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Fumigant Toxicity of Methyl Benzoate Loaded onto Different Carrier Materials Against the Rice Weevil, *Sitophilus oryzae*

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ABSTRACT

Methyl benzoate is a naturally occurring botanical compound that has insecticidal activity against a broad spectrum of insects and provides a potential alternative to synthetic insecticides. In this study, we evaluated the fumigant effectiveness of methyl benzoate (MB) loaded onto three simple carrier materials including filter paper disc, cotton disc and charcoal tablet against adults of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) with and without wheat grains. Also, the effects on germination and quality of stored wheat grains have been investigated. The results showed that after 12 h, MB-filter paper disc was the most effective treatment against *S. oryzae*. However, within 48 h MB-cotton disc achieved the highest activity both in the presence and absence of wheat grains. Throughout the 30 days of storage periods, MB-activated charcoal exhibited the highest activity against *S. oryzae* compared to other treatments. All MB-tested carrier materials had no negative impact on the germination, carbohydrate, and protein contents of stored wheat grains. These results suggest that using MB loaded onto some carrier materials like activated charcoal may significantly maintain its effectiveness over storage time in the fumigation process. Overall, this study provides good evidence that MB-activated charcoal tablets hold promise as a safe and effective alternative to hazardous synthetic fumigants such as phosphine and methyl bromide for stored product pest control.

KEYWORDS: Methyl benzoate, *Sitophilus oryzae*, fumigation, wheat grain, carrier materials.

1. INTRODUCTION

The infestation of stored products caused by insect pests completely decreases the quality and market value of these products. The most effective and adopted method to control storage pests is the use of fumigation and chemical insecticides (Weaver and Petroff, 2005).

Historically, stored products pest management has heavily relied on fumigants to control insects in bulk commodities, packaged materials, and structures. The two main fumigants have been methyl bromide and phosphine (Hagstrum and Phillips, 2017). Methyl bromide according to Environmental Protection Agency (EPA) report (2023) depletes the ozone layer so, the United State (US) has gradually stopped producing and using methyl bromide, except for critical uses and quarantine. Methyl bromide is a highly toxic substance that can cause serious harm to humans, particularly at fumigation sites where it is most dangerous. Fumigators and related workers who are exposed to high concentrations can lead to central nervous system and respiratory system failures, as well as harm to the lungs, eyes, and skin (Tomlin, 1997; Suwanlaong and Phanthumchinda, 2008). Phosphine is still a widely used fumigant for stored products and is typically available as aluminum or magnesium phosphide tablets (Navarro, 2006). Some stored product insects also developed resistance to phosphine (Rajendran and Gunasekaran, 2002; Benhalima *et al.*, 2004). Phosphine exposure can result in a variety of symptoms, including fluid in the lungs, nausea, vomiting, diarrhea, stomach discomfort, thirst, and muscle aches. Long-term and higher exposures have the potential to be extremely harmful (NIOSH, 2019). Therefore, it is important to research and develop new alternatives to synthetic pesticides for stored product pest control in order to reduce the hazards to humans and the environment.

In nature, many plant species release great amounts of volatile organic compounds (VOCs) into the atmosphere (Guenther *et al.* 1995 and Lerdau *et al*. 1997). Some of these VOCs may act as defensive compounds against insect herbivores and plant pathogens that attack plants (Penuelas and Llusia, 2001).

Methyl benzoate (MB) is a volatile compound naturally synthesized in many plants including snapdragons and petunias and has recently been discovered as an insecticide against several species of insect pests **(**Feng and Zhang, 2017 and Chen *et al*. 2019; Yang et al. 2020).

Methyl benzoate has been used as a fragrance in the perfume industry and a preservative in many personal care applications, such as shampoos, shower products and liquid soaps (Bickers *et al.* 2003 and European-Commission 2006). The most important chemical properties of this compound, it is slowly biodegradable in the atmosphere, and it has low to moderate human toxicity by ingestion and inhalation (Opdyke 1979 and Clayton and Clayton 1982).

