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Fumigant toxicity of neem formulations against *Sitophilus oryzae* and *Rhyzopertha dominica*

S. Michaelraj* **and R.K. Sharma**

Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012, India

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Fumigation toxicity of two neem formulations viz. Ware house neem I (mist and spray) and Ware house neem II (thermal fog) (Azadirachtin-1500 ppm in both) were studied against two major storage pests of maize, the rice weevil, *Sitophilus oryzae* and the lesser grain borer, *Rhyzopertha dominica* under laboratory conditions. The insects were confined in vials covered with cloth net (25 mesh) and were placed inside the fumigation chamber of 250 ml capacity. The lid was sealed by adhesive tape to create an air tight condition in the chamber. The adults were exposed to fumigants with and without grains. When the adults alone were exposed to WHN I, complete mortality of *Sitophilus oryzae* and *Rhyzopertha dominica* was observed at 15 µl and 30 µl / 250 ml and above doses, respectively at both the exposure periods (48 and 72 hours). Whereas in the case of WHN II, complete mortality of *Sitophilus oryzae* was observed at 50 µl in both exposure period and 93.33 per cent mortality of *Rhyzopertha dominica* observed at 250 µl with 72 hours exposure periods. Fumigation with grains required higher doses to cause the same level of mortality to that of fumigation with insects alone. Complete mortality of *Sitophilus oryzae* and *Rhyzopertha dominica* with WHN I was observed at 20 and 50 µl, respectively in 10 days of exposure period. In the case WHN II 100% mortality of *Sitophilus oryzae* was observed at 50 µl with 10 days exposure period, however only 50 per cent mortality of *Rhyzopertha dominica* was observed with 250 µl, which was five times higher than the former. Thus, *Sitophilus oryzae* was found more susceptible than *Rhyzopertha dominica* to fumigation of both formulations. The progeny emergence, percent damage and per cent weight loss were less in different doses of fumigants to both the pests when compared with untreated control. WHN I has more fumigation potential than WHN II to the test insects.

Key words: fumigation, maize, neem formulations, *Rhyzopertha dominica*, *Sitophilus oryzae,* stored grains.

Introduction

Fumigants are the most potent weapons in managing stored grain insect pests, not only because of their broad spectrum of activity but also their penetrating power and result in minimal or no residues on the treated products. Most effective fumigants are highly toxic to human beings and other non-

*Corresponding author: S. Michaelraj; e-mail: raak@vsnl.com

targeted organisms. Precautions to ensure the safe use of fumigants are necessarily and much more stringent than those required for most other insecticides. Methyl bromide and phosphine are widely used formulated chemical fumigants for disinfestations of commodities under storage conditions. Their usage will also be restricted in future due to their harmful effects. The use of methyl bromide has been highly restricted because of its ozone depleting potential, which leads to harmful effects of radiation on the organisms on the earth. Under the Montreal protocol the world has decided to restrict the use of these fumigants in 2005 in developed countries and in 2010 in developing countries (World Meteorological Organisation, 1995). So one of the few options for fumigation in the future will be phosphine. Many stored grain pests have developed resistance to phosphine (Bell and Wilson, 1995., Sayaboc *et al,* 1998., Daglish and Collins, 1999., Rahman and Shajahan, 2000., Benhalima *et al*, 2004). Thus, there is an urgent need to develop safe alternative fumigants for stored grain pest management.

