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Damage, Weight Loss and *Aspergillus* section Flavi Infection in Stored Maize Grains in Shumar Gewog, Pemagatshel

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ABSTRACT: Maize is grown as the staple food crop in Eastern Bhutan; however, the post-harvest practices are worrisome. The maize cobs are being heaped in the open field or in the house for days when there is labour shortage, leading to the fungal infection. Moreover, de-husked maize is stored in sacks after partial sun drying, favoring fungal growth. This study examined the incidence of *Aspergillus* section Flavi and also assessed grain damage and weight loss due to insect pests. 60 maize samples were collected from Nangkor, Shumar, Shali and Gamung villages under Shumar *Gewog,* covering low and mid altitudes maize production zones. *Aspergillus* fungi were isolated using Subaroaud Dextrose Agar and pure cultures of isolates were obtained with differential media to identify *Aspergillus* section Flavi. Based on their morphological characteristics, *Aspergillus* section Flavi was found to be predominant with 63.6% of *Aspergillus* fungi isolated. The rest of the isolates constitute unidentified sections of *Aspergillus* fungi. Insect pests belonging to genera *Sitophilus, Tribolium, Carpophilus* and *Sitotroga* were observed. Mean percentage of bored kernels and weight loss were 49.9% and 8.9% respectively. Therefore, good post-harvest practices need to be in place for the farmers to reduce grain losses, and concentration of mycotoxin in the maize needs to be assessed to ensure health safety.

Keywords: Aflatoxin; *Aspergillus*; Bhutan; grain damage; pest and storage.

INTRODUCTION: Maize (*Zea mays* L.) is a major food crop cultivated by 69% of the rural households and it ranks first in the extent of area cultivated amongst the food crops in Bhutan.[1] The maize production environment in the country is broadly categorized into three zones based on the altitude, namely, Sub-tropical maize production zone I (1800 metres (m) above sea level (asl)), or low altitudes; Sub-tropical maize production zone II (1200 -1800 m asl) or mid altitudes; and the Highland maize production zone (>1800 m asl).[2] In Bhutan, maize is cultivated both as a summer and winter crop depending on altitudes. During summer, maize is planted in February and harvested by August, while winter maize in lower altitudes is sown by August and harvested in December.[3] Maize is consumed in various forms such as grits (locally known as Kharang), flour, roasted or boiled cobs and popcorns. It is also pounded into flakes (locally known as Tengma), and brewed into a variety of local alcoholic beverages such as bangchang, shingchang and ara which are an indispensible part of the culture and tradition of Eastern Bhutan.[3] Moreover, it is regarded as one of the primary food sources for animals. The by-products such as grain, flour and solid residues left after brewing alcoholic beverages are used for feeding cattle, pigs and poultry.[3]

Though maize is considered as a staple crop, farmers in Bhutan still practice an age-old tradition of the post-harvest system. During the time of harvest, the maize cobs are heaped in the open field and then in the house for several days when farmers have a shortage of labourers. They remove the husk and then store in sacks, tins and baskets after partial drying in the sun. These practices lead to an increase in moisture content, which in turn increases the susceptibility to insect attack and fungal growth. Globally, 20–30% grain loss is particularly due to post-harvest insect pests and grain pathogens.[4] There are at least 37 species of arthropod pests associated with maize grains in storage.[5] The two most damaging insect pests for maize are the maize weevil (*Sitophilus zeamais*) and the larger grain borer (*Prostephanus truncates*). Other economically important storage pests of maize are Angoumois grain moth (*Sitotroga cereallela*) and the lesser grain weevil (*Sitophilus oryzae*).[6]

Maize is particularly susceptible to degradation by mycotoxin producing fungi during the post-harvest storages. Among the *Aspergillus* section Flavi, *A. flavus*, *A. parasiticus* and *A. nomius* are the most prevalent species which produce aflatoxin and can be detrimental to human health and animal productivity.[7] Aflatoxins are some of the most potent carcinogens produced, and aflatoxin B_1 is the most commonly found in maize.[8, 9&10] The U.S. Food and Drug Administration's standard for aflatoxins is 20 parts per billion (ppb) for human and animal consumption.[11]

The *A. flavus* infection and subsequent aflatoxin contamination of maize either during pre- or post-harvest is seen as a global issue, as it occurs virtually in every maize growing areas of the world.[9] Excessive heat, high humidity, a lack of ventilation in stores, and insect and rodent damage facilitate the proliferation and spread of fungal spores,[12] while most critical factor leading to aflatoxin contamination in stored grains is moisture.[13]

Therefore, the objectives of this study were to determine the occurrence of *Aspergillus* section Flavi and to assess grain damage and weight loss due to insect pests in Shumar *Gewog*, under Pemagatshel District.