It has been approved by the US Food and Drug Administration (21 CFR172.515; FDA, 2016) and the European Union (EU Regulation 1334/2008 & 178/2002) (European-Union, 2015) for food use as a food-grade flavor ingredient. So, MB could be considered as a green pesticide candidate in pest management due to its effective properties against insects and relative safety for humans and the environment. In this study, we investigated the potential fumigation effectiveness of MB loaded onto three carrier materials, including filter paper discs, cotton discs and activated charcoal tablets against *S. oryzae*, a major stored product insect. Moreover, we evaluated the impact of these carrier materials on MB persistence throughout the storage time of grains. This research aims to provide valuable insights into using MB as a fumigant and the role of different carrier materials in enhancing its efficacy.

2. MATERIALS AND METHODS

2.1. Chemicals

Methyl benzoate (99%) and acetone (99.5%) were purchased from Fisher Scientific Company.

2.2. Insects

Sitophilus oryzae, commonly known as the rice weevil, is a major stored product insect. The rearing process was as follows: about 100 adult insects were introduced into jars containing 500 g of sterilized wheat. The jars were then covered with muslin cloth and kept under laboratory conditions at 30 ± 2°C and 65 ± 5% relative humidity. The newly emerged adults, aged 1-2 weeks, were used for all subsequent tests and observations.

2.3. Fumigant toxicity of methyl benzoate against adults of *Sitophilus oryzae*

Two different bioassay methods were performed to evaluate the fumigation toxicity of MB loaded onto different carriers (MB-carrier material) against adults of *S. oryzae* both in the presence or absence wheat grains:

First, MB fumigation of *S. oryzae* (without grain): In this experiment, glass bottles (1L-Schott Duran) were sealed to serve as fumigation chambers. Three different carrier materials including filter paper disc, cotton disc, and activated charcoal tablet (each 1.5 cm diameter) were loaded with MB at series concentrations of 5, 10, 15, and 20 µg/L air. Each carrier material was loaded with 100µl of each concentration and placed at the bottom of the glass bottle. Twenty adults of *S. oryzae* were transferred into the jars and then sealed with Parafilm (Bemis Company Inc., Neenah, USA). Mortality rates were recorded at 6, 12, 24 and 48 h post-exposure. Three replicates were performed for each MB concentration and the control (without MB).

For the second bioassay method (with grain): Twenty gram of wheat grains were added to each jar. Series concentrations (10, 15, 20, and 25 µg/L air) of MB were used. Otherwise, the same methods and treatments as described above were followed.

2.4.Storage experiment

This experiment was conducted to evaluate the long-term effectiveness of MB against *S. oryzae* within specific period of storage (1, 2 and 3 months) in both presence and absence of wheat grains. After evaluating 24h-LC_{90} values for MB loaded onto the three carrier materials, jars were divided into two groups: the first group consists of 27 jars without grain and every 9 jars have a carrier material treated with MB at $LC_{90.}$ The second group of 27 jars has grains (20 g) and each 9 jars has a carrier material treated with MB at $LC_{90.}$ The wheat grains used in this study were previously sterilized by drying at 50ºC for 2 h to kill any prior infestation by insects. Untreated grains were served as control. After 1, 2, and 3 months, the two groups and the control were taken and infected by the tested insects. Mortality was recorded after one day for both bioassay treatments.

2.5.Grain quality assay

Carbohydrate and protein contents of treated (with LC_{90} value of MB) and untreated stored wheat grains for three months were determined according to the method of the Association of Official Analytical Chemists (AOAC 2000).

2.6.Effect of methyl benzoate on germination

After three months of storage, ten treated wheat grains (by LC_{90} of MB) were taken from each treatment and placed in two layers of filter paper in a 12 cm Petri dish. The filter paper was moistened with distilled water and kept at 25 ºC in the germination chamber. Each treatment has three replicates. Germination percentages were considered when exhibiting a radical extension of > 2 mm. Germination percentages were recorded after 7 days. The final germination percentage (FGP) was calculated according to the following equation (Anjum and Bajwa, 2005):

$$FGP = (Nt \times 100) / N$$

Where Nt = the proportion of germination seeds in each treatment for measurement; N = the total number of seeds in the bioassay.

