses: adefensive mutualism between plants and fungi. Ecology; 69: 10-16.
7. **Coleman, C.M and Neckameyer, W.S. (2005):** Sertonin synthesis by two distinct enzymes in Drosophila melanogaster. Arch Insect Biochem and Physiol. 59(1): 12- 31.
8. **Desire, M.H.; Bernard, F.; Forsah, M.R.; Assang, C.T. and Denis, O.N. (2014):** Enzymes and qualitative phytochemical screening of endophytic fungi isolated from Lantana camara Linn. Leaves. J. App. Biol. Biotech; 2(06):001- 006.
9. **Desbois, A.P. and Coote, P.J. (2012):** Utility of greater wax moth larvae G. mellonella for evaluating the toxicity and efficacy of new antimicrobial agents. Advanced Applied Microbiology, 78: 25- 53.
10. **Dubovskiy, I. M.; Grizanova, E, V.; Whitten, M.A.; Mukherjee, K.; Greig, C. and Alikina, T. (2016):** Immuno- physiological adaptations confer waxmoth G. mellonella resistance to Bacillus thuringenesis. Virul. 7: 860-870.
11. **Elias, K.S. and Cotty, P.J. (1996):** Incidence and stability of infection by double stranded RNA genetic elements in Aspergillus section flavi and effects on aflatoxigenicity. Can J. Bot, 74: 716- 725.
12. **Fallon, J.P.; Reeves, E.P. and Kavanagh, K. (2010):** Inhibition of neutrophile function following exposure to the A. fumigatus toxin fumagillin. J. Med Microbiol. 59:625-33.
13. **Gerin, D.; Garrapa, F.; Ballester, A. R; Candelas, L.G.; Angelini, R.M.; Faretra, F. and Pollastro, S. (2021):** Functional role of A. carbonarius AcOTAbZIP gene, a bZIP transcription factor within the OTA gene cluster. Toxins, 13(2), 111.
14. **Gobbi, A.; Budia, F.G. and Vinuela, E. (1998):** Effects of tebufenozide on S. littoralis larvae. Effects on different application techniques. Bol. de Sanidad Vegetal Plagas, 24(1): 41-56.
15. **Golla, S.K.; Rajasekhar, P.; Akbar, S.M. and Sharma, H.C. (2018):** Proteolytic activity in the midgut of Helicoverpa armigera (Noctuidae: Lepidoptera) larvae fed on wild relatives of chickpea, Cicer arietinum, J. Econ. Entomol. 111: 2409–2415.
16. **Harding, C.R.; Schroeder, G.N.; Collins, J.W. and Frankel, G. (2013):** Use of G. mellonella as a model organism to study Legionella pneumophila infection. J. Visualized Experiments (81).
17. **Jeyasankar, A.; Premalatha, S. and Elumalai, K. (2014):** Antifeedant and insecticidal activities of selected plant extracts against epilachna beetle, Henosepilachna vigintioctopunctata (Coleoptera: Coccinellidae). Adv. Entomol; 2(1): 14- 19.
18. **Juvvadi, R.; Lamoth, F. and Steinbach, W. (2014):** Calcineurin as a multifunctional regulator: unraveling novel functions in fungal stress responses, hyphal growth, drug resistance and pathogenesis. Fungal Biol Rev. 28(2-3): 56- 69.
19. **Kaur, M.; Chadha, P.;Kaur, S.; Kaur, A.; Kaur, R.; Yadav, A.K.; Kaur, R.(2018):** Schizophyllum commne induced genotoxic and cytotoxic effects in Spodoptera litura. Scientific reports, 8(1), 4693.

20. **Khedr, M. A. (2002):** Effect of some plant extracts and insect growth regulators applied to control cotton leaf worm on honey bees, Apis melifera L. M. Sc Thesis, Fac of Agric., Zagazig, Univ.
21. **Krzywinski, J. and Besansky, N.J. (2002):** Frequent intron loss in the white gene: A cautionary tale for phylogenetics. Mol. Biol. Evol. 19: 362- 366.
