#### **ORIGINAL ARTICLE**

## Assessment of some physical measures as safe and environmentally friendly alternative control agents for some common coleopteran insects in stored wheat products

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#### Abstract

Presently, finding effective, simple, inexpensive, hygienic and safe pest control agents are the biggest challenges in management of stored product insects, where those features are available in most physical factors. The insecticidal efficiency of four diversified physical control agents (ultraviolet and microwave irradiations, thermal remediation and silica nanoparticles) were assayed against the most common coleopteran insect species (Sitophilus oryzae L. and Tribolium castaneum Herbst) on stored wheat. Exposing tested insects to microwave irradiations (2450 MHz) for 25 sec gave preventive efficiency for stored material, which reached 97.68 and 99.02%, respectively. Sufficient exposure periods to kill 50% of the cole opteran adults (LT  $_{\rm 50\%}$  ) were 13 and 14 sec, respectively. For effective control with UV radiations, S. oryzae should be exposed for 12 h and T. castaneum for 24 h. An exposure period of 24 h caused progeny reduction 95.24 and 89.72% and gave preventive efficiency of 94.25 and 93.37%, respectively. Values of LT $_{\rm 50\%}$  were 56.76 and 74.04 h, respectively. Exposing infested samples of the tested species to 70°C for 10 min killed 100% of adults and caused complete cessation of egg laying. Furthermore, 65°C or 70°C caused full progeny reduction. The lowest level of stored product weight loss (1.15 and 1.35%, respectively) occurred at 70°C, where sufficient exposure temperatures to kill 50% of the coleopteran adults (LTD<sub>50%</sub>) were 60.95°C and 61.63°C, respectively. Synthetic silica nanoparticles (SSiNPs) were more toxic against the tested populations than bio-silica nanoparticles (BSiNPs) after 48–72 h. A concentration of 1.00 g  $\cdot$  kg<sup>-1</sup> of tested silica nanoparticles caused significant reduction in adult populations, saved wheat grain vitality and gave least lost weights of flour (3.35-6.85%).

**Key words**: microwave, nanosilica, *Sitophilus oryzae*, temperature, *Tribolium castaneum*, ultraviolet irradiation

## Introduction

Wheat is one of the world's most important staple grain products. Healthy wheat grains should undergo successful storage from the field to the consumer. Protecting stored grains from insect attack is important since most stored-grain pest insects are able to increase their population within a very short time and can destroy most stored grains especially during long-term storage (Pimentel 1991). Estimated annual losses of cereal grains due to insects and rodents are 30% in Africa and Asia (Hill 1990). Species of coleopteran insects in stored products have been researched more than other species. Red flour beetles *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and rice weevils *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) are considered to be the major insect pests in storage (Daglish *et al.* 1996; Campbell and Runnion 2003). Most coleopteran studies on stored products focus on these two species (Campolo *et al.* 2018).

Although the numbers of pesticides currently approved to protect stored grains are very limited and are chemical synthetics, such as methyl bromide and phosphine, they are commonly used worldwide as effective methods to control storage insects. Heavy and widespread use of chemical insecticides has resulted in negative effects on human public health, with serious environmental consequences such as depleting the earth's ozone layer (Leesch et al. 2000) and the occurrence of insecticide-resistant insect strains (Zhao et al. 2007). The above-mentioned reasons challenge researchers to find new, effective, hygienic, readily available, affordable and viable approaches to control stored product insect pests (Fields 2006; Mahdi and Rahman 2008; Collins and Kitchingman 2010) that are more environmentally friendly than synthetic chemicals.

Physical control involves physically changing the environment to make it hostile or inaccessible to insect pests. The physical environment of storage (temperature, relative humidity or grain moisture content and relative composition of atmospheric gases) or applying physical treatments (mechanical processing, physical removal, barriers, inert dusts, electric discharge, light, sound and ionizing irradiation) can be manipulated as hygienic physical control agents to manage insect pests in stored grain (Abd El-Aziz 2011).

There are many advantages of using physical agents in stored product insect control such as stored food products without toxic or undesirable chemical residues, no resistance development of pest insects, saving nutritive value, avoiding physicochemical changes of the treated products and inexpensive applications (Lapidot *et al.* 1991; Ahmed 2001; Zhao *et al.* 2007).

Using high-frequency electromagnetic energy in agriculture and pest control has been researched and reviewed by several authors (Highland and Wilson 1981; Moy 1993; Nelson 1996; Fields and Muir 1996). Microwaves are nonionizing irradiation that lack sufficient electromagnetic energy (Nelson 1967). Microwave radiation involved frequencies of more than 500 MHz with application frequencies of 869, 915, and 2450 MHz (Nelson 1996). Although, microwave radiation can kill pests existing inside or outside grain kernels (Halverson et al. 1999) with high penetrability, it does not have any harmful or severe impact on foodstuffs (Warchalewski et al. 2000; Vadivambal et al. 2007). A frequency of 2450 MHz can kill eggs and adults of Tribolium confusum (Halverson et al. 1996; Vadivambal et al. 2008; Vadivambal et al. 2010), S. oryzae (Zaho et al. 2007) and other insect species such as Plodia interpunctella (Shayesteh and Barthakur 1996; Vadivambal et al. 2010), eggs and pupae of Delia radicum (Biron et al. 1996), maize weevil Sitophilus zeamais (Motschulsky), S. granarium (Vadivambal 2009), S. zeamais (Hassan et al. 2010) and pulse beetle Callosobruchus chinensis (L.) (Bedi and Singh 1992; Singh

*et al.* 2012). In these species it caused deformed adults, missing legs, or decreased reproduction rates (Nelson 1996). The principle behind killing insects using microwaves is the dielectric heating of insects which depends on its electrical properties (Novotny *et al.* 2013).

Ultraviolet radiations (UV-rays) (UV-C) have been used in many experimental studies such as embryological and physiological studies (Bodenstein 1953). It was widely used in pest control applications, for instance, germicide, insect attractant traps (Bruce 1975), and superficial elimination of insect eggs (Guerra et al. 1968). As a result, ultraviolet is a less penetrating radiation than other ionizing radiations, and it is used only with bulk grains or with small amounts (Hasan and Khan 1998). Many authors have examined UV radiation effects on the development of stored product insects (Calderon et al. 1985; Faruki 2005). Increasing exposure time to UV-rays caused a significant reduction in egg hatchability and adult eclosion of T. castaneum, T. confusum (Duval) and Cadra cautella (Walker) (Lepidoptera; Pyralidae) (Faruki et al. 2007).