2.7.Statistical Analyses

For all laboratory bioassay tests control mortality (≤10%) was corrected according to Abbott's formula (1925), and LC_{50} values were calculated using probit analysis in SPSS software (version: 20). One-way analysis of variance (ANOVA) with a Tukey's post-test was used to analyze the results between treatment group means, with a $P < 0.05$ considered to be significant.

3. RESULTS

In order to evaluate the fumigation insecticidal activity of MB-loaded onto three different carrier materials against *S. oryzae*, we conducted bioassay tests with and without wheat grains. The mortality percentages and LC_{50} values have been calculated after 6, 12, 24 and 48 h of exposure. Based on the overlapping of fiducial limits, the LC_{50} values are not significantly different.

3.1.Insecticidal activity of methyl benzoate against *Sitophilus oryzae* in the absence of grains:

Table (1) shows the fumigation toxicity of MB-loaded onto the tested carrier materials against *S. oryzae* based on median lethal concentrations. The results revealed that the MB-filter paper disc had the highest insecticidal effect on *S. oryzae* adults, followed by MB-cotton disc and lastly MB-activated charcoal tablet with respective LC_{50} values of 10.08, 11.87 and 18.53 µg/L air after 6 h of treatment. Also, the same trend has been observed after 12

Table 1. Fumigation toxicity of methyl benzoate against *Sitophilus oryzae* using three different carrier materials without wheat grains after different exposure periods

MB-Carrier materials	LC_{50} µg/L air	Fiducial limits		Slope value	Chi-square
		lower	Upper		
6 h-post exposure					
Filter paper disc	10.08	8.78	11.21	5.97	2.96
Cotton disc	11.87	9.94	13.31	4.37	4.22
Charcoal tablet	18.53	15.70	24.72	3.9	1.86
12 h-post exposure					
Filter paper disc	9.77	7.47	10.33	5.85	4.14
Cotton disc	10.85	8.98	11.68	3.58	8.22
Charcoal tablet	16.43	13.76	21.54	3.32	1.33
24 h-post exposure					
Filter paper disc	8.87	7.84	10.25	5.69	2.09
Cotton disc	7.41	6.87	10.34	3.36	7.51
Charcoal tablet	13.43	13.04	19.44	3.46	2.49
48 h-post exposure					
Filter paper disc	8.43	7.56	10.21	3.19	2.71
Cotton disc	7.02	5.78	8.75	2.65	9.12
Charcoal tablet	7.92	6.62	9.56	4.70	2.98

h of exposure with LC_{50} values 9.77, 10.85 and 16.43 µg /L air, respectively. The treatment of MB-cotton disc exhibited the most potent insecticidal effect after 24 and 48 h of exposure with corresponding LC_{50} values of 7.41 and 7.02 µg /L air, respectively. While the least insecticidal effects on *S. oryzae* adults occurred by MB-activated charcoal tablet (13.43 µg /L air) after 24 h. The filter paper disc had also the lowest toxicity after 48 h of exposure with LC_{50} 8.43 µg /L air.

Figure 1 showed the mortality percentages over time due to exposure to the highest concentration (20 µg /L air) in the absence of wheat grains. The MB-cotton disc exhibited 100% mortality within 24 h, while the MB-filter paper disc and MB-activated charcoal tablet achieved the same percentage after 48 h. In the case of activated charcoal tablet, the mortality percentages increased gradually as time exposure increased. In general, we noticed that MB loaded onto the tested carrier materials had time and concentration-dependent toxicity against *S. oryzae*.

