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Evaluation of Some Grain Protectants and Ozone Gas against *Sitophilus Granarius* L. In Stored Wheat Grains

Ahmed M. A. Salman, Hany Ahmed Fouad, Abd –El aziz E. Abd –El aziz and Walaa A. A. Abazied

Abstract

This work was carried out in the laboratory at stored product pests at EL-Mattana Agric. Res. Station to determine the insecticide of plant powders (*S. aromaticum*, *P. nigrum* and cattle dung ash powder) at 0.5, 1, 1.5, 2, 3 % (W/W) concentrations. While ozone gas tested at 100, 200, 400 and 600 ppm against *S. granarius* L. Mortality percentage were registered after 1, 3, 5, 7 and 14 days. The results obtained that increasing in concentrations from 0.5 to 3 % and 100 to 600 ppm at exposure period 1-14 days. Mortality percentage attain to 100% at concentrations 2 and 3% (W/W) at clove and black pepper after 7days. Meanwhile, at concentrations 2 and 3% (W/W) in case of cattle dung ash were 81.7 and 90.0%, respectively. Black pepper was the most effective with LC_{50} of 0.327, while cattle dung ash was the least with LC_{50} of 0.867 (W/W). Reduction in F_1-progeny in all treatments were greatly significant. Obtained results showed that treatment a high dose 400 and 600 of ozone gas for 5days led to full mortality of *S. granarius* adult, but lows dose for 14 days some survival of *S. granarius* adults was found at all exposure time. Based on LT_{50} values at 400 ppm were 2.485 and 1.135, 2.15and 0.893 at 600 ppm, respectively. This mean that *S. granarius* mortality increased by increasing poseur time and period after treatment to ozone gas.

Keywords: Botanical powders, ozone gas toxicity, wheat grains, *Sitophilus granarius*.

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Ahmed M. A. Salman
Hany Ahmed Fouad
Abd –El aziz E. Abd –El aziz
Walaa A. A. Abazied

Plant Protection Department
Faculty of Agriculture
Sohag University
Sohag
82524
Egypt

Corresponding author:
Walaa Abd Ellatief A. Abazied
walaa.abdabdo@gmail.com

INTRODUCTION

Grains are infested and attacked by many insect pests during growth until storage in the store. (Shiferaw *et al*., 2011). It has many important to the live of human, grains have some carbohydrates, vitamins, minerals, fats, protein and fiber. The insects cause a lot of losses in store it was almost 20% in developing countries and almost 9% in developed countries (Pimentel 1991). Chemical pesticides has dangerous effect in the control of stored grain pests (Salem *et al*., 2007). It necessary to look for safety sources for mammals and environment (Udo, 2005). Plant powders had been using all over the world in old practice was protectants of stored products (Aslam *et al*., 2002) and mixing grains with plant-based protectants can protect in the generally involves the stored products (Tapondjou *et al.*, 2002). some of plant powders has strong effects the insects of stored grain (Emeasor *et al*., 2005;Nadra, 2006) such as powders of plant parts (leaves, roots and flowers) which had successful used in insect pest control (Akinneye *et al*., 2006). Fumigation process is the most cheap and effective way compared the others way often world-wide (Banks, 1989). Ozone control of stored grain pests was explored in more than a decade ago. Ozone can killed a wide range of microorganisms without leave any residues (Wu *et al.*,2006). Previous studies founded that ozone treatment had adversely affect on the insects stored from the order Coleoptera, (*Sitophilus* spp.) and (*T. castaneum* Herbst) and from the order Lepidoptera,(*Ephestia kuehniella* Zeller) and (*Plodia interpunctella* Hübner) (Strait,1998 ; Isikber and Öztekin, 2009). PCR based techniques are regularly used for identification, characterization and early diagnosis of microbes and pathogens. Random amplified Polymorphic DNA (RAPD) analysis (Williams et al., 1990; Miller 1996; Gupta et al. 2009 and Ingle et al., 2009) has been used for confirmation of identity among different isolates of fungi (Assigbetse et al., 1994 and Alves-Santos et al., 2002). It has been observed to have a high level of variability among many isolates (Chiocchetti et al., 1999; Edwards et al. 2002; Leslie and Summerell, 2006; Sabir 2006; Lievens et al., 2007; Bayraktar et al., 2008 and Steinkellner et al. 2008). In addition, RAPD is simple and relatively faster as compared with other molecular techniques (Wilson et al., 2004; Guleria et al., 2007 and Niessen 2007). Furthermore, RAPD markers can help to comprehend the mechanisms of pathogenic variation (Albores et al., 2014). So, RAPD markers demonstrated remarkable variation of bacteria, fungi and plants (Skaria et al. 2011 and Singh et al. 2011). There are several reports on characterization of fusarium species using RAPD markers. Gupta et al. (2009) reported the genetic polymorphism of six isolates of *F. solani* causing wilt disease in guava. Ingle and Rai (2011) reported genetic diversity of *F. semitectum* associated with mango malformation. In addition, similarly, a genetic variation in *F. oxysporum f. sp. Fragariae* causing wilt disease in strawberry was identification by Nagarajan et al. (2004). Therefore, the aim of the present study was to estimate the genetic variation among eight isolates of using R *F. oxysporum f.sp. lycopersici* APD-PCR marker to determine the relationship among them.

