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SHORT COMMUNICATION

Toxicity and antifungal activity of 2, 3-dimethylmaleic anhydride against stored product beetles and plant fungal pathogens

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Abstract

The present study explores the insect toxicity and antifungal activity of 2, 3-dimethylmaleic anhydride against mixed-age cultures of several stored product insects namely: the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae); the pulse beetle, *Callosobruchus chinensis* Fab (Coleoptera: Bruchidae) and the rust-red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and plant fungal pathogens namely: *Fusarium oxysporum* f. sp. *cepa* (Hypocreales:Nectriaceae) and *Sclerotium oryzae* Cattaneo (Magnaporthales: Magnaporthaceae), respectively. The study also revealed that the mixed-age-cultures of *C. chinensis* were slightly tolerant to 2, 3-dimethylmaleic anhydride, showing 80–90% mortality in 72-h exposure at 100 μg/L dose only. Whereas mixed-age-population of *S. oryzae* and *T. castaneum* were fairly susceptible, attaining 100% population extinction at 100 μg/L doses of 2, 3-dimethylmaleic anhydride in 24, 48 or 72 h of exposure. Further, the isolated compound also inhibits mycelia growth of the two filamentous fungi under study at 5 mg/plate and inhibition was dose-dependent and species-specific. This is first to report showing the antifungal activities of 2, 3-dimethylmaleic anhydride. Finally, results reveal that 2, 3-dimethylmaleic anhydride could be potential biopesticides for plant fungal pathogens and stored product beetles.

Keywords 2, 3-Dimethylmaleic anhydride · Biopesticides · Antifungal activity · Insect toxicity · Stored product beetles · Plant fungal pathogens

Introduction

The enormous post-harvest losses and quality deterioration caused by storage pests and fungal pathogens have been the major problems constantly faced by the agriculture sector, especially in developing countries (Fleurat-Lessard 2017), which consequently contributes to the inability of achieving food security in developing countries (Kesavan and Swaminathan 2008). Currently, conventional or chemical insecticides and fungicides are mostly used to protect insect infestation and fungal attacked on stored food commodities which necessitate evaluating the performance of biopesticides as safer alternatives to conventional insecticides and fungicides (Pirgozliev et al. 2002; Rajashekar et al. 2012). In view of the high costs of pesticides and their undesirable side effects, there is a need for a model shift in the development of non-chemical technologies which may eliminate the use of synthetic insecticides and could have health benefits to the applicators, consumers and the environment. In this regard, we can look at the prospect of biopesticides as attractive alternatives to synthetic chemical insecticides/fungicides for pest management (Isman 2006). For instance, plant-derived substances with insecticidal and antifungal properties (Kordali et al. 2008; Boulogne et al. 2012; Rajashekar et al. 2013;

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Miresmailli and Isman, 2014) that are eco-friendly, are being explored.

2, 3-dimethylmaleic anhydride bioactive molecules are isolated from the root stock of *Colocasia esculenta* (L.) Schott. The isolated bioactive molecule showed potentially toxic to various stored grains beetles and household pests, including American cockroach when applied as a fumigant (Rajashekar et al. 2016). There is no information on antifungal activity and fumigant toxicity of the isolated compound on the mixed—age population of stored grain pests with effective doses for 100% mortality. In view of these aspects, insecticidal activity of the isolated bioactive compound (2, 3-dimethylmaleic anhydride) on the mixed-age-population of rice weevil, *Sitophilus oryzae* (L.), rust-red-flour beetle, *Tribolium castaneum* (Herbst), and adzuki bean weevil, *Callosobruchus chinensis* (Fab), was investigated. Further, the antifungal activity of 2, 3-dimethylmaleic anhydride to fungal plant pathogens, namely *Fusarium oxysporum* f.sp.*cepae* (Hypocreales: Nectriaceae) and *Sclerotium oryzae* Cattaneo (Magnaporthales: Magnaporthaceae) was observed.

