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Proceeding Paper

Benzaldehyde Use to Protect Seeds from Foodborne Fungal Pathogens †

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Abstract: There is a limited efficacy with conventional seed sanitation methods, directly affecting food safety. The insufficient elimination of mycotoxin-producing fungi contaminating seed surfaces can result in a high mycotoxin contamination. In this study, a new seed sanitation formula was investigated by examining molecules repurposed from United States Food and Drug Administration (FDA)-approved food additives as active ingredients. Selected benzaldehyde, previously shown to inhibit mycotoxin production, could function as heat-sensitizing agent when co-applied with a mild heat. The co-application substantially enhanced the efficacy of sanitation against fungi contaminating crop seeds, whereas seed germination rate was unaffected. Therefore, benzaldehyde-based heat sensitization developed could be a promising tool to achieve safe and cost-effective pathogen control in agriculture/food production.

Keywords: antifungal; benzaldehyde; drug repurposing; food safety; heat sensitization; mycotoxins; seed sanitation

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Contamination of crops by pathogens, especially those resistant to conventional biocides, represents a major food safety/security concern [1]. Moreover, outbreaks of commodity-specific food sources, such as crop contamination by fungi-produced hepato-carcinogenic mycotoxins, directly affect the health and safety of the public.

Seed treatment enables healthy crop establishment in the fields through better seed preservation and crop protection against a wide variety of pathogens [2,3]. Compared to other protection measures, seed treatment can achieve a low-cost seed/crop protection since the treatment requires a relatively small amount of active ingredients, thus allowing sustainable disease control such as the prevention of mycotoxin contamination at the earlier stage of crop growth. The development of novel seed-treatment formulations that can be applied as alternatives to the conventional, toxic chemical compositions will also facilitate sustainable food production.

Heat treatment became one of the important strategies for the prevention of pathogen contamination in agricultural or food production [4]. Whereas, while heat treatment can ensure the reduction of pathogen contamination or postharvest decay, intensive heat treatment can result in deterioration of the quality of the crops or food products. Hurdle technology is an approach where combined application of different types of preservation method at reduced individual intensities could achieve increases in the effectiveness of antimicrobial treatments [5].

Drug or compound repurposing is the repositioning process of already marketed drugs/compounds which were previously applied for treating human diseases, foods, etc., to control microbial pathogens, weed or other pests [6]. The main merit of drug/compound repurposing is that the mode of action, molecular targets or safety of the commercial drugs/compounds have already been characterized. Considering the identification/development of entirely new anti-pest substances is an expensive and time-consuming process, the alternative approach, viz., drug/compound repurposing has been recently investigated [7]. We investigated the heat-sensitizing capability of a new seed sanitation formula as a new hurdle technology by using molecules repurposed from commercial food additives.

Antimicrobial assay: The heat-sensitizing effect of benzaldehyde currently used as food additives/derivatives [8] (Figure 1, basic structure) was examined using the mycotoxin-producing fungus *Aspergillus flavus*. Test samples were treated with mild heat (57.5 °C) or maintained at room temperature (RT; 22.0 °C), then entire samples were cultured further onto the recovery agar (potato dextrose agar (PDA)) plates for 48 hrs at 35 °C. Then, the most effective benzaldehyde was examined further at 0.1 to 0.3 mM to determine the optimum treatment condition (mild heat (57.5 °C) or (RT; 22.0 °C)).

Antifungal seed disinfection assay: Effect of benzaldehyde plus mild heat (hurdle technology) on seed sanitation was performed on *Brassica rapa* (cabbage) seeds. For seed treatments, co-application of benzaldehyde (3 mM) and mild heat (50 °C) (20 to 30 min) was investigated on *A. flavus*-contaminated seeds. The germination of seeds and fungal growth on the surfaces of germinated seeds were monitored for 7 days. Statistical analysis (student's *t*-test) was performed according to "Statistics to use" [9] where $p < 0.05$ was considered significant.

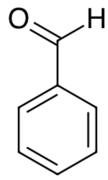

Figure 1. Structure of benzaldehyde tested in this study.

As shown in Figure 2, co-treatment of selected benzaldehyde (from above) at 3 mM and mild heat (50 °C) for 20 min completely inhibited the growth of *A. flavus*, while the germination frequency of the crop seeds was not affected when compared to the control. Either 2 mM or 4 mM benzaldehyde treatments were less effective compared to 3 mM treatment; namely, fungal contamination at 2 mM or less seed germination at 4 mM of benzaldehyde, respectively.

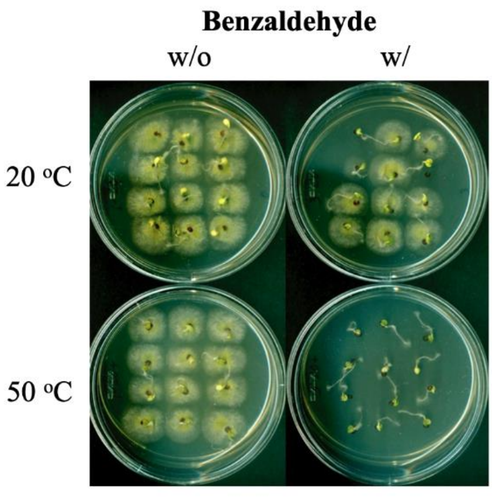

Figure 2. Representative assay showing enhanced seed sanitation via benzaldehyde-mediated heat sensitization (hurdle technology). Co-treatment of benzaldehyde at 3 mM and mild heat (50 °C) for

20 min completely inhibited the growth of *A. flavus* on the surface of seeds while the frequency of seed germination was unaffected.

In summary, new utility of repurposed benzaldehyde as heat-sensitizing agents has been identified; benzaldehyde exhibited potent heat sensitizing capability. Thus, results from this study provide means that can enhance the capacity of accepted intervention strategies, such as pasteurization/heat treatment or alternatives to toxic antifungal agents, for example, the seed disinfection agents Thiram, Ferbam and Ziram; the Pest Management Regulatory Agency (PMRA), Canada, announced the cancellation of the registration of these products in 2018 [10]. It is speculated that the prooxidant activity of benzaldehyde can disrupt the sensitive structures in microbes, thus effectively preventing the pathogen growth on seeds. Heat sensitization developed in this study will improve the efficacy of antimicrobial practices, and achieve safe, rapid, and energy/cost-effective pathogen elimination on seeds or during agriculture or food processing.

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