It has been suggested that fumigants from plant origins could have a greater potential in future on the basis of their efficacy, economic value and use in large-scale storage. Several types of aromatic plants are being investigated for their anti-feedant and insecticidal activity including their fumigant action (El- Nahal *et al*, 1989., Risha e*t al*, 1990., Lee *et al*, 2004., Rao *et al.,* 2005). Among these, neem products have shown outstanding insecticidal activities against different stages of insects and have different modes of action. Though the main insecticidal component in neem products is azadirachtin, some biologically active organosulfur volatile compounds with insecticidal property are also known to occur in neem oil (Balandrin *et al*, 1988). Several workers have tested neem oil for its efficacy as a grain protectant, however, its efficacy as a fumigant has not been studied. In this direction the Indian Tobacco Company (ITC) Limited of India developed two neem formulations *viz.* ware house neem I (WHN I) and WHN II exclusively for controlling stored grain pests. As maize is one of the staple foods of developing countries and also utilized in starch, oil, food and feed industries, improper storage conditions leads to severe attack by storage pests and grains become unfit for consumption. The present study was undertaken to study the efficacy of WHN I and WHN II as fumigants against two major storage pests of maize i.e. rice weevil, *Sitophilus oryzae* and lesser grain borer, *Rhyzopertha dominica* under laboratory conditions.

Materials and methods

Rearing of insects

The pure culture of rice weevil, *Sitophilus oryzae* and lesser grain borer, *Rhyzopertha dominica* were obtained from the Division of Entomology, Indian Agricultural Research Institute, New Delhi, India. The insects were reared on the susceptible maize variety, *Basi local*. Maize grains were sterilized in hot air oven at 60ºC for 4 hours. The sterilized grains were conditioned at 70% relative humidity using KOH solution as described by Solomon (1951). Approximately 100 adults were released in a jar (1 litre capacity) containing 250 g of conditioned grains. The adults were removed after two weeks and the grains were kept in BOD at 28 ± 1ºC and 70% rh for the development of progenies. Adults of different ages were used in the experiments according to the requirements.

Neem formulations

The neem formulations *viz.* Ware house neem I (Mist and Spray) and Ware house neem II (Thermal Fog) were received from the Indian Tobacco Company limited, India for the present study (Fig. 1). Both the formulations contain azadirachtin at 1500 ppm.

Experimental technique

Fumigation chamber

The fumigation chamber was designed by using a plastic jar of 250 ml capacity provided with screw lid (Rahman and Schmidt, 1999). Circular filter paper (5 cm diameter) was pasted on the inner surface of the lid with adhesive tape. The insects confined in vials were placed inside the fumigation chamber. The treatments were applied on the filter paper by using micropipette and the lid was closed and sealed by adhesive tape to create airtight condition in the chamber (Figs 2).

Fumigation bioassay without grain

Different concentrations of the neem formulations were tested against the test insects to decide their level of susceptibility. As both the test insects showed varied levels of susceptibility, four different concentrations were

Fig. 1. Neem formulations WHN I and II used in the present study. **Fig. 2.** Fumigation chamber containing glass vials covered with net and lid with filter paper. **Fig. 3.** Sealed fumigation chamber to create airtight condition.

selected for each species. One to two day old adults were used for the experiment. Ten adults were taken in a vial (5 cm × 1.2 cm) and the mouth of the vial was covered with net (25 mesh) to prevent the insects from escaping and being in contact with the treated filter paper. Three such vials containing insects was placed in the fumigation chamber (described in 3.1) and considered as three replications. The required doses of neem formulations were applied on the filter paper. A parallel untreated control was maintained with each experiment. One fumigation chamber without neem treatment was considered as control. The fumigation chambers were placed in a BOD incubator under optimum conditions of temperature at 28 ± 1ºC and 70% rh. Observations on the adult mortality were taken 48 and 72 hours after treatment as two sets of treatment were maintained separately. Insects showing any movements were considered to be alive.

Fumigation bioassay with grains

Fumigation bioassay with grain was conducted as described without grain except that vials of 25 ml capacity (5.5 cm × 2.5 cm) contained 10 g of maize grain. In this experiment five to seven days old adults were used, as the adult require a minimum of five days of maturation/ pre-oviposition before oviposition. Two sets of treatments were kept for each dose as the adult mortality was taken after five days and ten days of exposure period. The adults were separated out after the exposure period and the grains were kept under optimum conditions for the development of F_1 progeny. The progeny emergence was recorded once in two days from the starting of emergence and the adults were removed from the grains. This was continued until no adult emergence was observed. The damaged and undamaged grains were separated, counted and weighed for calculating per cent damage and per cent weight loss.