Materials and methods

Chemicals

The bioactive molecule, 2, 3-dimethylmaleic anhydride was isolated and identified from the edible part (root stock) of *Colocasia esculenta* var. *esculenta* (L.) Schott, as reported earlier (Rajashekar et al. 2016).

Preparation of mixed-age-cultures

Whole wheat flour with 5% dried yeast fortification was used for culturing *T. castaneum* (Herbst) while the whole wheat (*Triticum aestivum* L.) was used for rearing *S. oryzae* (L.) and culture were collected from Fo. *C. chinensis* (Fab) was maintained in whole green gram (*Vigna radiata* (L.) Wilczek). All three insect cultures were collected from warehouse of Food Corporation of India (FCI), Sangaiprou, Imphal West, India. The *S. oryzae*, *T. castaneum* and *C. chinensis* of mixed-age populations were prepared by adding 3–5-day-old adult insects into insect-rearing bottle at the rate of 300 adults/kg of the respective culture medium. One-to-two-week-old adults of respective species were shifted from the cultures and maintained continuously at temperature 30 ± 1 °C and 65 ± 5% RH. This process of transferring and maintaining of 1-week-old culture was continued for six successive weeks. Cultures from all the six successive weeks were pooled and it represents all developmental stages of the respective species. These populations of individual species now served as mixed-age cultures for toxicity tests (Rajashekar 2016).

Weighed 50 g of the mixed-age population of individual species and transferred into cloth bags. These bags were placed in airtight 0.85 L desiccators as a fumigation chamber and injecting isolated bioactive compound (2, 3-dimethylmaleic anhydride) to upper lid of desiccators provided with holes sealed by rubber septa.

Collection and maintenance of fungal pathogens

The fungal pathogens, *F. oxysporum* ITCC No. 6246 and *S. oryzae*, ITCC No. 4107 were obtained from the Microbial Repository Centre, Microbial Resources Division, Institute of Bioresources and Sustainable Development, Takyelpat, Imphal, Manipur and maintained on Potato Dextrose Agar medium (PDA) in BOD incubator at 25 ± 2 °C. The well-grown fungal colonies were subcultured for further studies.

Fumigation

The fumigant activity of the isolated 2, 3-dimethylmaleic anhydride was tested on *S. oryzae*, *T. castaneum* and *C. chinensis*. 50 g of the substrate with a mixed-age population of stored product beetles were placed in airtight desiccators. The different doses of 2, 3-dimethylmaleic anhydride (10–100 µg/L) were injected using syringes through rubber septum fitted to the desiccator's lid and for different exposure (24, 48 and 72 h) at 28 ± 2 °C and 70% RH. There were four replicates per dose with an equal number of untreated control replicates for each insect. After the end of fumigation exposure, the test insect bags were taken out of the desiccators and aired with the fan or fume hood. Both live and dead adult insects were removed and percentage mortality was calculated for each mixed-age population. Further, the immature mixed-age culture was returned back to respective experimental media culture and kept at the rearing temperature and humidity conditions for 56 days. The insects, which emerged from wheat (*S. oryzae*) or pulses (*C. chinensis*) or wheat flour (*T. castaneum*) as adults in their respective media were checked at 7 days intervals for 7–8 weeks along with counts were done in untreated control batches every week. Percentages of progeny emergence were calculated by using Abbott formula (Abbott 1925).

Antifungal activity

The antifungal activity of the bioactive compound was determined on the basis of the hyphal radial rate of filamentous fungi (*F. oxysporum* and *S. oryzae*). In this study, small wells were prepared on 9-cm-diameter potato dextrose agar (PDA) using sterilized cork borer. A fixed volume of isolated compound (dissolved in ethyl acetate) with different concentration ranging from 1 to 5 mg and a carrier solvent (solvent control) were loaded in the wells and allowed to diffuse at room temperature for 2 h and incubate at 25 ± 2 °C for 5–7 days. Fungal pathogens were placed at the center of the treated and solvent control plates. Similarly, the fungal pathogens were grown in the same way on fresh PDA plates where no treatment has been given (untreated control). The radial augmentation of mycelia in all plates was measured 5 days after inoculation. Each treatment point is average of three replicates measuring of a growing colony and percentage of mycelia growth inhibition by treatment was calculated using the following (Reyes et al. 1997).

