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Mycotoxin Contamination in Maize (*Zea Mays*): Prevalence and Management Strategies in Ethiopia: A Review

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Abstract

Mycotoxins are toxic secondary metabolites of certain fungi species which has a capable of contaminating and reduce crops quality and quantity. Maize is one of the most important crops which is subjected to contamination by many species of mycotoxigenic fungi both before and after harvest. The current review is designed with the objective of providing basic insights on the prevalence and available management options of mycotoxin contamination in Maize under Ethiopian condition. It is reviewed that mycotoxin contamination has an adverse economic effect in reducing the yield for food and fiber crops and food contamination which resulted in the huge and universal economic crisis. It can result in direct economic impact through limited yields, price discounts, restricted end markets and export rejections from importers. Mycotoxins are all heat-stable and not destroyed by cooking and normal industrial processing thereby it causes diseases in human and animals. The most important mycotoxigenic fungi involved in maize were Aspergillus spp. (*A. flavus, A. parasiticus, A. niger, A. candidus, A. fumigatus, A. glucatus, A. ramarii, A. versicolor, A wentii*), *fusarium* spp. (*F. verticillioides, F. proliferatum, F. oxysporium* and *F. graminearum*), penicillium spp. (*P. notatum* and *P. verrucosum*). It is also understood that mycotoxin of the greatest concern in maize are Aflatoxin, Ochratoxin, Fumonisins, Moniliformin, Deoxynivalenol, vomitoxin. Reduction of mycotoxin contamination and concentration was possible and found to be effective through mainly application of natural dietary spices (garlic and lemon); use of improved gotera storage structure. In addition, the use of two species of *Trichoderma* namely *Trichoderma harzianum* and *Trichoderma viride* were the promising biocontrol agent against aspergillus invasion of grains. Seed treatment with Carbendazim at 2 g/kg and Mancozeb 3 g/kg and were founded free of AFB1 and AFB2 but these AFB1 were detected in the negative control with highest concentration. Integration of FYM at a rate of 500 kg/ha integrated with *Trichoderma harzianum* seed treated at 5 g/kg had completely protect the invasion by *Aspergillus* spp. and AFB1 and AFB2 were not detected as compared to the negative control which has a concentration of 5704.4 and 2219.0 µg/kg in for AFB1 and AFB2 respectively in Eastern Ethiopia. Besides the application of farmyard manure at a rate of 500 kg/ha combined with seed treated with carbendazim fungicide at 2 g/kg resulted in fewer invasions by Aspergillus species as compared to the control.

Keywords

Aspergillus, Maize quality, Contamination, Aflatoxin

Introduction

Maize in Ethiopia is the leading crop both in terms of production and crop land coverage with 5.6 t ha^{-1} yield produced and 47.84% of total cultivated land in 2016 cropping season [1]. It is one of the principal cereal crops ranking first in total production and productivity, and second to teff in area coverage. Ethiopian farmers grow maize primarily for subsistence with 75% of all maize output consumed by farming households making it a key crop for overall food security and economic development in the country [1]. According to Abate, et al. [2], the national average maize yield in 2014 cropping season was 3.4 t ha^{-1} which is too low as compared to the world average productivity which is 5.6 t ha^{-1} [3].

The low yield is attributed to combination of biotic and abiotic yield limiting factors among which pre and post-harvest diseases play a major role. Maize grain is highly

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vulnerable to degradation by mycotoxigenic fungi which include *Aspergillus, Fusarium* and *Penicillium* [4]. Storage of maize grains under poor storage methods hastens the growth of fungi and promotes the production of mycotoxins [5]. Despite the fact that maize is a crucial food to Ethiopia, it is vulnerable to aflatoxin risk due to different geographical and climatic conditions and poor postharvest handling [6]. The traditional storage condition of maize in Ethiopia made up of mud, bamboo strips, and pits which hasten deterioration and fungal contamination [5].