3.2. Insecticidal activity of methyl benzoate against *Sitophilus oryzae* in the presence of grains:

Data in Table (2) show the same pattern obtained in the case of without wheat grains. The filter paper disc-MB possessed the highest fumigant toxic effect on *S. oryzae* followed by cotton discs and activated charcoal tablet after 6 h and 12 h. Their LC_{50} values were 20.18, 24.74 and 31.61 µg /L air after 6 h of exposure and after 12 h were 18.54, 22.46 and 27.56 µg /L air, respectively. Meanwhile, at 24 h and 48 h of treatment, MB-cotton disc induced the highest fumigant toxicity (LC_{50} =17.11 and 16.46 µg/L air, respectively). The least fumigant toxicity at 24 h was MB - activated charcoal tablet while the least one at 48 h was filter paper disc with LC_{50} values of 24.85 and 20.28 µg /L air, respectively.

Figure 2 illustrated the mortality percentages at the highest concentration (25 µg /L air) in the presence of wheat grains. After 48 h of exposure to highest concentration, MB-cotton disc exhibited the highest mortality percentages (95 %) followed by MB-activated charcoal (93.3%) and MB-filter paper disc (91%). The mortality percentages in the presence of wheat are less than their corresponding values in the absence of wheat grains.

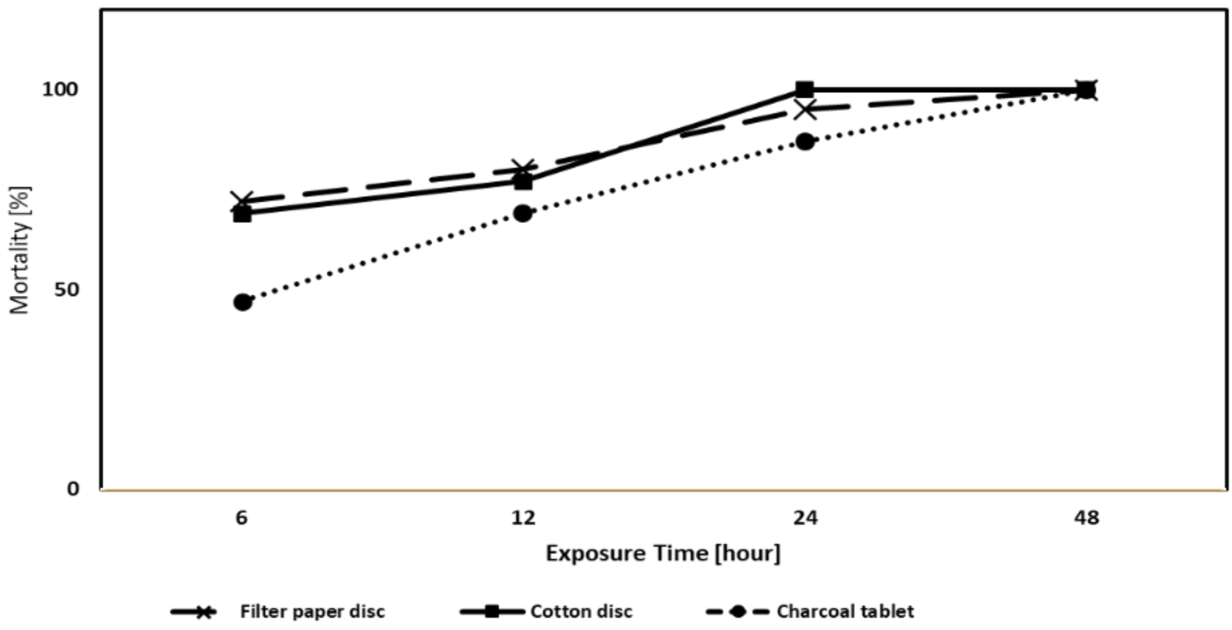

Figure 1. Mortality percentages over time of *Sitophilus oryzae* exposed to methyl benzoate (20 µg /L air) loaded onto three different carrier materials in the absence of grains.