MATERIALS AND METHODS:

Survey sites: The survey was conducted in Gamung, Shumar, Nangkor and Shali villages under Shumar *Gewog* (county), Pemagatshel, Eastern Bhutan, covering low and mid altitudes maize production zones (Figure 1). The study area has elevations ranging from 700 to 1500 m asl with an annual rainfall averaging between 1500 to 3000 millimetres (mm), and average annual temperature of 15ºC.

Sampling: Seventy five households were selected from each village and clustered into 5 households (HH). From each cluster, one HH was selected randomly for the maize grain sampling. From each storage facility of the selected HH, three samples (from top, center and bottom), each consisting 100 grams of maize kernels were taken using 25 mm diameter and 40 cm length sample spear. Samples were then mixed together and placed in a sterile plastic bag and labeled. The samples were collected as per the methods described by Sori and Ayana (2012) and Utomo (2013). From each village, 6 samples were randomly selected totaling to 24 samples for the laboratory analysis.

Isolation and identification of *Aspergillus*: The direct plating method was used to assay *Aspergillus* from each sample following the method of Pitt and Hocking (1997). Maize kernels were surface sterilized for 1 min in 2.5% NaOCl, washed in three changes of sterile distilled water and plated 5 kernels per petri plate directly on the surface of Subaroaud Dextrose Agar (SDA) supplemented with 0.1% streptomycin to suppress the bacterial growth (Figure 2). Three replicates from each sample were plated, incubated in the dark at 25°C for 5 days and then the fungal growth colonies from the maize kernels were single spored to obtain pure cultures. The pure cultures were subcultured on the Czapek Dox (CZ; Himedia Laboratories, Mumbai, India) agar for the identification of *Aspergillus* species by means of colony characteristics.[17] All isolates were cultured on Aspergillus Flavus Parasiticus Agar (AFPA; Himedia Laboratories, Mumbai, India) for 3 to 5 days at 27°C to confirm section identification by colony reverse colour.[16 & 18]

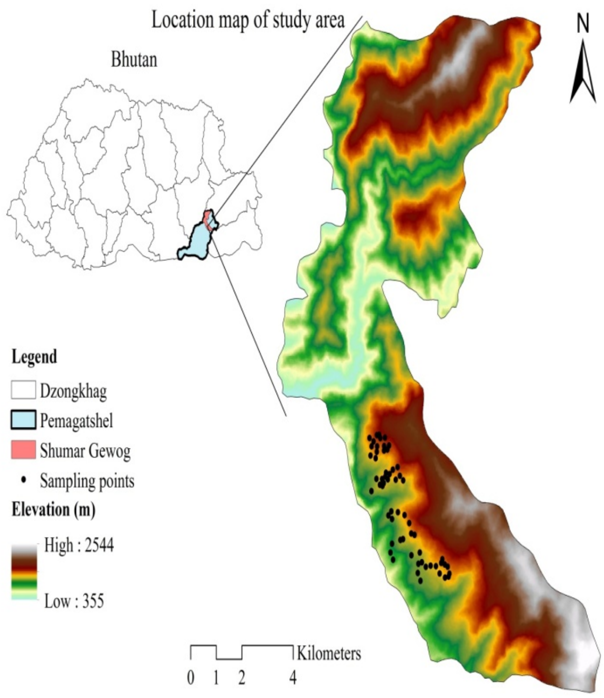

Figure 1: Study area depicting Shumar Gewog.

The colonies which developed yellow-green, green, deep green, shades of black and brown, white, bluish grey on CZ were identified as *Aspergillus* species (Figure 3). Isolates belonging to *Aspergillus* section Flavi had a bright orange colour on the reverse of the plate on AFPA media while negative isolates had either cream or black reverse colours (Figure 2).

Estimating grain damage and weight loss due to insect pests: The percentage of maize kernels damaged by insects was determined by the count method. A random sample of 100 maize kernels were taken from each sample of grains collected from the farmer's storage facilities and the number of bored and un-bored kernels were sorted for the presence of a clear exit hole in the grain. The percentage of bored kernels was calculated by formula:[19]

(Number of bored grains)/(Total number of grains counted)×100 = % bored grains

The kernels which were sorted (bored and un-bored) were categorized as damaged and undamaged respectively. The grains in each category were counted and weighed to calculate percentage weight loss using mathematical expression:[19]

%weight loss= ((UNd)-(DNu))/U(Nd+Nu) ×100

Where “U” is weight of undamaged kernels, “Nd” is the number of damaged kernels, “D” is the weight of damaged kernels, and “Nu” is the number of undamaged kernels.

