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Short-Exposure Biological Activity of Dichlorvos Insecticide Strips on Coleopteran Storage Pests under Two Evaporation Regimes: Can Slow-Release Dichlorvos Formulations Replace Aerosols?

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VS, GO and RA presented the concept and wrote the manuscript. RA and VS designed the methodology, administrated the project and acquired the funds.

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DDVP, Organophosphate, Aerosols, Evaporation rate, Grain stores, Storage arthropods.

ABSTRACT

Because of new restrictive DDVP regulations we investigated efficacy of short exposures of slow- release DDVP strips from the perspective of increasing the human safety. Therefore, we determined efficacy of 1-, 6-, and 24-h DDVP (evaporation rate: 0.15 ± 0.02 g.day^{-1}) strip exposures on 5 stored-product pests, namely, *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Rhyzopertha dominica* and *Sitophilus granarius* in an experimental chamber. We tested two DDVP evaporation regimes from the strips, namely, "preventive" and "repressive". In the "preventative" regime, the strips are introduced 168 h before pest exposure whereas in the "repressive" regime strips are introduced concurrently with pests. Based on our data, mortalities ranged from 0 to 100% depending on species. The most sensitive species was *O. surinamensis* whereas the most tolerant was *R. dominica*. At 1- and 4-h exposure periods, there were significant differences in mortality between repressive and preventive regimes. However, no differences between regimes existed for 24-h exposure. Our data show that short term exposure to DDVP strips have suppression effect for *O. surinamensis* but cannot fully replace long term exposure of strips or high dose DDVP aerosols for *T. castaneum*, *C. ferrugineus*, *R. dominica* and *S. granarius*.

INTRODUCTION

Fumigants and aerosol insecticides are key pesticide formulations for the control of urban and stored product pests (*e.g.* Arthur, 2008; Boina and Subramanyam, 2012; Subramanyam at *et al*., 2014; Aulicky and Stejskal, 2015; Aulicky *et al*., 2015a, b). Dichlorvos or 2,2-dichlorovinyl dimethyl phosphate (DDVP) is an organophosphate insecticide that is historically among the most efficient and successful pest control active ingredients; it is extremely effective against a wide range of glasshouse, veterinary, stored product, and urban pests (*e.g.* Jay *et al*., 1964; Lehnert *et al*., 2011). Its unique position among other insecticides is because of its high volatility which enables DDVP to penetrate hidden places thereby killing pests and its high toxicity at very low concentrations. Due to its effectiveness, DDVP has been used as a residual spray and direct protective admixture in grain stores (Sthong and Sbur, 1964; Arthur, 1996). Bins stored in warehouses have been subjected to space treatments with dichlorvos vapors from impregnated resin strips or using ultra-low volume application of DDVP with a fogger (Bullington and Pienkowski, 1993). In flour mills and seed stores DDVP has been used as a spray aerosol (Harein *et al*., 1971; Stejskal *et al*., 2014a, 2015, Subramanyam *et al*., 2014); it has also been used as slow release evaporative formulations. The DDVP evaporation formulations are based on either solid plastic or resin matrix formed as pellets or strips. Aerosols do not penetrate into solid materials like gas fumigants (Stejskal et. al., 2014b; Riaz *et al*., 2017) but evaporation formations compensate this deficiency by providing long lasting protection. The aerosol formulations are released from ULV and thermal FOG generators or cans/foggers (Tenhet *et al*., 1958; Childs *et al*., 1966; Harein *et al*., 1971; Cogburn and Simonaitis, 1975). Spray aerosols have high concentration and instant distribution over space and are used for quick pest repression (Arthur, 2008); for prevention and long term protection, slow release strips are used (Bengston, 1976).