% of mycelia growth inhibition = (mycelia growth in control − mycelia growth in treated/mycelia growth in control) × 100.

Statistical analysis

Mortality in 2, 3-dimethylmaleic anhydride treatments was estimated by taking survival of adults in the control as 100% and data's were expressed as mean ± standard error of four observations ($n = 4$). The mortality data of the different insects, after arcsine transformation, were subjected to ANOVA and Duncan's multiple range test to determine significant differences between treatments.

Results and discussion

The isolated plant-derived insecticidal molecule, 2, 3-dimethylmaleic anhydride was highly toxic to three stored grain beetles. The 100% population extinction was recorded at the dosage 100 µg/L within 72-h exposure for *S. oryzae* and *T. castaneum*, whereas 87% mortality was observed at the dose of 100 µg/L in 72-h exposure in pulse beetle, *C. chinensis* (Table 1). *C. chinensis* was found to be marginally or slightly tolerant to 2, 3-dimethylmaleic anhydride at 24- and 48-h exposure. These investigations revealed an increase in mortality with increasing exposure periods at the dosage of 100 µg/L in all three test insects (Table 1). Insignificant mortality was also observed in untreated control of all three species. The mean number of progeny emerges in untreated control batches of other species varied from 80.6 ± 1.2 (*T. castaneum*), 154.3 ± 6.2 (*C. chinensis*) to 202 ± 3.2 (*S. oryzae*) in 24-h exposure; 95.3 ± 1.2 (*T. castaneum*), 178.6 ± 5.2 (*C. chinensis*) to 264.2 ± 2.1 (*S. oryzae*) in 48-h exposure and 107.2 ± 1.8 (*T. castaneum*), 173 ± 4.8 (*C. chinensis*) to 283.8 ± 4.2 *(S. oryzae)* in 72-h exposure. These results of mixed-age culture experiment indicate that 2, 3-dimethylmaleic anhydride was highly effective at the dose of 100 µg/L at 28 ± 2 °C and having potential fumigant properties. Park et al. (2003) reported that several plant essential oils and volatile organic compounds were more susceptible to *C. chinensis* than *S. oryzae*. In the present study, only 87% mortality of *C. chinensis* was achieved at 100 µg/L × 72 h exposure, while 100% mortality was observed in case of *S. oryzae* at the same dose. This may be due to sorption of food commodities. The fumigant (phosphine) sorption is more in wheat flour rather than whole wheat (Reddy et al. 2007) and further, wheat flour (as other powders) forms many little compartments and may thus slow down the movement of fumigants (Brown's molecular movement) while *Sitophilus* larvae bore a hole into their grains to get rid of frass (feces). This and the large intergranular space in grain facilitates the movement of fumigants. These could be one of the major reasons for more susceptibility of the *S. oryzae* insects with 24- or 72-h exposure of 2, 3-dimethylmaleic anhydride in wheat flour.

Further, the antifungal activity of 2, 3-dimethylmaleic anhydride was evaluated against two agriculturally important fungal plant pathogens; *F. oxysporum* and *S. oryzae*. The growth inhibitory activities of the isolated bioactive compound against two filamentous fungal plant pathogens are summarized in Table 2. The result represents that the bioactive compound showed potent inhibition of the mycelial growth at 5 mg/plate in two filamentous fungi (Fig. 1). Mycelial inhibition was obtained as 83.22%, 70.99% and 46.28% at concentration 5 mg, 3 mg and 1 mg against *F. oxysporum* whereas in the case of *S. oryzae* the mycelial inhibition was 100% in both concentrations (5 mg and 3 mg) but no inhibition at 1 mg concentration (Table 2). These results reveal that the antifungal activity of 2, 3-dimethylmaleic anhydride was dose-dependent and species-specific.