Table 2. Fumigant toxicity of methyl benzoate against *Sitophilus oryzae* using three different carrier materials with grain after different exposure periods

MB-Carrier materials	LC_{50} µg/L air	Fiducial limits lower	Fiducial limits Upper	Slope value	Chi-square
		6 h-post exposure			
Filter paper disc	20.18	18.61	22.85	4.47	1.03
Cotton disc	24.74	21.06	26.40	9.02	0.45
Charcoal tablet	31.61	24.09	72.10	3.69	0.33
		12 h-post exposure			
Filter paper disc	18.54	15.38	21.86	5.13	1.51
Cotton disc	22.46	19.56	24.33	6.82	4.07
Charcoal tablet	27.56	22.93	44.66	4.04	0.48
		24 h-post exposure			
Filter paper disc	18.95	17.65	21.43	5.61	2.36
Cotton disc	17.11	16.22	20.43	5.38	3.63
Charcoal tablet	24.85	20.99	36.41	3.79	0.01
		48 h-post exposure			
Filter paper disc	20.28	17.96	24.00	4.70	0.40
Cotton disc	16.46	14.77	18.29	5.80	4.23
Charcoal tablet	17.09	15.44	19.41	4.98	1.73

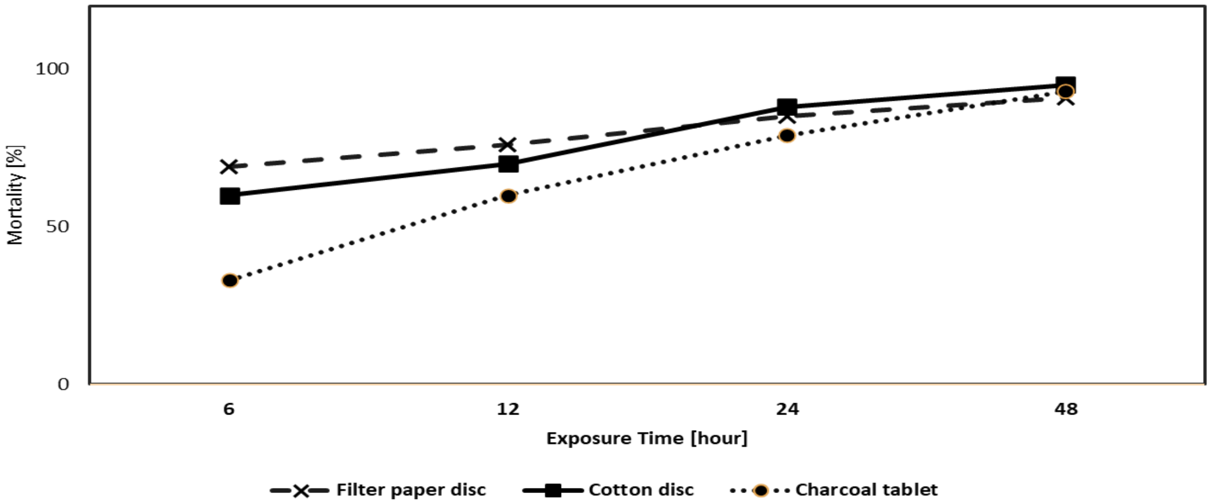

Figure 2. Mortality percentages over time of *Sitophilus oryzae* exposed to methyl benzoate (25 µg /L air) loaded onto three different carrier materials in the presence of grains.

3.3.Persistence of residual activity of methyl benzoate:

We stored the three carrier materials loaded with LC_{90} of MB, both with and without wheat grains to assess MB persistence. Subsequently, we exposed adults of *S. oryzae* to the stored treated jars at different time intervals.

Table (3) presents the mortality percentages of *S. oryzae* exposed to LC_{90} concentrations of MB-loaded onto carrier materials. It is evident from the data that the toxicity of MB decreased gradually over storage time. After 10 and 30 days of storage, the highest mortality percentages of *S. oryzae* were observed in MB-activated charcoal tablet treatment (100% and 88% without grains) and (93% and 65% with grains), respectively. The second highest mortality percentages were observed in MB-cotton disc treatment with 90% and 76% without grains, and 70% and 46% with grains, respectively. The lowest mortality percentages occurred in the MB-filter paper disc treatment, with 72% and 60% without grains, and 40% and 33% with grains, respectively. After 60 and 90 days of storage, there was no observed mortality of *S. oryzae* adults in any of the treatments. Overall, it was noted that the residual toxicity of MB stored with grain was less than that stored without grain.