Insects are poikilotherm organisms whose development and survival are affected easily by the surrounding temperature of the environment or microenvironment. In recent decades, high temperatures have been used extensively to manage storage insects with various methods such as radio frequencies, hot air, fluidized beds and microwave (Fields 1992; Nelson 1996; Roesli et al. 2003; Beckett et al. 2007; Kljajić and Andrić 2010). For effective and successful thermal control, the thermal tolerance of the host must be sufficiently lower than that of the target pest (Nelson 1967). High temperatures clearly affect physiological and biochemical aspects of storage insects (Fields 1992; Neven 2000) as well as survival (Beckett et al. 1998) or development of their different stages (Fields 1992; Beckett et al. 1998; Wright et al. 2002; Mahroof et al. 2003b; Loganathan et al. 2011). Many authors have discussed conventional heat treatment for disinfestation of stored grains from insects (Dosland et al. 2006; Phillips and Throne 2010; Alice et al. 2013). A temperature of 50°C has been suggested to be the most effective temperature to manage T. castaneum (Mahroof et al. 2003a; Campolo et al. 2013), S. oryzae (Golić et al. 2011; Campolo et al. 2013) and other insect species (Roesli et al. 2003).

Inert dusts (sands and other soil components, diatomaceous earth, silica aerogel, non-silica dusts and particle films such as kaolin and bentonite clays) are physical agents that have been used for centuries by Africans to control insects of stored grains (Fields and Muir 1996). Inert dusts are non-toxic to both humans and animals and can provide stored grains with continuous protection from insect infestations and save wheat quality (Abd El-Aziz 2011). Silica, which plugs up respiratory stomata of insects, can be used for controlling insect pests under field or laboratory

conditions (El-Samahy and Galal 2012; El-Samahy et al. 2014). Over the last 10 years nanotechnology has been incorporated into agriculture and crop protection as an innovative tool for pest control (Bhattacharyya et al. 2010; Khot et al. 2012; Cicek and Nadaroglu 2015; Kitherian 2017). Nanoparticles of silica are more active than their bulk counterpart because of their increased surface to volume ratio (Vani and Brindhaa 2013). Nanosilica or amorphous silica nanoparticles (SNP) have been used successfully to control a wide range of agricultural insect pests, ectoparasites of animals (Ulrichs et al. 2005) and stored product insects such as S. oryzae, T. castaneum and Rhizopertha dominica (F.) on wheat grains (El-Samahy et al. 2015). It caused more than 90% mortality for S. oryzae (Debnath et al. 2011) and 100% mortality for *Corcyra cephalonica* (S.) (Vani and Brindhaa 2013).

The current study was aimed to examine insecticidal efficiency of various, common, simple, inexpensive and safe physical control agents (ultraviolet rays, microwaves, high temperatures and silica nanoparticles) against Egyptian strains of *S. oryzae* and *T. castaneum* in stored wheat products.

## **Materials and Methods**

#### Target insect species and grain samples

Adult samples of Egyptian strains of rice weevil, S. oryzae and the rust red flour beetle, T. castaneum were picked from local stores of El-Behera Governorate, established and maintained in the laboratory of Stored Product Pests Department, Plant Protection Research Institute, Agricultural Research Center, Sabahia, Alexandria, Egypt under constant conditions:  $26 \pm 2^{\circ}$ C,  $65 \pm 5\%$  RH (relative humidity) and a 12 : 12 light : dark photoperiod, without exposure to insecticides. Wheat grains or wheat flour, mixed with yeast (10 : 1 w/w), were used as rearing media for target insects, respectively. Healthy adults of the tested species were picked 2 weeks after emergence and subjected to further bioassay tests. Untreated, clean, sterilized grains and flour of wheat Triticum vulgare variety (Giza171) were obtained from Etay El-Baroud, Agriculture Research Station, El-Behera Governorate, Egypt.

#### Preparing insects for bioassay

Jars of 250 ml were mounted with 20 gm of the obtained wheat grains or wheat flour. Twenty sexed individuals (sex ratio 1 : 1) of *S. oryzae* adults were transferred to hard wheat jars, while 20 unsexed adults of *T. castaneum* beetles were transferred to jars of wheat flour. Each treatment and control were replicated six times in the case *S. oryzae*; or three times for *T. castaneum*.

# Preparation of physical treatments for bioassay

#### **Microwave treatments**

The replicates of examined insect specimens were exposed to radiations of microwave energy using a household microwave system (LG Co. Korea – Model No: MS-2816 Mz). The power output of the generator was adjusted to 1,200 W and 2450 MHz. The samples were exposed for 5, 10, 15, 20 or 25 sec. The exposure period was determined using a stopwatch. Samples were investigated after 1 h, and mortality of adults was recorded.

#### UV-irradiation treatments

A wooden box  $(50 \times 40 \times 15 \text{ cm})$ , and a mobile base was designed. At a height of 12 cm from the base, it was supplemented with an 8 W UV germicidal lamp (T5 8 GL) that emitted irradiation at a wavelength of 254 nm (Faruki *et al.* 2007). The effects of UV-radiation were evaluated at different exposure periods (3, 6, 12 and 24 h). At the end of the exposure period the UV-lamp was turned off and the samples were removed immediately. Then, all treated samples were kept under standard rearing conditions, and mortality of adults was recorded 1 day later.

#### Thermal treatments

Tested samples were subjected to high temperatures using a Torre Picenardi Oven (Model: Panacea 430) made in Italy (V: 220, 50/60Hz, A: 3.7, W: 800). The samples were exposed to five temperatures (50, 55, 60, 65 and 70°C) for 10 min. They were then removed immediately, and kept under standard rearing conditions. Mortality of adults was recorded 1 day later.