The isolated plant-derived molecule, 2, 3-dimethylmaleic anhydride showed biopesticidal activity in both the three stored grain beetles and two agriculturally important fungal plant pathogens. Although, Mansfield (1983) reported that isolated bioactive molecules from natural origin with effect on growth inhibition of fungi play a role in plant defense mechanism, the in vitro (contact assay) tests may not always give an exact indication of the significance of these compounds in restricting growth in the plant. Plants have the ability to sense the presence of potential pests, including phytopathogens, insects and thereby escalating its defense mechanisms and can produce antifungal and insecticidal compounds to protect themselves from biotic stress that can be essential for insect and fungi infection resistance (Grayer and Kokubun 2001; Mithofer and Maffei 2016). A better understanding of the plant defense mechanisms would facilitate in achieving the target of developing an alternative to synthetic pesticides.

Table 1 Percentage mortality (mean ± SE)[a] of stored product beetles exposed for 24, 48 and 72 h on 2, 3-dimethylmaleic anhydride

Doses µg/L	% Population reduction in emergence (Mean ± SE)[a]								
	S. oryzae			*T. castaneum*			*C. chinensis*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
10	2.7 ± 1.1^{a}	4.3 ± 1.2^{a}	4.6 ± 1.9^{a}	1.6 ± 0.2^{a}	2.3 ± 0.4^{a}	2.2 ± 0.2^{a}	6.1 ± 3.2^{a}	8.6 ± 0.8^{a}	5.3 ± 1.2^{a}
25	74.9 ± 1.8^{b}	87.6 ± 1.2^{c}	76.5 ± 1.8^{b}	63.1 ± 2.9^{bc}	61.6 ± 1.2^{b}	69.8 ± 0.8^{b}	33.4 ± 1.2^{b}	34.4 ± 2.1^{bc}	37 ± 4.8^{b}
50	79.2 ± 1.4^{c}	84.3 ± 1.2^{b}	80.6 ± 3.2^{c}	69.4 ± 6.5^{bcd}	73 ± 3.4^{c}	73.3 ± 1.6^{c}	42.4 ± 3.4^{c}	34.3 ± 2.6^{bc}	55.9 ± 3.2^{c}
75	82.8 ± 1.9^{d}	91.1 ± 1.2^{d}	95.7 ± 1.2^{d}	74.7 ± 6.4^{cd}	79.3 ± 1.2^{d}	76.6 ± 1.4^{d}	55.5 ± 2.8^{d}	48.1 ± 3.8^{de}	68.5 ± 4.2^{d}
100	100^{e}	100^{e}	100^{e}	98.9 ± 1.0^{e}	100^{e}	100^{e}	66.6 ± 7.4^{e}	51.8 ± 11.2^{de}	87.4 ± 9.7^{e}
Control°	202 ± 3.2	264.2 ± 2.1	283.8 ± 4.2	80.6 ± 1.2	95.3 ± 1.2	107 ± 1.8	154.3 ± 6.2	178.6 ± 5.2	173 ± 4.8

The data were expressed within a column indicate that mean ± standard error of four replicates and significantly different ($P < 0.05$) by Duncan's multiple range test

[a]50 g of mixed-age population of stored product beetles media per replicate and there were 4 replicates per dose and in untreated controls. In untreated controls, the total numbers of survivors for four replicates given

Table 2 Radial growth of mycelia colony inhibition of 2, 3-dimethylmaleic anhydride against fungal pathogens *Fusarium oxysporum* f. sp. *cepa* and *Sclerotium oryzae* Cattaneo

Concentration (mg/plate)	% Inhibition of mycelia growth (mean ± SE)[a]	
	F. oxysporum	*S. oryzae*
Solvent alone	33.16 ± 1.4[a]	0.00 ± 0.0
1	46.3 ± 1.7[b]	0.00 ± 0.00
3	70.9 ± 1.5[c]	100 ± 0.00[ab]
5	83.2 ± 1.6[d]	100 ± 0.00[ab]

The data were expressed within a column indicate that mean ± standard error of four replicates and significantly different ($P < 0.05$) by Duncan's multiple range test

[a]There were three replicates per dose

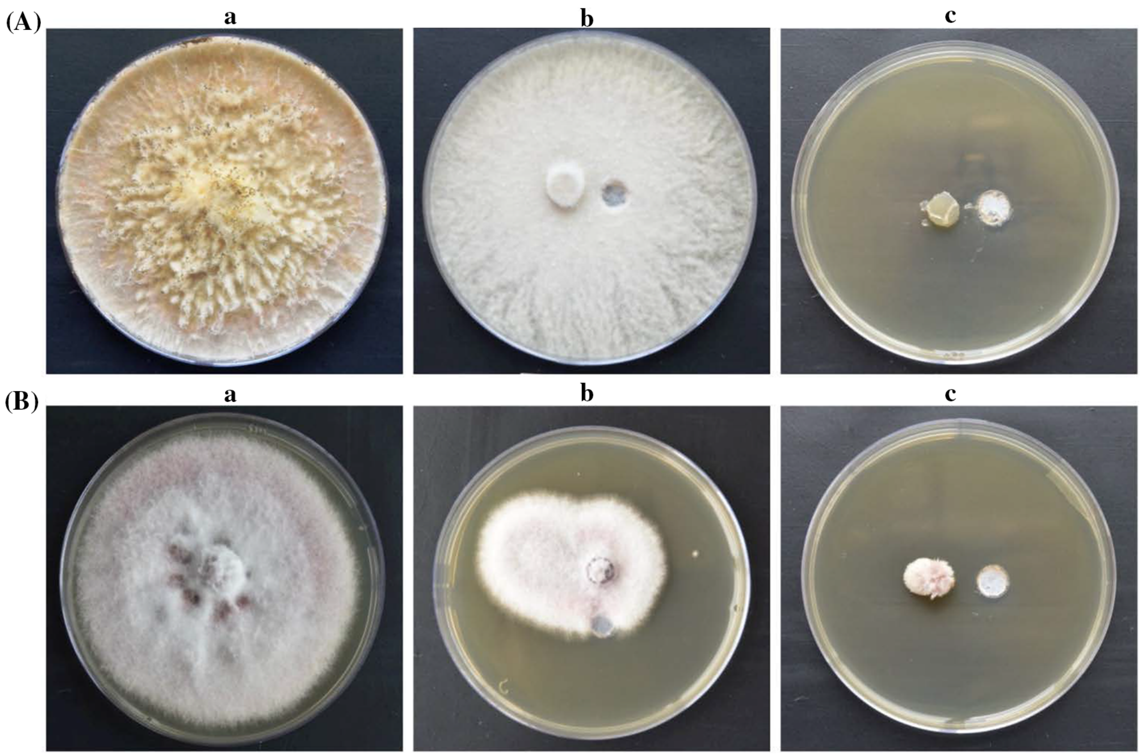

Fig. 1 Growth inhibition of fungal pathogens **A** *Fusarium oxysporum* **B** *Sclerotium oryzae* by 2, 3-dimethylmaleic anhydride. I) Untreated control, II) Solvent control, III) 5 mg/plate

The finding of the present study indicates that 2, 3-dimethyl maleic anhydride could be potential biopesticides/biofumigant. This is also first report of showing the antifungal activities of 2, 3-dimethylmaleic anhydride. However, comprehensive further research is required to authenticate the bio-safety aspects of 2, 3-dimethyl maleic anhydride to ensure environmental concerns and human health hazards.

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest.

Ethical approval All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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