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Original Article

Investigation of the Potential Effect of Insect Infestation on Mycotoxin Levels in Stored Rice

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ABSTRACT

Insect pest infestations of post-harvest grains encourage the establishment of fungi, leading to grain deterioration and mycotoxin production, threatening food safety, and their correlation remains insufficiently explored. Therefore, this study investigates the relationship between insect infestation and mycotoxin levels in stored rice. Twelve samples of stored rice were collected to identify insects and associated fungi. Accordingly, mycotoxins, specifically Aflatoxin and T-2 Toxin, were extracted from the samples and quantified using CD-ELISA. The study found that the highest percentage of insect infestation (81%) and weight loss (25.50%) were observed in samples infested with *Sitophilus zeamais* (L.) (SZ1). In comparison, the lowest infestation level (32%) and weight loss (9.6%) were recorded for *Trogoderma granarium* Everts (TG1). Generally, fungal contaminations were correlated positively with the severity of insect infestation, as the highest fungal colonies were isolated from samples infested with SZ1, with a total count of 27 colonies. Both *Aspergillus niger* and *Fusarium* sp., dominated the fungal population, accounting for 33.72% and 33.14%, followed by *Penicillium digitatum* and *Rhizopus stolonifer*, each contributing 11.63%. Additionally, mycotoxin detection confirmed the presence of Aflatoxin and T-2 toxins, with the highest concentrations (4.89 and 25.1 ppb) respectively, were detected in rice infested with SZ1, which exhibited the highest infestation rate (81%). This study underscores the complex relationship between insect infestation, fungal contamination, and mycotoxin production. This provides practical advice for farmers and grain storage managers on the importance of early monitoring and detection of common insect and fungal pests with effective integrated pest management strategies that can be implemented to protect post-harvest grains.

Keywords: Insect pest, Mycotoxin level, Fungal contamination, Stored grains, Percentage of rice infestation.

1 Introduction

Diverse studies have sought to address the fundamental challenge of feeding the world's growing population and concentrated on augmenting economic staple crops such as rice (*Oryza sativa* L.) that can provide food for over half of the global population to maintain global food security [1,2]. Global rice production is anticipated to increase to 567 million tonnes by 2030, produced in more than 100 countries, with Asia accounting for 90% of total production and over 110,000 cultivated rice varieties that exhibit variations in quality and nutritional composition [3,4].

Many abiotic and biotic factors affect grain quality after harvest, and it has been researched as a stored grain ecosystem. Grain respiration, contaminants, insects, temperature, and moisture constitute key factors that interact to promote mycotoxigenic fungi [5]. The quality deterioration might occur during storage due to mycotoxigenic secretion by fungal contaminants, predominantly represented by the genera *Aspergillus*, *Fusarium*, and *Penicillium* [6-8].

These three aforementioned important fungi inflict serious damage on stored rice, resulting in grain spoiling (i.e., weight reduction, loss of nutritional value, and poor milling quality) as well as the production of mycotoxins (MTs) (i.e., Aflatoxin (Afs), Trichothecenes (T-2 toxin)) [9,10]. Mycotoxin molecules are produced as secondary metabolites by specific fungal species, which can contaminate numerous agricultural commodities, particularly cereals and their products, and can pose a significant risk to humans [11,12]. According to the United Nations Food and

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Agriculture Organization, mycotoxins impacted at least 25% of the global food crop, a finding that was validated by a large number of data analyses [13].

The continuous global concern regarding mycotoxin contamination in food remains a key food safety challenge, as it is considered an inevitable and unpredictable issue despite the implementation of improved storage conditions and processing practices [14,15]. These toxic molecules have significant effects on the quality and safety of food and may cause death in humans and animals [16-18]. This probably refers to immunological dysfunction, growth impairment, organ toxicity, and DNA damage [11], Besides, the stability of many mycotoxins against physical and chemical treatments makes their elimination during food processing a complex task [19].

Furthermore, rice is susceptible to infestation by numerous insect pests during the storage phase, including rice weevil, *Sitophilus zeamais* (L.) (Coleoptera, Curculionidae), red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae), khapra beetle, *Trogoderma granarium* Everts (Coleoptera, Dermestidae), and saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) [20,21]. Beyond causing direct losses in both quantity and quality, insect infestation is also creating a condition (i.e., physical damage, an increase of moisture and temperature, poor aeration, and spreading fungal spores) that encourages the growth of fungal pathogens and is linked to mycotoxin contamination [22]. The dispersal of fungal spores and the establishment of conditions conducive to fungal growth lead to rapid multiplication during the storage period [23].

