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ORIGINAL ARTICLE

Fumigant toxicity of allyl isothiocyanate against phosphine-resistant populations of five major stored-grain insect pests

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Abstract. Given the development of resistance in stored-grain insect pests to phosphine (PH_3), the grain industry is seeking alternative methods for effective pest and resistance management. We evaluated the efficacy of allyl isothiocyanate (AITC), a potential alternative fumigant against adults of phosphine-susceptible (PH_3-S) and resistant strains (PH_3-R) of five major grain insect pests, including *Sitophilus oryzae* (Linnaeus), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (Fabricius), *Oryzaephilus surinamensis* (Linnaeus), and *Cryptolestes ferrugineus* (Stephens). Adult dose-mortality response curves were established for each species, and the mortality endpoints of post-fumigated adult *S. oryzae* and *T. castaneum* were compared. The effect of commodities on the efficacy of AITC was briefly investigated from the perspective of adult insect mortality. The PH_3-R strain of *S. oryzae* was the most tolerant and required the highest dose, LC_{50}: 1.75 µL a.i. L^{-1}, whereas the PH_3-R strain of *C. ferrugineus* was the most susceptible to AITC, requiring the lowest LC_{50}: 0.59 µL a.i. L^{-1}. Comparisons of $LC_{99.9}$ across the species and strains confirmed that AITC at 2.59 µL a.i. L^{-1} was adequate in achieving complete control of adults across all five insect species tested, irrespective of their resistance status to phosphine. These results suggest that phosphine-resistant insects fail to confer cross-resistance to AITC. Post-exposure endpoint mortality studies revealed a steady increase in mortality in *S. oryzae* (from 18% at 24 h to 100% at 168 h). In contrast, no such changes were recorded with *T. castaneum*, suggesting the existence of species-specific differences in responding to AITC. The presence of insect-infested commodities, such as rolled oats and cracked sorghum, reduced the efficacy of AITC, indicating that this fumigant could be sorptive.

1. INTRODUCTION

Disinfestation of stored products and processed commodities is becoming increasingly difficult due to the development of resistance to chemical treatments in major grain insect pests. Currently, the grain industry is relying heavily on phosphine, but strong levels of resistance to this key fumigant have been reported from across the globe (Lorini et al., 2007; Kocak et al., 2015; Cato et al., 2017; Nayak et al., 2020). This is a serious concern for the Australian grain industry, as nearly 80% of its harvested grain is treated with phosphine (Collins et al., 2001). Moreover, very high levels of resistance were reported in field populations of multiple grain insect pests, especially in the rusty grain beetle *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) (Nayak et al., 2013). Resistant populations of this species are difficult to control in many practical situations, consequently impeding the regular operation of the industry.

To attract premium prices for its grain commodities and maintain a competitive edge internationally, Australia follows a strict 'nil tolerance' for the detection of live insects in exporting grain (Nayak et al., 2020). Therefore, managing pest insects during storage is critical for maximising profitability and access to premium markets for Australian export grain with an estimated value of AU$ 30 billion annually (Rural Bank, 2024). In this context, the failure of current application rates of phosphine to manage the strongly resistant *C. ferrugineus* warrants the evaluation and development of potential alternative treatments (Nayak et al., 2020). Intensive research on sulfuryl fluoride and proactive collaboration with the grain industry in executing field efficacy trials confirmed that this fumigant can be judiciously applied to alleviate phosphine-resistant insects (Nayak et al., 2016; Jagadeesan & Nayak, 2017). Despite its success in overcoming phosphine resistance, sulfuryl fluoride appears to have some limitations that may hinder its practical application. First, it is relatively expensive (US$ 0.25/t for phosphine vs US$ 1.26–2.53/t for sulfuryl fluoride) (Nayak & Daglish, 2018) and leaves some level of fluoride residue in the treated commodities (Scheffrahn et al., 1989). Second, it is a greenhouse gas (Tsai, 2007), so emission reduction strategies are necessary

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(Cui et al., 2024). Therefore, there is an urgent need for alternative fumigants that are cost-effective, environmentally sustainable, and practical for use within the industry's operational framework (Nayak et al., 2020).

