Chemical Control of Certain Fusarial Mycotoxins Infected Wheat Grains during Storage by Sorbic Acid as An Alternative to Aluminum Phosphide

Mohamed Fouad Hussien[1], Mostafa Abdelazeem Amer[2], Saad Mahmoud Shama[2], Abdel-Rahman,T.F.M[3], Nesrine Hassan Youssef[4]

1 General Administration of Plant Quarantine, Alexandria, Egypt.
2 Agricultural Botany Department, Faculty of Agriculture (Saba-Basha), Alexandria University, Alexandria, Egypt.
3 Plant Pathology Research institute, Agricultural Research center, Giza,_ Egypt.
4 Microbiology lab, Regional Center for Food and Feed, Agricultural Research center, Alexandria, _ Egypt.

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ABSTRACT: Wheat (*Triticum aestivum*, L.) grass family *Poaceae* (*Gramineae*) is the most important strategic cereal crop for the majority of the world population. Storage fungi especially *Fusarium* species such as *Fusarium graminierum* and *F. verticilloides* infect grains before and after harvest and can grow on them during storage. These fungi produce mycotoxins that threaten human and animal health and cause severe illness or even death. The presence of insects augments the heat spots, the inoculum potential of storing fungi and the seed contamination of high amounts of mycotoxins, leading to high seed yield losses value during storage. The fumigation with aluminium phosphide tablets was usually used for controlling and killing these insects during storage. Unfortunately, this chemical was not safe for humans. This study aims to investigate the effect of aluminium phosphide against *F.verticilloides* and *F.graminearum* and their produced mycotoxins compared to sorbic acid as a safe and useful alternative.

Keywords: *Fusarium spp, mycotoxins, aluminum phosphide, sorbic acid, chemical control.*

INTRODUCTION

Wheat (*Triticum aestivum*, L.) grass family *Poaceae (Gramineae*) is the most important strategic cereal crop for most world population. Fungi play an important and dangerous role during storage operations compared to other microorganisms. The toxins produced by these fungi have significant economic effects in many agricultural crops, especially wheat, maize, and cotton seeds. Twenty-five percent of the world's crop production is contaminated with fungal compounds (**Kabak *et al.*, 2006).** In general, toxins reach the food of humans and animals through the contamination of food with fungi, which produce these toxins, causing illness which might lead to severe complications and death **(Ciegler and Bennett, 1980; Mohamed, 2011).**

Mycotoxins are toxic secondary metabolites produced by some specific species of filamentous fungi, mainly belonging to the genera of *Aspergillus, Penicillium, and Fusarium*, which invade crops in fields and during storage **(Daou *et al.*, 2021).**

Among the most investigated mycotoxigenic fungi in the field invaded wheat crop were *F. graminearum*, and *F. verticillioides.* Bad postharvest practices, including slow drying, hot spots, insects' occurrence, and inappropriate ventilation, can allow infection by mycotoxigenic fungi and further mycotoxins production **(Atanda *et al.*, 2011**). Deoxynivalenol (DON) is a sesquiterpenoid polar organic compound, which belongs to the type B trichothecenes, it contains carbonyl group in C-8.

DON, also known as vomitoxin, is a natural-occurring mycotoxin in wheat and other cereals produced at pre-harvest stage by several *Fusarium* species, mainly by *F. graminearum* according to its effects as a causative agent of vomiting in animals and humans **(Sobrova *et al.*, 2010).** DON affects animal and human health, causing vomiting, abdominal pain, acute temporary nausea, dizziness, headache, diarrhea, and fever. This mycotoxin and its derivatives also affected the reproduction process. **(Kushiro, 2008)**

