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# molecular studies of some entomopathogenic fungi

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In the present study, five fungal isolates namely *Metarhizium anisopliae*, *Metarhizium flavoviride*, *Beauveria bassiana*, *Beauveria brongniartii* and *Paecilomyces farinosus*, were used. These isolates were provided by Insect pathogen unit at Plant Protection Research Institute. These isolates were used as biological control agents for the Egyptian cotton leaf worm *Spodoptera littoralis*, which represents the most severe destructive cotton pest in Egypt and many other countries. The main points which were discussed in this study could be summarized in the following topics:-1- The fungi under study were grown on three different media where *M. anisopliae*, *M. flavoviride* and *P. farinosus* exhibit good growth on Dox medium, while *B. brongniartii* exhibit good growth on potato - dextrose agar medium. whereas *B. bassiana* exhibit good growth on complete agar medium. The medium constituents were prepared and adjusted at pH 5.5- 6.5. After sterilization, Petri- dishes were inoculated with the fungal strains and incubated for two weeks at 25°C and 50-60% RH. At the end of incubation period the conidia were harvested from the surface of the cultures by scratching using sterile solution of 0.01% tween-80. The resulting suspension was counted according to hemocytometer counts technique. Five concentrations were used for each isolates. The fungal metabolites were prepared by making broth medium. These media were inoculated with 142 entomogenous fungi and incubated for two weeks at 25°C and 50-60% RH. At the end of the incubation period the supernatant was separated from the mate. Five concentrations were used for each isolates.2- The second and fourth instar larvae of *S. littoralis* were exposed for 48h to treated castor bean leaves, *Ricinus communis* by using dipping technique with conidial suspension and metabolites. In case of conidial suspension effective host pathogen contact takes place while metabolites effective feeding takes place by cover the surface of castor bean leaves with five concentration of the entomogenous fungi. After an exposure time of 48h the infected larvae were placed in a sterile cups with cleaned castor bean leaves and filter paper. Mortality counts were recorded every two days. The results showed that spore suspension of *M. anisopliae* give the highly mortality percentage which ranged between 55% to the 4th instar larvae and 60% to 2'd instar larvae and LT50 was 7 days for 2'd instar larvae and 10 days for 4th instar larvae, while metabolites of *M. flavoviride* give the highly mortality percentage which ranged between 54% to the 4th instar larvae and 60% to 2'd instar larvae and LT50 was 7 days for 2nd instar larvae and 10 days for 4th instar larvae.3- The DNA of the five isolates

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-mentioned above were extracted according to hexadecyltri- methyl- ammonium bromide (CTAB) method. The PCR amplification was<sup>143</sup>performed by using five RAPD primers. The five primers amplified 227 DNA fragments including 45 non polymorphic fragments and 182 fragments were polymorphic. The number of polymorphic amplicons per primer ranged from 30 to 49. The amplification reaction with the 5 primers have shown the existence of a high polymorphism among the five isolates mentioned above, the polymorphism percentage (%) reached 83%.<sup>4</sup>- The protein of five isolates mentioned above was, extracted from their mycelium and fractioned by SDS- PAGE. SDS-PAGE revealed a maximum number of 19 bands, which were not necessarily present in all samples. The protein of *B. bassiana* was separated into 11 bands, while protein of *B. brongniartii* was separated into 13 bands. *B. brongniartii* has three positive markers distinguished it from *B. bassiana*. *B. bassiana* has only one positive marker distinguished it from *B. brongniartii*. The protein of *M. anisopliae* was separated into 5 bands, while protein of *M. flavoviridae* was separated into 11 bands. *M. anisopliae* has two positive markers distinguished it from *M. flavoviridae*, whereas *M. flavoviridae* has eight positive markers distinguished it from *M. anisopliae*. On the other hand the protein of *P. farinosus* was separated into 4 bands.<sup>144</sup>