Mycotoxins are toxic secondary metabolites produced by certain species of fungi viz., *Aspergillus, Fusarium, Alternaria* and *Penicillium* spp. in agricultural products that are susceptible to mold infestations [7]. Mycotoxins contaminate and reduce crops quality through discolorations and reduction of nutritional quality [8]. Regulations on mycotoxins have been set and strictly enforced by most agricultural commodities importing countries, thus affecting international trade. Center for Disease Control (CDC) has estimated that more than 4.5 billion people in the developing countries are exposed to aflatoxins [9]. According to Patten [10] and Munkvold [11] maize grains highly contaminated with aflatoxins that mostly caused by *Aspergillus flavus* and *Aspergillus parasiticus.* Aflatoxins can produce by fungal action during production, harvest, transportation, storage, and food processing [12].

Previous studies proposed that the occurrence of aflatoxins in food products mainly influenced by favorable conditions such as high moisture content and temperature [13]. The extent of contamination by aflatoxins also varies with different geographic location, agricultural and agronomic practices, storage condition of crops and more importantly processing of food materials under favorable temperature and humidity conditions [5]. In many developing countries of Africa continent, aflatoxins toxicity of food has been companion with increased risk of hepatocellular carcinoma in the presence of hepatitis B virus infection and esophageal cancer respectively [14].

In Ethiopia there is no study conducted on the amount of loss brought by mycotoxin contamination in agricultural commodities including maize. Aflatoxins toxicity has always remained a topic of debate in terms of international market as well as economic development of country which are part of trade market. To overcome these challenges many countries have set maximum acceptable levels of aflatoxins in food and food products and animal feed [15]. The total allowable level of aflatoxin (μg/kg) in human food in different countries were reported i.e., Australia, China, European Union, India, Kenya, Taiwan and USA which is 15, 20, 4-15, 30, 20, 50, and 20, respectively [16] but Ethiopia has no level of allowable mycotoxin standards.

Objectives

- To understand and review the current statues and prevalence of aflatoxin contamination in maize (*Zea mays*) in different maize growing regions of Ethiopia.
- To review the available efficient management strategies of mycotoxin contamination in maize under both pre harvest and postharvest condition.

Production and Importance of Maize in Ethiopia

Ethiopia is one of the largest maize producing country in Africa [3]. In Ethiopia maize accounts the largest share of production by volume and is produced by more farmers than any other crops [17]. Small holder farmers in Ethiopia almost in all regions of the country dominantly produce maize. It is also one of the strategic crops considered in the national agriculture center development plan of the country. Maize in Ethiopia is most important cereal crop both in terms of production level and cultivated area coverage. It is the cheapest source of calorie providing 16.7% of per capita calorie intake nationally [18]. It is particularly important for poor household as it is mixed flour with teff to make the national staple Injera, and the cost of maize is half that of wheat and teff. Three fourth of the maize produced is consumed at the household level by the small-scale producers themselves [19]. Despite of its importance as a principal food crop its average yield in Ethiopia is 3.2 tones which is lower than the world average (5.6 t ha^{-1}).

Maize Storage Conditions in Ethiopia

Ethiopia is situated in East Africa, which has several mountains, hills, plateau, plains, valleys, and gorges, and the topography and elevation vary from the lowest point at Dankali depression (126 m below sea level) to the highest point at Ras Dashen Mountain (4620 m above sea level). Based on temperature, moisture, and elevation conditions, Ethiopia has 18 main agro-ecological zones and owing to the country's wide and complex topography, the environmental and climate conditions vary from one region to the other [20]. These diverse agro-ecologies of Ethiopia allow production of a variety of crops as well as provide favorable environments for the growth of diversified mycotoxigenic fungi.

The common traditional grain storage structures in Ethiopia are gotera, gotta, polypropylene and jute bags. Gotera is an outdoor storage structure made from mud plastered basket work covered with thatched roofing and raised off the ground with stones or wooden platform. Gotta is an indoor grain storage bin made of mud plaster mixed with teff straw [21].