Fumonisins (FUM) is secondary metabolites produced mainly in cereals by pathogenic fungi, such as *F.verticillioides, F.proliferatum, Aspergillus niger* and *F.sacchari* **(Rheeder *et al.*, 2002; Cendoya *et al.*, 2018).** Fumonisin B1 (FB1) was the most toxic derivative of fumonisins which can exist alone or with other forms of fumonisins**. (Damiani *et al.*, 2019; Liu *et al.*, 2022)**. Researchers discovered a significant relation between the occurrence of fumonisins and the incidence of the following diseases in animals and humans: esophagus cancer, toxic effects on the liver and nephron in all the tested animals, incidences of

hepatocarcinoma **(Kamle *et al.*, 2019),** defects in the falloap tube and or neural-tube, stimulation and/or suppression of the immune system and nephrotoxicity all over the world **(Chu *and* L., 1994; Xue *et al.*, 2018).** The international agency for research on cancer **(IARC, 2002)** characterized fumonisins, especially FB1as a carcinogenic agent for human several animals such as rabbits**. (Burgess, 1981; FAO, 2019).** Both the toxigenic fungi and their produced mycotoxins are potential problems for both health and economic perspectives. Aluminum phosphide (AlP) is a cheap and highly toxic inorganic compound. It is very effective and commonly used as a pesticide , It was very toxic for any biotic cells and laid to death **(Ghonem *et al.*,2020 and Deraz et al.,2022)** . Unfortunately,it is now one of the most common causes of poisoning among agricultural pesticides. It liberates lethal phosphine gas when it reacts with water or acids or when it comes in contact with either atmospheric moisture or hydrochloric acid in the stomach **(Gurjar *et al.*, 2011).**

Phosphine is a strong reducing dose dependent agent that causes oxidative stress, the exposure to phosphine can cause dizziness, headache, vomiting, nausea, diarrhea, drowsiness, cough, and chest tightness. More severe exposure can cause shock, convulsions, coma, irregular heartbeat, and liver and kidney damage **(Sciuto *et al.*, 2016; Bekhier, 2021)**

Sorbic acid, or 2, 4-hexadienoic acid, is a natural organic compound used as a food preservative in food and drinks. Sorbic acid and its salts were considered as an antimicrobial agent that was used to prevent the growth of mold, yeast, and fungi. The sorbic acid was more effective and active than salts. The optimal pH for the antimicrobial activity is below pH 6.5.

It is a colorless solid, slightly soluble in water and sublimes readily. It was first isolated from the unripe berries of the *Sorbus aucuparia* (rowan tree). The world produced sorbic acid about 30,000 tons annually (**Lück *et al.*, 2000**). Sorbic acid and sorbate salts have a very low mammalian toxicity and carcinogenicity. The U.S. Food and Drug Administration consider sorbic acid to be safe for regular use, as it is not linked to cancer or other major health side effects.

The food additive sorbic acid (E200) is considered as an effective preservative for certain cereal products, e.g., bread and fine bakery wares. It has been postulated that sorbic acid may be safely used as antimicrobial agents **(European Parliament and Council European Union (2008).** Sorbic acid salts are widely used as food preservatives **(Mroueh *et al*., 2007; Heflish *et al.*, 2020)** and prevention and control of mold growth and mycotoxin production in cereals **(Eeckhout *et al.*, 2013)**. This study aimed to investigate the effect of this substance against *Fusarium verticilloides* and *Fusarium graminearum* and their produced mycotoxins and compared its effects with sorbic acid as safe and useful substance.

MATERIALS AND METHODS:

Preparation of the fungal isolates: Two isolates of fusaria are purchased from Moubasher Mycological Center (AUMMC), Assiut University. The code number of *F. verticilloides* (Sacc.) isolate was AUMC 14795 whereas, the code number of *F.graminearum Schwabe was* AUMC 1262(=CBS104.09).