Numerous studies have examined the impact of insect pests, and fungal pathogens with their toxins on post-harvest products over the past years [24-26]. Nevertheless, a dearth of research on the correlation between these factors could be particularly detrimental. Therefore, the objective of this investigation was to ascertain the link between insect infestation and mycotoxin levels in stored rice to apply appropriate control measurements during post-harvest storage.

2 Materials and methods

2.1 Sample collection

Rice grain samples (1 kg) infested with various stored product insect pests were collected randomly from market stores and warehouses that have been stored for more than six months and were within the expiry range in room conditions (Table 1). Samples were brought to the laboratory to detect infestations and to identify insect pests. Furthermore, the samples were investigated for the presence of associated fungi.

Table 1: Rice samples collected in houses and stores in Erbil City.

Sample No.	Type	Symbol	Insect infestation
1	Kurdi-Akre	OS1	Infested with *Oryzaephilus surinamensis*
2	Kurdi-Akre	OS2	Infested with *Oryzaephilus surinamensis*
3	Kurdi-Akre	OS3	Uninfected with *Oryzaephilus surinamensis*
4	Basmati-Taiwan	TG1	Infested with *Trogoderma granarium*
5	Basmati-Taiwan	TG2	Infested with *Trogoderma granarium*
6	Basmati-Taiwan	TG3	Uninfected with *Trogoderma granarium*
7	Basmati-Vietnam	SZ1	Infested with *Sitophilus zeamais*
8	Basmati-Vietnam	SZ2	Infested with *Sitophilus zeamais*
9	Basmati-Vietnam	SZ3	Uninfected with *Sitophilus zeamais*
10	Basmati-Vietnam	TC1	Infested with *Tribolium castaneum*
11	Basmati-Vietnam	TC2	Infested with *Tribolium castaneum*
12	Basmati-Vietnam	TC3	Uninfected with *Tribolium castaneum*

2.2 Isolation and detection of associated fungi

Half of each rice sample (500g) was randomly designated to isolate fungi with the potential to produce mycotoxins on the grains. Two major methods, the agar plate and the dilution methods, were followed to isolate potential toxigenic fungi. In both methods, the count of fungal colonies or the total plate count (TPC) on each plate was enumerated.

2.2.1 Agar plate method

Isolation was conducted on potato dextrose agar (PDA) to detect fungal incidence on rice. Each 100 g of collected rice samples was measured, and then spontaneously selected seeds were surface sterilized with 70% ethanol alcohol for 2 minutes. The samples were then rinsed two times in sterilized distilled water and left to dry on sterilized filter paper under controlled conditions in the hood chamber. Every ten seeds were plated on the surface of fresh agar plates, with a total of 60 plates and five replications. To prevent bacterial contamination, streptomycin and penicillin were added to the liquid media before being poured into the plates at a rate of 50 mg/L. Subsequently, the plates were

placed in an upright position to avoid damage from water droplets to the fungal colonies and incubated at 25 ± 3 °C for 5 - 8 days. After that, the number of rice seeds yielded colonies was recorded, and the number of colonies was also counted and then sub-cultured for identification. The frequency of infected seeds was expressed as percentages.

2.2.2 Dilution method

The dilution plate method followed the approach described by [27]. Five grams of ground rice from each sample were placed in 500 ml of sterilized distilled water in a flask and hand-shaken to homogenize for 15 minutes. One milliliter of seed suspension was placed into each Petri plate. Five plates were used for each sample. Plates were incubated at 25± 3 ^{O}C for 5- 8 days. As with the plate method, the number of colonies was counted and then sub-cultured for identification, and then the frequency of infected rice samples was expressed as percentages.

2.3 Identification of fungi

After enumerating the colonies on each medium, they were sub-cultured and purified. The newly grown pure colonies of fungi isolated from rice samples were identified based on macroscopic features (Zeiss, Germany), observed on fungal growth, and their characteristics. To support the identification, keys from textbooks were used [28,29].