#### Silica nanoparticle applications

To evaluate the efficacy of silica nanoparticles (SiNPs) against both tested insects, two different sources of SiNPs were examined. The first source of SiNPs was obtained from rice husks (RHs) and named "bio-silica" (BSiNPs), while the second source was synthetic silica (SSiNPs) according to Kotoky and Dolui (2004) with 99.99% purity obtained from Nanotech Egypt Company Limited, Cairo. A transmission electronic microscope (TEM) was used to examine the shape and size of bio and synthetic silica nanoparticles as shown in Figure 1. The two nanoparticles were almost spherical and ranged in size from 10.95 to 23.90 nm for BSiNPs and 13.12-18.81 nm for SSiNPs. Effects of the SiNPs on 2 week old adults of both tested insect species were determined by contact toxicity assay at four rates: 0.25, 0.50, 1.00 and 1.50 g of SiNPs  $\cdot$  kg<sup>-1</sup> of wheat or flour. After application, samples were kept under standard rearing conditions and adult mortality was checked after 1, 2, and 3 days. Medium lethal concentration  $(LC_{50})$  was estimated.



Fig. 1. Electron micrographs for silica nanoparticles. A – TEM image of bio-silica nanoparticles (BSiNPs), B – TEM image of synthetic silica nanoparticles (SSiNPs)

#### Estimating and calculating biological aspects

After 10 days of the above-mentioned treatment, egg laying numbers of surviving *S. oryzae* adults were recorded daily according to Frankenfeld (1948) and Howe (1952). After 6 weeks of treatment, egg hatchability percentages (emergence) of both tested insects were calculated according to the following equation of Moumouni *et al.* (2015):

Emergence (%) = 
$$E/G \times 100$$
,

where: E – of emerged adults, G – no. of laid eggs. Infestation reduction percentage was also calculated according to the following equation of Aldryhim (1990):

Reduction (%) = 
$$EC - ET/EC \times 100$$
,

where: EC – mean number of emerged adults in control, ET – mean number of emerged adults in treatment.

After 3 months, grain or flour weight losses were calculated according to the following equation of Ahmadi *et al.* (2009):

Loss (%) = 
$$(W_1 - W_2) \times 100/W_1$$

where:  $W_1$  – weight of the sample before treatment,  $W_2$  – weight of the sample after treatment. Then the efficiency percentage of each treatment was calculated according to the following equation of Mohamed *et al.* (2009):

$$E(\%) = (LC - LT) \times 100/LC$$

where: LC – grain or flour weight losses in the control, LT – grain or flour weight losses in treatment.

Vitality of tested grain was also determined by applying the germination test according to Qi and Burkholder (1981). Germination of seeds was recorded 4 days after planting and germination percentages were calculated according to the following equation:

%Germination =

$$= \frac{\text{Number of germinated grains}}{\text{Total number of planted grains}} \times 100.$$

#### **Data analysis**

Values of medium lethal exposure time  $(LT_{50})$  for microwave and UV-irradiation treatments, medium lethal temperature  $(LTD_{50})$  of thermal treatments and medium lethal concentration  $(LC_{50})$  of SiNPs treatments were estimated by using Log dose Probit software (LdP) Line (Ehabsoft, Cairo, Egypt) according to Finney (1971). All obtained data of adult mortality, laid eggs, adult emergence, sample weight loss and seed germination were subjected to one-way analysis of variance (ANOVA) and means were compared by Duncan multiple range test at 5% probability level (Steel and Torrie 1980). The SPSS statistical software (Version 12) was used.

## **Results and Discussion**

#### **Effects of microwave radiations**

Adult mortality, numbers of laid eggs and egg hatchability of S. oryzae adults treated with microwave for 5, 10, 15, 20 and 25 sec are presented in Table 1. Although exposing adults to microwave for 5 sec did not cause any significant difference in adult mortality and weight loss of wheat grain percentage as well as the control, it saved the vitality of stored wheat grains (91.65%). In contrast, the former case caused significant depletion in egg laying and adult emergence in comparison to the control. The mortality was significantly increased while egg laying and emerged adults were decreased with increased microwave exposure periods. In the case of exposing the adults for 25 sec, full mortality and complete cessation of egg laying were observed, achieving the highest level of insect pest population reduction (100%). Exposing adults to microwave for 25 sec gave preventive efficiency for stored wheat grains which reached 97.68%. The weight loss of wheat grain was not more than 1.67%. To kill 50% of adults ( $LT_{50\%}$ ) (upper limit – lower limit) exposure for 12.99 (11.97-14.03) sec was sufficient.

Exposure periods [sec]	Mortality of adults [%]	Mean no. of laid eggs ±SE	Mean no. of emerged adults ±SE	Emergence [%]	Reduction [%]	Weight loss of wheat grain [%]	Efficiency [%]	Germination [%]
Control (0)	$0.00 \pm 0.00 \text{ e}$	183.33 ± 1.45 a	150.33 ± 0.88 a	82.00	00.00	71.17 ± 0.12 e	-	100.00 ± 0.00 a
5	6.65 ± 0.88 e	175.67 ± 1.20 b	142.00 ± 1.15 b	80.67	5.54	70.83 ± 0.15 e	$00.42 \pm 0.15$ a	91.65 ± 0.33 a
10	$33.35\pm0.33~\text{d}$	100.67 ± 1.45 c	63.00 ± 1.15 c	62.58	58.09	$59.83 \pm 0.15 \text{ d}$	15.88 ± 0.15 b	$78.35\pm0.88~\text{b}$
15	56.65 ± 0.88 c	90.33 ± 0.88 d	50.33 ± 1.45 d	56.00	66.52	35.17 ± 0.15 c	$50.60 \pm 0.15$ c	$70.00\pm0.58~b$
20	75.00 ± 1.15 b	23.00 ± 1.00 e	9.67 ± 0.88 e	42.33	93.57	$20.83\pm0.12~b$	$70.70 \pm 0.12 \text{ d}$	56.65 ± 0.88 c
25	$88.35 \pm 0.67$ a	$00.00\pm0.00~\text{f}$	$00.00\pm0.00~\text{f}$	00.00	100.00	1.67 ± 0.09 a	97.68 ± 0.09 e	21.65 ± 0.88 d

**Table 1.** Impact of microwaves on biological aspects of *Sitophilus oryzae* adults and seed vitality, and their expected preventive efficiency for stored wheat grains at different exposure periods

Values are mean ± standard error; n = 6; column values followed by different letter(s) are significantly different at 0.05 levels

Table 2. Impact of microwaves on biological aspects of *Tribolium castaneum* adults and their expected preventive efficiency for stored wheat flour at different exposure periods

Exposure periods [sec]	Mortality of adults [%]	Mean no. of emerged adults [±SE]	Reduction [%]	Weight loss of wheat flour [%]	Efficiency [%]
Control (0)	$0.00\pm0.00~\text{e}$	129.00 ± 1.53 a	00.00	$66.35 \pm 0.09  f$	-
5	$5.00\pm0.58~\text{e}$	112.33 ± 1.45 b	12.92	$64.85 \pm 0.09  \text{e}$	$2.26\pm0.09~\text{a}$
10	$31.65 \pm 0.88 \text{ d}$	77.33 ± 1.45 c	40.05	$55.65 \pm 0.09 \text{ d}$	$16.13 \pm 0.09 \text{ b}$
15	53.35 ± 1.20 c	37.33 ± 1.45 d	71.06	$33.85 \pm 0.12$ c	48.98 ± 0.12 c
20	$70.00\pm1.00~\text{b}$	12.67 ± 1.45 e	90.18	$24.85 \pm 0.09$ b	$62.55 \pm 0.09 \text{ d}$
25	$83.35 \pm 0.33$ a	$00.00\pm0.00~\text{f}$	100.00	00.65 ± 0.03 a	99.02 ± 0.03 e

Values are mean ± standard error; n = 3; column values followed by different letter(s) are significantly different at 0.05 levels

In the case of *T. castaneum* adult treatments, it was shown that exposing adults to microwave irradiation for 5 sec did not cause any significant difference in adult mortality as well as in the control, but it significantly decreased percentages of stored flour weight loss and progeny production (Table 2). All microwave exposure periods significantly decreased the emerged adults compared to the control. No progeny emerged on wheat flour that was microwaved for 25 sec. To kill 50% of *T. castaneum* adults ( $LT_{50\%}$ ) exposure for 13.95 (12.85–15.11) sec was sufficient. Moreover, exposing adults to microwave for 25 sec protected 99.02% of treated wheat flour.

Many researchers are interested in using microwave treatment as an alternative to chemical fumigation in pest control of post-harvest pests on agricultural products such as wheat (Shayesteh and Barthakur 1996), sorghum (More *et al.* 1992), rice (Xiong *et al.* 2004) and some food materials such as cherries (Ikediala *et al.* 1999) because of its high penetrability and safety. Microwave irradiation has obvious effects on some biological aspects of insects such as reduction of reproductive rates, malformation and body weight loss (Nelson 1996).

The obtained results agree with the results of Halverson et al. (1996), who found that the high power of microwave and source operating at a frequency less than 2.45 GHz gave mortality percentages of more than 93% for S. zeamais and other insects of stored products. Exposure to microwave energy not only kills the insects but also can cause physical injuries and reduced reproduction rates in surviving insects (Nelson 1996). Earlier studies showed that eradication of insects increased with increasing microwave energy, while the seed viability, germination capacity, and seedling vigor decreased (Campana et al. 1993; Bhaskara-Reddy 1998). Singh et al. (2012) also concluded that germination capacity and seed viability of chickpea, pigeon pea, and green gram were affected by microwave exposure periods and power level.

Complete mortality of all life stages of *T. castaneum* can occur by using a power level of 400 W and an exposure time of 56 sec or at 500 W for 28 sec. Increasing power levels or exposure periods or both can significantly increase mortality. Germination of barley seeds was decreased with an increase in power level or longer exposure periods (Vadivambal *et al.* 2008). Complete mortality was achieved for adults of *T. castaneum* 

and Oryzaephilus surinamensis (L.) insects and larvae of T. castaneum when the power was 800 W and the exposure time was 30 or 40 sec. Mortality of T. castaneum larvae was also 100% when the power and exposure period were 600 W and 40 sec, respectively (Manickavasagan et al. 2013). The medium lethal time  $(LT_{50})$  of exposure to microwave irradiation indicated that the adult stage of S. zeamais was more tolerant to microwave irradiation than the other developmental stages (Tungjitwitayakul et al. 2016). An output of 840 watt power and an exposure period of 50 sec caused the highest mortality (90%) of T. castaneum and khapra beetle Trogoderma granarium Everts. Mortality increased with increasing concentrations or exposure periods or both (Agha et al. 2017). It was evident that increasing power and exposure periods significantly increased the mortality of the Mediterranean Flour Moth Ephestia kuehniella Zeller (Azizoglu et al. 2011), which may be caused by water present in the body fluid (Shayesteh and Barthakur 1996).

#### **Effects of UV-radiations**

Obtained data (Table 3) showed that the mortality of *S. oryzae* adults increased with longer ultraviolet exposure periods. For effective control with UV radiations,

infested samples should be exposed for at least 12 h since there were no significant differences between adult mortality of treated samples for 3 or 6 h and untreated samples at p < 0.05 (LSD < 0.05 = 1.88). All UV exposure periods caused significant reductions in the number of laid eggs and emerged adults in comparison to the control. An exposure period of 24 h decreased an adult emergence percentage to 17.67%. Moreover, treated samples with UV for 24 h was sufficient to give preventive efficiency up to 94.25% and weight loss of stored wheat did not increase than 2.30%. UV radiation for 24 h didn't have any significant negative effects on vitality of stored wheat grains. To kill 50% of adults (LT<sub>50%</sub>) an exposure period of 56.76 (35.5–145.29) h was sufficient.

The impacts of UV-radiation exposure periods on levels of infestations with *T. castaneum* are given in Table 4. Exposing the adults to UV-radiations for 12 h did not cause any significant mortality in comparison to the control or shorter exposure periods. The numbers of emerged adults were significantly decreased with increasing the exposure period. All of the ultraviolet exposure periods significantly reduced weight loss of wheat flour in comparison to the control at p < 0.05 (LSD < 0.05 = 0.23). Infested samples should be exposed to UV-irradiation for a period of not less than

**Table 3.** Impact of UV-radiation on biological aspects of *Sitophilus oryzae* adults and seed vitality, and their expected preventive efficiency for stored wheat grains at different exposure periods