Allyl isothiocyanate (AITC) naturally occurs in a range of cruciferous vegetables, including mustard, radish, horseradish, and wasabi, and is responsible for their pungent taste (Brone et al., 2008; Blazevic et al., 2020). Commercially produced AITC, known as 'synthetic mustard oil', is obtained through the reaction of allyl chloride and potassium thiocyanate (Emergon, 1971). AITC is a strong repellent and holds pesticidal properties against a range of arthropods (Wu et al., 2009; Hashimoto et al., 2020), nematodes (Zanda & Ferris, 2003), and microorganisms (Park et al., 2000; Dhingra et al., 2004). Additionally, AITC has undergone rigorous reviews by the European Food Safety Authority for a range of safety considerations and was certified as harmless for human health, non-target organisms, and the environment (European Food Safety Authority, 2010).

Although AITC has demonstrated excellent fume insecticidal properties against a range of stored-product pests, its full potential is yet to be established as an alternative to the existing primary postharvest commodity fumigants, such as phosphine and sulfuryl fluoride. The toxicity of AITC against adults of many stored-product pests has been explored previously (Santos et al., 2011; Mansour et al., 2012; Vilela et al., 2020b), however, the established information is limited. No efficacy data is available yet for two of the most economically important pest species, such as *C. ferrugineus* and the saw-toothed grain beetle *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera: Silvanidae). Moreover, the information regarding AITC's efficacy against strongly phosphine-resistant populations is scarce. So far, only one study involving a single species, the red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), has been published on this aspect, and it indicated that the phosphine-resistant populations of this species are susceptible to AITC (Santos et al., 2011). Therefore, we aim to generate detailed efficacy data of AITC against phosphine-resistant (PH_3-R) and susceptible strains (PH_3-S) of five major grain insect pests. These include the rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae), the lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), *T. castaneum*, *C. ferrugineus* and *O. surinamensis*. This study also established preliminary information on post-exposure mortality endpoints on two selective species, *S. oryzae* and *T. castaneum*, and the effect of infested commodities on the efficacy of this fumigant was briefly investigated against *C. ferrugineus* alone with the perspective of establishing standard efficacy protocols. Thus, the overall information generated in this study provides the foundational efficacy data that can be used for performing more practical and applied research on AITC in the future, which is essential for its registration and use in the industry.

2. MATERIAL AND METHODS

2.1. Insect strains

In the dose-mortality response study, the PH_3-R and PH_3-S strains of five key stored-grain insect pests were tested, including *S. oryzae*, *C. ferrugineus*, *R. dominica*, *O. surinamensis*, and *T. castaneum*. The resistant strains were characterised previously as strongly resistant to phosphine based on the reported resistance levels relative to their susceptible counterparts: *S. oryzae* (52×), *C. ferrugineus* (930×), *R. dominica* (481×), *O. surinamensis* (42×), and *T. castaneum* (89×) (Jagadeesan & Nayak, 2017). The test insects were maintained in different species-specific dietary regimes in laboratory conditions of 25–30°C and 60 ± 5% RH. The dietary media used were whole organic wheat for *S. oryzae* and *R. dominica*, oats + dried yeast (96% + 4% w/w) for *O. surinamensis* and *C. ferrugineus*, and whole meal wheat flour + dried yeast (95 % + 5% w/w) for *T. castaneum*.

2.2. Standardisation of fumigant

The pure technical ingredient of AITC in liquid formulation (96.9% purity) was supplied by New Commander SL, Europe. Different working standards of AITC were prepared by diluting the technical ingredient in dimethyl sulfoxide (DMSO), and thus, 10-fold diluted AITC was used for all the toxicity bioassays. The source concentration of AITC was quantified in a gas chromatograph (GC) (Agilent Technologies, model: Agilent 8890) equipped with a flame ionisation detector (FID). Conditions and methods of GC used for the compound identification and quantification were adopted from the previous study with some modifications (Vilela et al., 2020a). The temperature of the FID detector was adjusted to 300°C, with an airflow rate of 400 mL/min. The chromatographic separation took place in an HP-5 capillary column (Agilent Technologies) with dimensions of 30 m × 320 µm × 0.25 µm. The temperature and pressure at the injector, SSL-Back with a 5 : 1 split ratio, were set at 280°C and 10.90 psi, respectively.