Statistical analysis

The data obtained were analysed in completely randomised block design by using the AgRes statistical software, version 3.01. Appropriate transformation of data was done according to the requirements before subjected to statistical analysis.

Results

Fumigation bioassay without grain

Sitophilus oryzae

Fumigant toxicity of Ware house neem I (WHN-I) and Ware house neem II (WHN-II) to the adults of *Sitophilus oryzae* are presented in Figs 4, 5 respectively. Complete mortality of adults was observed at 15 µl /250 ml and above levels at 48 and 72 hours of fumigation with WHN-I. There was no mortality using 5 or 10 µl and in the control. In the case of WHN-II there was a gradual increase in mortality with increase in the dose between 10 and 60 µl. However, doses of 50 µl and above caused complete mortality. This showed that WHN- I (15 µl) was more than three times toxic to *Sitophilus* adults than WHN-II (50 µl). There was slight increase in the mortality as the exposure time increased from 48 to 72 hours in the case of WHN-II.

Fig. 4. Fumigation toxicity of WHN I against the adults of *S. oryzae*

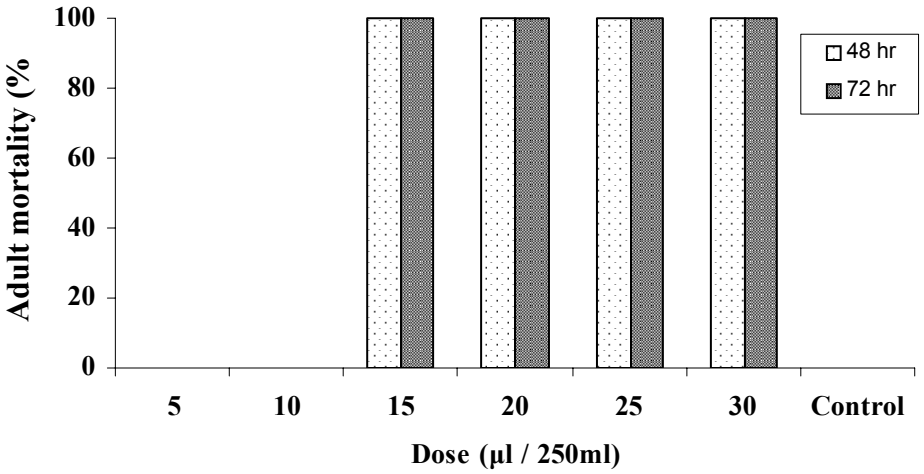
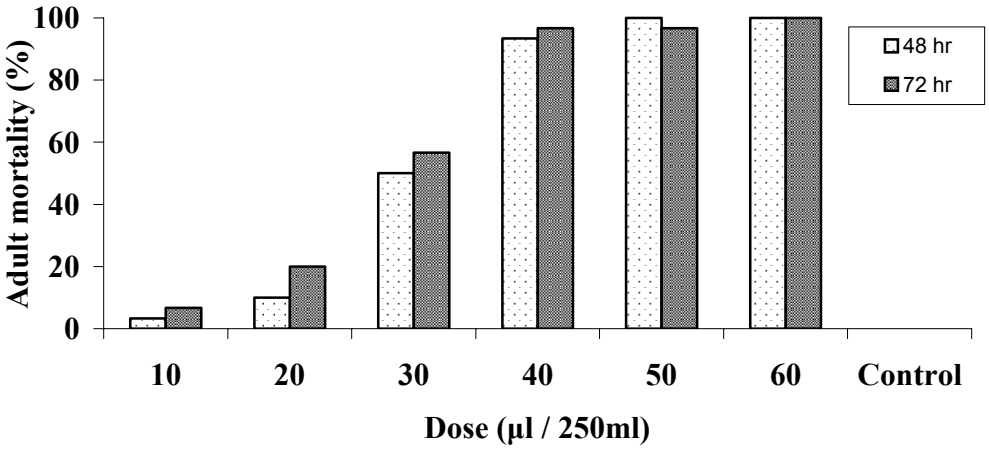