Table 3. Residual effect of methyl benzoate against *Sitophilus oryzae* over different intervals

Time (days)	Mortality (%)					
	Without grain			With grain		
	MB-Filter paper disc	MB-Cotton disc	MB-Charcoal tablet	MB-Filter paper disc	MB-Cotton disc	MB-Charcoal tablet
10	72	90	100	40	70	93
30	60	76	88	33	46	65
60	0	0	0	0	0	0
90	0	0	0	0	0	0

3.4.Effect on grain germination:

Based on the germination test results presented in Table 4, it is evident that there were no significant differences in the germination rates between the treated grains from each treatment and the control group. Specifically, the MB-filter paper disc treatment exhibited a high germination rate of 98.66%, while the MB-cotton disc and MB-activated charcoal tablet treatments resulted in germination rates of approximately 97.32% and 97.68%, respectively. These findings indicate that the treatments did not have a substantial impact on the germination of the grains compared to the control group.

Table 4. Effect of LC_{90} values of methyl benzoate on wheat grain germination after three months of storage

Treatments	Germination (%)
Control	100±0.0a
MB-Charcoal tablet	97.68±6.62a
MB-Cotton disc	97.32±3.32a
MB-Filter paper disc	98.66±4.15a

In the same column values followed by the same letter are not significantly different

3.5.Effect of methyl benzoate on grain quality:

The impacts of MB loaded onto the carrier materials on the quality (carbohydrate and protein) of stored grains are presented in Table 5. In MB-treated grain treatments, carbohydrate and protein contents showed insignificant increase compared to untreated grains. MB-filter paper disc treatment had the highest effect on carbohydrate content (66.82%) compared to control (64.55%). On the other hand, MB-cotton disc had the highest effect on protein content (9.88%) compared with control (8.41%).

Table 5. Effect of LC_{90} value of methyl benzoate on wheat grain quality after three months of storage

Treatments	% carbohydrate	% protein
Control	64.55 ±14a	8.41±57a
MB-Charcoal tablet	64.76 ±18a	9.02±34a
MB-Cotton disc	65.39±72a	9.88±45a
MB-Filter paper disc	66.82 ±28a	9.03±78a

In the same column values followed by the same letter are not significantly different

4. DISCUSSION

The potential hazards of synthetic pesticides to humans and the environment led to a search for biorational pesticides. Methyl benzoate is a newly discovered botanical pesticide that occurs naturally in some plants and has relatively low toxicity to non-target organisms (Zhu *et al.*, 2019). Recently the insecticidal activity of this compound has been successfully studied against many species of insects. However, in this study, we evaluated the fumigation toxicity of MB- loaded onto simple carrier materials to enhance its effectiveness as long as possible and to facilitate its use during the fumigation procedure. In bioassay tests, our findings showed that MB-loaded onto tested carrier materials had a high toxicity against *S. oryzae* whether in the presence or absence of grains. Interestingly, the values of LC_{50} in the presence of grains were significantly higher than their values in the absence of grains. This indicated that loaded MB was more effective against this insect in the absence of food. The concentration of MB reached to insects (with grain treatment) is lower in (without grain) due to adsorption at the grain surface. This result is consistent with those of Yang *et al*., (2020), who reported that fumigation of rice weevil adults alone resulted in complete control within 16 hours. However, when rice weevil adults on rice were fumigated, complete control was achieved in 72 hours. Also, with Park *et al*., (2016), who found that MB showed the highest fumigation toxicity against adult weevils of *Callosobruchus chinensis* at a concentration of 11.76 mg/L of air with LC_{50} = 5.36 mg/L.