DDVP leaves residues in air and commodities (Harein

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et al., 1971; Collins and de Vries, 1973); however, their concentration diminishes due to low stability. Exposure to high DDVP concentrations or chronic exposure is harmful to human health. Therefore, there exists a serious international debate on the health risks of DDVP use (Gillett *et al*., 1972). For example, DDVP has been shown to cause no change in clinical parameters of blood and urine following exposure of pest control operators (Das *et al*., 1983). However, a total of 31 cases of acute DDVP pest strip-related illnesses have been reported in U.S.A. and Canada (Tsai *et al*., 2014). To clarify, a majority of these illnesses were found to have resulted from use of the product in violation of label directions; *i.e.* mostly use of DDVP pest strips in areas occupied by people for ≥4 h/day. The different approaches to pest and insecticide risk analysis that exist means there are different national regulations for DDVP. Many countries have refused to prolong DDVP registration. According to Ciesla and Ducom (2010), mills would be negatively impacted by a DDVP ban given that there are no alternatives that are equally effective and have similar desirable qualities. DDVP cannot simply be replaced by registered pyrethroid aerosols because DDVP has higher efficacy and penetration of hidden places (Boina and Subramanyam, 2012); there is also increasing resistance to pyrethroids (*e.g.* Horowitz *et al*., 1998). Despite lack of alternatives, DDVP sprays and aerosols are now banned or strongly restricted in most countries worldwide. Nevertheless, DDVP is still registered in U.S.A. and many African and Asian countries albeit with stricter new regulations. In U.S.A., foggers are generally no longer allowed but are permitted for veterinary (cattle) and poultry spray application for DDVP+tetrachlorvinphos. Several slow release strip formulations are also still registered. Slow release DDVP strips are allowed for homes, farms and stores with some agricultural commodities-including bulk storage of raw grains, namely, corn, soybeans, cocoa beans and peanuts, where DDVP strips can be hung in the store space above stored commodities. However, strips must not be used in any area where people will be present for extended periods of time.

The justification for the present study are the aforementioned new international DDVP regulations, *i.e.*, a ban on fast acting aerosols and new imperatives that decrease the length time of human exposure in order to increase safety during usage of DDVP strips. A practical concern arising from these new label restrictions is how effective the DDVP strips will be if they are used for short exposures. Therefore, we investigated effectiveness of short exposures of DDVP strips against five key (Stejskal *et al*., 2014, 2015) stored-product pests, namely, *Tribolium castaneum* (Herbst, 1797) (Tenebrionidae; Red flour beetle), *Oryzaephilus surinamensis* (Linnaeus, 1758) (Silvaniade; Saw-toothed Grain Beetle), *Cryptolestes ferrugineus* Stephens 1831 (Cucujidae; Rusty Grain Beetle), *Rhyzopertha dominica* (Fabricius, 1792) (Bostrichidae; Lesser Grain Borer) and *Sitophilus granarius* (Linnaeus, 1758) (Curculionidae; Grain Eeevil) in a standard experimental chamber. Additionally, we compared two evaporation regimes, namely, "preventive" and "repressive". In the "preventive" regime, the strips are introduced 168 h before pest exposure thereby limiting human exposure. For the "repressive" regime, strips are introduced concurrently with pests. The preventive regime is the traditional method for using DDVP strips in warehouses and storerooms and cannot be used according to the new regulatory rules which restrict human exposure to no more than 4 h. The repressive regime can be used under the new legislation imperatives to decrease human exposure time.

Fig. 1. Visualization of the experimental chamber with the internal replaceable plastic tent.

MATERIAL AND METHODS

Insects

Experiments were conducted in four separate experimental chambers (AB-3, AB-Cont s.r.o., Czech Republic). Three chambers were used for DDVP-related treatments whereas the remaining chamber was used as untreated control. Each chamber was equipped with an internal replaceable plastic tent (Fig. 1) in order to ensure high level of isolation (hermetic conditions), replication, and to prevent contamination of chamber walls by DDVP. The volume of each chamber was 20 m^3. Adults of five species of stored-product insect pests, namely, *S. granarius*, *T. castaneum*, *O. surinamensis*, *C. ferrugineus* and *R. dominica* were tested. The ratio of males to female for each species was 1:1. Strains of the beetles used were taken from cultures kept at the Crop Research Institute, Prague, Czech Republic. The strains used in the DDVP exposure experiments were collected in the Czech grain stores and kept in the laboratory no longer than 10 generations.

Fig. 2. Fragment (30 g) of the porous strip evaporating strip: Detail of porous structure.