2.4 Stored product insect identification

All species were morphologically diagnosed using identification keys from [30-32] and if needed, were reconfirmed and identified by Professor Dr. Nabil Abdulqadir, an expert taxonomist in the Plant Protection Department, College of Agricultural Engineering Science, Salahaddin University, Erbil.

2.5 Mycotoxin detection

Mycotoxins (Aflatoxin and T-2 Toxin) were extracted from 12 rice samples for detection and to isolate the related seed-borne fungi. The MTs were extracted by a competitive direct enzyme-linked immunosorbent assay (CD-ELISA) in the Central Veterinary Laboratory of the Southern Region of Kurdistan in Erbil.

2.5.1 Preparation of seed extracts

The seeds from each sample were ground using an electronic grinder. Representative portions of five grams from each sample were placed in conical flasks. For the extraction of Aflatoxin 25 mL of 70% and for T-2 Toxin, 25 mL of 35% methanol was utilized, following a 3-minute shaking of the samples, extraction was carried out using filter paper (Watman No. 1). Finally, five milliliters of the supernatant was placed into test tubes for aflatoxin, and one milliliter of the supernatant was mixed with four milliliters of sterilized distilled water for T-2 toxin and they were stored inside the refrigerator for future use.

2.5.2 Mycotoxin quantification

The mycotoxins in the prepared sample solutions were detected quantitatively using a competitive direct enzyme-linked immunosorbent test (CD-ELISA) following the procedure used by [27]. Hence, each red well of 96 microtiter plates received 100 µL of enzyme conjugate, as per the manufacturer's instructions. A pipette was used to thoroughly mix 100 µL of each control (free T-2 and aflatoxin) and the samples in the red-marked mixing wells.

The contents of the red-marked wells were mixed three times with a 12-channel pipette. The sample mixtures were then transferred to new microwell plates coated with antibodies that had been designed to function with plate readers. The plates were placed in a microwell plate reader, and the optical densities using a 650 nm filter were measured utilizing the Veratox kit for Aflatoxin and T-2 (Neogen Corporation, USA). The optical densities and the controls from the standard curve were displayed across the curve to ascertain the exact concentration of the toxins found in each stored rice specimen.

2.6 Data analysis

2.6.1 Calculation of associated fungi

The assessment of fungal species extracted from seed samples cultivated on potato dextrose agar was computed using equations from [27] as below:

Total count (TC) = number of each fungal species/seed

Total count percentage (TC%) = number of each fungal species / total count of all species × 100

Incidence (I): the occurrence of fungi in each number of specimens of rice

$$\text{Incidence percentage (I \%)} = \frac{\text{incidence(I)}}{\text{number of rice specimen}} \times 100$$

Tota1 fungal incidence percentage (TFI%) = number of all isolated fungi / sample × 100

2.6.2 Calculation of insect infestation

The percentage of insect infection was calculated for each sample following the equation below:

Percentage of infestation = Number of infested rice × 100 / Total number of grains.

The percentage of weight loss due to infection was calculated by counting and weighing damaged and undamaged rice in a sample of 1000 grains. Accordingly, the damaged and undamaged rice samples were compared with the control according to the following equation used by [33].

$$\text{Weight Loss}(\%) = \frac{(\text{UNd}) - (\text{DN4})}{\text{U}\,(\text{Nd} + \text{N4})} \times 100$$

Where: U = Weight of undamaged grains, N4 = Number of undamaged grains, D = Weight of damaged grains, Nd = Number of damaged grains.

2.6.3 Calculation of the correlation between insect infestation with mycotoxin production and weight loss in rice grains

A Pearson correlation analysis was conducted using the statistical package of social science IBM SPSS Statistics software, version 23, to assess the relationship between the percentage of insect infestation with mycotoxin release from associated fungal species and the percentage of weight loss in rice grains. The Pearson correlation coefficient (r) was used to determine the strength and direction of the linear association between the two variables, and the statistical significance was set at $P \leq 0.05$.

2. 6. 4 Calculation of Mycotoxins

Neogen's Veratox software version 3.0 was used to calculate and quantify the concentration of mycotoxin present in rice samples.

3 Results

3.1 Isolation and detection of associated fungi

The results of fungal isolation revealed that most of the associated fungi belonged to the genera *Aspergillus* and *Penicillium*. In general, rice samples not infested with insects had lower fungal contamination than those infested with stored-product insects (Table 2). The highest fungal colonies were isolated in the imported rice samples (SZ1 and TC1) that were infested with SZ and TC, with total fungal colonies of 27 and 25, respectively, followed by the samples SZ2, OS1, and TC2 with the total fungal colonies of 24, 21, and 21, respectively.