Through the first 12 h of the bioassay tests, MB-filter paper disc was the most effective treatment against *S. oryzae* than others in the absence and presence of grains. It might be because the ability of filter paper (made from cellulose) to hold the MB molecules is lower than cotton and activated charcoal. Consequently, the concentration of MB released from filter paper at the beginning of the fumigation process is higher than cotton and activated charcoal. While, after 48 h the cotton disc achieved the highest activity against *S. oryzae* in both with and without grains. In the storage experiments, we evaluated the long-term effectiveness of MB loaded in the tested carrier materials against *S. oryzae* after 1, 2, and 3 months of storage with and without grains. The findings provided that the MB-activated charcoal tablet was the most effective treatment followed by the MB-cotton disc through the first month of storage, but the least effective treatment was the MB-filter paper disc. The activated charcoal tablet was more efficient in extending the persistence of MB than other tested carrier materials. Because activated carbon effectively adsorbs many organic compound molecules and releasing them gradually (Zhang and Chuang 2001; Quinlivan *et al.,* 2005). To confirm the safety of using MB for stored product pest control in the presence of grains, we assessed the germination and quality of treated wheat grains with MB at the LC_{90} concentration after three months of storage. The results showed that the stored grains had no negative impact on germination or quality, suggesting that MB treatments poses no harmfull effect on wheat grains. Yang *et al*., (2020) showed that the visual quality of the tested varieties of apples was not adversely affected even four weeks after being subjected to a 24-h MB fumigation at 2°C.

5. CONCLUSION

Overall, the findings of this study demonstrated the possibility of loading MB with some carrier materials to persist its activity and make it easier to use. The MB-activated charcoal tablet proved to be an efficient fumigant for controlling stored product pests. Therefore, MB-activated charcoal tablet could be used as an effective and safe alternative to methyl bromide fumigation which has been phased out for stored product insect control. Methyl benzoate is a promising green pesticide, providing a potential substitute for some synthetic pesticides. Therefore, further studies are required to develop MB formulations to enhance its insecticidal efficacy under field applications.

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الملخص العربي

التأثير التبخيرى السام للميثيل بنزوات المحمل على مواد حاملة مختلفة ضد حشرة سوسة الارز، سايتوفيلاس اوريزا

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الميثيل بنزوات هى مادة طبيعية مستخلصه حديثا من أصل نباتى ولها تأثير ابادى على كثير من الحشرات وتعتبر بديلا فعالا للمبيدات الكيميائية الخطرة المستخدمة في عمليه التبخير. فى هذة الدراسة، تم تقييم فاعلية الميثيل بنزوات المحمل على ثلاث مواد حاملة هى ورق الترشيح، القطن و اقراص الفحم ضد حشرة سوسة الارز فى وجود او عدم وجود حبوب القمح. ايضا تم دراسة تاثير مثيل بنزوات على كلا من عملية انبات وجودة حبوب القمح المعامله. حيث اظهرت نتائج الدراسة ان معاملة ورق الترشيح كانت الاكثر تاثيرا على حشرة سوسة الارز بعد ١٢ ساعة من المعاملة، لكن بعد ٤٨ ساعة كانت قطع القطن المعاملة هى الاكثر فاعلية وذلك فى وجود او عدم وجود حبوب القمح. خلال ثلاثون يوما من التخزين كانت اقراص الفحم الاكثر فاعلية على الحشرة مقارنة بالمعاملات الاخرى. اظهرت كل المعاملات عدم التاثير على انبات حبوب القمح وكذلك على محتوى الكربوهيدرات والبروتين للحبوب المخزنة. تقترح هذة الدراسة باستخدام الميثيل بنزوات المحمل على بعض المواد الحاملة خاصه اقراص الفحم في عمليات التبخير اثناء فترات التخزين. حيث ان اقراص الفحم المحملة بالميثيل بنزوات يمكن اعتبارها بديلا امننا وفعالا لمواد التبخير الضارة مثل الفوسفين و بروميد الميثيل المستخدمة فى مكافحة افات المواد المخزونة.

الكلمات المفتاحية: