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Original Article

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

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Introduction: Nutritional requirements of fungi are essential for their successful growth in laboratories. Some fungi feed on the compounds present in the insects' bodies. The aim of this study was to evaluate the effect of insects' body powder (IBP) as a supplement for the fungi growth in culture media. **Methods:** We used 10 mg of IBP, obtained from *Anopheles superpictus* and *Culex theileri* mosquitoes, as the optimum amount for the enrichment of Sabouraud Dextrose Agar medium containing chloramphenicol (SC). The growth rate of 27 species belonging to saprophytes and yeasts on this medium was compared with the control SC medium containing no IBP. **Results:** From a total of 27 species of saprophytic and yeast fungi, 23 showed a positive response to the addition of IBP in the culture media, and there was a significant difference between the growth rate in the culture media containing IBP and the controls ($P<0.001$). The fungi *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Trichoderma* spp., *Chrysosporium* spp., *Mucor* spp., *Syncephalastrum* spp., *Scopulariopsis* spp., *Rhizopus* spp., and *Stachybotrys* spp. showed the highest positive response to the addition of IBP as a supplement ($P<0.001$). **Conclusion:** IBP can be used as a supplement in fungal medium cultures for enhancing fungi growth to isolate the pathogenic fungi more rapidly. The use of IBP would be economical in the industry for making culture media. Also, it would be possible to use the fungi tested in the current study for biological control of insects' populations serving as vectors of infectious diseases. *J Med Microbiol Infec Dis, 2016, 4 (3-4): 57-61.*

Keywords: Entomophaga, Culture Media, Insects' Body Powder, Growth.

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 for 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 hair, horns, forks, and coating surface of the mammals [5-7]. Hydrolases, a group of enzymes produced by some microorganisms such as fungi are capable of hydrolyzing the chitin from various sources including insects' bodies [5, 7-9]. Thus, insects' body powder (IBP) with a high amount of the two mentioned elements can be used as a nutritional source for *in vitro* culture of some fungi species. IBP addition to culture media might shorten the time required for growth of fungi obtained from the clinical samples. Also, these fungi can be used in biological control of insects' populations, especially those responsible for transmission of infectious disease. There is not much data on the effects of IBP on fungi growth in culture media. The aim of this study was to evaluate the effect of IBP as a supplement in culture media for enhancing fungi growth.

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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*, C*hatomium*, *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 base 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 1. Growth rate of fungi in IBP-supplemented SC media and controls (SC media without IBP)

Fungi type	Growth rate after 7 days		Fungi type	Growth rate after 7 days	
	Control medium (SC)	Experimental medium (SC with IBP)		Control medium (SC)	Experimental medium (SC with IBP)
A. niger	1+	3+	*Acremonium* spp.	1+	2+
A. flavus	1+	3+	*Paecilomyces* spp.	1+	2+
A. fumigatus	1+	3+	*Cladosporium* spp.	1+	2+
Scopulariopsis spp.	1+	3+	*Alternaria* spp.	1+	2+
Chrysosporium spp.	1+	3+	*Nigrospora* spp.	1+	2+
Mucor spp.	1+	3+	*Hendersonella* spp.	1+	2+
Rhizopus spp.	1+	3+	*Bipolaris* spp.	1+	2+
Stachybotrys spp.	1+	3+	*Aurobasidium* spp.	1+	2+
Trichoderma spp.	1+	3+	*Chaetomium* spp.	1+	2+
Syncephalastrum spp.	1+	3+	*Rhodotorula* spp.	1+	1+
A. nidulans	1+	2+	*Sporothrix* spp.	1+	1+
A. versicolor	1+	2+	*Geotrichum* spp.	1+	1+
Penicillium spp.	1+	2+	*C. albicans*	1+	1+
Fusarium spp.	1+	2+	***P < 0.05***		

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 C*hatomium* spp., *Aureobasidium* spp., *Bipolaris* spp., *Hendersonella* spp., *Nigrospora* spp., *Alternaria* spp., *Cladosporium* spp., *A. nidulans*, *A.*

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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.