Because of the traditional post-harvest practices and the prevailing environmental conditions in Ethiopia, the risk of maize grain contamination is expected to be high. Still, available information regarding occurrences of mycotoxins in maize produced in the country is fragmented: They vary in age of grain (duration from harvest to sampling), targeted stage of production and supply chain for sampling, coverage of high producing areas, or number and type of mycotoxins investigated [5,22,23].

Major Mycotoxins

Mycotoxins are toxic secondary metabolites mainly produced by *Aspergillus, Fusarium, Alternaria* and *Penicillium* spp. of fungi in agricultural produce [7]. Mycotoxins contaminate and reduce crops quality through discolorations and reduction of nutritional quality [8]. The extent of contamination by mycotoxin varies with different geographic location, agricultural and agronomic practices, storage condition of crops and more importantly processing of food materials under favorable temperature and humidity conditions [5]. Mycotoxins contaminate food and feed and affect food security worldwide, and their effect is the major bottleneck of agricultural productions, especially in low and middle-income countries [24].

Researchers have isolated and characterized more than 400 mycotoxin types. The most important and highly toxic mycotoxins include; aflatoxin, ochratoxin A, trichothecenes, zearalenone, fumonisins B1 and B2, tremorgenic toxins, and ergot alkaloids [25]. The major fungi causing frequent and problematic contamination of foods and feeds with mycotoxins are members of the fungal genera *Aspergillus, Fusarium* and *Penicillium* [26].

Aflatoxins

Are poisonous mycotoxin which are carcinogenic interfering with the immune system and are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* [27]. Aflatoxins grow in soil, decaying vegetation, hay, and grains of primarily found in hot, humid climates, colonizing mostly the aerial parts of plants [28]. There are about 20 known types of aflatoxins based on their structure, chromatographic and fluorescent characteristics which are mainly classified into aflatoxin B1, B2, G1, G2, M1 and M2 [29]. Fungi species such as *Aspergillus flavus* and *Aspergillus parasiticus* are the two essential members of the genus *Aspergillus*, which produce different kinds of aflatoxins like AFB1, B2, G1, G2 and AFM1 [30,31].

Both *Aspergillus flavus* and *Aspergillus parasiticus* are most frequently detected in agricultural products because of their widespread distribution [32]. Drought and stress increase aflatoxin spread in the field and can be produced due to insufficient drying of contaminated crops before storage or stored under humid conditions [27]. Due to their stability to severe processes of roasting, extrusion, baking, and cooking, aflatoxins also induce a great problem in processed foods, such as roasted nuts and bakery products and it can be found alone or simultaneously, as well as co-occurring with other mycotoxins such as OTA [28].

Ochratoxin A

Is the most toxic member of the ochratoxin which is structurally similar to the amino acid phenylalanine. It is produced by *Aspergillus Circumdati, Aspergillus Nigri, Penicillium verrucosum*, and Penicillium nordicum [33]. Ochratoxin A has an inhibitory effect on a number of enzymes that use phenylalanine as a substrate, particularly Phe-tRNA synthetase, resulted in the inhibition of protein synthesis. It is a mitochondrial poison, which causes cellular damage, oxidative burst, lipid peroxidation, and oxidative phosphorylation. Furthermore, it increases cell apoptosis, and it is a stable and heat resistant which is not damaged by common food preparation temperature (above 250 ℃ for several minutes reduce its concentration [28]. *Aspergillus* spp., mainly *Aspergillus ochraceus*, *Aspergillus niger*, and *Aspergillus carbonarius*, can produce different kinds of ochratoxins, with OTA known to be more toxic and the most frequently detected [34].

Fumonisins

Are fusarium toxins and constitute the large family of compounds which are produced by a number of fungi most dominantly *Fusarium verticillioides* and *Fusarium proliferatum* [24]. Other fungal species, including *Fusarium dlamini, Fusarium nygamai* and *Fusarium napiforme* also produce fumonisins [35]. There are about 12 types of known fumonisins types, and the most important ones are FB1, FB2, and FB3 of which FB1 is most toxic. They are mostly found in maize grown in warmer areas. They are fairly heat-stable, and toxicity can be minimized only during processes where temperature is beyond 150 ℃ [28].