Table 2: Fungi isolated and associated with rice samples collected in rice stores.

Sample ID	Isolated fungus	No. of colonies	Total
OS1	*Aspergillus* spp. *Penicillium* spp. *Fusarium* sp.	9 5 7	21
OS2	*Aspergillus* spp. *Penicillium* spp. *Cercospora* sp. *Fusarium* sp.	12 1 1 4	18
OS3	*Aspergillus* spp. *Penicillium* sp.	2 1	3
TG1	*Aspergillus* spp. *Penicillium* spp. *Fusarium* sp.	2 2 6	10
TG2	*Aspergillus* spp. *Penicillium* spp. *Fusarium* sp.	2 1 8	11
TG3	*Aspergillus* spp. *Penicillium* spp.	1 2	3
SZ1	*Aspergillus* spp. *Penicillium* spp. *Rhizopus* sp. *Fusarium* sp.	11 6 2 8	27
SZ2	*Aspergillus* spp. *Penicillium* spp. *Rhizopus* sp. *Fusarium* sp.	7 9 2 6	24
SZ3	*Aspergillus* spp. *Penicillium* spp.	0 0	0
TC1	*Aspergillus* spp. *Penicillium* spp. *Fusarium* sp.	5 9 11	25
TC2	*Aspergillus* spp. *Penicillium* spp. *Fusarium* sp.	9 5 7	21
TC3	*Aspergillus* spp. *Penicillium* spp.	8 1	9

3.2 Isolation with agar plate method

The results of fungal isolation on PDA revealed that the total count (TC) of *Aspergilus niger* reached the highest level (58) and the TC percentage was 33.72 followed by *Fusarium* sp., (57) with

a TC percentage of 33.14. Both *Penicilium digitatum* and *Rhizopus stolonifera* had a TC of 20 for each of them, with a TC% of 11.63 (Table 3). The TC of *Aspergillus Oryzae* was 15 with a TC% of 8.72. However, other species of isolated fungi showed less TC and TC%.

Table 3: isolated fungi from rice samples using the agar plate method.

Fungus	TC	TC%	I	I %
Aspergillus Oryzae	15*	8.72	5	41.66
Aspergilus niger	58	33.72	8	66.66
Penicilium digitatum	20	11.63	4	33.33
Penicilium chrysogenum	1	0.58	1	08.33
Cercospora janseana	1	0.58	1	08.33
Penicillium oxalicum	5	2.91	2	16.66
Rhizopus stolonifer	20	11.63	2	16.66
Penicillium fellutanum	4	02.33	1	08.33
Penicillium frequentans	5	02.91	1	08.33
Fusarium sp.	57	33.14	8	66.66
Total	172	100	33	

*Calculated from total colonies (172) diagnosed on the samples.

3.3 Isolation with the dilution method

The results of fungal isolation using the dilution method revealed that *Rhizopus stolonifer* had the highest total count (TC) of 142, followed by *P. digitatum* (135) and TC% of 19.29 and 18.34, respectively. However, other associated fungi were less frequent (Table 4). Both isolated fungi, *R. stolonifer*, and *Fusarium* sp., showed the highest incidence and incidence percentages.

3.4 Mycotoxin detection

The results of the mycotoxin detection showed that both T-2 toxin and Aflatoxin exist in rice samples. However, the amount of toxins in infected samples was higher in samples infected with insects (Table 5). The highest T-2 toxin (25.1 ppb) was detected in the Basmati rice sample infected with *Sitophilus zeamais* (SZ1), which had the highest insect infestation rate of 81% followed by the rice sample (TC1) infected with *Tribolium castaneum* with the T-2 toxin rate of 21 ppb and insect infestation of 51%. Another Basmati rice infested with the same insect (SZ2) also had a high T-2 toxin (19.33 ppb), which had an insect infestation rate of 66% (Table 5). The highest Aflatoxin rate (4.89 ppb) was also associated with the rice sample infected with *Sitophilus zeamais* (SZ1), which was the highest insect infestation, followed by TC1 and TC2 rice samples with Aflatoxin rates of 4 and 3.2 ppb, respectively. The results also displayed that the samples with high insect infestations showed high weight losses.

