Screening of Propylparaben (PP) for control in situ populations of Aspergillus flavus and AFB₁ contamination on stored chilli powder

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ABSTRACT

Food spoilage and their contamination with mycotoxins are a significant issue for the food industry, leading to economic losses and a negative impact on public health all over the world. The objective of this study was to examine the preventive effect of different concentrations of Propylparaben (PP) for control of fungal populations and aflatoxin b₁ (AFB₁) contamination of stored chilli powder in both artificially and naturally contaminations. These treatments were examined at two different water activity (aw) levels (0.90 and 0.95 aw) in stored chilli powder at 30°C for 20 days. The total populations of A. flavus isolated from both artificially and naturally contamination of stored chilli powder at 30°C were significantly reduced by using PP treatments especially, with 2000 ppm. In additions, the AFB₁ production was reduced when increased PP concentration compared to the untreated control. In conclusion, the economic and health impacts related to Aspergillus and AFB₁ contamination could be minimised by adding PP as a food-grade preservative to stored chilli powder; Results show From a human health perspective, the use of PP is allowed as a food preservative by the (IARC) and (WHO). It must use according to legislation doses (0.1%) introduced by the law of (GRAS) regulations.

INTRODUCTION

Chillies use in Iraqi cuisines in a wide range as a type of food additives and also, used as herbal in integrative medicine. Iraq imports up to 70% of chillies from other countries because of insufficient domestic production. Chillies are prone to fungal infection, especially mycotoxigenic fungi during the ripening, handling, storage and transporta-
Table 1: Summarise statistical analyses of effects of PP (ppm) on colonisation and AFB<sub>1</sub> contamination on chilli powder inoculated with A. flavus spores at 30°C and two <i>a</i><sub>w</sub> levels for 20 days.

| Factors                  | ppm    | Time | <i>a</i><sub>w</sub> | ppm x time | ppm x <i>a</i><sub>w</sub> | ppm x time x <i>a</i><sub>w</sub> |
|--------------------------|--------|------|----------------------|------------|--------------------------|----------------------------------|
| Population               | Sb     | Sb   | Sb                   | Sb         | Sb                       | Sb                               |
| AFB<sub>1</sub> production | Sb     | Sb   | Sb                   | Sb         | Sb                       | Sb                               |

NS = not significant  S = a significant effect (P ≤ 0.05)
a) ANOVA test. b) Shapiro-Wilk test

Table 2: Shows statistical analyses of effects of PP (ppm) on in situ populations and AFB<sub>1</sub> production in natural contaminated chilli powder stored at 30°C at two <i>a</i><sub>w</sub> levels over 20 days.

| Factors                  | Effect | Action |
|--------------------------|--------|--------|
| ppm                      | S<sup>b</sup> | Population (Log<sub>10</sub> CFU g<sup>-1</sup>) |
| time                     | S<sup>a</sup> | AFB<sub>1</sub> production (Log<sub>10</sub> AFB<sub>1</sub> (µg g<sup>-1</sup>) |
| <i>a</i><sub>w</sub>     | S<sup>b</sup> | Population (Log<sub>10</sub> CFU g<sup>-1</sup>) |
| ppm x time               | S<sup>a</sup> | AFB<sub>1</sub> production (Log<sub>10</sub> AFB<sub>1</sub> (µg g<sup>-1</sup>) |
| ppm x <i>a</i><sub>w</sub> | S<sup>a</sup> | Population (Log<sub>10</sub> CFU g<sup>-1</sup>) |
| ppm x time x <i>a</i><sub>w</sub> | NS<sup>b</sup> | AFB<sub>1</sub> production (Log<sub>10</sub> AFB<sub>1</sub> (µg g<sup>-1</sup>) |

S = significant NS = non-significant effect a) ANOVA test  b) Shapiro-Wilk test

Figure 1: Effect of PP (ppm) on populations of A. flavus isolated from stored chilli powder at 30°C at two <i>a</i><sub>w</sub> levels (0.90-0.95 <i>a</i><sub>w</sub>) after 20 days. Bars indicate Standard deviation Mean.

Figure 2: Effect of PP (ppm) on AFB<sub>1</sub> accumulation on chilli powder inoculated by A. flavus at 30°C at 0.90-0.95 <i>a</i><sub>w</sub> after 20 days storage. (--) = EU legislation limits for AFB<sub>1</sub> in chillies. Bars = SDM.