Fig. 5: Fumigation toxicity of WHN II against the adults of *S. oryzae*



Rhyzopertha dominica

Fumigation toxicity of Ware house neem I (WHN-I) and Ware house neem II (WHN-II) to the adults of *Rhyzopertha dominica* are presented in Figs 5, 6, respectively. In the case of WHN-I, complete adult mortality was observed at doses of 30 µl and above. Mortality increased with increase of exposure period. At 10, 20 and 30 µl dosage with 48 hours of exposure, the mortality rate was 53, 73 and 93% respectively and 80, 77 and 100% at 72 hours of exposure. In case of WHN-II, though the doses were higher than WHN-I, maximum mortality of 93% was observed at 250 µl dosage with 72 hours exposure. With the increase of exposure time, a corresponding increase in mortality was observed. This clearly indicated that WHN-I possessed a greater fumigant toxicity than WHN-II.

Fumigation bioassay with grains:

Sitophilus oryzae

Fumigant toxicity of ware house neem formulations against *Sitophilus oryzae* with grains are presented in Table 1. WHN-I formulation caused adult mortality between 27 and 100% at the doses of 5, 10, 15 and 20 µl at five days of exposure. All doses were significantly different from untreated control. Mortality at 15 and 20 µl was similar and significantly better than the other treatments. Mortality increased with increase in exposure time. Ten days of exposure to WHN-I caused higher mortality than five days of fumigation. At 10 days exposure the mortality ranged between 70 and 100% in different doses and was significantly superior over the untreated control. In both the exposure periods the weight loss to the grains was significantly less in all doses than the control. No progeny emerged in all the treatments except in the control which clearly indicated that WHN-I either inhibited oviposition or the development of young stages.

WHN-II was relatively less toxic to *Sitophilus oryzae* as higher doses were required to cause mortality. The mortality also increased with exposure time. In the case of five days exposure, all the treatments caused significantly higher mortality than the untreated control. At 40 and 50 µl dosage the mortality was significantly higher than the other doses. Complete mortality was observed at 50 µl dosage over 10 days and similar to 40 µl. The grain weight loss was significantly higher in control than in other treatments at both exposure times. It was between 0.5 and 1.5% in both the cases as against 46

