Non-Aflatoxigenic Aspergillus flavus Control Aflatoxins

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Aflatoxins (AFs) are carcinogenic compounds causing liver cancer in humans and animals. Several methodologies have been developed to control AF contamination, yet; they are usually expensive and unfriendly to the environment. Consequently, interest in biocontrol agents has increased, as they are convenient, advanced, and friendly to the environment. Using non-aflatoxigenic strains of *A. flavus* (AF−) as biocontrol agents is the most promising method to control AFs' contamination in cereal crops.

Keywords: aflatoxins ; biocontrol ; non-aflatoxigenic Aspergillus flavus ; biotic and abiotic factors

1. Introduction

Aflatoxins (AFs) are secondary metabolites produced by *Aspergillus flavus*, *A. parasiticus*, *A. nomius*, and *A. pseudotamarii* [1][2]. AFs are organic compounds with lower molecular weight, typically produced by fungal mycelia and accumulated in conidia and sclerotia. AFs contaminate various crops, including corn, oilseeds, rice, and nuts [3][4][5][6]. AFs contamination in cereals may occur during pre- or post-harvest stages [7][8]. Hot temperature and high humidity stimulate fungal growth in fields and storage. Contamination by AFs is responsible for substantial commercial losses throughout the world [9][10][11]. AFs are among the most toxic compounds that adversely affect humans and animals' health [12][13][14][15][16][17]. AFs are mutagenic, teratogenic, genotoxic, and carcinogenic compounds, causing severe diseases in humans, poultry, fishes, and cattle under long-term exposure [18][19]. AFs can penetrate the feed and food chain, posing a threat to even newborns [20][21]. While several AFs were currently identified, AFB_1, AFB_2, AFG_1, and AFG_2 are the four most significant AFs. The IARC (International Agency for Research on Cancer) classifies AFB_1 as the most toxic, mutagenic, and Group 1 human carcinogen [22][23][24], causing chronic and acute diseases in children and the elderly. AFB_1 carcinogenicity has long been linked to the liver; however, recent epidemiological studies revealed that it was also carcinogenic to the pancreas, kidney, bone, bladder, and central nervous system [25][26][27][28].

2. Advantages of Biocontrol of Aflatoxins Using Non-Aflatoxigenic *Aspergillus flavus*

Biocontrol methods are more effective and innovative in controlling AF contamination in crops. The application of biocontrol agents (AF^-) carries some adaptations in fungal populations, which persist throughout the food chain. These adaptations prevent the grains from AF contamination during storage and transport; even environmental conditions favor fungal growth. In biocontrol methods, AF− strain application in the field remarkably reduces AF contamination in crops [29][30]. Similarly, like air, AF can disperse Aspergillus spores-communities, improve safety within the treated, and positively affect neighboring fields [31]. The positive impacts of AF^- strains can benefit crops and other plants for several years. This means a single dose of AF^- strain could benefit the treated crop and the second season crop, which missed the treatment [32].

3. Selection of Non-Aflatoxigenic Strains

Biocontrol is a promising method to reduce AF contamination in crops. Recent studies reported reducing AF contamination by applying AF^- strain to the soil around growing plants. When the crop is vulnerable to fungal attack during drought conditions, these AF^- strains competitively exclude the AF^+ strains in the soil and reduce AF concentrations. Dorner [33] reported the reduction in AF contamination in a cornfield using AF^- strains. In other research, Dorner [33] assessed the efficacy of AF^- for AFs control in peanuts. AF^- strains can be found in air, soil, and plants. Usually, both AF^+ and AF^- strains mutually occur in different ecosystems. AF^- strains competing with AF^+ strains for nutrients provide an opportunity to use them as biocontrol agents. Different techniques have been developed to discover the suitable AF^- strain for biocontrol use. Some of them are based on phylogenetic features, while others on phenotypic characteristics such as sclerotial size. Based on sclerotial morphology and production, *A. flavus* can be divided into two distinct morphotypes: S-strain and L-strain. The S-strains produce a large number of small-sized sclerotia (>400 µm in diameter), whereas the L-strains produce a small number of large-sized sclerotia (<400 µm in diameter). Moreover, S-strains produce a higher concentration of AF compared to L-strains. Molecular techniques may describe the phylogenetic relationships between *A. flavus* strains successfully. Several polymerase chain reaction (PCR)-based pyrosequencing methods are currently being developed to detect genes responsible for AF production and discover suitable biocontrol agents [34]. Abbas et al. [35] isolated some AF^- strains, including K49, F3W4, NRRL 58,974, NRRL 58,976, and NRRL 58,988. The classification was based on their growth rate, pigmentation, fluorescence, and AF production.