2.3. Dose-mortality response bioassays

Based on the results obtained from the series of preliminary range-finding experiments, detailed dose-mortality response bioassays were conducted against adults of PH_3-R and PH_3-S strains of each species. The range of AITC concentrations tested includes 0.95 to 1.7 µL a.i. L^{-1} for *R. dominica*, 0.65 to 2.7 µL a.i. L^{-1} for *S. oryzae*, 0.45 to 1.5 µL a.i. L^{-1} for *C. ferrugineus* and *O. surinamensis*, and 1 to 2.2 µL a.i. L^{-1} for *T. castaneum*. Briefly, 50 adults of each strain in each species (2–4 weeks after eclosion) were placed in a set of individual transparent polystyrene vials (10 mL). These plastic vials with test insect cohorts were sealed with perforated lids and placed inside the gas-tight desiccators (4–6 L). Before sealing desiccators with lids, the required concentrations of AITC were dispensed directly onto a 45 mm Whatman white filter paper (Sigma-Aldrich Australia, Grade 1) using a standard micropipette (10 to 1000 µL) (Eppendorf South Pacific Pty. Ltd, NSW). Control bioassays for each species were run in parallel, involving the same steps as described above, except that filter paper in the untreated control contained an equivalent volume of DMSO. The fumigation exposure lasted 24 h and was maintained at 25°C and 60% ± 5 RH. Upon completion of the exposure period, the desiccators were opened inside a fume cupboard with a running exhaust fan and aerated for 30 min. Afterwards, a teaspoonful (10 g) of suitable diet was provided to treated and untreated insects (control) in each experimental vial representing different strains and species. These vials were then shifted to a recovery room at fixed environmental conditions of 30°C and 60 ± 5% RH. Mortality assessments were carried out on

the following day (24 h post-fumigation) by examining live and dead insects in each vial exposed to each test concentration of AITC and corresponding insects in untreated controls. The entire experiment was performed twice, with three sub-replications for each concentration. The mortality response data from both experiments were combined for statistical analysis.

2.4. Post-exposure endpoint mortality bioassays

This experiment was part of a continuation of the dose-mortality response bioassays (section 2.3.). The mortality assessment of post-fumigated adults of the PH_3-R strain of *T. castaneum* and *S. oryzae* was continued up to 168 h at every 24 h interval. Observations were recorded for 1 and 1.5 µL a.i. L^{-1} test concentrations for *T. castaneum* and *S. oryzae*, respectively. This was to determine species-specific differences between *T. castaneum* and *S. oryzae* in mortality endpoints after the fumigation exposures. We only used S. *oryzae* and *T. castaneum* as they both exhibited relatively increased tolerance to AITC (section 3.1.) among the others and also establishing efficacy information on these two species, can broadly represent a model for internal and external feeding categories of stored product pests, respectively.

2.5. Impact of the commodity on the efficacy of AITC

The impact of the commodity on the efficacy of AITC was evaluated against *C. ferrugineus* only, since the level of resistance to PH_3 recorded for this species was found to be the strongest among all the other species investigated (>1000-fold). Thus, established information on *C. ferrugineus* under this compound will provide clues for further research on controlling resistant populations. A PH_3- R strain of *C. ferrugineus* was used, and three different grain commodities were tested: whole wheat (including 5% cracked grains), cracked sorghum and rolled oats. Two sets of test insects were used: (a) 50 g of each test commodity infested with adult beetles (50) in 200 mL glass jars, and (b) 50 adult beetles in an empty plastic cup (without any commodity). For the first set of test insects, the infestation was confined to 48 h prior to the fumigation exposure, mainly to acclimatise the adults. Both sets of test insects were placed inside a desiccator (6 L) for fumigation with AITC. Two concentrations of AITC, 5 and 10 µL a.i. L^{-1} were applied with a 7 d exposure period at 25°C and 60 ± 5% RH. The fumigation was performed following the same procedure as described in section 2.3. Untreated control insects were maintained in the same environment that carried filter paper containing equivalent volumes of DMSO only. At the end of the exposure period, the treated test insects, (a) and (b), were transferred to the temperature-controlled room (30°C and 60 ± 5% RH) for recovery over 24 h. The mortality assessment of insects in both treatment and control jars/vials was undertaken after the 24 h post-fumigation recovery. Each treatment had three sub-replicates, and the experiment was performed once. Only *C. ferrugineus* was treated since resistant populations of this species are difficult to control in Australia, particularly in bulk storage. Thus, establishing efficacy information against this pest holds significant economic importance for the industry.