Fig. 6: Fumigation toxicity of WHN II against the adults of *S. oryzae*

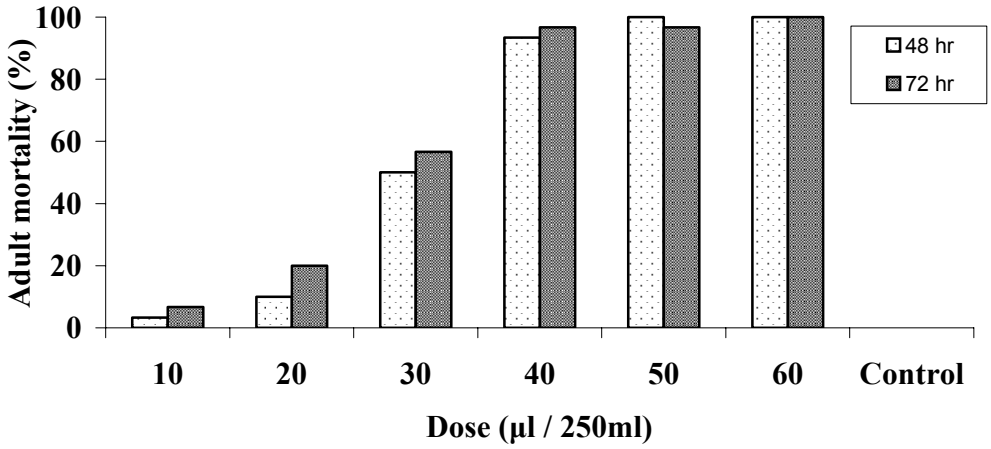
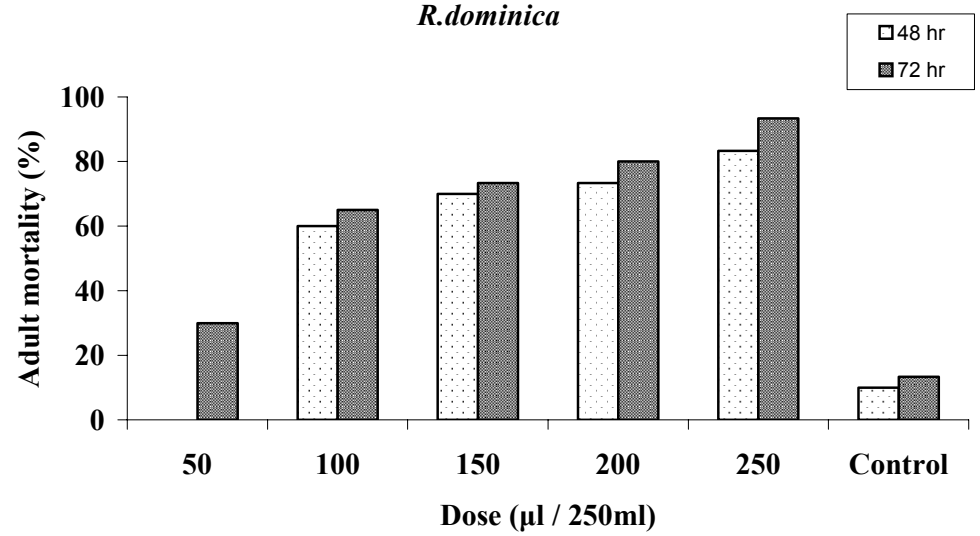


Fig. 4: Fumigation toxicity of WHN II against the adults of *R.dominica*



and 53% in the untreated control after five days and 10 days of exposure, respectively.

Rhyzopertha dominica

Fumigant toxicity of WHN I to *Rhyzopertha dominica* is presented in Table 2. At five days exposure, the maximum mortality of 76.67% was observed with 40 µl dosage, which is significantly different from other treatments. Mortality at 10 µl dosage was nil and 20% at 20 µl and both were similar to the untreated control. Mortality increased with increase in exposure time. At 10 days exposure the maximum mortality of 90% observed in 40 µl which was significantly different from other treatments. The progeny emergence was lowest in 40 µl at both the exposure time and was significantly different from other treatments including untreated control. Correspondingly the grain damage was 1.63 and 9% and weight loss was 2.30 and 13% at 5 and 10 days exposure, respectively and significantly different from the other treatments. In both the cases all treatments were significantly different from the untreated control. Although adult mortality at 10 µl dosage was at similar with the control, the percentage grain damage and weight loss was significantly lower than the untreated control.

WHN II required higher concentrations than WHN I to kill insects. At 250 µl WHN II caused 33 and 50 % mortality at 5 and 10 days of exposure and was significantly different from the other treatments including the untreated control (Table. 3). There was also a positive relation between the exposure time and mortality. At 5 days, in case of adult mortality, all treatments except 250 µl were similar to the control. However all treatments were significantly different from the control in the case of progeny emergence, percentage damage and weight loss. At 10 days, 250 µl dosage resulted in 5.5, 4.8 and 6.3 of progeny emergence, percentage damage and weight loss, respectively which were significantly different from other treatments including control.

