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Efficacy and Safety of Some Plant Extracts as Alternatives for *Sitophilus oryzae* Control in Rice Grains

[1]A.S. Derbalah, [1]A.M. Hamza and [2]A.A. Gazzy
[1]Department of Pesticides, Faculty of Agriculture, Kafr-EL-Shiekh University, 33516, Egypt
[2]Department of Biology, Faculty of Science, Kafr-EL-Shiekh University, 33516, Egypt

Corresponding Author: Dr. Aly Soliman Hamed Derbalah, Department of Pesticides, Faculty of Agriculture, Kafr El-Sheikh University, 33516, Kafr El-Sheikh, Egypt Tel: 20473255831

ABSTRACT

The rice weevil (*Sitophilus oryzae*) is a serious stored product pest which attacks several economically important crops. In an attempt to find alternative to control this insect, extracts of seven plant species (*Bauhinia purpurea, Caesalpinia gilliesii, Cassia fistula, Cassia senna, Chrysanthemum frutescens, Euonymus japonicus* and *Thespesia populnea acutiloba*) were evaluated under laboratory conditions for their ability to protect rice grains against *Sitophilus oryzae* insect. The efficacy of the tested plant extracts was evaluated on whole rice grains with respect to mortality and emergency of *S. oryzae* adults. Moreover, gas chromatography-mass spectrometry (GC-MS) analysis was carried to identify the chemical components of the most effective plant extract against *S. oryzae*. Furthermore, the safety of the most effective plant extract was evaluated with respect to biochemical and histological changes in treated rats relative to control. The results revealed that, the tested botanical extracts showed high efficiency against *S. oryzae* and *C. gilliesii* extract was the most effective plant extract against *S. oryzae*. The GC-MS analysis showed the presence of different bioactive chemical components that known by its insecticidal activity. The most effective plant extract showed low toxicity on rats relative to control with respect to biochemical and histological changes. The results suggest the ability of using these plant extracts in rice grains protection as a safe alternative to insecticides. Moreover, the presence of mixture of components with, apparently, different mechanisms of insecticidal activity of the tested plant extracts may be delay the resistance development by insect relative to the insecticide.

Key words: *Sitophilus oryzae*, rice, alternative, control, safety

INTRODUCTION

Pest insects affect food output directly by reducing the quality and quantity of the crop produced, or indirectly by serving as vectors of plant diseases. The use of synthetic compounds to control insect pests has lead to several adverse effects, including water and soil contamination, insect resistance and toxicity to non target species (Zettler and Cuperus 1990). Thus effective and safe alternatives to fungicides for controlling plant disease are in demand. Plant derived materials for potentially useful products as bio-insecticides are a source of major concern (Regnault-Roger and Hamraoui, 1994; Regnault-Roger, 1997; Oparaeke and Kuhiep, 2006; Sathyaseelan *et al*., 2008; Khorram *et al*., 2011; Mulungu *et al*., 2011). Moreover, resistance by pests and vectors against hese bio-insecticides has not been reported and likely to be difficult (Regnault-Roger, 1997).

Furthermore, these alternatives (bio-insecticides) supposed to have low persistence in the environment than chemical fungicides (Koul and Dhaliwal, 2001).

Essential oils derived from plants received much attention for using it as potential components of integrated pest management (IPM) due to its efficacy against pests and its environmental compatibility (Ikbal *et al*., 2007; Katz *et al*., 2008; Akinkurolere, 2007). Recently, many studies have focused on the possibility of using it for control of stored grains pests (Collins, 2006; De Carvalho and Da Fonseca, 2006).

In spite of the efficacy of botanical extract in pest's control, there are lack studies on the safety of these botanical extracts on human health. Although, the assessment of enzymes activity in the blood is generally a more sensitive measure of compounds toxicity than histopathological changes and can be assessed within a shorter time. The tissue alterations considered a confirmatory and supporting diagnostic role in the case of certain abnormalities in blood sampling (Massoud *et al*., 2010).

The use of plant materials can lead to the identification of new bio-insecticides for the benefit of agriculture production and human health. Therefore, this study attempted to evaluate insecticidal activity of some newly used plant extracts (*Bauhinia purpurea, Caesalpinia gilliesii, Cassia fistula, Cassia senna, Chrysanthemum frutescens, Euonymus japonicus* and *Thespesia populnea acutiloba*) against *Sitophilus oryzae* in rice grains with the respect to progeny and mortality of the insect adults. Also to identify the chemical components of the most effective plant extract against *S. oryzae*. Furthermore, to evaluate the toxicity of the most effective plant extract on rats with respect to biochemical and histological changes in treated rats relative to control.

