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Research Article

EFFICACY OF ACETIC ACID AND SODIUM BICARBONATE IN INHIBITING *ASPERGILLUS OCHRACEUS* AND REDUCING OCHRATOXIN A

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ABSTRACT

The current study aimed to identify safe products that could serve as biological control agents against fungi responsible for the accumulation of ochratoxin A in poultry feed. Both acetic acid and sodium bicarbonate exhibited inhibitory effects against *Aspergillus ochraceus* on culture media and significantly reduced ochratoxin A levels. When incorporated into Potato Dextrose Agar medium, acetic acid completely inhibited the growth of *A. ochraceus* (100% reduction). A similar effect was observed with sodium bicarbonate, which also achieved 100% growth inhibition when applied at a 5% concentration. Furthermore, ochratoxin A levels were reduced by 65.7% and 43.6% after one month of treatment with 0.5% acetic acid and 1% sodium bicarbonate, respectively. By the second month, both treatments achieved complete (100%) reduction of ochratoxin A. These findings suggest that both compounds are effective and safe options for mitigating the harmful effects of *A. ochraceus* in poultry feed.

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INTRODUCTION

Plant pests are among the most significant factors contributing to annual agricultural losses in Iraq (Adhab and Alkuwaiti, 2022; Khalaf et al., 2023; Obaid and Adhab, 2025). Corn is regarded as one of the most valuable crops globally, as it is a staple in both human and animal diets (Abbas and Shier, 2009). Cereal crops occupy the majority of cultivated land in Iraq (FAOSTAT, 2019); consequently, corn yields are negatively affected each season by the spread of various pathogens (Al-Ani et al., 2011a; Adhab et al., 2021; Radhi et al., 2021). Corn seeds are vulnerable to infection by numerous fungi that produce harmful mycotoxins, including *Aspergillus ochraceus*, which generates ochratoxin A both in the field and during storage (Mehboob et al., 2014; Hassan et al., 2018; Seerat et al., 2022). Corn seeds contaminated with mycotoxins pose a serious risk to the health of both humans and animals (Abbas and Shier, 2009; Anfossi et al., 2016).

The contamination of poultry feed by fungal pathogens and their associated mycotoxins represents a major threat to animal health and food safety. Among these, *A. ochraceus* is particularly concerning due to its production of ochratoxin A, a potent mycotoxin that adversely affects poultry, causes economic losses, and poses potential health risks to humans through the food chain (Hassan et al., 2018). There is an urgent need to develop safe and effective strategies to reduce the impact of such contaminants. In recent years, attention has shifted toward natural compounds for their antifungal properties and detoxification potential (Hussein et al., 2017, 2018, 2024; Al-Samaraee et al., 2019; Al-Tamimi et al., 2020).

Ochratoxin A (OTA), produced by *A. ochraceus* and *Penicillium* species, is a dangerous mycotoxin that threatens poultry health and food safety (Hassan et al., 2018). Biocontrol strategies, particularly those involving eco-friendly compounds such as acetic acid and sodium bicarbonate, have shown promise in mitigating OTA contamination in poultry feed.

To reduce mycotoxin contamination, several approaches have been employed for treating corn seeds and animal feed, including the use of various chemical agents. Sodium bicarbonate, hydrogen peroxide, sodium hydroxide, ammonia, nanoparticles, ammonium phosphate, formaldehyde, and a variety of acids and bases have been tested to inhibit mycotoxin production (Paskevicius et al., 2006; Scott and Trucksess, 2009; Freitas-Silva and Venancio, 2010; Karlovsky, 2011). For example, treating corn seeds with formic acid and propionic acid for three hours has been shown to result in complete mycotoxin degradation (Lerda, 2011).

Given that acetic acid and sodium bicarbonate are commonly used as food preservatives and pH regulators, their antifungal activity has attracted increasing interest. Although several studies have explored the antifungal effects of various acids and sodium bicarbonate, this research uniquely investigates their combined use in poultry feed to address the critical issue of *A. ochraceus* contamination and OTA detoxification. Unlike previous work, this study provides in-depth analysis of their practical application in agricultural settings.

The objective of this study was to evaluate the effectiveness of acetic acid and sodium bicarbonate in inhibiting the growth of *A. ochraceus* on culture media and detoxifying ochratoxin A in poultry feed. By exploring the potential of these compounds, this research aimed to enhance feed safety, promote poultry health, and support food security through improved feed management practices.

MATERIALS AND METHODS

Extraction of ochratoxin A

A specific isolate of *A. ochraceus*, known for its ability to produce ochratoxin A, was obtained from the Mycotoxin Laboratory, Department of Plant Protection, University of Baghdad. The isolate was cultured in a liquid medium containing yeast extract. After cultivation, the fungal suspension was filtered through Whatman No. 4 filter paper. Fifty milliliters of the resulting filtrate were transferred to a 250 ml separating funnel and mixed with 100 ml of chloroform. The mixture was shaken and left undisturbed for 15 min to allow phase separation. The chloroform layer, which settled at the bottom, was collected and passed through Whatman paper containing sodium sulfate (Na_2SO_4) to remove moisture. The filtrate was then evaporated to dryness in a water bath at 50°C. The dried extract was dissolved in 5 ml of chloroform and stored under freezing conditions.

Detection of ochratoxin A

Ten microliters of the extract were spotted onto a pre-activated thin-layer chromatography (TLC) plate (20 × 20 cm), with spots placed 2 cm apart. The TLC plate was pre-activated by heating at 110°C for 1 h. A standard spot of ochratoxin A was applied to the left side of the plate as a control. The plate was developed in a glass chamber containing a solvent mixture of toluene, ethyl acetate, and formic acid (60:30:10 v/v/v) to a depth of 1 cm. Once the solvent front had migrated within 2 cm of the top edge, the plate was removed and air-dried at room temperature. Visualization was carried out under UV light at a wavelength of 366 nm using a UV viewing cabinet (Asensio et al., 1982). The recovery rate of ochratoxin A, determined by spiking samples with known concentrations, was 98% ± 2%. The limit of detection (LOD) was 0.1 ng/g, and the limit of quantification (LOQ) was 0.5 ng/g, confirming the sensitivity and reliability of the method.

Production of ochratoxin A

The *A. ochraceus* isolate was cultivated on rice seeds. A total of 150 g of rice was mixed with 100 ml of distilled water in a glass container and sterilized by autoclaving at 121°C under 1.5 kg/cm^2 pressure for 20 min on two consecutive days. After sterilization, the seeds were inoculated with two 9 mm discs from an *A. ochraceus* culture grown on potato dextrose agar (PDA). The mixture was thoroughly shaken for homogenization and incubated at 25°C for 21 days. After incubation, the contaminated seeds were oven-dried at 50°C, packed in paper bags, and ground into a powder, following the method described by Abbas et al. (1984). The concentration of ochratoxin A in the powdered rice was determined using high-performance liquid chromatography (HPLC), according to the method described by Căpriţă et al. (2007).

Effect of acetic acid and sodium bicarbonate on the growth of *A. ochraceus*

Before the solidification of the PDA medium, acetic acid (at concentrations of 0.2%, 0.5%, and 1%) and sodium bicarbonate (at concentrations of 1%, 2%, and 5%) were added at a temperature of 40-45°C. The medium was poured into 9 cm petri dishes. Each plate was inoculated with a 7 mm disc from the fungal culture (one disc per plate) and incubated at 25°C for two days. The percentage inhibition of fungal growth was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{C - T}{C} \times 100$$

C = Colony diameter in control,

T = Colony diameter in treatment

This formula quantified the degree of fungal growth inhibition due to treatment, compared to the control. The control consisted of PDA medium without any additives and served as the baseline for comparison.

Effect of vinegar and baking soda on reducing ochratoxin A in poultry feed

A total of 500 g of poultry feed was placed in a desiccator and mixed with a known amount of ochratoxin A-contaminated powdered rice to achieved a concentration of 2 mg/kg of feed. Acetic acid (0.5%) and sodium bicarbonate (1%) were added to the contaminated feed, followed by thorough mixing to ensure even distribution. The treatments were randomized and conducted in triplicate. The ochratoxin A concentration in the feed was monitored using HPLC at three intervals: week 1, week 2, and week 4, to assess degradation over time.

Statistical analysis

Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Least significant difference (LSD) values were reported for pairwise comparisons.

RESULTS AND DISCUSSION

Inhibition of *A. ochraceus* growth on PDA

The results showed that acetic acid application at concentrations of 0.2% and 0.5% reduced *A. ochraceus* growth on PDA medium by 90.22% and 98.77%, respectively. However, increasing the acetic acid concentration to 1% completely inhibited fungal growth (Table 1, Figure 1).

A similar trend was observed with sodium carbonate-treated PDA. Fungal growth was reduced by 81% and 91.5% at concentrations of 1% and 2%, respectively, while a 5% concentration resulted in complete (100%) inhibition of *A. ochraceus* growth (Table 1, Figure 1).

Table 1. Inhibitory activity of different concentrations of acetic acid and sodium bicarbonate against *A. ochraceus*.

Treatment	Inhibition Zone (mm) ± SE	% Growth Inhibition ± SE
Control	0.00 ± 0.00 a	0.00 ± 0.00 a
Acetic Acid (0.5%)	18.34 ± 0.55 b	64.23 ± 1.20 b
Acetic Acid (1%)	24.12 ± 0.48 c	82.70 ± 1.03 c
Sodium Bicarbonate (1%)	19.56 ± 0.43 b	68.40 ± 0.95 b
Sodium Bicarbonate (2%)	25.77 ± 0.60 c	85.12 ± 1.11 c

Values represent the mean of three replicates ± standard error (SE). Means followed by the same letter within the same column are not significantly different according to the LSD test at P ≤ 0.05.

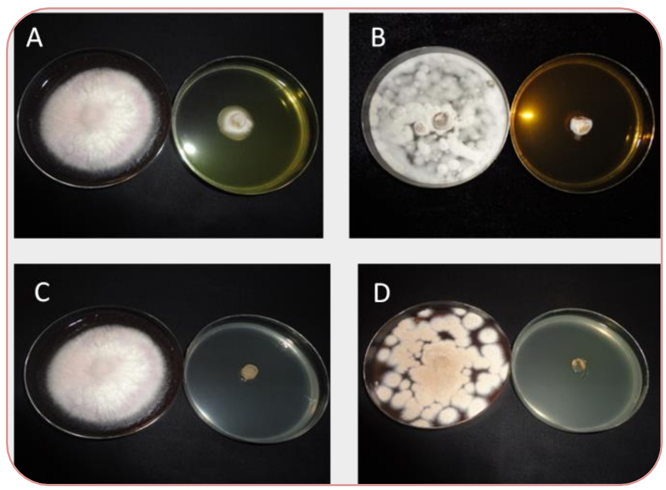

Figure 1. Effect of different concentrations of acetic acid and sodium bicarbonate on the growth of *A. ochraceus* on culture media. (A) *A. ochraceus* with 2% acetic acid, (B) 0.5% acetic acid, (C) 5% sodium bicarbonate, (D) 2% sodium bicarbonate.

Ochratoxin A detoxification in the poultry diet

The results showed that the poultry diet treated with 0.5% acetic acid exhibited a significantly lower concentration of ochratoxin A (879.9 ng/g) in the first month compared to the control (2566.1 ng/g), representing a 65.7% reduction. Similarly, treatment with 1% sodium bicarbonate reduced ochratoxin A levels to 1446.4 ng/g in the first month, corresponding to a 43.6% reduction compared to the control. In the second month, ochratoxin A levels continued to decline, reaching complete detoxification (100% reduction) for both acetic acid and sodium bicarbonate treatments (Figure 2).