Preservative in foodstuffs as an antifungal agent to control fungal populations and mycotoxin production in a variety of commodities, including beer, jams, desserts, sauces and soft drinks. Also, used in 90% water-based cosmetics such as shampoos and creams (Rahman, 2007; Panfili et al., 1992).
Propylparabens is a stable, non-volatile compound, widely used as preservatives in food, drugs because of their low toxicity to humans (Soni et al., 2001). According to the Unit State Food and Drug Administration (USFDA), PP is a synthetic chemical widely used as safe (GRAS) in food, medicine and cosmetics but should be used in concentrations below the recommended legislative limits (0.1%) and not affect nutrient values and taste (WHO, 2001). PP classified as Endocrine Disrupting Chemicals (EDC) when it has been raised in blood and alter the activity of hormones results in Breast cancer (Hadir and Hessa, 2020; Golden et al., 2005).

To my knowledge, there are no previous studies on the efficacy of PP for control of chilli contamination by mycotoxigenic fungi and AFs (Costa, 2019; Sultan, 2010). At the same time, there are a few studies on other food products. For examples, (Furukawa et al., 2016) found PP inhibited AFs production of A. flavus in corps more strongly than propyl syringate and propyl gallate.

The overall objective of this study was examining the protective effects of the PP on A. flavus colonisations and AFB\textsubscript{1} accumulation in chilli powder with two a\textsubscript{w} levels (0.90 and 0.95 a\textsubscript{w}) inoculated with A. flavus conidia and naturally contaminated chilli powder stored at the same a\textsubscript{w} conditions for 20 days at 30°C.

**MATERIALS AND METHODS**

**Collection of samples and chemical agents**

Three kg of red chilli powder was purchased from Iraqi markets and stored at 4°C until used. The AflaStar\textsuperscript{TM} – Immunoaffinity Column (IAC) were purchased from Romer Labs PLC (Tulln, Vienna, Austria). All chemicals solvents and culture media were obtained from (Fisher Scientific, UK). All solvents were of HPLC grade.

**Stock solution preparation of Propylparaben (PP)**

One g of PP was dissolved in 10 ml absolute Ethanol (HPLC grade) to the prepared stock solution (50.000 ppm). Final stock solutions werefiltrated through a 0.22 μ filter cartridge using a sterile syringe into a sterile 25 ml plastic Universal and kept at 4ºC until use.

**Preparation of fungal conidia**

The spore suspension used in this study prepared from the AFB\textsubscript{1} producing strains of A. flavus (DAJ\textsubscript{1}) were isolated previously from Iraqi chilli samples.

**Preparation of Media**

Preparation of Malt Extract Agar (MEA) has been previously described

**Preparation of chilli samples**

Effect PP and a\textsubscript{w} on total populations and AFB\textsubscript{1} accumulation in artificially contaminated chilli powder

Thirty grams of chilli powder was weighed into nine reliable culture vessels and then closed with plastic lids with a permeable membrane. The samples divided into two groups of a\textsubscript{w} conditions (0.90 and 0.95 a\textsubscript{w}). Each group of chilli samples adjusted by adding the appropriate volume of distilled water based on the moisture absorption curve of chilli powder to obtain the required target levels.

For studying the effect of PP (ppm) on the A. flavus fungal population on the chilli powder, two different concentrations were dissolved in distilled water. All the chilli treatments were treated with the appropriate doses of the preservative to obtain the target concentrations (1000 and 2000 ppm). Controls prepared by adding water only. All chilli treatments
were stored at 4°C for 24 hrs with periodic shaking to allow absorption and equilibration. After 24 hr, controls and treated samples inoculated with 0.25 ml of a 10^4 spores/ml of A. flavus (DAJ1). Each treatment was divided into three replicates replicated an each a_w levels and stored in plastic chambers at 30°C for 20 days. Each plastic chamber also contained on 500 mL beaker containing a glycerol/water solution of the same a_w, as the treatment to maintain the ERH. The A. flavus population present initially and after 10 and 20 days were quantified at each treatment. The samples were also stored for AFB_1 analyses at each treatment condition.

Efficacy of PP and a_w on the total population and AFB_1 in naturally contaminated chilli powder

Thirty grams of chilli powder was weighed into nine solid culture vessels as described previously in section (i) and all samples adjusted by adding the appropriate amount of water-based on the adsorption curve and treated with preservatives and incubated at 30°C for 20 days without inoculated with spores.

Extraction of AFB_1 from chillies using HPLC

Immune-affinity column technique (IAC)

Statistical analysis

The effects of the PP and a_w treatments on fungal populations and AFB_1 production on chilli powder were determined by using the Shapiro Wallis Test (non-normality data) and one-way ANOVA (Normality data). The significance level was at P < 0.05 for all factors.

RESULTS AND DISCUSSION

Effect of PP (ppm) and a_w on in situ control of populations and AFB_1 production on stored chilli powder inoculated with A. flavus spores

Figure 1 showed the effect of treatment with two concentrations of PP on the isolation of A. flavus conidia from chilli powder stored at two different a_w levels for 10 and 20 days. In general, the total populations of A. flavus isolated from stored chilli powder were increased with incubation time and a_w level. Also, there was gradually inhibition in the A. flavus populations isolated from the control treatment when compared with control, especially at 2000 ppm. However, there was some effect of the 1000 ppm treatment in the 0.95 a_w treatment.