4. Efficacy of Non-Aflatoxigenic Strains as Biocontrol Agents

AF^- strains have been suggested as biocontrol agents hoping that they would inhibit the growth of AF^+ and thereby reduce AFs contamination. Previous studies conducted by Erhlich [36] revealed that co-inoculation of AF^- strains with AF^+ substantially reduced the production of AF in corn under in vitro conditions. The potential for biocontrol of AFs using AF^- strains has been demonstrated under field conditions in cotton [37], peanuts [38], and corn [39][40]. These scientists have applied the AF^- strain to the soil as infested grain cultures of barley, rice, or wheat, whereas [41] inoculated corn ears directly by injection. In the cotton studies performed by Cotty [42], the AF^- strains failed to suppress AFs contamination when sprayed on the cottonseed immediately before the bolls formed but were effective when sprayed on the soil later.

5. Factors Affecting the Efficacy of Biocontrol Agents

5.1. Inoculation Method

For many years, AF^- strains have been used on cornfield soil. Although the use of K49 in the soil can reduce AF levels by 65% [43], the direct use of AF^- strain on corn ears is immensely more efficient. A clay-based water-dispersible granule system was also developed to spray AF^- strain on corn silk directly. This management decreased AF production by up to 97%.

5.2. Inoculum Rate

Inoculum concentration is an essential factor for the effective control of AF contamination. Recent studies have revealed a direct relationship between the inoculum rate and AF's efficacy$^-$ strain decreasing AF concentrations [44]. Studies demonstrated a significant reduction in AF concentration in peanuts when AF^- inoculum increased from 2–50 g/L. In the USA, research was conducted in which an AF^- strain (NRRL 21,368) with different quantities (0, 2, 10, and 50 g) was applied to the cornfield [45].

5.3. Optimal Time for Non-Aflatoxigenic Strains Application

Research showed that with the concentration of AF^- strains, the time of its application significantly affects their efficacy. The application of AF^- strain at earlier stages significantly reduced AF levels in cotton. Similarly, Kabak and Dobson [46] suggested the co-inoculation of AF^+ and AF^- strains (TX9-8) to reduce the AF contamination; however, if the AF^- strain is applied one day later, AF^+ strains, fewer or no reduction in AF concentrations will be achieved.

5.4. Abiotic Factors

The time for the application of AF^-, depends on the significant environmental conditions. Abiotic factors such as water activity and temperature directly affect AF^- strains' efficacy by controlling spore germination, hyphal growth, and spore-production [47]

5.5. Biotic Factors

Low temperature and high water content in storage provide favorable conditions for insects, mites, and other microorganisms to grow. Insects' respiration process produces hot spots in seeds, causing grain charring that affects seed quality and germination. In grains, insects' activities increase the surrounding bulk's temperature and water content, providing favorable mold growth conditions. Studies have shown that seeds damaged by insects are highly susceptible to fungal contamination [48]. Some fungi absorb insects and boost their populace, while others repel pests by secreting harmful toxins.

5.6. Physiological Manipulation of Non-Aflatoxigenic Strains

Most fungal niches are not persistent as they modify their features according to the external environment [49]. In unfavorable environments, xerophilic fungi produce small polyols, which allow their enzymatic systems to work efficiently. Similarly, *A. flavus* accumulates glycerol and erythritol in their conidia during unfavorable conditions [50]. Therefore, fungal propagules used for biocontrol must be resistant to environmental stresses [51]. According to Magan [52], agricultural management could improve the resistive performance of biocontrol agents.

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