Exposure periods [h]	Mortality of adults [%]	Mean no. of laid eggs [±SE]	Mean no. of emerged adults [±SE]	Emergence [%]	Reduction [%]	Weight loss [%]	Efficiency [%]	Germination [%]
Control	$00.00 \pm 0.00$ b	78.00 ± 1.53 a	63.00 ± 1.15 a	80.67	00.00	$40.00 \pm 0.12$ d	-	100.00 ± 0.00 a
3	1.65 ± 0.33 b	67.67 ± 1.2 b	51.33 ± 1.86 b	76.00	18.52	$38.00 \pm 0.15 \text{ d}$	5.00 ± 0.15 a	$93.35\pm0.88~ab$
6	$8.35 \pm 0.33$ b	50.67 ± 1.2 c	$30.33\pm0.88~c$	59.67	51.86	$28.30\pm0.12~c$	29.25 ± 0.12 b	$95.00\pm0.58~ab$
12	18.35 ± 0.88 a	35.33 ± 1.45 d	14.67 ± 1.45 d	41.51	76.71	$22.30\pm0.15~b$	44.25 ± 0.15 c	$88.35 \pm 0.67 \text{ b}$
24	26.65 ± 0.88 a	16.67 ± 0.88 e	3.00 ± 0.58 e	17.67	95.24	2.30 ± 0.03 a	94.25 ± 0.03 d	$88.35 \pm 0.88$ b

Values are mean ± standard error; n = 6; column values followed by different letter(s) are significantly different at 0.05 levels

Table 4. Impact of UV-radiation on biological aspects of *Tribolium castaneun* adults and their expected preventive efficiency for stored wheat flour at different exposure periods

Exposure periods [h]	Mortality of adults [%]	Mean no. of emerged adults [±SE]	Reduction [%]	Weight loss of wheat flour [%]	Efficiency [%]
Control	$0.00\pm0.00~\text{c}$	71.33 ± 2.03 a	0.00	34.70 ± 0.09 e	-
3	1.65 ± 0.33 c	55.33 ± 1.45 b	22.43	$30.30\pm0.09~d$	12.68 ± 0.09 a
6	$6.65 \pm 0.33 \text{ cb}$	36.33 ± 1.76 c	49.07	23.70 ± 0.15 c	31.70 ± 0.15 b
12	$13.35 \pm 0.88  b$	24.67 ± 1.86 d	65.41	18.30 ± 0.09 b	47.26 ± 0.09 c
24	$23.35\pm0.88a$	7.33 ± 1.20 e	89.72	$2.30\pm0.09~\text{a}$	93.37 ± 0.09 d

Values are mean ± standard error; n = 3; column values followed by different letter(s) are significantly different at 0.05 levels

24 h to obtain the highest mortality of adults (23.35%) and progeny reduction (89.72%) that could save 93.37% of wheat flour. To kill 50% of adults ( $LT_{50\%}$ ) an exposure time of 74.04 (42.07–256.54) h was sufficient.

The effects of UV-rays were reported by Sharma and Dwivedi (1997) who found that UV-rays inhibited oviposition and emergence of C. chinensis. Earlier, Guerra et al. (1968) reported that UV-radiations of a short wavelength (2537A°) could completely inhibit egg hatchability of Heliothis virescens (Fabricius) and H. zea (Boddie). Percentages of egg hatchability were gradually decreased with increasing periods of exposure and eggs were completely killed after an exposure period of 20 min. Knipling (1970) suggested that ionizing radiation could be used to control economically important insects that cause chromosomal translocations and then fertility reduction. Irradiation of late-stage larvae of Indian meal moth, Plodia interpunctella with UV-rays caused inhibition of adult emergence (Beard 1972). UV-rays can be used to suppress the population of insects (Calderon et al. 1985). Irradiation of Exorista sorbillans pupae with UV-rays significantly reduced adult emergence and caused deformation of larvae and adults (Hasan et al. 1998).

UV-exposure of larvae (Faruki *et al.* 2005) and pupae of *A. diaperinus* (Parween 2004) significantly reduced reproduction. When Faruki (2005) exposed larvae of *T. castaneum* to UV-rays, he noticed that adult emergence was significantly decreased. Moreover, egg-hatching and adult emergence were significantly reduced in *T. castaneum*, *T. confusum* and *Cadra cautelladue* when eggs were exposed to UV-rays (Faruki *et al.* 2007). The mortality of *S. oryzae* was highest when ultraviolet radiation was 254 nm for 20 min. The combination of ultraviolet radiation and Chinese wingnut oils gave the best control and mortality rates which reached 100% after 4–5 days of treatment (Zhihua

and Yin 2007). Finally, exposing adults of astigmatid mites, *Tyrophagus putrescentiae* (Schrank, 1781) to UV-C (260 nm) and UV-B (315 nm) and exposure periods ranging from 3 to 15 min, caused mortality rates between 85–100% for UV-C and 62–83% for UV-B (Bakr 2013).

### **Effects of thermal remediation**

The results of exposing S. oryzae adults and stored wheat grains to different thermal treatments with ascending temperatures are presented in Table 5. It is clear that the mortality of S. oryzae was increased with increasing temperatures. To obtain significant mortality, adults should be exposed to 60°C for at least 10 min. Exposing adults to 70°C for 10 min killed 100% of adults and caused complete cessation of egg laying. Treating adults at 50°C did not cause any significant reduction of laid eggs, while exposing adults to 55°C or more could cause significant reduction of laid eggs. No adult emergence was noticed at 65°C or more, so it was considered to be the optimal temperature for full progeny reduction (PR %). Thermal remediation had a significant negative effect on adult emergence; however it did not have any significant effect on wheat grain vitality. At 70°C the lowest level of wheat grain weight loss (1.15%) and the highest level of preventative efficiency (98.48%) were observed. To kill 50% of adults (LTD<sub>50%</sub>) exposure at 60.95°C (60.29–61.63) was efffective.