Statistically, the total populations of A. flavus (DAJ1) isolated from chilli powder were significantly affected (P<0.05) by PP (ppm), time, a_w, interaction between ppm x time, ppm x a_w, time x a_w, and ppm x time x a_w (Table 1).

Figure 2 showed the effect of different concentrations of PP (ppm) on AFB_1 production in stored chilli powder inoculated with fungal spores under two a_w levels at 30°C for 20 days. The results of this study revealed that no production of AFB_1 by A. flavus at 0 days for both a_w levels. The production was gradually decreased when increased PP treatment (ppm) at 10 and 20 days at two a_w levels especially, with 0.90 a_w there was complete inhibition at 1000 and 2000 ppm over 10 and 20 days. Table 1 shows that there were significant effects (P<0.05) of PP (ppm), a_w, time, time x a_w, ppm x a_w, x time, ppm x a_w and ppm x time.

In situ efficacy of PP (ppm) on fungal populations and AFB_1 production in naturally contaminated chilli powder after treatment and storage

Figure 3 reported the effect of PP treatments on the natural occurrence of A. flavus on naturally contaminated chilli powder stored for 20 days with 0.90 and 0.95 a_w at 30°C after treatment with two doses 1000 and 2000 ppm of PP. The results found that the populations of A. flavus on stored chilli powder increased with the incubation time at both a_w conditions and affected by PP (ppm) at two a_w levels especially, with 2000 ppm reported no population under two a_w levels. Statistically, there were significant effects of ppm, a_w, time and their interactions on populations (Table 2).

Figure 4 shows the control effect of PP (ppm) on a natural accumulation of AFB_1 in stored chilli powder at two a_w levels and at 30°C for 20 days. The results of this experiment reported that the AFB_1 production in natural contamination of stored chilli powder affected by PP treatment at both a_w levels especially, with 0.90 a_w and no toxin at 2000 ppm over ten days for 0.95 a_w level. Statistically, Table 2 showed the production of AFB_1 was significantly affected by all factors except ppm x time x a_w.

The present study revealed that the treatment with different concentrations of PP had protective effects on total populations and AFB_1 production by A. flavus in stored chilli powder at 30°C with two a_w levels (0.90-0.95 a_w) over 0, 10 and 20 days in both conditions of natural and artificial contamination. Also, the results were obtained in the current study recorded that there was the significant impact of PP (2000 ppm) on total populations in stored chilli powder with A. flavus conidia spores and naturally contaminated stored chilli powder at 30°C at both two a_w levels for 20 days. Also, there was an apparent effect of PP (2000 ppm) on AFB_1 presence in both artificially and naturally contaminated chilli powder except at 0.95 a_w over 20 days.

The overall aim in this study was to minimise the
AFB$_1$ amount in stored chillies less than EU the legislative limits five $\mu g.kg^{-1}$ in chillies samples (Commission, 2006). Based on this fact and obtained data from the current study, the protective effect of PP was better, especially, at 2000 ppm for reducing economic losses and health impacts consequence from AFB$_1$ contamination in stored chillies. To my knowledge, there were no previous studies conducted on the use of PP in preventing the incidence of the fungal populations and AFB$_1$ accumulation in chilli samples (Sultan, 2010; Costa et al., 2019).

In the present study, it is very important to understand the mechanism action of PP as antifungal action. The available information on the effect of most preservatives as antifungal and control mycotoxins in food is limited and has not well understood yet (Maris et al., 2004). But in general, the mode of action of most preservatives to effect on microbial cell occur through several targets in the cell include cell membrane, nucleic acids (DNA and RNA) and key enzymes, for example, interfere with cell membrane functions and lead to inhibit transport system including transport of nutrients (Brul, 1999) Also, inhibit the action of key enzymes and interfere with the synthesis of nucleic acids (Bredin et al., 2005). For the mode of action of PP as antifungal through disrupting membrane transport systems or by inhibiting the synthesis of protein, DNA and RNA or interfering with ATPases (Sangamwar et al., 2008).

CONCLUSIONS

The total populations and AFB$_1$ production of A. flavus in both artificially and naturally contamination of stored chilli powder at 30°C and 0.90-0.95 a$_w$ levels over 20 days were significantly reduced by PP (1000, 2000 ppm). There was an apparent protective effect of PP at 2000 ppm compared with 1000 ppm, especially at 0.90 a$_w$. So, the doses used in present work consider as safe for the consumer and without health concerns. Finally, recommended carry out many studies on the efficacy of PP for control fungal colonisation and mycotoxins contamination in other foodstuffs.

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Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHER in Iraq

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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