Prevalence of \textit{Aspergillus} spp. on Shell Eggs

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(Received 30.3.2011; accepted 20.11.2011)

\textbf{ABSTRACT}

The prevalence of fungi on the egg samples collected from the local market and nearby areas of Palampur, Himachal Pradesh was studied with special emphasis on \textit{Aspergillus} spp. because of public health importance. The fungal species were differentiated on the basis of their cultural and microscopic morphology. The fungi were present in all the egg samples (100\%) and the \textit{Aspergillus} spp. were found in 61.70\% of the egg samples. The \textit{Aspergillus} spp. was exclusive in 27.66\% of egg samples and the \textit{Aspergillus} spp. was found along with other fungal species in 34.04\% of egg samples. The species of \textit{Aspergillus} which were identified on the basis of macroscopic and microscopic morphology in this work were \textit{A. niger}, \textit{A. fumigatus}, \textit{A. flavus}, \textit{A. parasiticus} and \textit{A. ochraceus} in their order of prevalence.

The high prevalence of \textit{Aspergillus} spp. on the egg samples points towards the risk for human health.

\textbf{Keywords:} \textit{Aspergillus} spp., chicken eggs, prevalence, public health significance.

Fungi are ubiquitous in nature and have great public health importance due to their wide contamination in various feed ingredients or foods used for human and animal consumption. Fungal contamination of foods can occur at any stage of food processing i.e. right from harvesting operations until it reaches the consumer’s table. The fungi growth is generally favoured by the high temperature (27°C) and high relative humidity (85\%), which normally prevails in tropical countries like India (Murthy \textit{et al.}, 2009).

The consumption of foods contaminated by fungi and their toxic metabolites can represent a relevant source of danger to humans (Reddy \textit{et al.}, 2010). The occurrence of toxin production strains isolated from foods and animal feed does not necessarily imply the presence of mycotoxins. However, it indicates potential risk for the possible contamination with mycotoxins (Arenas \textit{et al.}, 2005). \textit{Aspergillus} causes fungal infection in birds and occasionally in other animals including man. The genus \textit{Aspergillus} includes over 185 species and around 20 species have been so far reported as causative agents of opportunistic infections in man. The aflatoxin-producing \textit{Aspergillus flavus} and \textit{A. parasiticus}, and ochratoxigenic \textit{A. niger}, \textit{A. ochraceus} and \textit{A. carbonarius} species are frequently measured in agricultural commodities (Leong \textit{et al.}, 2007). Besides having the ability to produce carcinogenic and mutagenic aflatoxins in man \textit{Aspergillus} spp. can infect vital organs of man leading to asthma, endocarditis and meningitis (Patton, 2006). \textit{A. fumigatus} is involved in about 90\% of human aspergilloses, followed by \textit{A. flavus}, \textit{A. terreus}, \textit{A. niger}, \textit{A. nidulans} and \textit{A. ochraceus} (Denning, 1998; Bertout \textit{et al.}, 2001). Though molecular methods continues to improve and become more rapidly available, the morphological characteristics of the colony and microscopic examination

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Many *Aspergillus* strains are very close in their morphological characters and chances are very high to misidentify them. Therefore, accurate identification of *Aspergillus* spp. is important to develop proper management practices to control these toxigenic fungi and their mycotoxins in food grains (Reddy et al., 2010). Many workers employed the morphological characters for the identification of *Aspergillus* spp. from clinical samples (Kim et al., 2009; Diba et al., 2007), kitchen samples (Alwakeel, 2007), soils (Morya et al., 2009) and coffee beans (Moslem et al., 2010).