Impacts of thermal remediation on adult mortality, progeny production, adult emergence reduction of *T. castaneum* and weight loss of wheat flour are presented in Table 6. No significant difference was detected for adult mortality between treatments of 50 or 55°C and the control. Complete mortality of an examined population was achieved when samples were

Table 5. Impact of thermal treatment on biological aspects of *Sitophilus oryzae* adults and seed vitality, and their expected preventive efficiency for stored wheat grains

Temperature [°C]	Mortality of adults [%]	Mean no. of laid eggs [±SE]	Mean no. of emerged adults [±SE]	Emergence [%]	Reduction [%]	Weight loss [%]	Efficiency [%]	Germination [%]
Control	$00.00 \pm 0.00 \text{ d}$	117.67 ± 1.20 a	92.00 ± 1.53 a	78.18	00.00	$76.00 \pm 0.15  f$	-	100.00 ± 0.00 a
50	3.35 ± 0.67 d	116.00 ± 1.00 a	72.67 ± 1.45 b	62.65	21.01	64.65 ± 0.18 e	$14.93\pm0.18$	$100.00 \pm 0.00$ a
55	5.00 ± 0.58 d	96.33 ± 1.76 b	58.00 ± 1.53 c	60.21	36.96	52.65 ± 0.15 d	$30.72\pm0.15$	96.65 ± 0.33 a
60	40.00 ± 1.15 c	75.33 ± 1.45 c	31.67 ± 1.76 d	42.04	65.58	$44.00\pm0.12c$	42.11 ± 0.12	95.00 ± 0.58 a
65	85.00 ± 1.00 b	17.00 ± 1.73 d	$00.00\pm0.00~\text{e}$	00.00	100.00	8.65 ± 0.23 b	$88.62\pm0.23$	100.00 ± 0.00 a
70	100.00 ± 0.00 a	00.00 ± 0.00 e	$00.00 \pm 0.00 \text{ e}$	00.00	100.00	1.15 ± 0.03 a	$98.49\pm0.03$	100.00 ± 0.00 a

Values are mean ± standard error; n = 6; column values followed by different letter(s) are significantly different at 0.05 levels

Temperature [°C]	Mortality of adults [%]	Mean no. of emerged adults [±SE]	Reduction [%]	Weight loss of wheat flour [%]	Efficiency [%]
Control	$00.00\pm0.00~d$	95.33 ± 1.45 a	00.00	$70.85 \pm 0.12 \text{ f}$	_
50	1.65 ± 0.33 d	$78.00 \pm 1.00 \text{ b}$	18.18	$60.15 \pm 0.09 \text{ e}$	15.10 ± 0.09 a
55	3.35 ± 0.33 d	67.67 ± 1.45 c	29.02	51.00 ± 0.15 d	28.02 ± 0.15 b
60	38.35 ± 0.88 c	43.33 ± 1.76 d	54.55	45.65 ± 0.09 c	$35.57 \pm 0.09 \text{ c}$
65	76.65 ± 0.88 b	$0.00\pm0.00~\text{e}$	100.00	$6.65 \pm 0.20 \text{ b}$	90.61 ± 0.20 d
70	100.00 ± 0.00 a	$0.00\pm0.00~\text{e}$	100.00	1.35 ± 0.07 a	98.09 ± 0.07 e

Table 6. Impact of thermal treatment on biological aspects of *Tribolium castaneum* adults and their expected preventive efficiency for stored wheat flour

Values are mean ± standard error; n = 3; column values followed by different letter(s) are significantly different at 0.05 levels

exposed to 70°C for 10 min. It was clear that the numbers of emerged adults were significantly decreased in treatments compared to the control. At 65 and 70°C the highest level of progeny reduction (100%) was achieved, while 50°C achieved the lowest. Temperatures of 65 or 70°C were sufficient to protect approximately 93.35 and 98.65% of wheat flour from *T. castaneum* infestation, respectively. Exposure temperature, sufficient to kill 50% of adults (LTD<sub>50%</sub>) was 61.63°C (60.92–62.39).

It is clear that insect pest manipulation with extreme temperatures provides a useful tool for the control of S. oryzae and T. castaneum in an Integrated Pest Management Program. Insects die when exposed to high temperatures because of their limited physiological capacity to thermoregulate (Fields 1992). Rapid mortality of insects exposed to high temperatures may be due to increased respiration and metabolic rates (Adler et al. 2000; Mbata and Phillips 2001). For most stored product insects, 25 to 33°C is considered to be optimal for development. Moreover, from 13 to 25°C or from 33 to 35°C, insects can develop completely and produce offspring. At less than 13°C or higher than 35°C, stored product insects will die. Lethal temperatures will vary depending on the species and life stage of the insect (Fields 1992; Ali et al. 1997). Increasing temperatures significantly increased egg mortality rate. One hundred percent mortality was achieved after 180, 120, 60, 40 and 30 sec at temperatures of 54, 60, 65, 70 and 75°C, respectively. Mortality is generally due to physiological and biochemical changes (Neven 2000).

There is a wide variation between species in their ability to survive at high temperature (Strang 1992). Coleopteran species of insects have different susceptibilities to high temperature treatment (49°C). Here, the survival of different species is arranged ascendingly (O. surinamensis or T. confusum), (O. mercator or Cathartus quadricollis), (Gibbium psylloides or S. granarium), (Trogoderma variable, T. castaneum or S. oryzae), (R. dominica or C. pusillus) then Lasioderma serricorne (Kirkpatrick and Tilton 1972). In the current study increasing temperatures had significant effects on adult mortality, which agrees with Baker et al. (1956) who detected that a temperature of 70.4°C was lethal to T. confusum and S. granaries after 1 week of exposure, and a temperature of 80.6°C for 18 sec was also lethal to 100% of T. confusum larvae. Only 2-3% of T. castaneum and Rhyzopertha dominica (F.) adults survived at 57°C for 32 sec (Fields 1992; Fields and Muir 1996). Young larvae of T. castaneum were tolerant to temperatures over 50°C while exposure to 50°C for 72 h caused 99% mortality. Moreover, temperature had a negative impact on progeny production of T. castaneum adults (Mahroof et al. 2003b). Exposing all developmental stages of T. castaneum and T. confusum and adults of T. castaneum, S. oryzae and R. dominica to 51°C and 50°C, respectively, for less than 2 h could cause 100% mortality (Arthur 2000; Tilley et al. 2007). Temperatures of 54 and 55°C and an exposure time of 0.06 h were sufficient to kill 90% of young larvae of S. paniceum, whereas increasing exposure time to 1.3, 0.63 and 0.25 h, killed 99% of T. castaneum, L. serricorne and T. confusum, respectively (Abdelghany et al. 2010).

Exposing adults of *S. oryzae* to 70°C for 10 min was very effective and 100% mortality was achieved. These results agree with Zewar (1993) who found that 70°C for 20 min gave complete mortality for *S. granaries* adults. Eggs laid on wheat grains tolerate higher temperature better than adults and needed 70°C for 15 min to inhibit the emergence of adults. In addition, emergence of *T. castaneum* decreased with increased temperature and exposure time (Boina and Subramanyam 2004). When exposing pupae and adults of *T. castaneum* to 50°C for 39 and 60 min, respectively, there was a significant reduction in oviposition, survival of developmental stages, and progeny production (Mahroof *et al.* 2005).