2.6. Data analysis

Statistical analyses were performed using the software GenStat version 22 (VSN International, 2022). The dose-mortality response data were subjected to probit analysis under generalised linear regression models. If required, the percentage of adult mortality from treatments and untreated controls was compared and corrected using Abbott's formula (Abbott, 1925). The chi-square values were estimated for the overall mortality responses, and the linearity of response data was tested for goodness of fit test. Key lethal parameters, such as LC_{50} and $LC_{99.9}$ values, were determined, and the tolerance level between corresponding strains of each species was also determined (Sims & Stone, 1991).

3. RESULTS

3.1. Dose-mortality response bioassays

Results from this set of bioassays revealed that AITC was highly effective against adults of all five insect species tested, and there were no substantial differences in mortality responses between PH_3-S and PH_3-R strains across species. A comparison of the estimated LC_{50} and $LC_{99.9}$ values over the 24 h exposure period indicated that *S. oryzae* was the most tolerant species, followed by *T. castaneum*, *R. dominica*, *O. surinamensis* and *C. ferrugineus* (Table 1). For example, the estimated LC_{50} and $LC_{99.9}$ values were 1.75 and 2.59 µL L^{-1} for PH_3-R strain of *S. oryzae* and 0.59 and 0.87 µL L^{-1} for the least tolerant species, *C. ferrugineus* (PH_3-R strain), respectively (Table 1). Additionally, the tolerance levels for the PH_3-R strains fell within the close vicinity of 1.0, indicating that there were no substantial differences in mortality responses to AITC between the two PH_3-R and

Table 1. Probit dose-mortality responses of PH_3-R and PH_3-S strains of five key stored-grain insect pest species to AITC. The fumigation bioassays were performed over a 24 h exposure period at 25°C and 60 ± 5% RH.

Species and strain	Slope	LC_{50} (95% FL) (µL L^{-1})	$LC_{99.9}$ (95% FL) (µL L^{-1})	TL at LC_{50}	*df*	χ^2	*P* value
Cryptolestes ferrugineus							
PH_3- S	18.81	0.60 (0.59–0.61)	0.89 (0.87–0.92)	–	4	0.15	0.90
PH_3- R	15.96	0.59 (0.58–0.60)	0.87 (0.85–0.89)	0.98	4	0.58	0.60
Oryzaephilus surinamensis							
PH_3- S	20.81	0.61 (0.60–0.62)	0.91 (0.89–0.93)	–	4	3.50	0.16
PH_3- R	16.70	0.64 (0.63–0.65)	0.94 (0.92–0.97)	1.04	5	0.31	0.75
Rhyzopertha dominica							
PH_3- S	32.29	1.01 (1.0–1.03)	1.50 (1.47–1.53)	–	4	1.35	0.28
PH_3- R	20.89	1.08 (1.07–1.10)	1.60 (1.57–1.64)	1.06	4	3.69	0.09
Tribolium castaneum							
PH_3- S	17.08	1.08 (1.07–1.10)	1.61 (1.57–1.64)	–	6	0.44	0.96
PH_3- R	20.32	1.28 (1.26–1.30)	1.90 (1.86–1.95)	1.18	5	0.40	0.72
Sitophilus oryzae							
PH_3- S	21.30	0.80 (0.78–0.81)	1.18 (1.16–1.21)	–	5	5.38	0.17
PH_3- R	26.12	1.75 (1.72–1.77)	2.59 (2.54– 2.64)	2.18	5	0.80	0.97

LC – lethal concentration; FL – fiducial limit at 95% probability; TL – tolerance level (LC_{50} of PH_3-R strain/LC_{50} of PH_3-S) of each species; *df* – degree of freedom; χ^2 – Chi-square; *P* value – probability at 0.05 significance.