MATERIALS AND METHODS

The insect rereading: *S. oryzae* (Egyptian strain) was obtained from the Department of Stored Products Pests Control, Research Institute of Plant Protection, Sakha, Kafr-El-Shiekh. This strain was continuously reared free of insecticidal contamination for several years at 30±2°C and 70±5% R.H. The cultures were maintained under the same conditions in the Pesticide Department, Faculty of Agriculture, Kafr-El-Shiekh University, Egypt and 200-400 adults from the pervious culture were added in 1000 mL glass jars containing 400 g of rice as a culture medium. The mouth of the jars was covered with muslin cloth. Then, 7-14 d old adults were used for experimental work.

The stored product: Rice grains were used to culture *S. oryzae* and to evaluate the efficacy of the tested plant extracts against the same insect as well. Rice grains stored in airtight tins until required for experiments. The experiments were carried out in a room kept at a constant temperature of 25°C and 70% RH.

Plants and preparation of its crude extracts: The leaves of seven medicinal plant species (*C. senna, C. gilliesii, T. populnea var. acutiloba, C. frutescens, E. japonicus, B. purpurea* and *C. fistula*) were collected from a local nursery at Kafr El-Sheikh, Monofia, Gharbia and Alexandria Governorates, Egypt. *C. senna* (Alexandrian senna) belonging to the family Fabaceae and cultivated in Egypt and Sudan. *C. gilliesii* (bird of paradise) belonging to the family Fabaceae, is native to tropical America, mainly Argentina and Uruguay. *T. populnea var. acutiloba* (Portia tree) belonging to the family Malvaceae, is native to South Africa. *C. frutescens* (marguerite daisy) belonging to the family Asteraceae, is native to the Canary Islands. *E. japonicus* (Japanese spindle) belonging to the family Celastraceae, is native to Japan, Korea and China. *B. purpurea* (purple camel's foot) belonging to the family Fabaceae, is native to South China. *C. fistula* (cassias)

belonging to the family Fabaceae, is native to southern Asia. The different leave samples were oven dried for 24 h at 70°C and then, finely powdered using a blender. Each sample (25 g) was extracted twice with 300 mL of methanol at room temperature for 2 days. The extracts were filtered through filter paper (No. 15, Whatman, Inc., Piscataway, NJ, USA) and the combined filtrates from the twice-extracted leaves were concentrated to dryness by rotary evaporation at 40°C.

Effect of tested plant extracts and malathion on progeny of *S. oryzae*: The tested plant extracts at concentration levels of 100, 200 and 300 ppm were used to evaluate its efficacy against *S. oryzae*. Malathion was used as recommended compound against *S. oryzae* at concentration levels of 10, 20 and 30 ppm. Each concentration was applied in three replicates and in each replicate there were 20 g of rice grains. The treatment of rice grains was carried out by dipping rice grains in water solution of malathion and botanical extracts at tested concentration levels twice consecutively for 5 sec and subsequently spread on top of plastic sheets to dry for 90 min. The control treatment was carried using water only and replicated three times. Then, 10 adults of *S. oryzae* were transferred to treated rice grains which putted in a 85×75 mm plastic jar and kept at 30±2°C and 70±5% RH according to the method described by Kestenholz *et al.* (2007). The emerged adults from the hatched eggs were recorded after 6 weeks of treatment. These adults were used to calculate the reduction percentages in *S. oryzae* progeny from the use of the tested plant extracts as well as malathion compared to the control as shown in Eq.1 as described by El-Lakwah *et al.* (1992).

$$\% \text{ Reduction } = \frac{\text{MNEC-MNET}}{\text{MNEC}} \times 100 \quad (1)$$

Where:
MNEC = Mean No. of those which emerged in the control
MNET = No. of those which emerged in the treatment

Efficiency of the tested plant extracts and malathion on adults of *S. oryzae* by mean mortality: Efficiency of the tested plant extracts and malathion on adults mortality of *S. oryzae* was evaluated according to the method described by Kestenholz *et al.* (2007) as mentioned before. The number of dead insects in each jar was counted after one and two weeks and the percentage of insect mortality was recorded and corrected using the Abbott formula (Abbott, 1925).

Chemical composition of the most effective plant extract: GC/MS analysis was carried to identify the components of the most effective plant extract *(C. gilliesii)* according to the method described by Duarte-Almeida *et al.* (2004).

Toxicity assessments

Animal treatment: Toxicity assessments were performed using 8-week-old 80-100 g Wistar male rats (*Rattus norvegicus*) obtained from Faculty of Medicine, Tanta University. Wister rats were housed in wire cages under standard conditions with free access to drinking water and food. The rats were kept in temperature-controlled room with 14 h light and 10 h dark cycles. The rats were given a standard diet as describe by Romestaing *et al.* (2007). Before treatment, rats were left two weeks during feeding for adaptation. The animals were randomly divided into two groups each comprising of three animals. One groups for the treatment with the most effective plant extract (21 days) and the second group for control. The most effective plant extract (*C. gilliesii*) against the tested insect were administered to rats orally at concentration level of 500 mg kg^{-1} body weight.