Six times higher dosage (250 µl) of WHN II caused only 50% mortality as compared with 90% caused by WHN I (40 µl). This shows that WHN I has a greater fumigation potential than WHN II. Alhough increase in exposure time increased the mortality, it also increased the possibility of more oviposition as the adult mortality was less in the case of WHN II. This resulted in higher progeny emergence, percentage damage and weight loss over 10 days exposure as compare to 5 days. In case of 5 days exposure period the progeny emergence ranged between 8.39 and 13% in different treatments, which was less than the exposure period of 10 days (5.55 to 26%). At 10 days of exposure, although there was 10% increase in adult mortality than over 5 days, the damage and

weight loss was 40% in both the cases, which was higher than 20.45 and 20.37 observed over 5 days of exposure, respectively.

Discussion

Neem formulations WHN I and II showed fumigant toxicity to both *Sitophilus oryzae* and *Rhyzopertha dominica*. When the adults were exposed to fumigation chamber with WHN I, complete mortality of *Sitophilus oryzae* and *Rhyzopertha dominica* was observed at 15 µl and 30 µl / 250 ml and above doses, respectively at both the exposure periods (48 and 72 hours). Complete mortality of *Sitophilus oryzae* was observed at 50 µl doage of WHN II at both exposure periods and 250 µl at 72 hours exposure period caused the maximum of 93% mortality of *Rhyzopertha dominica*. When the adults were fumigated with grains higher doses were required to cause the same level of mortality as without grain. Complete mortality of *Sitophilus oryzae* was observed at 20 µl dosage for WHN I at both 5 and 10 days of exposure. However, more than double the dose (50 µl) with 10 days of exposure was required to cause the same level of mortality in the case of *Rhyzopertha dominica.* In the case of WHN II, 100% *Sitophilus oryzae* mortality was observed at 50 µl over 10 days exposure, however only 50% mortality of *Rhyzopertha dominica* was observed with 250 µl which was five times higher than the former. Dose and time dependent increase in the mortality were observed in most cases. These findings are in agreement with the other reports, which showed that neem volatiles possessed fumigant toxicity to various insect pests. Balandrin *et al* (1988) reported that di-n-propyl disulfide; a major volatile constituent of neem seeds was toxic to larvae of *Aedes aegypti, Heliothis virescens and H. zea.* Neem seed volatiles also showed toxicity to eggs, grubs and adults of *Callosobruchus maculates* at different doses with different exposure periods (Reddy and Singh, 1998). Khatavkar *et al* (2005) observed that hydrodistilled neem leaf volatile oil showed fumigant activity against *C. maculates* and *T. castaneum*. Ravi Dhar *et al*. (1996) hypothesized that organosulfur constituents of neem volatiles could enter either through the cuticle or through the spiracle. The probable reason for the death of insects when exposed to neem volatiles could be either due to interference in gaseous exchange in respiration or asphyxiation.

The present study revealed that though both the formulations WHN I and II contain 1500 ppm azadirachtin, the former exhibiting greater fumigant potential. Among the two insects tested, *Sitophilus oryzae* was more susceptible to fumigation than *Rhyzopertha dominica.* The sensitivity differences may also be due to inherent variation in the susceptibility of both

the insects to neem volatiles. This view is supported from numerous reports, which show great variations in the susceptibility of insects to neem products, irrespective of size, genera or species. Large variation in the sensitivity of stored grain insect pests to fumigation toxicity of volatiles of other plants have been reported by several workers (Klingauf *et al*., 1983; El-Nahl *et al*. (1989); Huang *et al.,* 1997; Schmidt,1991; Shaaya *et al*.,1997; Tripathi *et al*., 2002). El-Nahl *et al*. (1989) observed that the declining order of pest susceptibility to vapours of *Acorus calamus* was *Callosobruchus chinensis, Sitophilus granarius* and *Sitophilus oryzae*, with *Tribolium confusum* and *Rhyzopertha dominica* being tolerant to all the doses and period of exposure tested. Klingauf *et al*. (1983) compared the results obtained with 16 essential oil vapours as fumigants against the adults of *Sitotroga cerealella* and *Acanthoscelides obtectus* and concluded that former is more susceptible than the latter. Schmidt (1991) reported that the adults of *Tribolium confusum* were less sensitive than the adults of *Rhyzopertha dominica* to the vapours of *Acorus calamus* oil.