MATERIALS AND METHODS

1. Insect culture of *S. granarius* L.

Insect, was reared in plastic jar containing 1kg of sterilized wheat kernels which treated by freezing at -18°C for 2 weeks before application to eliminate any possible infestation by any insect species. 800 adults of *S. granarius* were introduced into the plastic jars contain 2kg for laying eggs and covered with muslin cloth and fixed with rubber bands. For (1-2 weeks), then kept it under controlled conditions at the rearing laboratory room.

2. Botanicals powders

2.1. Natural plant species used and cattle dung ash powder:

Two plant species belonging to two different families were used during the studied: Clove flowering buds, *Syzygium aromaticum* (Family: *Myrtaceae*) and black pepper seeds , *Piper nigrum* (Family: *Piperaceae*) obtained from herb-shop, grounded well with an electric mill sieved using mesh sieve and kept in a clean glass jar. The pulverized seed powder was tested at 0.5, 1, 1.5, 2 and 3 % (W/W) concentrations. And cattle dung ash powder at 0.5, 1, 1.5, 2 and 3 % (W/W) concentrations.

4. Ozone gas:

Ozone gas was product from air using an ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada (OZO Max Ltd, Shefford, Quebec Canada) from purified extra dry oxygen feed gas at the laboratory of Food Toxicology and Contaminants, National Research Center, Egypt. The amount of ozone output was controlled by a monitor- controller having a plug-in sensor on board which is changed for different ranges of ozone concentration and a belt pan in the monitor-controller allows controlling the concentration in a selected range. 3 different exposure times (treatment) of 1, 2 and 3 hours at 100, 200, 400 and 600 ppm concentration.

5. Bioassay tests

5.1. Natural materials and cattle dung ash powder:

20 adults of *S. granarius* were introduced into each plastic jar with 20 gm wheat grains. 3 replicates for each treatment and control. The replicates were kept at the same rearing conditions. All jars were covered with muslin cloth and fixed with rubber band and then kept in the laboratory. Mortality data were recorded after 1, 3, 5, 7 and 14 days from exposure. %mortality was taken after 24hr and were calculated and corrected according to Abbott's formula (1925), Finney (1971). After 14 days adults were removed from all replicates and kept under laboratory conditions for 45 days to inspect the number of F_1- progeny. Ldp line program was used in statistical analysis.

5.2. Ozone gas:

50g of wheat grains for 40 adults of *S. granarius* were put into plastic jars and let for 10 days. 3replicates were carried out for each treatment and control. After 10 days, 40 adults of *S. granarius* with wheat grains were introduced into small cloth bags, then closed well with rubber band. All bags of the same concentration were put into large flask closed with rubber. Three different exposure times (treatment) of 1, 2 and 3 hours at 100, 200, 400 and 600 ppm concentration. After exposure, treatments were transferred carefully into plastic jars and covered with muslin cloth and secured with rubber bands and kept at the laboratory conditions. Mortalities were recorded at 1, 3, 5, 7,10 and 14 days after treatment. After 14 days adults in all insects were removed from all replicates and kept under laboratory conditions for 45 days and inspect the number of F_1-progeny. Ldp line program was used to obtain the toxicity regression lines, the lethal times LT_{50} and LT_{90} were determined.