Ecological Requirements of Mycotoxigenic Fungi

Fungal growth and mycotoxin production depend on temperature, relative humidity, and water activity [36,37]. Mycotoxin-producing fungi usually belong to the genera *Aspergillus*, *Penicillium,* or *Fusarium* [38]. The growth of fungi usually occurs at temperatures of 10 ℃ - 40 ℃ [39]. Besides, grain having moisture content of 16-30% and a temperature of 25 ℃ - 32 ℃ favor fungal growth and mycotoxins production in stored grains [40]. Many species in the genera *Penicillium* and *Aspergillus* can be capable of growing under conditions of reduced water activity (0.93) and are capable of growth down to at least 0.78 water activities. *Aspergillus* spp. can adapt to higher temperatures ranging 30 ℃ - 40 ℃ and higher relative humidity of greater than 80% [41].

Fusarium is one of the main plant pathogenic mold genera widely distributed around the world, which causes a wide range of plant diseases in tropical and moderate climate zones [42]. *Fusarium* spp. require higher relative humidity of 70-90% [41] and temperatures ranging 20 ℃ - 30 ℃, and water activity of 0.97-0.995 for their effective growth and mycotoxins production [43]. However, these fungi could also generate mycotoxins even at lower temperatures close to 0 ℃, without significant fungal proliferation [39]. Infections of crops by *Fusarium* spp. are usually accompanied by mycotoxins contamination, which could cause health risks in humans. These fungal genera can produce several toxins such as FUMs (B1, B2), ZEN, trichothecenes (DON, nivalenol, T-2 toxins, H-T2), and other toxins [44].

Occurrence and Prevalence of Maize Mycotoxin in Ethiopia

The maize grain mycotoxigenic fungi contamination started from the fields before harvest and continued across storage, consumptions and marketing. According to Solomon, et al. [45] several fungal species have been isolated from the maize grain sampled in three districts of West Shewa zone of Oromia regional state, *Aspergillus* spp. were the most predominant mycotoxigenic fungi with 50.7% frequency of occurrence. About 3.3% and 7.7% maize samples had aflatoxin B1 higher than those recommended by Food and

Drug Administration (FDA; 20 µg/kg) and European Union (EU; 4 µg/kg) regulatory levels respectively [45] (Table 1).

Similarly, Garbaba [46] revealed that three major mycotoxigenic fungal species were obtained from stored maize with different frequency and relative frequency in southern Ethiopia. Among those *aspergillus* species were reported to be the higher frequency of occurrence from six month time period stored maize. The higher frequency of fungal infection specifically *Aspergillus* spp. was due to poor storage types and longtime storage greater than two years as similarly reported by Habtamu, et al. [47] (Table 2).

Besides, Chauhan, et al. [5] reported that mean aflatoxins concentration for a two year stored maize grain samples was 53 ppb with 100% contamination in aflatoxin. Moreover, Admasu, et al. [48] founded that maize samples collected from Toke kutaye, Chelia, Halaba wemberma and Merawi districts were 100% positive for AFT and 32.7% of the samples were contaminated with FUM ranging 0.2 to 6.5 µg/g. DON was detected in 7.3% of the collected samples in trace concentration ranging 0.27-1.98 µg/g and only two of the 11 positive samples exceeded the 0.75 µg/g European Commission MTL in cereals intended for direct human consumption [48] (Table 3).

Management Strategies of Mycotoxin

Recent research performed in the development and improvement of mycotoxin control technologies focused on both prevention and good storage and manufacturing practices that can be applied in the feed and food chain to reduce aflatoxin exposure, but these efforts are not always satisfactory to ensure food safety [51]. Recent research activities shifted towards reducing the aflatoxin contents already present in feeds and foods, and several biological, physical, and chemical methods have been tested and evaluated in the mitigation of aflatoxin in this way.