Table 4: Isolated fungi from rice samples using the dilution method.

Fungus	TC	TC%	I	I %
Penicilium chrysogenum	76	10.32	5	41.67
Penicillium oxalicum	111	15.08	4	33.33
Rhizopus stolonifer	142	19.29	8	66.66
Penicillium fellutanum	76	10.32	3	25.00
Aspergillus niger	23	3.13	3	25.00
Aspergillus falvus	9	1.22	2	16.66
Penicilium chrysogenum	76	10.32	5	41.67
Penicilium digitatam	135	18.34	7	58.33
Fusarium sp.	88	11.96	8	66.66
Total	736	100	40	

*Calculated from total colonies (648) diagnosed on the samples.

Table 5: Detection of mycotoxins in rice samples with their infection rates and weight losses.

Symbol	Infestation status	T-2 toxin (ppb)	Aflatoxin (ppb)	% of infestation	Weight loss %
OS1	Infested	13.5	2.7	71	18.19
OS2	Infested	9.5	3.4	68	16.3
OS3	Control	3.5	0.4	0	0
TG1	Infested	7.2	1.7	32	9.6
TG2	Infested	11.2	2.3	54	14.9
TG3	Control	0	0	0	0
SZ1	Infested	25.1	4.89	81	25.50
SZ2	Infested	19.33	2.1	66	17.35
SZ3	Control	4.7	0	0	0
TC1	Infested	21	4	51	16.37
TC2	Infested	18.5	3.2	38	11.51
TC3	Control	0	0.5	0	0

3.5 Correlation between insect infestation with mycotoxin types and weight loss in rice grains

A Pearson correlation revealed that the percentage of insect infestation strongly positively correlated with T-2 toxin, r = 0.827, P <.0001, and strongly positively correlated with Aflatoxin, r=0.886**,** p <.0001, and highly strongly positively correlated with percentage weight loss in the grains, r = 0.981, P <.0001, the percentage of insect infestation (M=38.45. 42, SD=31.42), T-2 toxin (M=11.13, SD=8.32) and Aflatoxin (M=2.09, SD=1.71)**,** and percentage weight loss (M=10.82, SD=8.86)**.**

Table 6: Pearson correlation between insect infestation with mycotoxin types and weight loss

		% Infection	T-2 toxin	Aflatoxin	Weight loss %
% Infection	Pearson Correlation	1	0.827	0.886	0.981
	Significant level	_	P <.0001	P <.0001	P <.0001
	Mean	38.45	11.13	2.09	10.82
	Std. Deviation	31.42	8.32	1.71	8.86

4 Discussion

The results of our study on fungal isolation from stored rice samples revealed a significant prevalence of species belonging to *Aspergillus*, *Penicillium*, and *Fusarium* among the identified fungi. This finding aligns with existing literature that highlights the role of these genera (*Aspergillus*, *Penicillium*, and *Fusarium*), particularly under conditions conducive to fungal growth (i.e., moisture and the presence of insects) [34-36].

One of the most substantial observations from the data is the correlation between insect infestation and fungal contamination levels [37]. Hence, it is worth mentioning that the levels of fungal infection were significantly lower in either uninfected or low-infested rice samples with insects than in highly infested samples with stored insect pests. Insects can act as fungal vectors, but their presence alone does not guarantee increased contamination unless they are causing significant damage to the crop, which would create an environment that would promote the growth of fungi [38].

In this regard, the isolation techniques used in this experiment, both the dilution and Potato Dextrose Agar, might provide adequate information about the fungal communities found in the stored rice [39]. Even though both above-mentioned methods yielded significant results, they draw attention to variations in the frequency and distribution of specific fungal species as the total count of 58 and 57 were recorded for *Aspergillus niger* and *Fusarium* that accounted for 33.72% and 33.14% of the total fungal population respectively, making them the most common fungi obtained via PDA method. The PDA media probably contains various elements (i.e., nutrient availability and moisture content) that enhance and favor the growth of these specific fungi [40]. *Penicillium digitatum* and *Rhizopus stolonifer* were isolated with equal TCs of 20 after *A. niger* and *Fusarium* sp., each of which contributed 11.63% to the total count. Despite their importance, these species may not be as competitive in this specific habitat given their lower frequency compared to *A. niger* [41].