The neem formulations also have the effect on oviposition, development of young stages and progeny production. They also reduce the damage of grains caused by the insects. No progeny produced in the case of *Sitophilus oryzae* in both the formulations. This clearly shows that they either affect the oviposition or the development of young stages. In the case of *Rhyzopertha dominica* though there was emergence of progeny in fumigated grains, this was significantly less than the untreated control. These findings are in conformity with the findings of several workers who reported that vapours of plant oils reduce the fecundity, egg hatchability and increased neonate larval mortality (Risha *et al.,* 1990; Stamopoulos, 1991; Rahman and Schmidt; 1999). Rahman and Schmidt (1999) observed a significant reduction in oviposition of *Callosobruchus phaseoli* when exposed to vapours *Acorus calamus* oil. In the case of WHN II, when the adults were exposed with grains, the mortality of *Rhyzopertha dominica* was less than 50% in most of the doses tested. At lower doses tested, for example at 100 µl over 10 days of fumigation the mortality increased to 10% from no mortality at 5 days of fumigation. However the progeny emergence, damage and weight loss also increased (26, 40 and 40% respectively) at 10 days of fumigation as comparedto 5 days (13, 20 and 20% respectively). This may be due to the fact that an increase in exposure time of the live adults on grain, favours adult feeding and oviposition. Thus, a correspondence increase in percentage damage, weight loss and progeny production occurs, though a higher mortality is recorded with the increase in the exposure period. These findings are in agreement with the studies of Rahman and Schmidt (1999). They tested the effect of vapours of essential oils of *Acorus calamus* from different origins and observed that at lower doses

increase in exposure time increased the oviposition and progeny production of *Callosobruchus phaseoli.*

The present studies show that the neem formulations WHN I and II affect different stages of the *Sitophilus oryzae* and *Rhyzopertha dominica* and can be used as effective fumigants. However further studies under bulk storage conditions should be carried out before recommending the large-scale use of these funigants.

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Table 1. Fumigation toxicity of WHN I and WHN II to *S. oryzae* at different exposure period.

Dose (µl / 250 ml)	WHN I				WHN II			
	Adult mortality (%)		Weight loss (%)		Adult mortality (%)		Weight loss (%)	
	5 DF	10 DF	5 DF	10 DF	5 DF	10 DF	5 DF	10 DF
5	26.67 (30.29) [b]	70.00 (57.00) [b]	1.17 [a]	1.67 [a]	--	--		
10	30.00 (33.21) [b]	86.67 (76.43) [ab]	1.83 [a]	0.83 [a]	--	--		
15	93.33 (77.47) [a]	96.67 (83.36) [a]	2.17 [a]	2.00 [a]	--	--		
20	100.00 (89.26) [a]	100.00 (89.26) [a]	1.50 [a]	1.83 [a]	43.33 (41.07) [b]	73.33 (59.71) [b]	1.17 [a]	1.50 [a]
30	--	--			36.67 (37.22) [b]	76.67 (61.22) [b]	1.50 [a]	0.50 [a]
40	--	--			86.67 (72.05) [a]	93.33 (77.47) [a]	1.00 [a]	0.83 [a]
50	--	--			93.33 (77.44) [a]	100.00 (89.26) [a]	1.17 [a]	1.00 [a]
Control	6.67 (12.53) [c]	20.00 (26.07) [c]	46.00 [b]	53.33 [b]	6.67 (12.53) [c]	20.00 (26.07) [c]	46.00 [b]	53.33 [b]
CD (0.05)	(14.63)	(21.42)	5.49	7.49	(23.43)	(13.82)	5.45	7.41

Figures in parentheses are arcsine-transformed values
CD (0.05) significant at 5 % level
DF – Days of fumigation
Figures followed by the same letter in the same column are not significantly different at 0.05 levels as determined by the LSD

Table 2. Fumigation toxicity of WHN I to *R. dominica* at different exposure period.

