Using Insects' Body Powder as a Complementary Growth Factor in Fungal Culture Media

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INTRODUCTION

Fungi are aerobic or facultative anaerobic eukaryotic organisms categorized in a separate kingdom. Some fungi species invade insects in natural environments and are considered as important biological agents in order to control the insects’ populations. In fact, some of the fungi are known as naturally entomopathogenic species. The members of the genera, Metarhizium, Beauveria, Lagenidium, and Verticillium are among the most important pathogenic fungi of insects [1-4]. Many pathogenic fungi have been isolated from various species of insects, including members of the genera Anopheles, Culex, and Aedes [1-4]. Some of the pathogenic fungi of insects are available in the market with commercial names of Ago Bio, Bassiana and Bioblast (The Ministry of Primary Industries-MPI, New Zealand). Some fungi can attack and consume the insects’ body and finally kill them, while some merely feed on the dead insects [1-4]. These fungi use some compounds of the insects’ bodies as nutrients for their growth, among them carbon and nitrogen are the main elements, and their presence in the culture media is critical [5]. The fungi culture media are prepared based on the specific requirements of the species to these two primary nutrients, and peptone and glucose are commonly used to supply these elements [1-5]. There are a variety of sources for nitrogen and carbon in nature including chitin (polymers of N-acetyl glucosamine) of insects’ body and keratin in

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MATERIAL AND METHODS

Insects' body powder (IBP). In this study, we used the IBP obtained from Anopheles superpictus and Culex theileri mosquitoes for the enrichment of Sabouraud Dextrose Agar medium containing chloramphenicol (SC) (Merck, Germany). The insectary of Tehran University of Medical Sciences (Culicidae section) provided non-engorged mosquitoes. The insects' bodies were powdered to the particles ≥2 mm by a stone mortar. The IBPs were transferred to screw-cap glass tubes and autoclaved for 30 min at 121°C and the pressure above 75%.

Optimizing the amount of IBP. To obtain the optimum amount, initially 2 mg of the IBP was added into the 10 cm diameter culture medium in which fungi were cultured. The amount of powder was increased in subsequent cultures, and finally, the best fungal growth was obtained with 10 mg of IBP.

Fungal samples. In this study, we used 27 saprophyte fungi and yeasts species obtained from human clinical specimens. The species included Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus versicolor and the species belonging to the genera Penicillium, Scopulariopsis, Fusarium, Acremonium, Paecilomyces, Chrysosporium, Mucor, Rhizopus, Syncephalastrum, Cladosporium, Alternaria, Nigrospora, Hendersonella, Bipolaris, Eurobasidium, Chatomium, Stachybotrys, Trichoderma, Sporothrix, Rhodotorula, Geotrichum, and Candida albicans. These fungi were obtained from clinical isolates and are kept in distilled water in the fungal collection of Medical Mycology Department in Tehran University of Medical Sciences.

Fungi culture. The fungi (27 species) were cultured in the SC culture media containing 10 mg of IBP (the experiment group). Similar cultures were performed with no IBP as controls. All of the cultures were carried out in triplicate. Equal number of samples were cultured on the media in both experimental and control groups. After incubating the media for 7-10 days at room temperature (25-30°C) with a humidity of 20-50%, the grown colonies were examined with a microscope at a magnification of 400 X. The increase in the growth rate, colony and color changes, increase in colonies volume and morphology of fungi such as conidia formation in both groups were evaluated as described elsewhere [5, 10]. The growth rate was measured based on the fungi mycelium volume that occupied the plates and defined as three degrees, 1+ (normal growth on the SC medium without IBP), 2+ (two-time more than normal growth), and 3+ (three-time more than normal growth) (Table 1).

Statistical analysis. The data analysis of growth rate in both groups was performed by SPSS software version 22.0 [11]. The ordinal growth rate variable in two groups could be considered as variable in Two-dependent or Two-independent groups. Thus, the study was assessed by using standard Wilcoxon signed ranks test or Mann-Whitney-U-Test with 95% confidence intervals (CI). P-value <0.05 was considered as statistically significant.