22. **Lee, S.C.; Li, A.; Calo, S.; Heitman, J. (2013):** Calineurin plays key roles in the dimorphic transition and virulence of the human pathogenic zygomycetes Mucor circinelloids. PLoS Pathog 9: e1003625.
23. **Maurer, E.; Browne, N.; Surlis, C.; Jukie, E.; Moser, P. and Kavanagh, K. (2015):** G. mellonella as host model to study A. terrus virulence and amphotericin B resistance. Virulence. 6(6): 1-8.
24. **Moubasher, A. H. (1993):** Soil fungi in Qatar and other Arab countries. The center for Scientific and Applied Research, Doha, Qatar, pp. 566.
25. **Negi, P. Chand, S. Thakur, N. Nath, A.K. (2018):** Biological activity of serine protease inhibitor isolated from the seeds of Phaseolus vulgaris, Agric. Res. 7:265–270.
26. **Pinkerton, R. and Strobel, G. (1976):** Serinol as an activator of toxin production in attenuated cultures of Helminthosporium sacchari. Proceedings of the National Academy of Sciences of the united status of America, 73: 4007- 4011.
27. **Raber, K.B. and Fennel, D.I. (1965):** The genus Aspergillus Wiliams and Wilkins Co. Baltimore, Maryland, USA.
28. **Raviraja, N.S. (2005):** Fungal endophytes in five medicinal plant species from Kudermukh Range, Western Ghats of India. J. Basic Microbiol; 45: 230-235.
29. **Renwick, J.; Reeves, E.P.; Wientjes, F.B. and Kavanagh, K. (2007):** Translocation of proteins homologus to human neutrophile P 47 Phox and P 67 Phox to the cell membrane in activated hemocytes of G. mellonella. Dev Comp Immunol. 31: 347- 59.
30. **Saad, M.M.; Ghareeb, R.Y. and Saeed, A.A. (2019):** The potential of endophytic fungi as biocontrol agents against the cotton leafworm, Spodoptera litura. Egyptian journal of biological pest control, 29: 7.
31. **Schrettl, M.; Bignell, E.; Kragl, C.; Joechl, C.; Rodgers, T. and Arst, A.N. (2004):** Siderophore biosynthesis but not reductive iron assimilation is essential for A. fumigatus virulence. J. EXP. Med, 200: 1213-9.
32. **Schulz, B.; Guske, S.; Dammann, U.; Boyle, C. (1998):** Endophyte- host interaction II. Defining symbiosis of the endophyte host interaction. Symbiosis, 25: 213-227.
33. **Singh, B.; Kaur, T.; Kaur, S.; Manhas, R.K. and Kaur, A. (2015):** An alpha glucosidase inhibitor from an endophytic Cladosporium sp. with potential as a biocontrol agent, Appl. Biochem. Biotechnol. 175:2020–2034.
34. **Singh, B.; Kaur, T.; Kaur, S.; Manhas, R.K. and Kaur, A. (2016):** Insecticidal potential of an endophytic Cladosporium velox against Spodoptera litura mediated through inhibition of alpha glycosidases, Pestic. Biochem. Physiol. 131:46–52.

35. **Slater, J. L.: Gregson, L.: Denning, D. W., and Warn, P. A. (2011).** Pathogenicity of A. fumigatus mutants assessed in G. mellonella matches that in mice. Med. Mycol. 49, S107–S113.
36. **Van Diepeningen, A.D. (2008):** Mycoviruses in the Aspergilla: **Varga, J.** and **Samson, R.A.**, Aspergillus in the genomic era, Wageningen, the Netherlands, 133- 176.
37. **Vierstraete, E.; Verleyen, P.; Baggerman, G.; Hertog, W.; Van den bergh, W.; Arckens, L. (2004):** A proteomic approach for the analysis of instantly released wound and immune proteins in Drosophila melanogaster hemolymph. Proc Natl. Acad Sci, 101: 470-5.
38. **Yuan, Q.; Joiner, W.J. and Sehgal, A. (2006):** Asleep promoting role for the Drosophila serotonin recepto1-A. Current biology, 16(11): 1051- 62.