Testing the ability of the fusaria isolates to produce mycotoxins

The ability of the two tested isolates to produce mycotoxins was tested individually in their seven days culture using plug method agar according to **(Frisvad , 1995; Balouiri *et al.*, 2016)** then, the produced mycotoxin was quantified and determined in their specific broth medium potato sucrose (PS) using HPLC apparatus according to **(Turner *et al.*, 2009).**

Preparation of fusarial culture for chemical control experiment:

The two fusaria (F.*graminearum & F.verticillioides*) cultures were grown on potato sucrose agar (PSA)**.**

Chemical Control experiment:

The chemical control experiment was conducted to study the effects of both studied chemical agents on the behavior of the two tested fusaria in growing and producing mycotoxins. This experiment was carried out using the disk diffusion method (DDM) by the Kirby-Bauer method as a standardized method with certain modifications according to **(Christensen and Relich, 2018),** where the sterilized filter paper disks were impregnated with each tested chemical agent at three tested concentrations. Petri dishes filled with PDA medium, three replicates each. Both disk and *Fusarium* inoculums were putted onto PDA medium. Each control was prepared using an imbibed disk with sterilized water. All petri dishes were incubated at 25 ˚C for seven days. At the end of the petri dishes the fungal radial growth at each treatment was measured then the mycotoxins produced in medium were detected using Plug method agar and HPLC according to **(Frisvad , 1995; Balouiri *et al.*, 2016).**

Statistical analysis:

The experiment was accomplished in a completely randomized design with three replicates. The obtained data were analyzed by one-way ANOVA according to **(SAS, 1999),** and

the results were compared by the least significant difference (LSD) according to Duncan's Multiple Range test **(Duncan, 1955**) using "Costat,

Cohort" computer software package.

RESULTS AND DISCUSSION:

Mycotoxin production ability of the isolated fungi

The ability of the isolated fungi for the production of mycotoxins was carried out using a qualitative method (Agar plug), according to **(Frisvad and thrane, 1995; Balouiri *et al.*, 2016)** with certain modifications the tested two fusaria can produce mycotoxins in their cultures.

Detection of fumonisin B1 and deoxynevalenol in fungal solid cultures (confirmatory test):

Fumonisin (FB1) and deoxynevalenol **(DON)** were quantitatively detected in their solid cultures using HPLC-UV technique according to **(Azcarat *et al.*, 2008; Brzonkalik *et al.*, 2011**) and at regional centre for food and feed RCFF. Mycotoxins Lab. Cairo. The obtained FB1 and DON standards were brought from Sigma Aldrich, local provider, Cairo, Egypt.

Chemical control of the tested fusaria by aluminum phosphide and sorbic acid:

The experiment was carried out using the disk diffusion method (DDM) by the Kirby-Bauer method as a standardized method with minor modifications according to **(Christenson and Relich, 2018)**. After the incubation period for seven days at 25°C the petri dishes were observed, the radial growth of each fungus was measured, then each produced mycotoxin was detected using HPLC-UV technique according to **(Balouiri *et al.*, 2016).** The resulted data were registered and presented Tables 1&2 and Figures 1, 2 and 3.

Effect of chemical control on radial growth and mycotoxins produced by the tested fusaria

Table (1): Chemical control effects on *F. graminearum* radial growth and deoxynevanol inhibition.

Treatments	Deoxynevalenol production in ppm	Radial growth in cm	Efficacy ratio of the inhibition rate of the deoxynevalenol production%	Efficacy ratio of the inhibition rate of the radial growth %
F.graminearum control	34.52 [a]	40 a	----	0
F.graminearum +Sorbic1%	0.992 [e]	27.95 d	97.13	30.12
F.graminearum +Sorbic2%	0.950 f	34 b	97.25	15
F.graminearum +Sorbic4%	0.288 g	30.4 c	99.16	24
F.graminearum + Al.ph1%	4.347 d	0 e	87.41	100
F.graminearum + Al.ph2%	7.184c	0 e	79.19	100
F.graminearum + Al.ph4%	21.35 b	0 e	38.157	100
L.S.D$_{(0.05)}$	**0.01**	**0.86**		

N.B. The difference between data with same letters is not significant

Table (2): Chemical control effects on *F.verticilloides* radial growth and fumonisin inhibition.