<table>
<thead>
<tr>
<th>Fungi type</th>
<th>Growth rate after 7 days</th>
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<th>Growth rate after 7 days</th>
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<td></td>
<td>Control medium (SC)</td>
<td>Experimental medium (SC with IBP)</td>
<td>Control medium (SC)</td>
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<tr>
<td>A. niger</td>
<td>1+</td>
<td>3+</td>
<td>Acremonium spp.</td>
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<tr>
<td>A. flavus</td>
<td>1+</td>
<td>3+</td>
<td>Paecilomyces spp.</td>
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<td>A. fumigatus</td>
<td>1+</td>
<td>3+</td>
<td>Cladosporium spp.</td>
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<td>Scopulariopsis sp.</td>
<td>1+</td>
<td>3+</td>
<td>Alternaria spp.</td>
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<td>Chrysosporium sp.</td>
<td>1+</td>
<td>3+</td>
<td>Nigrospora spp.</td>
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<td>Mucor spp</td>
<td>1+</td>
<td>3+</td>
<td>Hendersonella spp.</td>
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<td>Rhizopus spp.</td>
<td>1+</td>
<td>3+</td>
<td>Bipolaris spp.</td>
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<td>Stachybotrys sp.</td>
<td>1+</td>
<td>3+</td>
<td>Eurobasidium spp.</td>
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<td>Trichoderma sp.</td>
<td>1+</td>
<td>3+</td>
<td>Chatomium spp.</td>
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<td>Syncephalastrum sp.</td>
<td>1+</td>
<td>3+</td>
<td>Rhodotorula spp.</td>
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<td>A. nidulans</td>
<td>1+</td>
<td>2+</td>
<td>Sporothrix spp.</td>
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<tr>
<td>A. versicolor</td>
<td>1+</td>
<td>2+</td>
<td>Geotrichum spp.</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>1+</td>
<td>2+</td>
<td>C. albicans</td>
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Table 1: Growth rate of fungi in IBP-supplemented SC media and controls (SC media without IBP)

Note: IBP denotes insects' body powder, 1+ (average growth on the SC medium without additive substances), 2+ (two-time more than average) and 3+ (three-time more than average).

RESULTS

From a total of 27 species of saprophytic and yeast fungi, 23 species showed a positive response to the addition of IBP into the culture media, and there was a significant difference between the growth in the media containing IBP and the controls without IBP (P<0.001). The fungi A. niger, A. flavus, A. fumigatus, Trichoderma spp., Chrysosporium spp., Mucor spp., Syncephalastrum spp., Scopulariopsis spp., Rhizopus spp., and Stachybotrys spp. showed the highest positive responses (3+) to the addition of IBP as a supplement and a significant difference was determined between the growth in media containing IBP and the controls (P<0.001). The average responses (2+) were observed in the fungi Chatomium spp., Eurobasidium spp., Bipolaris spp., Hendersonella spp., Nigrospora spp., Alternaria spp., Cladosporium spp., A. nidulans, A.
versicolor, Penicillium spp., Fusarium spp., Acremonium spp., Paecilomyces spp., and the difference between the growth in media containing IBP and the controls was significant ($P<0.001$). Regarding the fungi Sporothrix spp., Rhodotorula spp., Geotrichum spp., and C. albicans, there were no significant differences in the growth rate of these fungi in media containing IBP and the controls; the amount of colony growth in both media was the same (1+), and no significant difference was observed ($P=0.4$). Figure 1-A shows the macroscopic features of A. niger in a medium culture containing IBP and the controls with no IBP. The IBP-supplemented medium turned black due to excessive growth of conidia. In microscopic observation of this fungus in Figure 1-B, the difference in vesicles size or diameter of growth in the two media is evident.

In macroscopic observation of fungus A. flavus (Fig. 2-A), the growth rate in the media containing IBP was more than the control. The color of the colony in the medium with IBP was observed as dark green, but in control as yellow to yellow-greenish. In the dark green color medium, due to the provision of a more favorable condition, sporulation or conidia formation was more than the control. In microscopic observations (Fig. 2-B), the size of vesicles diameter in the IBP-supplemented medium was greater than the control. Figure 3-A shows the growth of Penicillium spp. (2+) in a medium containing IBP in comparison to the control (1+). As it is seen in Figure 3-B, there is no significant difference in fungus growth rate in experimental medium with IBP and the control.