Proper Handling and Improved Storage

Traditionally in Ethiopia, grains are stored in gotera, gotta, dibignt, sacks, polypropylene and jute bags having

Table 1: Mycotoxigenic fungi occurrence in relation to storage types in number and percentage.

Fungal type	Occurrence	OAG	SOA	SIH	OSS	IG	IHG
A. flavus	38	10(26.3)	7(18.4)	8(21.1)	6(15.8)	2(5.3)	5(13.2)
A. parasiticus	28	7(25)	3(10.7)	4(14.3)	7(25)	1(3.6)	6(21.4)
F. verticillioides	19	5(26.3)	7(36.8)	1(5.3)	4(21.1)	0	2(10.5)
P. notatum	17	6(35.3)	2(11.8)	3(17.6)	2(11.8)	1(5.9)	3(17.6)
P. verrucosum	16	5(31.3)	3(18.8)	2(12.5)	3(18.8)	0	3(18.8)
F. proliferatum	10	4(40)	2(20)	2(20)	1(10)	0	1(10)
F. graminearum	10	5(50)	3(30)	0	2(20)	0	0
A. niger	9	2(22.2)	0	3(33.3)	1(11.1)	1(11.1)	2(22.2)
T. Spp	1	0	0	0	0	0	1(100)
Total	148	44(29.7)	27(18.2)	23(15.5)	26(17.6)	5(3.4)	23(15.5)

Source: Solomon, et al. [45]

Where: OAG: Open above Ground; SOA: Sack in Open Air; SH: Sack in House; OSS: Open Sorghum Stalk; IG: Improved Gotera and IHG: In House Ground Storages

Table 2: Frequency of occurrence major fungal genera associated to stored maize under wholesaler condition in Southwestern Ethiopia.

Mycotoxigenic fungi	Frequency of occurrence in storage duration (month)						
	1	2	3	4	5	6	Mean
Penicillium	2.2	3.4	12.4	34.1	26.2	36.3	19.1
Aspergillus	1.1	1.5	2.7	11.2	12.7	17.6	7.8
Fusarium	67.4	29.1	27.0	66.6	72.6	32.6	48.

Table 3: Mycotoxin concentration range of stored maize sample from three agro ecologies of Ethiopia.

Mycotoxin Concentration range (ppb)					
Sample Area	**AFT**	**FUM**	**DON**	**Ochratoxin**	**References**
Adama	2.1-27.1	0-300	60-140	nd	
Ambo	2-3.1	0-300	50-700	nd	Ayalew [49]
Dire Dawa	2-3.2	700-2400	nd	nd	
Merawi	6.5-150	0.27-6.52	0.2-1.98	2-186.5	Worku, et al. [50]
Toke Kutaye	12.3 ± 2.6	0.27-6.5	nd	nd	
Ilu Gelan	0.002-17.43	nd	nd	nd	Solomon, et al. [45]
Bako Tibe	0.0002-5.09	nd	nd	nd	
Gobu Sayo	0.00003-1.7	nd	nd	nd	
Southern Ethiopia	0-290	0-8246	nd	0-1725	Alemu [6]

Where nd = Not detected

different capability in favoring the growth and proliferation of mycotoxigenic fungi. Proper initial drying to the recommended moisture content and subsequent moisture-proof storage is crucial for reducing the growth of toxigenic fungi and toxins development [52]. According to Solomon, et al. [45], the highest occurrence of mycotoxigenic fungi were observed in open above ground (OAG) storage type which has been accounted about 44(29.7%) and few mycotoxigenic fungi 5(3.4%) was isolated from improved gotera (IG), thus storing the commodity in the improved gotera minimize the risk of aflatoxin contamination in maize. Similarly, Chauhan [5] reported that the highest aflatoxin B1 concentration were recorded in open ground storage types (18.03 ppb) whereas the lowest aflatoxin B1 concentration were observed in grain stored in improved gotera (0.16 ppb). So, storing grain in improved gotera is disfavoring aflatoxin production thereby the use of improved gotera for grain storage in combination with other suitable management aspects can significantly manage the risk.