Identification of insect pest species: Insect pests from the grain samples were isolated with the 2 mm-sized mesh sieves, observed under the light microscope and identified with the identification keys and descriptions provided by Bousquet & Branch (1990), and Haines (1991).

RESULTS AND DISCUSSION:

***Aspergillus* section Flavi profile of maize kernels:** Out of 29 fungal strains isolated, 22 colonies were identified as *Aspergillus* species out of which 14 showed bright orange reverse colouration on AFPA medium indicating the presence of *Aspergillus* section Flavi with an incidence level of 63.6%. The incidence of *Aspergillus* section Flavi in the maize samples collected from Gamung, Shumar, Shali and Nangkor villages were 6 (42.9%), 2 (14.3%), 3 (21.4%) and 3 (21.4%) respectively and may be attributable to the high temperature, high humidity, lack of aeration in stores, and insect and rodent damage as they facilitate the proliferation and spread of fungal spores.[22] Insects and rodents feed on grain kernels and also injure them thereby leading to fungal infection. These allow the spread of fungal spores in grains and subsequently increase the moisture content of the stored grains through their respiration.[13]

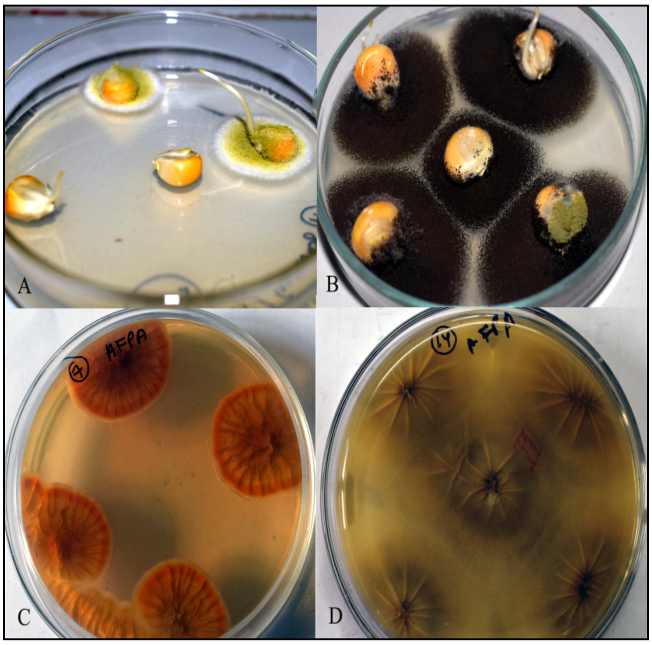

Figure 2: A & B: Plated kernels on the SDA; C: Positive isolate; D: Negative isolate of *Aspergillus* section Flavi cultured on AFPA.

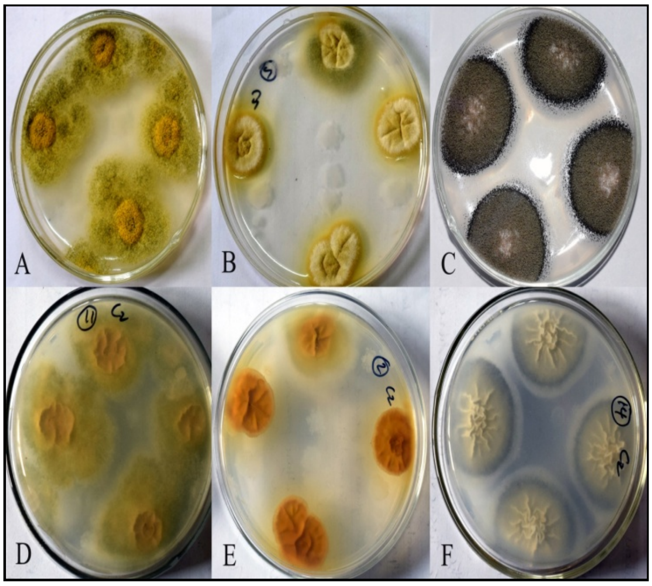

Figure 3: Morphological characters of different *Aspergillus* species on CZ (A-C); and D–F: reverse side of colony.

According to Wright (1991), strong correlation between the pattern of insect infestation and *A. flavus* infection to high densities of weevils (live or dead) and other secondary species were observed. Beti, Phillips, and Smalley (1995) also reported that maize weevils facilitate the growth of *A. flavus* and aflatoxin production by increasing the surface area, making maize kernels susceptible to fungal infection and increasing moisture content as a result of weevil metabolic activity.

Table 1: Numbers of *Aspergillus* section Flavi isolated from maize kernels collected from the villages of Shumar Gewog.

Village	**No. of samples (N)**	**No. of fungal isolates**	***Aspergillus* Species in the sample**	***Aspergillus* section Flavi in the sample**
Gamung	6	9	7	6
Shumar	6	5	4	2
Shali	6	7	5	3
Nangkor	6	8	6	3
Total	**24**	**29**	**22**	**14**

Insect pests found in maize sample: Three genera of coleopteran beetles, namely, maize weevil (*Sitophilus* sp.), an adult red flour beetle (*Tribolium* sp.), and *Carpophilus* sp. beetle, and one angoumois grain moth (*Sitotroga* sp.) of order Lepidotera were found in the stored maize (Figure 4). Among all, *Sitotroga* sp. and *Sitophilus* sp. were the dominant pests.

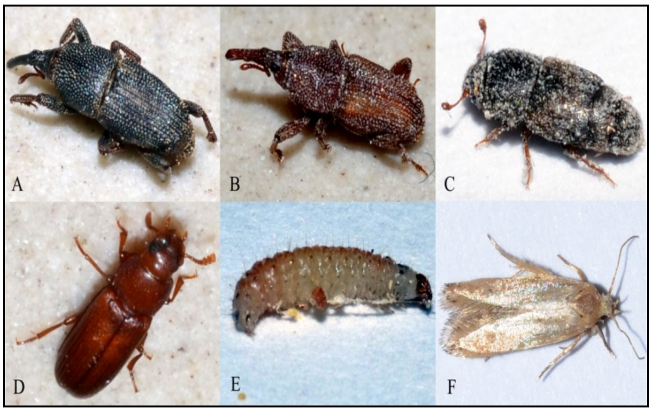

Figure 4: A & B: *Sitophilus* sp.; C: *Carpophilus* sp; D: *Tribolium* sp.; E: *Sitotroga* sp. (larvae); and F: *Sitotroga* sp.

Grain damage and weight loss: Mean percentage of bored kernels and weight loss caused by insect pects in the stored mazie kernels were 49.9% and 8.9% respectively (Table 2). The highest bored kernels were observed in the maize samples of Shumar village with a mean value of 84.7% (79-93%), and the corresponding weight loss was with a value mean of 16.0% (10.86-21.56%). Lowest bored grains were observed in the maize samples of Gamung with a mean value of 26.7% (10-39%) and its corresponding wight loss was 5.5% (2.27-7.76%). Losses due to insect pests could be influenced by the storage time and population of insects involved in infestation.[14] Therefore, the highest weight loss in Shumar village could be attributed to the storage time and density of insect pests.

Table 2: Percentage (%) bored kernels and weight loss of stored kernels collected from villages of Shumar Gewog, Pemagatshel.

Village	Number of samples (N)	Mean and range of bored kernels (%)	Mean and range of weight loss (%)
Gamung	6	26.7 (10-39)	5.5 (2.27-7.76)
Shumar	6	84.7 (79-93)	16 (10.86-21.56)
Shali	6	31.5 (18-65)	5.1 (2.16-9.84)
Nangkor	6	57.0 (26-94)	8.9 (6.1-16.92)
Mean (Range)		49.9 (10-93)	8.9 (2.16-21.56)

CONCLUSION: This study observed the incidence of afltoxin producing fungi in the traditionally stored maize in Shumar Gewog. Moreover, there is significant grain damage and weight loss caused by insect pects in the stored maize kernels besides other post harvest losses. The occurrence of aflatoxin-producing *Aspergillus* species in agricultural commodities such as maize should be of great concern as maize is a staple food and feed source for domestic animals in Eastern Bhutan. Post-harvest loss due to insect pests and growth of fungi such as *Aspergilus* not only results in substantial economic loses threatening the food security of subsistence farmers but also poses potential health hazards to both human beings and animals. Grain damage due to insect pests and contamination with aflatoxin reflects the need to have good post-harvest systems in place for the farmers. Nevertheless, the concentration of mycotoxin in the maize needs to be quantified to ensure health safety for both human and animal consumption.

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