Treatments	FumonisinB1 production in ppm	Radial growth in cm	Efficacy ratio of the inhibition rate of the fumonisin production%	Efficacy ratio of the inhibition rate of the radial growth %
***F. verticilloides* control**	34.55 a	32.00a	----	0
F. verticilloides+ Sorbic1%	3.44 e	22.5b	90.04	29.68
F. verticilloides+ Sorbic2%	0.355 g	22.2b	98.97	30.62
F. verticilloides+ Sorbic4%	3.69 d	21.00c	89.32	34.37
F. verticilloides+ Al.ph1%	3.265 f	0 d	90.55	100
F. verticilloides+ Al.ph2%	5.772 c	0 d	83.29	100
F. verticilloides+ Al.ph4%	8.752 b	0 d	74.67	100
L.S.D$_{(0.05)}$	**0.01**	**0.84**		

N.B. The difference between data with same letters is not significant

As shown in **Table (1)**, the treatment with sorbic acid 1%, and 4% were the best treatments in falling radial growth of *F. graminearum* with efficacy ratios 30.12% and 24% respectively whereas, the best treatment for inhibiting the deoxynevalenol production was with sorbic 4% with efficacy ratio 99.16%. Our findings are coincided with those of (**Lopes *et al.*, 2012**) who announced that sorbic acid has a wide range of antibacterial and antifungal effects.

In case of aluminum phosphide: the same reaction occurred, indicating that aluminum phosphides affect the growth of any fusaria and subsequently no mycotoxin was produced. Our findings were in agreement with those of **(Kabir *et al.*, 2020**) who reported that aluminum phosphide inhibited the growth of *F. graminearum*

As presented in **Table (2),** the treatment with sorbic acid 4% and 2% were the best treatments in reducing radial growth of *F. verticilloides* with efficacy ratios 34.37% and 30.62% respectively. Whereas, the most effective treatment for inhibiting the fumonisin B1 production was with sorbic 2% with efficacy ratio 98.97 %. Our findings are coincided with those of (Tang and Wu, 2005; Stratford et al., 2009, Huang et al., 2010) who reported that sorbic acid possess an antimycotic effects against molds and yeasts.

The treatment with aluminum phosphide completely inhibited the fungal growth and consequently no fumonisins was produced. Our findings were consistent with those of **Gerez *et al.* (2016)** who mentioned that mycotoxin was not detected when the fungal growth was 100% inhibited.