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**Fig. 1.** Macroscopic comparison of A. niger growth in SC culture medium containing IBP and the control (A); microscopic comparison of A. niger sporulation in SC culture medium containing IBP and the control (B).

**Fig. 2.** Macroscopic comparison of A. flavus growth in SC culture medium containing IBP and the control (A); microscopic comparison of A. flavus sporulation in SC culture medium containing IBP and the control (B).

**Fig. 3.** Macroscopic comparison of Penicillium growth in SC culture medium containing IBP and the control (A); macroscopic comparison of C. albicans growth in SC culture medium containing IBP and the control (B).
DISCUSSION

In this study, we introduce IBP as a new economic supplement for enhancing the growth rate of fungi in the culture media. The insects’ body contain different nutrient compounds for fungi growth including nitrogen and carbon sources [2-5, 7], so IBP can be used to enrich the culture media to enhance fungi growth rate. In this study, we used IBP obtained from the members of the family Culicidae to enrich the culture media. For sure, this method can be extended to other insects as well. In Brazil, 13 species belonging to the 7 genera, Aspergillus, Acremonium, Alternaria, Penicillium, Paecilomyces, Beauveria, and Curvularia, which were able to grow and produce keratinases in the media containing powdered chicken feather were isolated (12). The most keratinolytic reaction, after 4-6 days, belonged to Alternaria tenuissima, Acremonium hyalinulum, Curvularia brachyspora, and Beauveria bassiana. This finding showed that with regard to the fungi keratinolytic and chitinolytic activities a significant amount of poultry and animals wastes, as well as insects, can be utilized as nutrients in fungi culture media [12]. In another study, 196 soil samples were collected from 43 parks in Shiraz, and by using the children’s sterile hair as a baiting method, common keratinophilic fungi including Fusarium spp., Chrysosporium spp., Acremonium spp., Penicillium spp., Microsporum gypseum spp., and Chatomaria were isolated from the soils [13]. Some fungi are a serious threat to the arthropods as pathogens. Some insects are in continuous contact with the soil searching for food and shelter. Thus, in the ground, the fungi can easily attack the insects, especially dead ones, and feed on components of their body. In a study in the Gheareh Penhan Mountains of Iraqi Kurdistan, from 149 dead insects belonging to 7 species, 226 fungi species were isolated from 12 fungal colonies [14].

In the current study, the IBP from the Culicidae mosquitoes increased the growth rate of some fungi including Aspergillus spp. (especially A. niger, A. flavus, and A. fumigatus), Penicillium spp., Chrysosporium spp., Fusarium spp., Rhizopus spp., Mucor spp., and black fungi such as Alternaria spp., Aureobasidium spp., and Nigrospora spp. Our results showed that these fungi species have different enzymes for hydrolysis of IBP that make them capable of consumption of IBP elements such as nitrogen and carbon for their growth. Since Chitin is a significant component of IBP, it can be said that in addition to keratinase, most of the fungi have chitinase for hydrolysis of chitin in different sources such as insects body [5, 7]. Basseri and Hashemi (2011), introduced A. niger as a natural entomopathogenic fungus for controlling Anopheles Dhlali mosquitoes (the secondary malaria vector in Iran). This fungus could destroy not only the larval stage, but also the adult mosquitoes in a few days. The cause of the adult mosquitoes death was mainly due to the tight attachment of fungus spores to different parts of the insects' body including head, trunk, and the cuticle, and the fast mycelia growth, and wide dissemination of long Conidiophores in various parts of the mosquitoes body [15]. In another study, A. niger was successfully used as an entomopathogenic agent for controlling Anopheles stephensi mosquitoes, the main malaria vector in the south of Iran [16]. According to the results of our study and the two previous reports [15, 16], it would be possible to take advantage of this fungus as a biological agent to control Anopheles mosquitoes. The results of present study showed that the IBP contains the essential nutrients for the fungi growth. Our results also suggest using IBP as a supplement in laboratory culture media in the industry. Regarding the limited number of fungi available commercially for controlling the mosquitoes populations and considering the ability of the fungi used in this study to grow faster in IBP-supplemented media, the possibility of using these fungi as biological agents for controlling the insects responsible for mosquito-borne diseases should be investigated.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES