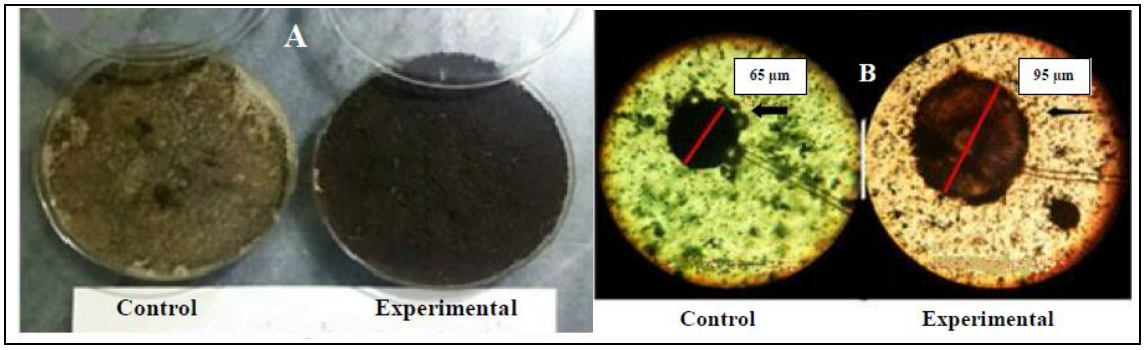


Fig. 1. Macroscopic comparison of *A. niger* growth in SC culture medium containing IBP and the control (A); microscopic comparison of *A. niger* sporulation in SC culture medium containing IBP and the control (B).

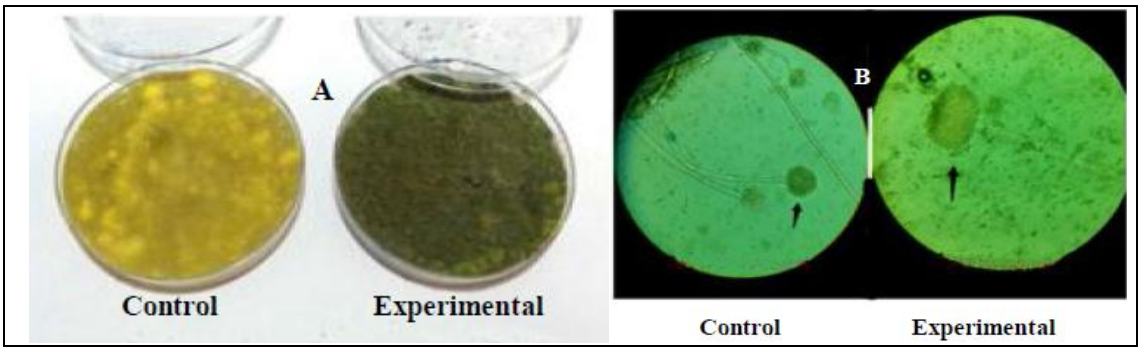


Fig. 2. Macroscopic comparison of *A. flavus* growth in SC culture medium containing IBP and the control (A); microscopic comparison of *A. flavus* sporulation in SC culture medium containing IBP and the control (B).

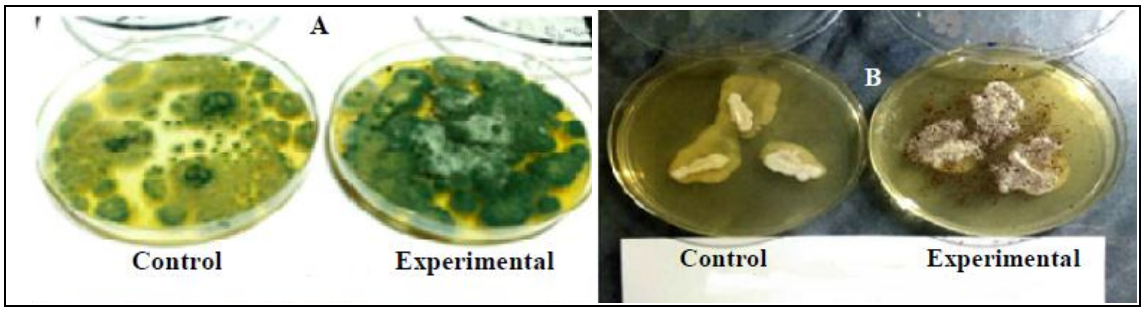


Fig. 3. Macroscopic comparison of *Penicillium* growth in SC culture medium containing IBP and the control (A); macroscopic comparison of *C. albicans* growth in SC culture medium containing IBP and the control (B).

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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 tenuissina*, *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 *Chatomium* 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 Ghareh 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 Dthali* 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.

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