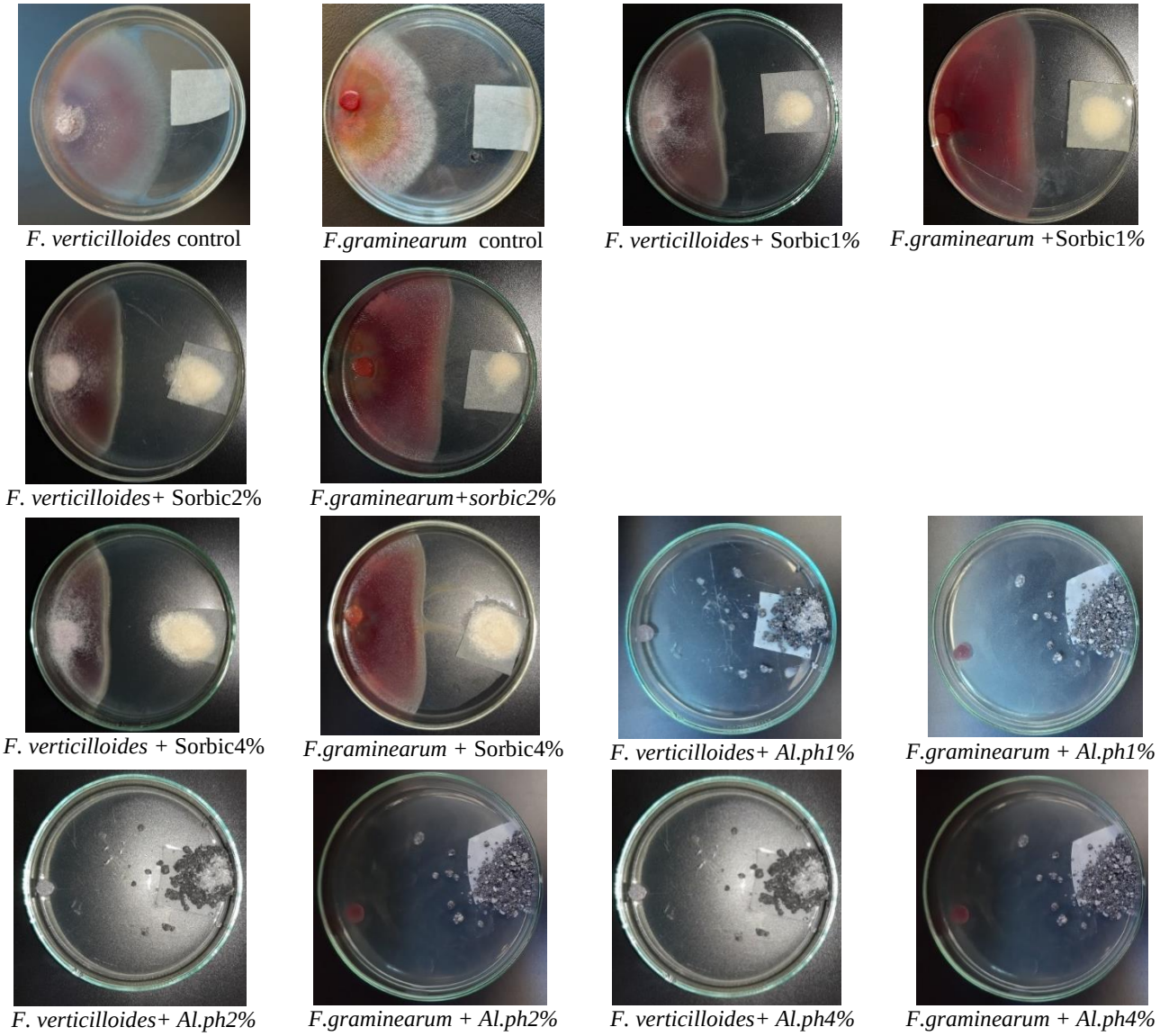

Fig. (1): Chemical control effects of sorbic acid and aluminum phosphide on *F. verticilloides* and *F. graminerarium* radial growth

As illustrated in **Fig(1),** both sorbic acid and aluminum phosphide had inhibitory effects against the two tested fusaria. Our findings were in harmony with those of (**Plumridge *et al.*, 2004; Razavi-Rohani and Griffiths, 2007)** who mentioned that sorbic acid inhibits mycelial growth and conidial germination of *Aspergillus niger*, *Fusarium spp*, *Candida*, and *Penicillium spp*