Bioassays

Beetles were transferred to 6-cm diameter Petri dishes containing 1 piece of oat flake (27.7±1.8 mg) the day before experiment. The inner wall of each Petri dish was coated with Fluon to prevent insects from escaping during the experiment. Ten unsexed insects of each species were separately placed in each Petri dish and 5 Petri dishes were placed in each experimental chamber. The placement of Petri dishes was conducted in a regular pattern with 4 dishes near the walls (30 cm from the walls) of the chamber while one dish was placed in the center of the chamber. Each exposure time (1, 4, or 24 h) and evaporation regime (preventive or repressive) combination was replicated three times, *i.e.,* each treatment was replicated three times. After exposure, insects were transferred to the clean dishes containing 10 pieces oat flakes (238.4 ± 6.0 mg) and then placed in a thermo-box (TB 300, AVIKO Praha, s.r.o., Czech Republic) maintained at 23°C and 75 % r.h. for 48 h. According to Boina and Subramanyam (2012), mortality due to DDVP aerosol exposure becomes pronounced after 24 h after exposure. Therefore, we assessed mortality after 48 h – this was even more justified by the fact that exposure periods used were quite short (1, 6, and 24 h).

Dichlorvos (DDVP) evaporation strip and exposure regimes

The brand of commercially available dichlorvos evaporating strips used was Detmol-strip (Frowein, GmbH, Albstadt, Germany); these contained 360 g of dichlorvos.kg^{-1} of strip. The label rate for strips of this particular formulation is 1 strip.50 m^{-3}. To achieve the label dosage for a 20-m^3 chamber, pieces of strips containing 30.66±0.59 g DDVP were cut accordingly (Fig. 2). Prior to the experiment, the daily DDVP evaporation rate (Fig. 3) of the strips was estimated gravimetrically under conditions identical to those that prevailed during the experiment, *i.e.* in the center of 20-m^3 chambers with inner plastic tents (Fig. 1) and maintained at 23°C and 75 % r.h. Two DDVP evaporation regimes, namely, "preventive" and "repressive" were tested. In the "preventative" regime, the strips are introduced 168 h before pest exposure whereas in the "repressive" regime strips are introduced concurrently with pests. The preventative regime is the traditional method for using DDVP strips in warehouses and storerooms and cannot be used according to the new regulatory rules which restrict human exposure to no more than 4 h (*e.g.* Otto *et al*., 2016). The repressive regime can be used under the new legislation imperatives to decrease human exposure time. The exposure and dosage of dichlorvos from the strip are based on experimental hypothesis and are not supported by the label of the product used.

Statistical analysis

Data were subjected to non-parametric tests using the Kruskal-Wallis ANOVA and Mann-Whitney U test, using the statistical program Statistica 10.0 (released November 2010; StatSoft - Dell Software, USA).

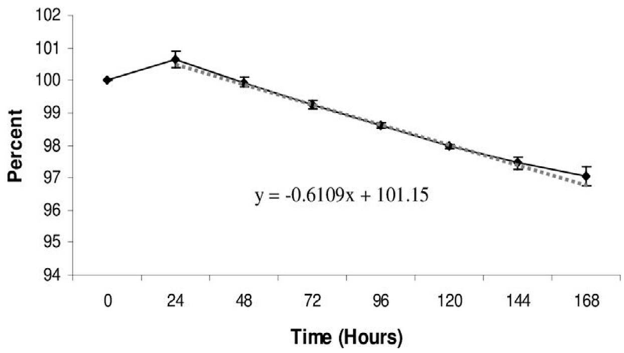

Fig. 3. Temporal percentage change in weight by DDVP after their opening in the experimental chamber. The black line shows change in weight from the initial 30.66 ± 0.59 g of DDVP strips (n =3) which is the result of gradual evaporation of the active ingredient in the strips. The dotted line represents the linear regression line that describes DDVP evaporation rate (data for the first day is excluded because of weight increase caused by initial moisture absorption from air into the porous matrix of the strip).

Table I.- Mortality (%) of five species of stored product beetles (Coleoptera) caused by 1, 4 and 24 h exposures under two DDVP evaporative regimes namely, preventive and repressive. Preventative DDVP evaporation regime (Prev) is where strips were introduced 168 h before pest exposure whereas the repressive evaporation regime (Repr) is where the strips were introduced concurrently with pests. Different letters within columns indicate significant differences at α < 0.05. Mortality was determined 48 h after exposure.

	Exposure					
	1 h		6 h		24 h	
	Prev.	Repr.	Prev.	Repr.	Prev.	Repr.
Oryzaephilus surinamensis	87.33±2.67b	41.33±9.95b	100.00±0.00b	90.00±2.18b	100.00±0.00b	99.33±0.67b
Tribolium castaneum	39.30±3.16b	0.00±0.00a	81.33±3.63bc	70.67±4.41bc	90.67±2.84b	74.00±3.63b
Cryptolestes ferrugineus	1.33±0.91a	0.00±0.00a	50.67±5.11ac	26.00±2.73cd	87.33±3.30b	82.67±4.08b
Sitophilus granarius	5.33±1.33a	0.00±0.00a	9.33±2.28ad	0.00±0.00a	42.67±3.58a	30.67±3.96a
Rhyzopertha dominica	2.67±1.53a	0.67±0.67a	6.67±1.59ad	2.00±1.07ad	35.33±3.36a	24.00±2.89a

Prev., preventive evaporation regime; Repr., repressive evaporation regime.