It is evident from the present study that high temperatures provided adequate protection for stored products against infestation by *S. oryzae* and *T. castaneum*. It offers a great prospect for successful protection of stored material against attack by the mentioned insects for small and medium storage and does not require any extra financial expenses. In the present study temperature did not have any significant effect on the vitality of wheat grains which is in agreement with Murdock and Shade (1991) who found that the temperatures and exposure times necessary to disinfest the cowpeas did not have a significant effect on cooking time and germination of cowpea seeds. But it does not agree with the findings of Purohit *et al.* (2013) who observed that the germination of treated mung bean seeds was reduced significantly due to microwave exposure and increasing temperature and exposure times.

#### Physical toxicity of silica nanoparticles

Adult mortality of exposed S. oryzae and T. castaneum to different concentrations of synthetic or bio silica nanoparticles is shown in Table 7, where the mortality was dependent on concentrations and duration of exposure. Mortality of the two tested coleopteran insect species increased with increasing concentrations and exposure periods. Toxic responses of the two tested insects to silica nanoparticles were similar. After 48-72 h of exposure, synthetic silica nanoparticles (SSiNPs) were more toxic than bio-silica nanoparticles (BSiNPs) against S. oryzae. Treatment of 1 kg of infested wheat grains with 0.86 g of SSiNPs was sufficient to kill 50% of exposed adults in less than 72 h. On the other hand, no significant differences were observed between entomotoxicity of SSiNPs and BSiNPs against T. castaneum adults until 48 h of exposure. Even though the entomotoxic effect of the tested silica nanoparticles increased significantly after 72 h, the toxicity of SSiNPs against T. castaneum was higher than BSiNPs by approximately 4.34 fold.

Biological changes of *S. oryzae* and *T. castaneum*, resulting from silica nanoparticle application, are presented in Tables 8 and 9, respectively. It was ob-

vious that exposing adults to different concentrations (0.25, 0.50, 1.00 and 1.50 g  $\cdot$  kg<sup>-1</sup>) of silica nanoparticles caused significant reduction in the numbers of eggs laid by the tested insects, however, no significant differences were detected between examined sources of silica nanoparticles. Observed numbers of eggs laid by S. oryzae adults exposed to 0.25 or 0.50 g of SSiNPs were significantly less than when BSiNPs were used. No significant difference was detected between the toxicity values of the tested silica nanoparticles in higher concentrations (1.00 or 1.50 g  $\cdot$  kg<sup>-1</sup>), which caused a significant reduction of egg laying. Numbers of emerged adults of S. oryzae or T. castaneum were completely frustrated especially in higher concentrations of both tested materials. Concentrations of 1.00 g  $\cdot$  kg<sup>-1</sup> or more caused significant reductions in the adult populations of both species, and reached 100%. The least weight loss of wheat grains (2.35–4.35%) was observed when adults of S. oryzae were exposed to SSiNPs concentrations of 1.00 g or more, or BSiNPs with a concentration of 1.5 g for kg wheat gains. Both tested silica nanoparticles saved wheat grain vitality and then seed germination. On the other hand, the least losses of flour weight (3.35-6.85%) were observed in samples treated with 1.00 g or more of SSiNPs or BSiNPs.

Amorphous silica is the active component of the diatomaceous earth dusts that are classified as desiccant dusts and are recognized as "safe products". It was approved by the US Food and Drug Administration and it is harmless for humans and mammals (Subramanyam *et al.* 1994; Erb-Brinkmann 2000). The recommended application rate for diatomaceous earth dusts is dependent on the source or origin of the dust, species of stored grain insect and exposure conditions (Erb-Brinkmann and Straube 2002; Stathers *et al.* 2002). An inert dust, based on silica, has been used as a stored grain protectant (Owolade *et al.* 2008). The nanoparticles exhibit unique properties compared to

**Table 7.** Physical toxicity of silica nanoparticles against newly emerged adults of Sitophylus oryzae and Tribolium castaneum after different exposure periods

			Silica nanoparticles treatments								
To	Exposure		Synthetic (SSiNPs)						Bio (BS	iNPs)	
larget insect	[h]	LC 50	confiden	ce limits	slope value	2	LC <sub>50</sub>	confide	nce limits	slope value	2
		$[\mathbf{g} \cdot \mathbf{kg}^{-1}]$	lower	upper	(± SE)	X	[g · kg⁻¹]	lower	upper	(± SE)	X
	24	1.65	1.20	3.06	1.17 ± 0.23	3.11	6.26	2.54	270.94	$0.75 \pm 0.24$	1.35
S. oryzae	48	0.86	0.65	1.24	$1.06 \pm 0.22$	2.62	2.43	1.34	22.95	$0.69\pm0.22$	1.87
	72	0.23	0.13	0.31	$1.30\pm0.23$	1.31	0.86	0.63	1.35	$0.92\pm0.22$	4.57
	24	2.15	1.53	4.15	$1.36 \pm 0.25$	4.23	5.33	2.49	61.88	$0.89 \pm 0.25$	1.95
T. castaneum	48	1.28	0.94	2.26	$1.04 \pm 0.22$	1.93	3.46	1.85	23.17	$0.86 \pm 0.23$	1.80
	72	0.29	0.16	0.40	$1.12 \pm 0.22$	0.50	1.26	0.93	2.17	$1.06 \pm 0.22$	3.79

 $LC_{so}$  – concentration [g · kg<sup>-1</sup>] that causes 50% of adult mortality; SE – standard error;  $\chi^2$  – Chi-square value