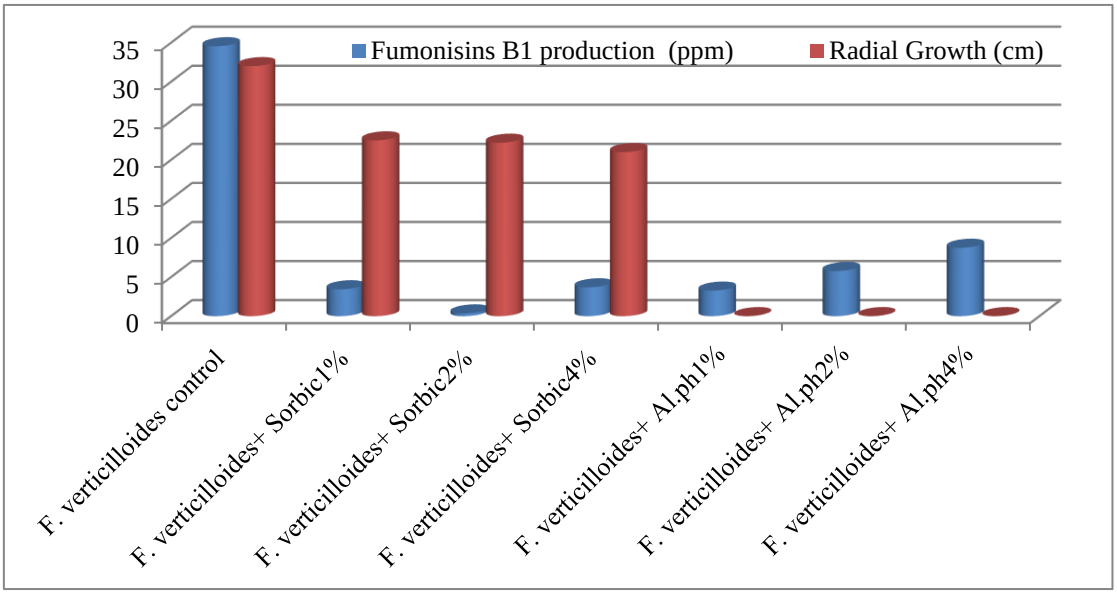

Fig. (2): Chemical control effects on *F. verticilloides* radial growth and fumonisinB1 inhibitions

Our results exhibited in **Figs. 2, 3** showed that the comparison between the effects of sorbic acid on the radial growth of *F. verticilloides* and *F. graminearum* illustrated that *F. verticilloides* behavior was different from *F. graminearum* at the same sorbic acid concentration. The sorbic acid inhibition rate of *F. verticilloides* radial growth was higher than those of *F. graminearum*, which indicated that *F. verticilloides* was more susceptible to sorbic acid than *F. graminearum*. Our findings re in harmony with those of **(Gerez *et al.*, 2016),** who reported that the sensitivity of *Aspergillus* species against sorbic acid was not similar and it depended on the fungal strain. On the other hand, in case of mycotoxins, both of the tested mycotoxins were highly inhibited by sorbic acid at the three tested concentrations but they are dose dependent. Our findings were similar to those of **(Gerez *et al.*, 2016**), who announced that the response of *Aspergillus niger* against sorbic acid was dependent on pH and sorbic dose. Furthermore, neither of the produced mycotoxins has the same inhibition efficacies.

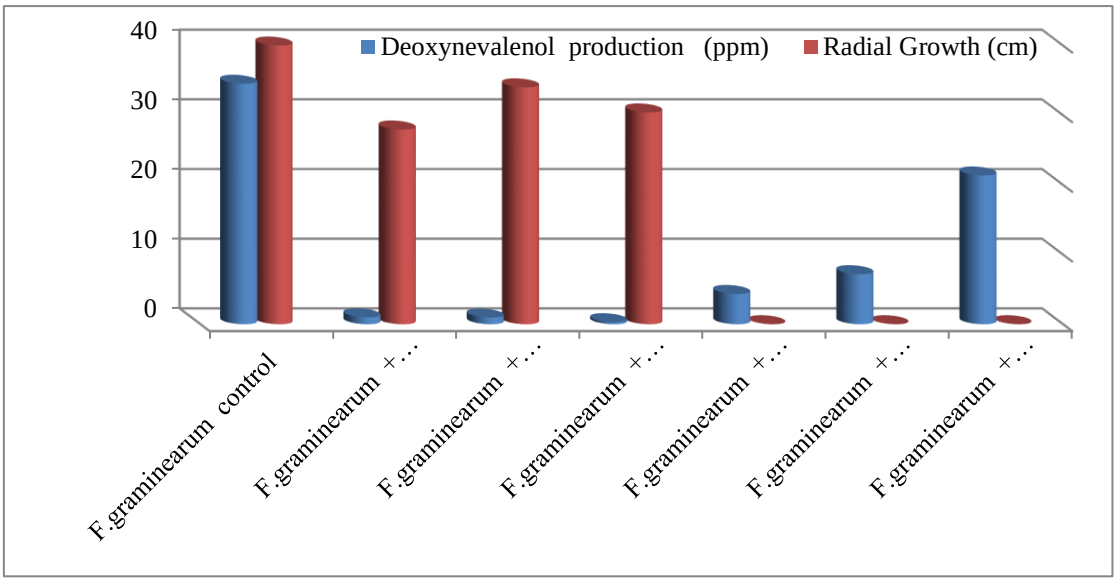

Fig. (3). Chemical control effects on *F. remuneration* radial growth and deoxynevanol inhibitions

Deoxynevalenol was more sensitive to be degraded by sorbic acid than fumonisin B1 which indicated that the difference between the structures of the tested mycotoxins play an important role in its response against the same treatment. Our findings are coincided with those of **(Youssef *et al.*, 2014),** who reported that citrinin was more sensitive to sorbic acid at the same concentration than alternariol.