RESULTS AND DISCUSSION

1. Effect of the plant powders on the adults of *S. granarius*:

Biological activity of tested materials, clove, black pepper and cattle dung ash dust as grain protectants against *S. granarius* under laboratory condition are presented in Table (1). The results indicated that the mortality was increased with increasing concentration and exposure period. This result reveals that black pepper had toxic on *S. granarius* at various concentration after 5, 7 and 14 days than clove and cattle dung ash at the same time. While the clove had effect on *S. granarius* after 1 and 3 days at all the concentrations than black pepper and cattle dung ash. At the lowest concentration of the grain protectants 0.5 and 1% give complete mortality with black pepper after 7days, While give 48.3, 75.55% and 63.3, 65.0% for clove and cattle dung ash at the same concentration, respectively. Also at 3% (w/w) concentrations after 5 days from treatment the mortality % were 100, 100 and 83.3% with clove, black pepper and cattle dung ash dust, respectively. Mortalities increased after 14 days to reach (53.3,81.7, 100.0, 100.0, and 100%), (100 -100%) and (68.3, 76.7, 86.7, 90.0 and 95.0 % w/w) for clove, black pepper and cattle dung ash powder, respectively against *S. granarius* at various concentrations, respectively. The results obtained that black pepper dust inhibited adult emergence at all concentration. While with clove and cattle dung ash give inhibition rates (51.7, 93.5, 100, 100 and 100%) and 79.7, 86.1, 87.6, 90.3 and 92.1% for all concentrations, respectively. These results, agreed with that obtained by, Choden et al. (2021) evaluated the efficacy of *Piper nigrum* L. seed powder against *S. zeamais*. They showed that mortality of *S. zeamais* was100% after 8 days at 1.5% concentration g/ kg of maize. Mona et al. (2021) Studied the effect of clove powder against *S. granarius* at 0.125, 0.25, 0.5 and 1% concentration. Who revealed that after

1, 3 and 7 days post treatment mortality percent was 20, 78 and 100%, respectively. As well as Rani *et al.* (2021) studied the effect of powders of cow dung ash, silver *nanoparticles*, malathion and sweet flag rhizome powder against *S. oryzae*. They showed that malathion was more effective than sweet flag rhizome powder followed by cow dung ash and the last one was *s. nanoparticles*.

Table (1) Effect of botanical powders on the adults of S. *granarius* and reduction in F_1-progeny under laboratory conditions.

Plant Powder Treatment	Conc. (W/W) %	(%) Adult mortality after indicated days					Average NO. of emerged Adults after 45 days	% reduction in F_1-Progeny
		1	3	5	7	14		
Control		0.0	0.0	0.0	0.0	0.0	26.3	0.0
clove	0.5	18.3±0.6	38.3±0.6	43.3±0.6	48.3±0.6	53.3±0.6	12.7	51.7
	1	33.3±0.6	60.0±0.0	68.3±0.6	75.0±0.0	81.7±0.6	1.7	93.5
	1.5	46.7±1..2	73.3±0.6	81.7±0.6	90.0±0.0	100±0.0	0.0	100
	2	63.3±0.6	85.0±0.0	91.7.±0.6	100±0.0	100±0.0	0.0	100
	3	76.7±0.6	90.0±0.0	100±0.0	100±0.0	100±0.0	0.0	100
Control		0.0	0.0	0.0	0.0	0.0	28.0	0.0
black pepper	0.5	0.0±0.0	25.0±1.0	71.7±1.2	100±0.0	100±0.0	0.0	100
	1	0.0±0.0	40.0±1.0	81.7±0.6	100±0.0	100±0.0	0.0	100
	1.5	0.0±0.0	50.0±1.0	93.3±0.6	100±0.0	100±0.0	0.0	100
	2	0.0±0.0	61.7±0.6	100±0.0	100±0.0	100±0.0	0.0	100
	3	0.0±0.0	66.7±0.6	100±0.0	100±0.0	100±0.0	0.0	100
Control		0.0	0.0	0.0	0.0	0.0	38.0	0.0
cattle dung ash	0.5	0.0±0.0	13.3±0.6	36.7±0.6	63.3±0.6	68.3±0.6	7.7	79.7
	1	0.0±0.0	21.7±0.6	51.7±0.6	65.0±0.0	76.7±0.6	5.3	86.1
	1.5	0.0±0.0	35.0±1.0	63.3±0.6	75.0±0.0	86.7±0.6	4.7	87.6
	2	5.0±0.0	51.7±0.6	73.3±0.6	81.7±0.6	90.0±0.0	3.7	90.3
	3	6.7±0.6	58.3±0.6	83.3±0.0	90.0±0.0	95.0±0.0	3.0	92.1

Lethal concentration of clove, black pepper and cattle dung ash powders against the adults of *S. granarius*.