Dose	Adult mortality (%)		F_1 Progeny emergence		Damage (%)		Weight loss (%)	
(µl / 250 ml)	5 DF	10 DF	5 DF	10 DF	5 DF	10 DF	5 DF	10 DF
10	0.00 (0.74)[c]	6.67 (12.53)[c]	18.67[bc]	17.67[b]	28.99 (31.96)[b]	30.65 (33.57)[b]	30.50 (33.02)[b]	25.73 (30.35)[b]
20	10.00 (15.24)[bc]	16.67 (23.36)[bc]	21.67[bc]	17.00[b]	33.10 (34.78)[b]	21.13 (26.76)[ab]	32.10 (34.15)[b]	18.33 (25.15)[ab]
30	26.67 (30.29)[b]	43.33 (40.78)[b]	16.35[ab]	12.67[b]	26.05 (29.74)[b]	21.91 (27.68)[ab]	25.80 (29.80)[b]	19.97 (26.53)[ab]
40	90.00 (74.76)[a]	76.67 (61.93)[a]	0.33[a]	2.67[a]	1.63 (7.04)[a]	9.31 (16.02)[a]	2.30 (8.18)[a]	12.63 (20.02)[a]
Control	0.00 (0.74)[c]	0.33 (6.64)[c]	30.00[c]	32.00[c]	66.00 (54.39)[c]	68.18 (55.82)[c]	67.40 (55.21)[c]	71.03 (57.54)[c]
CD (0.05)	(16.25)	(20.19)	15.61	7.59	(15.90)	(11.22)	(14.75)	(8.89)

Figures in parentheses are arcsine-transformed values
CD (0.05) significant at 5 % level
DF – Days of fumigation
Figures followed by the same letter in the same column are not significantly different at 0.05 levels as determined by the LSD

Table 3. Fumigation toxicity of WHN II to *R. dominica* at different exposure period.

Dose	Adult mortality (%)		F_1 Progeny emergence		Damage (%)		Weight loss (%)	
(µl /250 ml)	5 DF	10 DF	5 DF	10 DF	5 DF	10 DF	5 DF	10 DF
100	0.00 (0.74) [b]	10.00 (15.24) [b]	13.37 [a]	26.00 [c]	20.45 (25.60) [a]	40.27 (39.39) [c]	20.37 (26.81) [a]	40.27 (39.38) [b]
150	0.33 (6.64) [b]	26.67 (30.99) [a]	12.67 [a]	16.33 [b]	19.22 (25.64) [a]	31.78 (33.78) [bc]	23.20 (28.49) [a]	30.93 (33.26) [b]
200	10.00 (15.24) [ab]	30.00 (33.00) [a]	12.67 [a]	13.33 [b]	18.92 (25.35) [a]	23.79 (28.93) [b]	20.60 (26.61) [a]	25.30 (29.95) [b]
250	33.33 (34.14) [a]	50.00 (45.00) [a]	8.39 [a]	5.55 [a]	13.85 (18.80) [a]	4.78 (12.21) [a]	16.30 (21.83) [a]	6.23 (14.13) [a]
Control	0.00 (0.74) [b]	0.33 (6.64) [b]	30.00 [b]	32.00 [c]	66.00 (54.38) [b]	68.18 (55.72) [d]	67.40 (55.21) [b]	71.03 (57.53) [c]
CD (0.05)	(18.92)	(14.86)	10.97	9.23	(15.15)	(10.44)	(13.63)	(10.58)

Figures in parentheses are arcsine-transformed values
CD (0.05) significant at 5 % level
DF – Days of fumigation
Figures followed by the same letter in the same column are not significantly different at 0.05 levels as determined by the LSD