Silica nanoparticles treatment	Concentration $[g \cdot kg^{-1}]$	No. of laid eggs	No. of emerged adults	Emergence [%]	Loss of wheat [%]	Reduction [%]	Germination [%]
	0.25	67.67 ± 1.45 c	36.67 ± 2.03 c	54.00 ± 0.02 b	37.85 ± 0.23 e	56.35	100.00 ± 0.00 a
Synthetic	0.50	38.33 ± 1.20 e	$6.67 \pm 0.88  f$	17.00 ± 0.01 c	17.50 ± 0.12 c	92.06	95.00 ± 0.58 b
(SSiNPs)	1.00	20.33 ± 1.45 f	$0.00 \pm 0.00 \text{ e}$	0.00 ± 0.00 d	3.15 ± 0.27 a	100.00	95.00 ± 0.00 b
	1.50	7.00 ± 1.15 g	$0.00 \pm 0.00$ e	0.00 ± 0.00 d	2.35 ± 0.09 a	100.00	96.65 ± 0.33 ab
	0.25	72.00 ± 1.53 b	40.67 ± 1.76 b	56.00 ± 0.01 b	$40.65 \pm 0.12  f$	51.58	96.65 ± 0.33 ab
Bio	0.50	42.67 ± 0.88 d	9.00 ± 1.73 d	$21.00 \pm 0.04$ c	19.85 ± 0.09 d	89.29	95.00 ± 0.00 b
(BSiNPs)	1.00	24.33 ± 1.45 f	$0.00 \pm 0.00 \text{ e}$	0.00 ± 0.00 d	7.35 ± 0.09 b	100.00	98.35 ± 0.33 ab
	1.50	8.33 ± 1.45 g	$0.00 \pm 0.00$ e	0.00 ± 0.00 d	4.35 ± 0.09 a	100.00	96.65 ± 0.33 ab
Control	0.00	101.67 ± 1.76 a	84.00 ± 2.08 a	83.00 ± 0.03 a	56.50 ± 0.12 g		100.00 ± 0.00 a

**Table 8.** Physical toxicity of silica nanoparticles against newly emerged adults of Sitophilus oryzae and Tribolium castaneum afterdifferent exposure periods

Values are mean ± standard error; n = 6; column values followed by different letter(s) are significantly different at 0.05 levels

**Table 9.** Effects of ascending concentrations of synthetic and bio-silica nanoparticles on adult emergence of *Tribolium castaneum* adults and associated flour weight loss

Silica nanoparticles treatment	Concentration [g · kg <sup>-1</sup> ]	No. of emerged adults	Adult no. reduction [%]	Flour weight loss [%]
	0.25	40.67 ± 1.45 c	42.72	33.65 ± 0.15 f
Synthetic	0.50	7.00 ± 1.73 e	90.14	$14.50 \pm 0.12 \text{ d}$
(SSiNPs)	1.00	$0.00\pm0.00~\text{f}$	100.00	$5.15\pm0.03$ bc
	1.50	$0.00\pm0.00~\text{f}$	100.00	$3.35\pm0.09a$
	0.25	44.67 ± 1.76 b	37.08	$36.35 \pm 0.15$ g
Bio	0.50	12.00 ± 1.53 d	83.10	16.85 ± 0.15 e
(BSiNPs)	1.00	$0.00\pm0.00~\text{f}$	100.00	$6.85\pm0.09~c$
	1.50	$0.00\pm0.00~\text{f}$	100.00	$4.00\pm0.15~ab$
Control	0.00	71.00 ± 2.08 a		49.15 ± 0.12 h

Values are mean ± standard error; n = 3; column values followed by different letter(s) are significantly different at 0.05 levels

their bulk material, including higher toxicity (Anjali *et al.* 2010). Nanosilica may be useful against stored grain insects, animal parasites, household pests, worms, fungal organisms, etc. (Ghormade *et al.* 2011). The toxicity of  $SiO_2$  and  $Al_2O_3$  nanoparticles is due to their binding to the insect cuticle, followed by physical sorption of waxes and lipids, leading to insect dehydration (Benelli 2018). SiNPs have high mobility, enabling better penetration into insect tissues by direct contact through the insect's cuticle, or by ingestion and penetration through the digestive tract (Margulis-Gosh and Magdassi 2012). They have been used in insect pest management as molecule delivery, release control improvement and toxic material (Cáceres *et al.* 2019).

Exposing adult *S. oryzae* and *T. confusum* to different diatomaceous earth formulations (Silicosec, Biofa GmbH, Germany) at four dose rates: 0.25, 0.5, 1 and 1.5 g  $\cdot$  kg<sup>-1</sup>, showed that *T. confusum* was less susceptible to SilicoSec than *S. oryzae*. In general, the rates of

1 and 1.5 g  $\cdot$  kg<sup>-1</sup> of wheat provided a satisfactory level of protection against the two examined species. Mortality was higher at longer exposure intervals (Athanassiou et al. 2005). Tribolium castaneum was more susceptible to hydrophobic synthetic amorphous silica (SAS) powder. When exposing S. oryzae adults, no significant difference was detected between LT<sub>95</sub> values of hydrophobic and hydrophilic SAS (Li et al. 2020). Silica nanoparticles were found to be highly effective against S. oryzae which caused mortality rates of more than 90%, indicating the effectiveness of SiNPs as a stored product insect pest control agent (Debnath et al. 2011). Silicon dioxide nanoparticles could also be applied to protect stored grains against adults of R. dominica and T. confusum at low concentrations (Ziaee and Ganji 2016). It has the ability to prevent development of progeny and reduce the emergence of adults of the F1 generation, when parents and progeny of S. oryzae or both are treated; no new progeny was found in rice treated

with SiNPs even after 2 months of treatment (Debnath *et al.* 2011). Zinc oxide nanoparticles (ZnO NPs) and hydrophilic silica nanoparticles (SiO<sub>2</sub> NPs) exhibited a significant toxic effect against *S. oryzae* and *C. maculatus* while *T. castaneum* showed high resistance against the two tested materials at the highest concentrations (0.3, 2 and 8 g  $\cdot$  kg<sup>-1</sup> of SNPs), which caused adult mortality percentages of 81.6, 98.3 and 58.3%, respectively. Moreover, they caused high reductions in F1-progeny (%) with *C. maculatus* and *S. oryzae* (Haroun *et al.* 2020).

Silica nanoparticles can also kill larvae of Spodoptera litura, so they can be considered to be nanocides and an alternative to commercial insecticides, with physical action (Debnath et al. 2012). In addition, hydrophobic nanosilica at 112.5 ppm was effective against some mosquito species. Hydrophobic nanosilica had high larvicidal and pupicidal effects against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus (Barik et al. 2012). Finally, application of nanosilica as dust spray had higher significant entomotoxic effects against larvae of Plutella xylostella than larva dipping, leaf dipping, or solution spray. Mortality rate increased with increasing time exposure and concentration. It reached 58 and 85% at 24 and 72 h after treatment, respectively, with an application rate of 1 mg  $\cdot$  cm<sup>-2</sup> (Shoaib et al. 2018).

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