The confidence limits and lethal concentration of the tested clove, cattle dung ash and black pepper powder against the adults of *S. granarius* after exposure different periods are shown in Table (2) Results obtained that clove was the most toxic (0.717 -0.623%) and black pepper (1.438-0.327%), after 3and 5days, respectively followed by cattle dung ash (2.240 and 0.867 % w/w) after 3 and 5 days, respectively. Also, the toxicity of cattle dung ash, clove and black pepper powder were significantly increased with the increasing of the exposure times.

The toxicity index of various powders tested against the adults of *S. granarius*.

The toxicity index of various tested powders against the adults of *S. granarius* under laboratory conditions were shown in Table (3). Results showed that cattle dung ash had the least efficacy, whereas black pepper was the highest toxic compound, followed by clove powder.

2. Effect of ozone gas against *S. granarius* adult:

Mortality of *S. granarius* generally increased with increasing exposure time (1, 3, 5, 7,10 and 14 days) and injection time ppm (1, 2 and 3hrs) at (100, 200, 400 and 600 ppm) results presented in Table (4). The data cleared that corrected percentage of mortality increased with increasing exposure time to ozone gas as well as the days post treatment. At 100ppm significant differences were observed between various exposure times after 1, 3, 5, 7,10 and 14 days of ozone, %mortality of *S. granarius* adults were (0.0, 3.3, 8.3, 15.0, 20.0 and 24.2%) , (0.8, 10.8, 15.0, 24.2, 30.0 and 36.7) and (1.7, 15.8, 23.3, 33.3, 40.0 and 47.5%) for 1, 2 and 3 hrs injection, respectively. The reduction of F_1-progeny it them treatments high compared to mortality it was (44.0, 49.3 and 58.0) for 1, 2 and 3 hrs, respectively. At 200ppm the mortality% was moderate high compared to 100ppm at the same periods.

Table (2) confidence limits and lethal concentrations of cattle dung ash, clove and black pepper powder against the adults of *S. granarius* after different exposure periods.
N.B:

Plant Powders	Exposure period (days)	LC_{50}	Slope
Clove	Day 3	0.717 a (0.534-0.873)	2.092
	Day 5	0.623 a (0.487-0.741)	2.764
Cattle dung ash	Day 3	2.240 b (1.833-3.030)	1.854
	Day 5	0.867 a (0.629-1.075)	1.672
Black pepper	Day 3	1.438 b (1.132-1.853)	1.479
	Day 5	0.327 a (0.183-0.449)	2.424

CL: confidence limits, toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) x100]. Sun (1950).
LC_{50} values within the same row having different letters are significantly different (95% FL did not overlap). Finney (1971).

Table (3) Toxicity index of various tested powders against of *S. granarius* adults**.**

Treatments	Lethal concentrations at 5 days (w/w %)		
	LC_{50}	**Slope**	**Toxicity index**
Clove	0.623	2.764	52.5
Black pepper	0.327	2.424	100
Cattle dung ash	0.867	1.672	37.7

N.B:
CL: confidence limits, toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) x100]. Sun (1950).

These values were 32.5, 47.5, and 63.3% after 14days post treatment for 1, 2 and 3 hrs, respectively. Also, the reduction of F_1-progeny was slowly high compared to mortality were 53.8, 62.7 and 68.2% for 1, 2 and 3 hrs, respectively. At 400ppm the mortality% after 1hr injection was 5.8, 34.2, 40.0, 42.5, 50.0 and 74.2% at 1, 3, 5, 7,10 and 14 days. Mortality was complete after 5days at 2 and 3hrs injection of ozone gas. On the other hand, the reduction in F_1-progeny is low the mortality at all time injection (58.8, 68.6 and 74.5%) for 1, 2 and 3 hrs, respectively. At 600ppm the mortality% after 1hr injection was 10.8, 60.8, 69.2, 75.0, 77.5 and 93.3% after 1, 3, 5, 7,10 and 14 days. These values were 97.5 and 100% after 3days at 2 and 3hrs injection gas. But the reduction in F_1-progeny is very decreased compared the mortality at various the injection time (67.0, 75.1 and 79.6%) at 1, 2 and 3 hrs, respectively. These results showed that the adults of *S. granarius* is very sensitive to ozone gas compared the egg. In similar studies, Ahmed *et al.* (2017) studied the effect of ozone against the adult of *T. castaneum* and *S. oryzae* at 500 ppm. Results observed that after 7 days exposure time was adequate to eliminate adults of *S. oryzae* and *T. castaneum*. Xinyi *et al.* (2017) evaluated the effect of ozone gas against *S. zeamais*, *O. surinamensis*, *T. castaneum* and *S. oryzae* at concentration of 200 ppm after 1, 2, 3, 5, 6, 8, 10 and 12 hrs. They showed that mortalities at 5d were 100% for *Sitophilus spp*. and *O. surinamensis* after 8 and 12hrs exposure time and for *T. castaneum* mortalities were 90%. As well as, Lemic *et al.* (2019) found that mortality of *S. granarius* at 2.5 g/m3 (0.001ppm) ozone applied for 120 min reached 100% at 7days after ozonztion.