CONCLUSION:

The effect of aluminum phosphide against both tested fungi was not comparable with the effect of sorbic acid because aluminum phosphide was very toxic for any biotic cells and laid to death, so the fungi are dead before it produces mycotoxins, whereas, sorbic acid was not toxic. It was used to preserve food and beverages and cannot lead to death; it only inhibited the fungal growth and reduced the mycotoxins production safely. Although the effectiveness of aluminum phosphide as a fungicide, the use of sorbic acid as antifungal and antimycotoxic agents and as an alternative to aluminum phosphide is recommended.

Conflicts of Interest: The authors declare no conflict of interest

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الملخص العربي

المكافحة الكيميائية لبعض السموم الفيوزاريومية الملوثه لحبوب القمح أثناء تخزينها بواسطة حامض السوربيك كبديل لفوسفيد الألومنيوم

محمد فؤاد حسين[1]، مصطفى عبد العظيم عامر[2]، سعد محمود شمه[2]، تغريد فكرى محمد عبدالرحمن[3] ،نسرين حسن يوسف[4]

1 الإدارة العامة للحجر الزراعى الإسكندرية – مصر

2 قسم النبات الزراعي– كلية الزراعة (سابا باشا) – جامعة الإسكندرية– مصر

3 معهدبحوث امراض النبات – مركز البحوث الزراعية – الجيزة – مصر

4 مختبرالميكروبيولوجي بالمركز الإقليمي للأغذية والأعلاف – مركز البحوث الزراعية – الاسكندرية– مصر.

يعد القمح (*Triticum aestivum*)، التابع للعائله النجيلية من أهم محاصيل الحبوب الإستراتيجية لغالبية سكان العالم. تصيب فطريات المخزن وخاصة أنواع الفيوزاريوم الحبوب بعد الحصاد التى تتطور فى نموها أثناء التخزين.حيث تنتج هذه الفطريات سمومًا فطرية تهدد صحة الإنسان والحيوان وتسبب الامراض والتى قد تنتهى أحياناً بالوفاة.

ويؤدى وجود الحشرات إلى زيادة البقع الحرارية وزيادة الظروف الملائمه لتكاثر فطريات المخزن وزيادة تلوث البذوربكميات عالية من السموم الفطرية مما أدى إلى ارتفاع قيمة خسائر إنتاجية البذور أثناء التخزين. وعادة ما يستخدم التبخير بأقراص فوسفيد الألومنيوم لمكافحة وقتل هذه الحشرات أثناء التخزين. ولم تكن هذه المادة آمنة للإنسان. لذا هدفت هذه الدراسة إلى معرفة تأثير هذه المادة ضد نمو كل من *Fusarium verticilloides* و *Fusarium graminearum* والسموم الفطرية المنتجة لهما ومقارنة تأثيرها مع حمض السوربيك كمادة آمنة ومفيدة للانسان والحيوان معا. وقد اوضحت النتائج المعمليه امكانية استخدام حمض السوربيك لتقليل النمو الفطرى للفيوزاريوم فيرتسيليودس والفيوزاريوم جرامينيرم والتثبيط الفائق لانتاج السم الفطرى دى اوكسنيفالينول يليه الفيومنسين ب1برغم التفوق التام لفوسفيد الالومنيوم الذى يبيد تماما الفطر وبالتالى لايوجد اى انتاج للسم الفطري إلا ان خطره على صحة الانسان يجعل لهذه الكفاءه محاذير صحيه كثيره لذا فاننا نوصى باستخدام حمض السوربيك بديلا آمنا لفوسفيد الألومنيوم للتحكم والمكافحه الكيميائية للفيوزاريومات محل الدراسه والتثبيط الفائق لسمومها الفطريه الدى اوكسى ليفالينول والفيومنسين ب1.