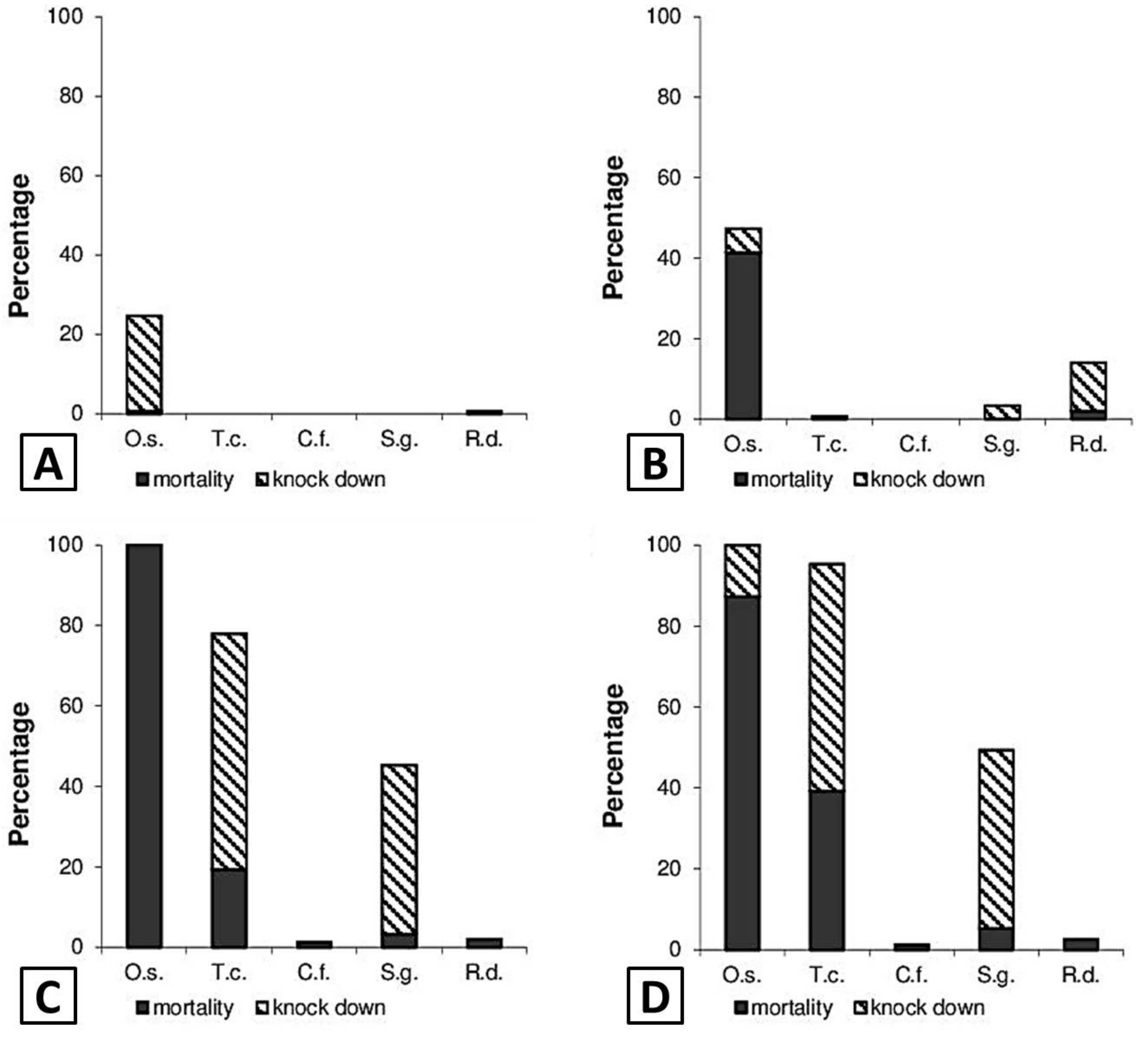

Fig. 4. Efficacy of DDVP strip on five species of stored product beetles (Coleoptera): 1 h exposure under repressive evaporative regime: A, mortality estimated 24 h after exposure; B, mortality estimated 48 h after exposure; and preventive evaporative regime: C, mortality estimated 24 h after exposure; D, mortality estimated 48 h after exposure.