Lethal time values of mortality to ozone gas against the adults of *S. granarius* adults.

Confidence limits and lethal time values of ozone gas against the adults of *S. granarius* under laboratory conditions are shown in Table (5-6). Results observed that, at 100 ppm LT_{50} values were 6.79 % and 3.44%, at 7 and 14 days, respectively. At 200 ppm LT_{50} were 4.07% and 1.98%, at 7 and 14 days, respectively. In case of 400 ppm LT_{50} values were 2.485% and 1.135%, at 1 and 3days, respectively. While at 600ppm the LT_{50} values, for the adults were 2.154% and 0.893% at 1-3 days, respectively.

Table (4): The efficacy of 100, 200, 400 and 600 ppm ozone gas against the adults of *S. granaries* and reduction in F_1-progeny under laboratory conditions.

Ozone doses (ppm)	Exposure time (hours)	(%) Adult mortality after indicated days						Average NO. of emerged Adults after 45 days	% reduction in F_1-Progeny
		1	3	5	7	10	14		
100	1	0.0 ±0.0	3.3 ±0.6	8.3 ±0.6	15.0 ±1.0	20.0 ±1.0	24.2±0.6	136.3	44.0
	2	0.8 ±0.6	10.8 ±0.6	15.0 ±0.0	24.2 ±0.6	30.0 ±1.0	36.7±0.6	123.3	49.3
	3	1.7 ±0.6	15.8 ±0.6	23.3±0.6	33.3±1.2	40.0±1.0	47.5±1.0	102.3	58.0
200	1	0.0 ±0.0	7.5 ±0.0	13.3 ±0.6	20.8 ±0.6	27.5 ±1.0	32.5±1.0	112.3	53.8
	2	1.7 ±0.6	14.2 ±0.6	24.2 ±0.6	33.3 ±0.6	40.8 ±0.6	47.5±0.0	90.7	62.7
	3	3.3 ±0.6	22.5 ±0.0	33.3±0.6	43.3±1.5	51.7±1.2	63.3±1.5	77.3	68.2
400	1	5.8 ±0.6	34.2 ±1.2	40.0 ±1.7	42.5 ±1.7	50.0 ±1.7	74.2±0.6	100.3	58.8
	2	30.8 ±1.2	96.7 ±0.6	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	76.3	68.6
	3	65.8 ±1.5	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	62.0	74.5
600	1	10.8 ±0.6	60.8 ±2.1	69.2 ±2.1	75.0 ±1.0	77.5 ±1.0	93.3±1.2	80.3	67.0
	2	38.3 ±0.6	97.5 ±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	60.7	75.1
	3	75.8 ±0.6	100.0 ±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	49.7	79.6
Control		0.0	0.0	0.0	0.0	0.0	0.0	243.3	0.0

Table (5) Lethal time values and confidence limits for the *S. granarius* adults at 100 and 200ppm.

Ozone doses	Exposure period (days)	T_{50}	confidence limits LT_{50}		LT_{90}	lope
			Lower	Upper		
100 ppm	7	6.79	4.06	45.00	61.76	1.261
	10	5.03	3.31	21.27	56.48	1.22
	14	3.44	2.59	7.25	22.42	1.326
200ppm	7	4.07	2.93	10.15	36.53	1.344
	10	2.86	2.24	5.00	26.37	1.329
	14	1.98	1.63	2.48	12.14	1.626

Table (6) Lethal time values and confidence limits for the *S. granarius* adults at 400 and 600ppm.

Ozone doses	Exposure period (days)	LT_{50}	confidence limits LT_{50}		LT_{90}	Slope
			Lower	Upper		
400 ppm	1	2.485	2.287	2.743	5.015	4.204
	3	1.135	1.062	1.208	1.688	7.424
600ppm	1	2.154	1.983	2.355	4.478	4.034
	3	0.893	0.777	0.983	1.514	5.592

CONCLUSION

From the previous results of the various plant powders and ozone gas against *S. granarius*, while the least effective was in cattle dung ash. Using ozone gas high doses 400 and 600 was the most effective controlling the adult of *S. granarius*

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