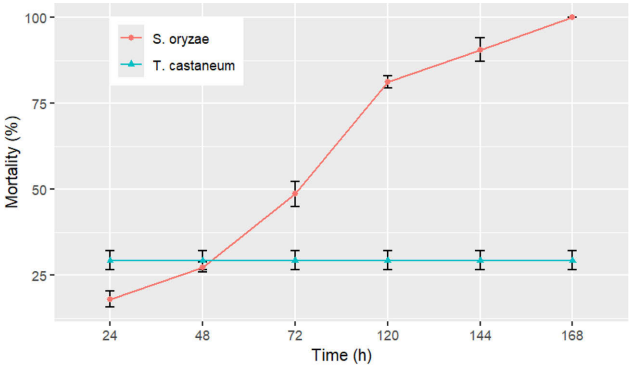

Fig. 1. Cumulative mean percent adult mortality ± standard error (SE) recorded with PH_3-R strains of *Sitophilus oryzae* and *Tribolium castaneum* at 24 h intervals over 168 h (150 adult beetles per species, n = 3).

PH_3-S strains for all tested species and, hence, confirming that phosphine-resistant insects fail to confer resistance to AITC (Table 1). Although only *S. oryzae* showed a 2.18-fold tolerance level to AITC in the current study, the entire dose-mortality response curve of the PH_3-R strain resembled that of the PH_3-S strain; thus, observed differences could be due to species-specific responses to the fumigant. The estimated fiducial values were also very narrow, and therefore, the obtained values for some species did not overlap, especially for *S. oryzae* and *T. castaneum*. Slope values across the species ranged from 15.96 (PH_3-R strain of *C. ferrugineus*) to 32.29 (PH_3-S strain of *R. dominica*), demonstrating variations in mortality responses among species or strains to AITC were lower.

3.2. Post-exposure endpoint mortality bioassays

The results of post-exposure endpoint mortality bioassays indicated that percent mortality in *S. oryzae* increased progressively throughout the assessment period. The initial mortality recorded was 18% at 24 h and reached 100% at 168 h, and hence, significant differences were observed among the mortalities recorded at different mortality assessment times (Fig. 1). However, such changes in mortality were not observed for *T. castaneum* between the initial (24 h) and final mortality assessment points (168 h) (Fig. 1). Test insects in untreated controls remained normal over the entire assessment period.

3.3. Impact of the commodity on the efficacy of AITC

Among the tested commodities, *C. ferrugineus* adults in rolled oats had the lowest mortality. It was 15.41% at 5 µL L^{-1} and 21.87% at 10 µL L^{-1}. AITC at 5 µL L^{-1} fumigated on cracked sorghum caused a relatively higher mortality of 85.32%, and a high dose of 10 µL L^{-1} caused complete insect mortality in the same commodity. However, complete control was achieved with the insects treated in whole wheat and empty cups without grains at both doses. No mortality was observed in the control, confirming that the observed effects are due to treatments alone (Table 2).

Table 2. Impact of commodities on the efficacy of AITC against adults of PH_3-R strains of *Cryptolestes ferrugineus* (n = 3, mean ± SE). The fumigation exposure lasted 7 days at 25°C and 60 ± 5% RH.

Experimental parameters		Adult mortality (%)
Commodity	Dose µL L^{-1}	(mean ± SE)
No grains (Insects alone)	0	0
	5	100
	10	100
Whole wheat grains + Insects	0	0
	5	100
	10	100
Cracked sorghum + Insects	0	0
	5	85.32 ± 3.26
	10	100
Rolled oats + Insects	0	0
	5	15.41 ± 2.28
	10	21.87 ± 1.88