Use of Natural Dietary Spices (Aflatoxin Detoxification)

Natural plant extracts are of interest as a source of safer or more effective alternative to biological agents for aflatoxin detoxification. The use of plant products for aflatoxin control has been reported by several authors [53]. Much emphasis has been given to inhibition of the plant extracts against growth of aflatoxigenic fungi. Besides, many of these plants are not suitable to be used in foods, as the resultant products cannot be consumed by humans. The use of natural dietary spices provides an attractive opportunity as a community-based (suitable for large-scale implementation), safer, cost-effective, and practical method for aflatoxin control. According to Negera, et al. [54], garlic (*Allium cepa*) showed the highest (61.7%) degradation of AFB1 (Aflatoxin B1) followed by lemon (56.0%) during 1 hour exposure of aflatoxin standards to the spice extract at 25 ℃ as it was investigated using LC-MS/MS and electrochemical methods. All natural dietary spices namely. Cumin (*Nigela sativa*), clove, basil, lemongrass, lime, lemon, garlic, thyme, ginger, black cumin and fenugreek can reduce AFB1 levels to certain extents compared to non-treated samples after 24 hr exposure at 25 ℃ [54].

Biological detoxification

The use of bio control agents for toxigenic fungi control has focused on the efficacy in terms of control of germination, growth and colonization by the fungi to raw or processed food commodities and reduction in the production of the associated mycotoxin by often targeting the biosynthetic genes involved in toxin bio-synthesis [55]. Biological detoxifications are advantageous in terms of the sensory and nutritional values of food and represent a safer option to choose considering food safety aspects [56]. According to Sahile, et al. [57], the dual culturing of pathogen with 18 Isolates of Trichoderma and 26 of other fungi revealed clearly potential of control in some of the isolates of Aspergillus species. Thirteen Isolates of Trichoderma produce 4 mm or higher inhibition zone on agar medium [57]. Moreover, Abdi, et al. [58] revealed that plots treated with *Trichoderma hazarium* and *Trichodermia viride* had a minimum invasion by aspergillus species. Mycotoxin B1, B2 G1 and G2 were not detected in seeds of ground nut treated with *Trichoderma hazariumin* both 2014 and 2015 cropping season at Babile, Eastern Ethiopia [58]. Seeds treated with *Trichodermia viride* are also free of mycotoxin B1, B2, G1 and G2 in 2014 main cropping season although B1 and B2 are detected in a very trace amount in 2015 cropping season (Table 4 and Table 5).

Furthermore, different bacterial strains such as *Azotobacter armeniacus*, B. subtilis, Bacillus spp., Burkholderia cepacia and others act against *F. verticillioides*, *F. verticillioides*, and *F. proliferatum* [59]. Additionally, many bacterial strains belonging to Streptococcus, Lactobacillus, Saccharomyces, Bacillus and Acinetobacter genera have more than 95% OTA degradation and some have shown detoxifying properties [60].

Chemical detoxification

Nowadays, there are strict regulations on the use of pesticides, and there is political pressure to remove the most hazardous chemicals from the market. However, in order to protect food quality and the environment, low persistent synthetic fungicides are still relevant at present to prevent diseases of food crops [61]. Abdi, et al. [58] reported that plots treated with carbendazim at 2 g/kg of seed had less invasion

Table 4: Effect of *Trichoderma* species against *Aspergillus* species seed invasion in log CFU/g seed in Babile District, Eastern Ethiopia.

Aspergillus species				
Treatments	***A. flavus***	***A. flavus S strain***	***A. parasiticus***	***A. niger***
Negative control	1.08	0.00	0.00	0.00
Trichoderma hazarium 5 g/kg	0.00	0.00	0.00	0.00
Trichodermia viride 5 g/kg	0.48	0.30	0.00	0.00

Source: Abdi, et al. [58]

Table 5: Aflatoxin concentration (µg/kg) in Babile district.

		2014				**2015**		
Treatments	**AFB1**	**AFB2**	**G1**	**G2**	**B1**	**B2**	**G1**	**G2**
Negative control	651.3	71.0	nd	nd	5704.4	2219.0	nd	nd
Trichoderma hazarium 5 kg	nd	nd	nd	nd	nd	nd	nd	nd
Trichodermia viride 5 kg	nd	nd	nd	nd	50.0	2.3	nd	nd

Source: Abdi, et al. [58]; Where nd = Not detected

Table 6: Effect of fungicides on Aflatoxin concentration (µg/kg) at Babile district, eastern Ethiopia.

		2014				2015		
Treatments	**B1**	**B2**	**G1**	**G2**	**B1**	**B2**	**G1**	**G2**
Negative control	651.3	71.0	nd	nd	5704.4	2219	nd	nd
Carbendazim 2 g/kg	nd	nd	nd	nd	3.8	0.1	nd	nd
Mancozeb 3 g/kg	nd	nd	nd	nd	588	50.1	205.2	24

Source: Abdi, et al. [58]; Where, nd = Aflatoxin not detected

Table 7: Combined effect of fungicides, Trichoderma species and Farmyard Manure on AFB1 and AFB2 concentration (in µg/kg seed) at Babile District.

Treatments	**Aflatoxin Concentration**	
	B1	**B2**
Negative Control	5704.4	2219.0
FYM 2.5 tons/ha + Carbendazim 2 g/kg	6.6	0.3
FYM 2.5 tons/ha + Mancozeb 3 g/kg	Nd	nd
FYM 2.5 tons/ha + *T. harzianum* 5 g/kg	2.5	0.1
FYM 2.5 tons/ha + *T. viride* 5 g/kg	16.2	3.4
FYM 5 tons/ha + Carbendazim 2 g/kg	6.1	0.5
FYM 5 tons/ha + Mancozeb 3 g/kg	3.8	2.7
FYM 5 tons/ha + *T. harzianum* 5 g/kg	Nd	Nd
FYM 5 tons/ha + *T. viride* 5 g/kg	76	9
FYM 7.5 tons/ha + Carbendazim 2 g/kg	306.8	11.1
FYM 7.5 tons/ha + Mancozeb 3 g/kg	22.4	1.3
FYM 7.5 tons/ha + *T. harzianum* 5 g/kg	15	1.2
FYM 7.5 tons/ha + *T. viride* 5 g/kg	96	5.7

Source: Abdi, et al. [58]; Where, nd = Aflatoxin not detected; FYM = Farmyard Manure

as compared with samples from control plots. Seeds treated with Carbendazim at a rate of 2 g/kg and Mancozeb 3 g/kg showed free from B1, B2, G1 and G2 mycotoxin as compared to control which showed a concentration of 651.3 and 71 µg/kg seeds for B1 and B2 mycotoxin type respectively (Table 6).

Integrated management

The effectiveness of integrated soil organic amendments of FYM with biocontrol agent (*T. harzianum*) and fungicide (carbendazim) against Aspergillus invasion was evaluated [62]. According to Abdi, et al. [58] the samples obtained from plots treated with FYM at a rate of 500 kg/ha integrated with *Trichoderma harzianum* seed treated at 5 g/kg had completely prohibit the invasion by *Aspergillus* spp. and no AFB1 and AFB2 type of aflatoxin were not detected as compared to the negative control which has a concentration of 5704.4 and 2219.0 µg/kg seed in for AFB1 AND AFB2 respectively tested for ground nut in Eastern Ethiopia. Besides the application of farmyard manure at a rate of 500 kg/ha combined with seed treated with carbendazim fungicide at 2 g/kg resulted in fewer invasions by Aspergillus species as compared to the control [58] (Table 7).

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