RESULTS

Daily evaporation rate in the chambers was determined to be 0.15±0.02 DDVP grams of the initial weight of 30.66±0.59g (Fig. 3). Figures 4, 5 and 6 showed efficacy of DDVP strips on five species of stored product beetles (Coleoptera) after 1 h and 6 or 24 h of exposure under repressive evaporative regime or preventive evaporative regime, respectively. Mortalities ranged from 0 to 100% depending on the species, exposure time, and evaporation regime (Table I). The most sensitive species was *O. surinamensis* whereas the most tolerant was *R. dominica*. For the pooled data for all species and exposure times, there were significant difference in mortality between repressive and preventive treatments ($Z = 4.01$, $p = 0.01$) and among exposure times ($N = 450$, $H = 135.29$, $p = 0.01$). Multiple comparisons showed significant differences among the exposure times 1, 4, and 24 h. For two of the three exposure times, there were significant differences in mortality between repressive and preventive treatment, *i.e.,* at 1 h ($Z = 4.14$, $p = 0.01$) and 4 h ($Z = 2.22$, $p = 0.03$) there were differences, but not 24 h ($Z = 1.82$, $p = 0.07$). Differences between preventive and repressive evaporation regimes were not practically important, despite being statistically significant.

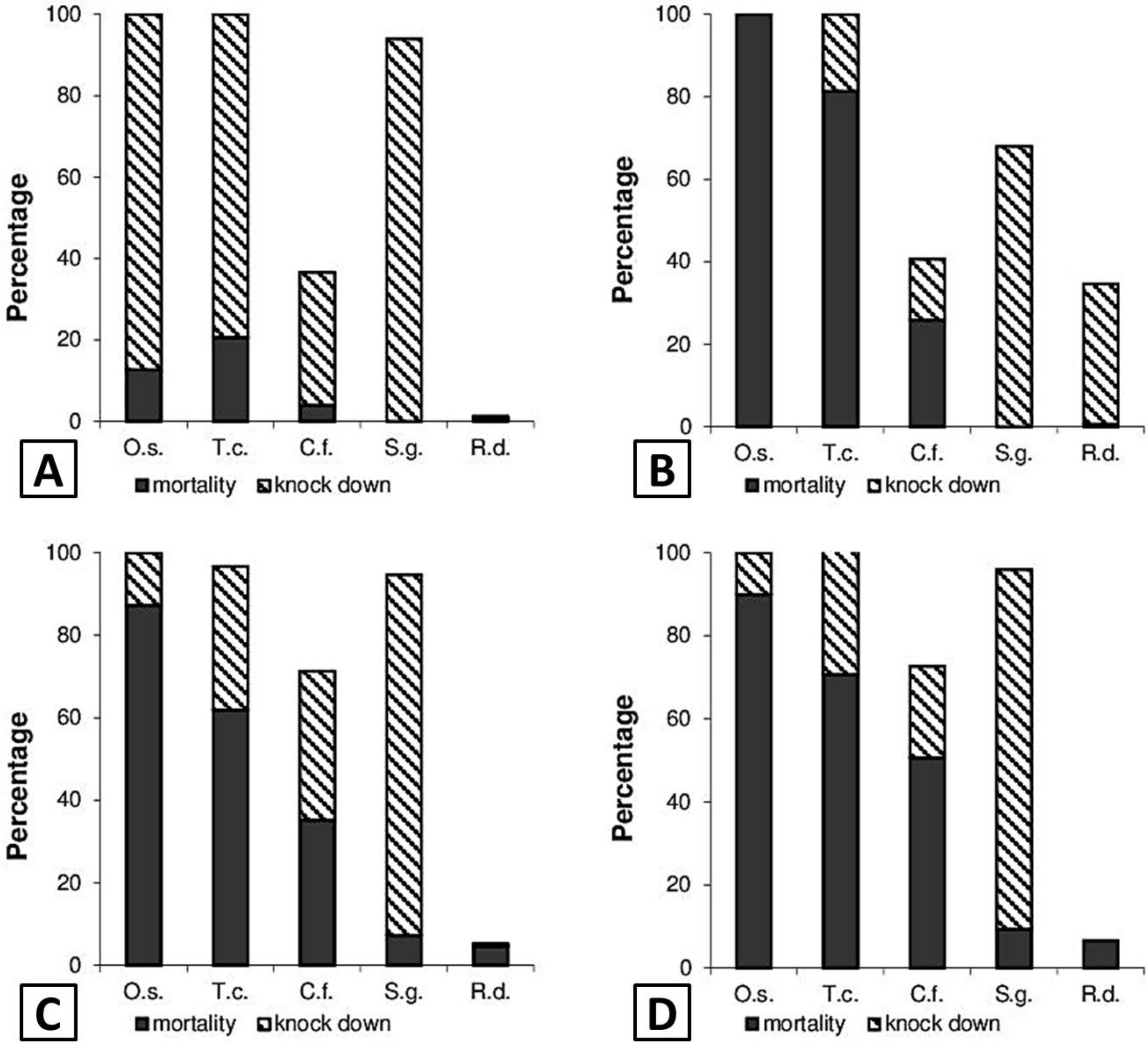

Fig. 5. Efficacy of DDVP strip on five species of stored product beetles (Coleoptera): 6 h exposure under repressive evaporative regimes: A, mortality estimated 24 h after exposure; B, mortality estimated 48 h after exposure; and preventive evaporative regimes: C, mortality estimated 24 h after exposure; D, mortality estimated 48 h after exposure.

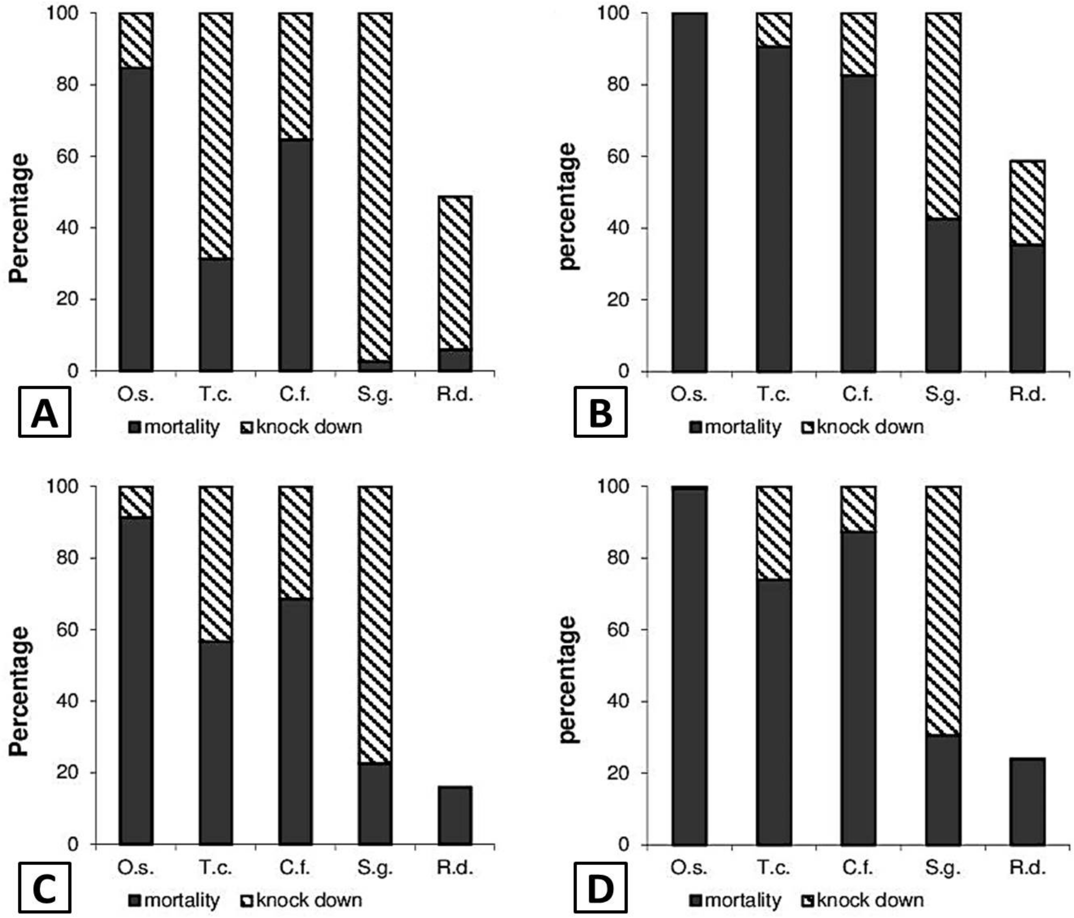

Fig. 6. Efficacy of DDVP strip on five species of stored product beetles (Coleoptera): 24 h exposure under repressive evaporative regimes: A, mortality estimated 24 h after exposure; B, mortality estimated 48 h after exposure; and preventive evaporative regimes: C, mortality estimated 24 h after exposure; D, mortality estimated 48 h after exposure.