4. DISCUSSION

In the current study, as part of exploring the potential of AITC as a postharvest grain fumigant, we established detailed dose-mortality response data against adults of strongly phosphine resistant (PH_3-R) and susceptible (PH_3-S) strains of five major stored-product pest species. Our results demonstrated that, irrespective of differences in their susceptibility status to phosphine, AITC was very effective against strains of all five major pest species tested. Complete adult mortality in all five insect species was achieved at very low concentrations of AITC, 0.87–2.59 µl L^{-1} over a 24-h exposure period, suggesting that this fumigant could be used as a rapid disinfestation tool targeting highly mobile adult stages. The tolerance hierarchy among the species based on the established LC_{50} values is as follows: *S. oryzae* > *T. castaneum* > *R. dominica* > *O. surinamensis* > *C. ferrugineus*. No substantial differences in tolerance to AITC were observed between the PH_3-R and PH_3-S trains of each species, as the estimated tolerance levels were close to 1.0 for the four tested species, confirming that there was no cross-resistance to AITC in phosphine-resistant insects. Although a slightly increased tolerance level of 2.18-fold was observed with *S. oryzae*, the mortality response curves of both PH_3-S and PH_3-R strains at multiple concentrations remained almost the same. It confirms that the observed differences in tolerance levels could be transient and related to other biological attributes. Similar results on the lack of cross-resistance to AITC in PH_3-R strains were also reported in previously published studies (Santos et al., 2011; Freitas et al., 2016), corroborating the key findings of our study.

The lethal LC_{50} and $LC_{99.9}$ values estimated for *T. castaneum* in this study are more or less similar to the values reported in China for the flour beetle, *Tribolium ferrugineum* (Fabricius) (LC_{50}: 1.61, LC_{90}: 2.82 g mL^{-1}), even though the bioassays of this study were slightly different and used a different formulation of AITC, extracted from the horseradish plant (*Armoracia rusticana*) and a longer exposure period of 72 h (Wu et al., 2009). Conversely, the lethal values reported for multiple field populations of *T.*

castaneum from Brazil were 3-fold higher and in the range of 3.74–4.66 and 4.86–7.57 μL L^{-1} for LC_{50} and LC_{95}, respectively (Santos et al., 2011). Similarly, the lethal values estimated for a Chinese strain of the confused flour beetle *Tribolium confusum* (Jacquelin Du Val) (LC_{50}: 4.7 μL L^{-1}, LC_{95}: 9.3 μL L^{-1}), a closely related species to *T. castaneum*, exhibited 4-fold higher tolerance than the values estimated for *T. castaneum* in the current study (Mansour et al., 2012). These reported differences in toxicity values can be attributed to the usage of different AITC formulations between the studies. It is also evident that substantial differences in the susceptibility to AITC exist among insect populations of different geographical origins, emphasising the importance of establishing regional toxicity data utilising native insect populations.

Like *T. castaneum*, the overall toxicity patterns of *S. oryzae* and *R. dominica* were consistent with previous studies. Still, some variations were found due to the potential differences in the bioassay protocols and insect inherent tolerances. For instance, the lethal parameters estimated for *S. oryzae* in the current study are slightly lower than those in previously published data for *S. oryzae* (LC_{50}: 2.6 μL L^{-1}, LC_{95}: 4.7 μL L^{-1}) from China (Mansour et al., 2012). However, the toxicity values reported for the maize weevil *Sitophilus zeamais* (Motschulsky) remained more or less equivalent (LC_{90}: 1.83 g mL^{-1}) (Wu et al., 2009) to the results obtained in the present study. Interestingly, some populations of *S. zeamais* from Brazil were reported to have extremely high tolerance to AITC, and the registered LC_{95} values were 3-4-fold higher (6.8 to 9.56 μL L^{-1}) than the reported values from China (Wu et al., 2009) and the current study (Freitas et al., 2016; de Souza et al., 2018). Comparison of the multi-geographical toxicity reports, mainly from Australia, China, and Brazil, indicated that the populations of *Sitophilus* spp originating in Brazil tended to have a higher intrinsic tolerance to AITC than those from other countries. Thus, the differences observed between *S. zeamais* and *S. oryzae* may not be exclusive to species variation, but also due to geographical impacts. Our results reaffirm the conclusion that the geographical variation among the pests of stored products plays a critical role in determining the toxicity of AITC, and therefore, establishing efficacy data against the local populations is mandatory before the registration of new insecticides/fumigants can be considered (Santos et al., 2011). In addition to geographical and species-driven differences, other key factors, such as methods and approaches to performing AITC fumigation bioassays, could contribute to the differences in the observed results on efficacy. For example, in the present study, dimethyl sulfoxide (DMSO) was used to deliver the required concentration of AITC in the fumigation assays, whereas some studies used different diluents such as soybean oil and methanol (Paes et al., 2012; de Souza et al., 2018; Vandicke et al., 2020).