For the present study, a total of 47 egg samples were collected from the local market and nearby areas of Palampur (Himachal Pradesh). The egg samples were collected in sterile plastic bags and brought to the laboratory for further processing. The isolation of yeast and moulds was done from the egg shell as per the method outlined by Reu et al. (2004). Ten milliliters of buffered peptone water (BPW) was added to each plastic bag containing individual egg. The bag containing egg and BPW was gently rubbed for one minute. One ml of the above egg washing was used for making serial dilution in BPW. One ml of each dilution was poured in different petri-plates. Then molten (45°C) potato dextrose agar (PDA) containing 10% tartaric acid was poured into the Petri-plates. After proper mixing of media, the Petri-plates were allowed to solidify and then were transferred to the incubator maintained at 25±1°C for 5-7 days. After 5-7 days yeast and mould counts of each egg shell was done and fungal species were identified. The differentiations of fungal species were made on the cultural characteristics and microscopic morphology. For macroscopic morphology- colony diameter, texture, exudate and colony colour on PDA were noted. For the microscopic identification of *Aspergillus* spp., wet mount technique using lactophenol cotton blue stain was employed. Then the prepared slide was examined microscopically for the colour of asexual spore, hyphae, vesicles, conidiophores, conidiospore and phalides.

The aim of the present study was to observe the prevalence of *Aspergillus* spp. on eggs due to the public health importance of this fungus. The prevalence of *Aspergillus* spp. in market eggs collected from in and around Palampur (Himachal Pradesh) was observed on intact shell eggs. The fungus was found to be in the range of 5 – 70 moulds at 1:100 dilution per egg shell. The prevalence of *Aspergillus* spp. on shell eggs is presented in (Table 1). Out of the 47 eggs, 29 (61.7%) were found to be positive for *Aspergillus* spp., while 18 (38.3%) samples were found to be dominated by *Penicillium* spp. Out of 29 positive samples the *Aspergillus* spp. showed exclusive presence on 13 (27.66%) samples and in remaining 16 (34.04%) samples the *Aspergillus* spp. was present along with other fungal species including *Penicillium* spp. Out of 18 egg samples containing non-*Aspergillus* fungal species, 11 were found to be positive for *Penicillium* spp., of which 8 samples showed presence of *Penicillium* spp.

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Total <em>Aspergillus</em> isolates</th>
<th>Percent to total</th>
<th>Percent to isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. fumigatus</em></td>
<td>9</td>
<td>19.15%</td>
<td>31.03%</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>5</td>
<td>10.64%</td>
<td>17.24%</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>11</td>
<td>23.4%</td>
<td>37.93%</td>
</tr>
<tr>
<td><em>A. parasiticus</em></td>
<td>3</td>
<td>6.38%</td>
<td>10.34%</td>
</tr>
<tr>
<td><em>A. ochraceus</em></td>
<td>1</td>
<td>2.13%</td>
<td>3.49%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>29</strong></td>
<td><strong>61.7 %</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

Table 1: Prevalence of different *Aspergillus* spp. isolated from the shell eggs
spp. exclusively and in other 3 samples it was mixed with other fungal species.

In the present study, among the 29 isolates, 11 (37.93%), 9 (31.03%), 5 (17.24%), 3 (10.34%) and 1 (3.49%) fungal isolates were selected, characterized and identified as *A. niger*, *A. fumigatus*, *A. flavus*, *A. parasiticus* and *A. ochraceus*, respectively. In earlier study, Reddy et al. (2010) reported predominance of *Aspergillus flavus* (45), followed by *A. niger* (32), *A. fumigatus* (8), *A. ochraceus* (7), and *A. tamarii* (8) based on the morphological characters. Earlier various researchers (Pattron, 2006; Denning, 1998; Bertout et al., 2001) reported that *Aspergillus fumigatus* to be most commonly isolated species, followed by *Aspergillus flavus* and *Aspergillus niger*.

The presence of *Aspergillus* spp. on the egg shell indicates that improper sanitary practices are being followed at poultry farms which can be hazardous to both occupational workers and consumers of egg. Occurrence of *Aspergillus* spp. on eggs poses a serious threat to human health because of its disease producing ability in human beings as well as due to the production of aflatoxins that have been found to be carcinogenic, teratogenic and mutagenic in both humans and birds (Obi and Igbokwe, 2009). The findings of present study reveals that there is an urgent need of regular inspection of poultry farms, feeds, sanitary and management practices so as to reduce the prevalence of *Aspergillus* spp. in eggs and in turn safeguard the human health.

References