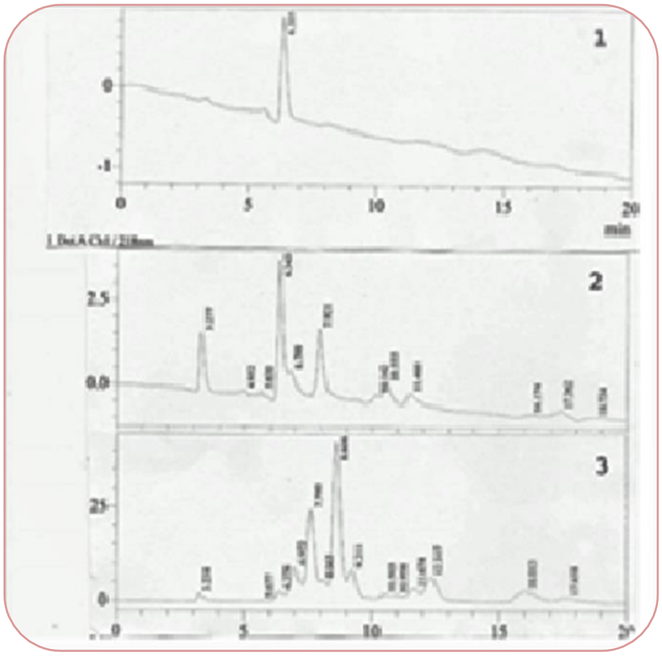

Figure 2. Effect of acetic acid and sodium bicarbonate on ochratoxin A degradation in poultry diets.

The use of environmentally safe compounds has become essential in various procedures due to the importance of maintaining a clean and healthy environment. These compounds may include plant extracts or safe chemicals used for controlling different microbes (Al-Ani et al., 2011b; Al-Ani and Adhab, 2013; Al-Ani et al., 2013; Azeem et al., 2025a, b).

This study demonstrated the effectiveness of environmentally safe compounds, specifically, acetic acid and sodium bicarbonate, in inhibiting the growth of *A.chraceus* on PDA and their high efficiency in degrading ochratoxin A in poultry diets.

The inhibition of fungal growth may be attributed to the ability of these compounds to penetrate fungal cells and interact with cell membranes, leading to deformation of cellular components and disruption of membrane selectivity. Ultimately, these effects may result in fungal cell death. Several studies have reported that propionic acid and acetic acid exhibited strong antifungal activity, significantly reducing fungal growth on corn seeds and in culture media (Valle et al., 2024).

Acetic acid and sodium bicarbonate might interact with active functional groups in mycotoxins, leading to the degradation of ochratoxin A and a reduction in its toxicity. Numerous studies have shown that various compounds can inhibit fungal growth in stored seeds. For example, the application of 0.5% citric acid and 0.75% lactic acid was found to inhibit *A. parasiticus* growth on bread wheat (Singh et al., 2024).

The mechanism by which sodium bicarbonate reduces ochratoxin A toxicity involves breaking the lactone ring structure of the toxin, producing two degradation products, OP-OA and OA-OH, which exhibit lower toxicity than the parent compound (Kuhn et al., 2024).

Compared to commercial antifungal agents such as propiconazole and other synthetic fungicides, acetic acid and sodium bicarbonate offer advantages such as lower toxicity and greater environmental sustainability. Other natural alternatives, like essential oils, have also shown antifungal activity, though challenges related to their cost and stability persist (Abdul-Karim and Hussein, 2025; Hussein et al., 2025).

Although the results obtained on PDA medium are promising, it is acknowledged that such a simplified system does not fully replicate the complexity of poultry feed matrices. Therefore, future studies should evaluate the performance of acetic acid and sodium bicarbonate in actual poultry feed formulations, taking into account potential interactions with other feed components.

Although acetic acid and sodium bicarbonate show promising results in reducing ochratoxin A contamination, further research is required to assess their stability in feed over extended periods. Moreover, factors such as their effects on feed palatability, nutritional quality, and economic feasibility should be considered before large-scale application.

This study provides preliminary evidence for ochratoxin A degradation; however, structural confirmation of the degradation products using LC-MS/MS is essential to validate the detoxification process. Future studies should incorporate this analytical technique to confirm the breakdown products. Moreover, further investigations should explore the mechanisms of action in greater depth, including the use of electron microscopy to examine fungal cell morphology and detailed characterization of ochratoxin A degradation products via LC-MS/MS.

AUTHORS' CONTRIBUTIONS

HH and MA designed, formulated and laid out the study; AA and HH conducted the experiments, collected, analyzed the data, wrote and proofread the manuscript.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED
SDG 3: Good Health and Well-being
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

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