Regarding *R. dominica*, the published lethal estimates, LC_{50} and LC_{95} of the Chinese strain, were 0.41 and 1.17 g mL^{-1}, respectively (Wu et al., 2009), which are in alignment with the results of the current study. Limited research in this area restricted us from making broader comparisons and contrasts, emphasising the necessity of generating more detailed information for this pest species.

The efficacy data reported on *C. ferrugineus* and *O. surinamensis* in the current study is new information. As far as we know, no prior information is available for these two species globally. Thus, our research strengthens the overall efficacy profile established for AITC as a potential quick disinfestation tool for controlling adults. The fact that strongly phosphine-resistant *C. ferrugineus* was the most susceptible species to AITC among the other insect species tested in this study carries significant importance for the grain industry globally, as resistant adults of this species exhibit very high levels of resistance (>1000-fold) to phosphine and require a lengthy phosphine fumigation period for complete control (Nayak et al., 2013; Jagadeesan et al., 2021). These findings for *C. ferrugineus*, along with the lack of cross-resistance in the other four grain insect pests investigated in the present report, suggest that AITC holds potential for the management of phosphine-resistant pests.

Insecticides/fumigants of different groups act at different speeds on target organisms, and thus, the mortality evaluation timing (endpoint) could affect predicting the dosage-mortality relationship (Beard, 1949). To address this and to complement the adult efficacy data for AITC, we determined the endpoint mortality of post-fumigated adults of the two most important species, *S. oryzae* and *T. castaneum*. Comparisons of endpoint mortality assessments from 24 h to 168 h confirmed that the toxicity of AITC was immediate with adults of *T. castaneum*. In contrast, the mortality increased incrementally over time (cumulative) for *S. oryzae*. This difference was substantially high (from 18 to 100%), indicating that post-exposure mortality assessment times need to be standardised for AITC against all the insect species. This result also hinted that the observed higher tolerance level in *S. oryzae* (2.18-fold) was transient and did not last after 48 h post-fumigation. Winks (1986) warned that early mortality assessments for insects fumigated with phosphine could mislead the estimation of the overall dose-mortality relationship.

The current study also pointed out that the type of grain commodities used in the AITC bioassays influences adult mortality in *C. ferrugineus*. Complete mortality was achieved with adults fumigated with and without wheat, suggesting that lethal concentrations of AITC were available in the atmosphere or intergranular space inside the desiccator in both scenarios. However, a significant proportion of *C. ferrugineus* adults survived the AITC treatment when they were placed on rolled oats and cracked sorghum (5 μL L^{-1} dose only), indicating that most of the applied AITC might have been bound to these two commodities, perhaps due to sorption. Such scenarios of differential sorption of fumigants on multiple commodities were well documented in previous studies (Reddy et al., 2007; Daglish & Pavic, 2008; Hwaidi et al., 2015). Thus, the observed differences in the efficacy of AITC against adults in different dietary media could be due to the sorption of this fumigant in selected commodities, and more exclusive studies involving

a range of commodities, exposure periods, and multiple-dose rates and temperatures are essential to establish practical fumigation protocols for AITC.

The current study confirms that AITC is effective against adults of five major stored-grain insect pests, including strongly phosphine-resistant individuals. The fact that it caused complete adult mortality at very low concentrations (2.59 µL L^{-1}) over a 24-h exposure period indicates that AITC holds the potential for postharvest fumigation treatments, at least for the disinfestation of actively moving insect life stages. Preliminary studies on the effects of AITC on grain commodities indicate that AITC is more sorptive in processed grains like rolled oats and cracked sorghum than in wholegrain wheat. Detailed efficacy and sorption studies of AITC utilising all developmental life stages of insects are currently underway, with the perspective of establishing comprehensive efficacy information for this fumigant.

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