The existence of *Aspergillus oryzae*, which has a TC of 15 (8.72%), indicates the diversity within the fungal community, albeit still being overcome by both dominant fungi, *A. niger* and *Fusarium* sp. On the other hand, fewer numbers of the other isolated fungi were found, suggesting that certain significant ecosystem members may thrive under these conditions, whereas others have less chance for survival [42].

A distinct hierarchy has been established by the results of the diluting approach, as *R. stolonifer* was the most prevalent species here with an exceptional TC of 142, closely followed by *P. digitatum* at 135. The consistent TC percentages of 18.34% for *P. digitatum* and 19.29% for *R. stolonifer* demonstrate a more equal representation of these two species in comparison to the PDA data. This shift in dominance may have been caused by the dilution method's that impose multiple selective pressures and hence encourage the growth of *P. digitatum* and *R. stolonifer* over *A. niger* [39].

The results of mycotoxin detection analysis demonstrated that the highly insect-infested rice samples had a much higher quantity of both aflatoxin and T-2 toxin than the other rice samples [43]. The T-2 toxin and aflatoxin in rice samples were directly correlated with the presence of *Aspergillus* species and *Fusarium* [44]. The intricate association between insect infestation with mycotoxin production on the grain quality might be revealed through this correlation [45].

The highest concentrations of T-2 toxin (25.1 ppb) and aflatoxin (4.89 ppb) were observed in rice samples infested with *S. zeamais*. Consequently, the positive connection between elevated mycotoxin levels and high rates of insect infestation (81% in the SZ1 sample) suggests that insect damage could foster a fungal colonization process, which in turn could result in higher mycotoxin synthesis [46]. As a result, the growth of fungi that produce mycotoxins as secondary metabolites can be encouraged by the damage caused by a variety of insect pests [47].

Furthermore, the highest infestation rate of 81% was not only positively correlated with the fungal colonization but also with the highest weight loss, which was 25.5 % in stored rice samples infested with *S. zeama*. Identifying the common species that cause significant damage are probably linked to the variety of fungal species that inflict the highest total number of colonies could help by offering appropriate advice regarding when and how quickly control measures should be implemented. This is in line with previous research indicating that *Sitophilus* species can cause significant damage to stored cereals, possibly leading to 30% weight loss [48,49].

The discovery of T-2 toxin in TC1 and SZ2-infested rice samples will further illustrate the correlation between increased toxin levels and higher insect infestation frequencies [50]. Having said that, this correlation would likely be dependent on the insect species since *T. castaneum*-infested TC1 sample exhibited a T-2 toxin concentration of 21 ppb and a 51% infestation rate, whereas the SZ2 sample displayed a rate of 19.33 ppb and a 66% infestation rate. This pattern emphasizes the perception that different insect species can vary in their capacity to facilitate fungal growth and subsequent mycotoxin production [51]. Determining the potential risk related to different insect infestations in grains that have been stored requires an understanding of these processes [52].

Conclusions

This study has highlighted the possible health concerns associated with stored grains, especially in conjunction with a variety of stored insect pests, with common fungal species such as *Aspergillus*, *Fusarium*, and *Penicillium* that have been detected in significant quantities in stored rice samples, and therefore emphasizing their roles in grain deterioration. Insects can act as vectors for the spread of fungi, but their presence alone does not always lead to higher contamination levels unless they damage the grains and cause significant weight loss. Nevertheless, a strong correlation between the level of insect infestation and fungal contamination has been detected. Additionally, the discovery of significant mycotoxin levels in stored rice, specifically both aflatoxin and T-2 toxin, presumably draws attention to the risks posed by fungal contamination, particularly from highly infested grains with post-harvest insect pests. Hence, the data revealed a direct relationship between high insect infestation rates and elevated mycotoxin levels, indicating that insect damage not only facilitates fungal colonization but also promotes mycotoxin production. Thus, storage management strategies should be placed into practice, starting with a preliminary inspection for insect pest infestations in stored rice stacks to prevent the growth of fungi and the spread of mycotoxin contamination, with further studies on other types of mycotoxins.

Conflicts of interest

The authors declare that they have no conflict of interest.

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Author contribution

Sahand K. Khidr and Qasim A. Marzani designed the study, performed the data analysis and writing, and approved the final manuscript. Other authors collected samples and performed laboratory experiments.

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