DISCUSSION

Dichlorvos (DDVP) has high contact, stomach and inhalation toxicity against a wide range of insects. It has high volatility and activity (*i.e.* rapid knockdown) in a vapor phase (Inhdris and Sullivan, 1958). There are no specific published data on DDDP repellency on storage insects. But generally vapors of pyrethroids are more repellent than vapors of most of organophosphate insecticides (*e.g.* Mondal, 1984). Dichlorvos has excellent and unique insecticidal properties but increasing concerns in relation to human safety necessitates that it is used more carefully. Additionally, better ways to decrease quantities of the chemical people are exposed to and/or exposure times need to be sought. Traditional use of DDVP strips inherently results in long exposure periods not currently permitted under new regulatory rules. Therefore, our study investigated efficacy of short exposure times through the use of repressive use of strips. There are reports suggesting that the use of short exposure times may be different from the longer exposure under laboratory conditions (Stejskal *et al*., 2009; Arthur, 2012). However, differences between short and long exposures have not been investigated under field conditions. Based on the current study, efficacy of short exposures of 1, 4, and 24 h in chambers on five species of stored-product pests under two DDVP evaporative regimes did not produce 100% efficacy for all species except *O. surinamensis*. For *S. granarius*, *T. castaneum*, *C. ferrugineus* and *R. dominica*, the short exposure resulted in low efficacy for *S. granarius* and *R. dominica* whereas it produced moderate efficacy in *C. ferrugineus* and *T. castaneum*. Our data shows that DDVP strips cannot simply replace spray DDVP aerosol formulations that provide 100 % control for many species even under relatively short exposures (*e.g.* Childs *et al*., 1966; Boina and Subramanyam, 2012; Aulicky and

Stejskal, 2013). For example, spray DDVP aerosol based on 2 h exposure resulted in complete control of adult of tobacco beetle (*Lasioderma serricore)* (Childs *et al*., 1966). According to Boina and Subramanyam (2012), complete mortality of *T. confusum* is attained within 24 h in open and obstructed mill locations as a result of exposure to spray aerosol. Manzoor and Sattar (2013) state that *R dominica* is generally tolerant to many organophosphates (including DDVP), however, a field warehouse experiment by Aulicky and Stejskal (2013) showed that aerosol mixtures of DDVP + natural pyrethrins provided instant 100% mortality in most of the geographical strains of *R. dominica* that they tested. The experiment conducted by Aulicky and Stejskal (2013) indicated that a combination of DDVP strip and pyrethroid or pyrethrin spray aerosol is highly effective against stored-product pests. This strategy may also be beneficial for sustainable usage of pyrethroids that constitute prevalent active ingredient of currently used insecticide sprays or aerosols due to deregistration of many insecticide classes. Therefore the DDVP strip may serve as a resistance management tool to slow down the evolution of resistance to pyrethroids after new regulatory rules governing the use of DDVP strips take effect.

Concerns in relation to human safety necessitates that DDVP should be used carefully and every user should strictly follows the updated label instructions. It is seems (from the current scientific debates) that there is a trade-off between human exposure safety and efficacy of DDVP. Our work showed that short term exposure using evaporation strips cannot fully replace highly effective and quick acting spray DDVP aerosols or long term evaporation strip exposures. Nevertheless, short exposures may help control more susceptible strains such as *O. surinamensis* and contribute to reducing populations of *T. castaneum and C. ferrugineus* in situations where there are no other options or highly pyrethroid resistant insects exist. However, primary pests (*S. granarius* and *R. dominica* – that generally make more damages - are more tolerant and therefore their control may not reach the required level. It is proposed that a combination of short- term strips exposure and pyrethroid aerosols should be explored in order to increase efficacy of DDVP short exposure and to slow down the evolution pyrethroid resistance.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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