Control group rats were orally administrated with equal amount of almond oil. After 21 days the rats were sacrificed under anesthesia. Then, the blood samples were taken under anesthesia by cardiac puncture in vials containing heparin. Moreover, specimens from kidney and liver were taken from each treatment and kept in neutral buffered formalin 10% for histopathological tests.

Enzymes assays: Blood samples were centrifuged at 4500 rpm for 15 min at 4°C and the blood serum was used to determine Creatinine, Alkaline Phosphatase (ALP) and Glutamate Pyruvate Transaminase (GPT) according the methods described by Reitman and Frankel (1957), Wilkinson *et al*. (1969) and Barham and Trinder (1972), respectively.

Histopathological test: The histopathology test was carried out at Histopathology laboratory, Department of Histopathology, Faculty of Veterinary Medicine, Kafr El-Sheikh University according to the method described by Bancroft *et al*. (1996).

Statistical analysis: Data from the experiments were statistically analyzed using one-way repeated measurement analysis of variance. Duncan's multiple range test (Duncan, 1955) were used to separate means using SAS program (Version 6.12, SAS Institute Inc., Cary, USA).

RESULTS

Effect of tested plant extracts and malathion on progeny of *S. oryzae*: The numbers of emerged adults of *S. oryzae* were significantly decreased after treatments compared to the untreated

Table 1: Effect of the tested plant extracts and malathion on progeny of *S. oryzae*

Treatments	Concentration (ppm)	Reduction (%)
Chrysanthemum frutescens	100	43.0d
	200	53.5e
	300	78.8j
Euonymus japonicus	100	39.0c
	200	51.0e
	300	69.0g
	100	38.0c
Bauhinia purpurea	200	54.6e
	300	73.0h
	100	50.8e
Caesalpinia gilliesii	200	69.2g
	300	79.5j
	100	31.0b
Cassia fistula	200	46.7d
	300	73.5h
	100	35.0b
Cassia senna	200	44.0d
	300	59.0f
	100	33.0b
Thespesia populnea var. acutiloba	200	48.0d
	300	69.0g
	10	66.0g
Malathion	20	78.0j
	30	86.0k
Control	0	00.0a

Letters in columns indicate separation of means according to the Duncan's multiple range test ($p<0.05$)

control, as shown in Table 1. Increasing the concentration level of all tested treatments reduced the emergence of *S. oryzae* even more (concentration dependent) malathion (86%) followed by *C. gilliesii* (79%) extract and *C. frutescens* (73%) were the most effective treatments on reduction of *S. oryzae* progeny while *C. senna* extract was the lowest effective one.

Efficiency of tested plant extracts and malathion on *S. oryzae* adults determined by mortality values: The efficacy of the tested plant extracts and malathion against *S. oryzae* adults by means mortality are presented in Table 2. The results showed that, *C. gilliesii* (100%) was the most effective treatment against S. oryzae adults followed by *C. frutescens* (95.6%), T. populnea var. *acutiloba* (88%), *E. japonicus* (85%) , *B. purpurea* (75%), *C. senna* (80%), and *C. fistula* (70%), respectively. Malathion (100%) and *C. gilliesii* (100%) extract were the most effective treatments on adult's mortality of *S. oryzae* after two weeks while *C. fistula* extract (70%) was the lowest effective one. The morality percentages of S. oryzae were significantly increased in the second week as compared to the first week at all tested treatments. Increasing the concentration level of all tested treatments increased the mortality of *S. oryzae* adults even more (concentration dependent).

Table 2: Effect of the tested plant extracts and malathion on adult's mortality of *Sitophilus oryzae*

		Mortality (%)	
Treatments	Concentration (ppm)	After one week	After two weeks
Chrysanthemum frutescens	100	40.0hi	73.3e
	200	46.7h	83.3d
	300	67.6de	95.6d
Euonymus japonicus	100	33.3g	55.0d
	200	57.6bc	75.0d
	300	61.0b	85.0d
Bauhinia purpurea	100	27.6ef	50.0c
	200	40.0de	63.5b
	300	57.6bc	75.0b
Caesalpinia gilliesii	100	29.0j	80.0c
	200	31.3ij	93.5b
	300	38.0hi	100b
Cassia fistula	100	26.0fg	53.7b
	200	43.3ef	61.0b
	300	53.3cd	70.0b
Cassia senna	100	21.3k	63.3d
	200	38 hi	77.5d
	300	43.3g	81.0d
Thespesia populnea var. acutiloba	100	35.0j	70.0d
	200	55.0hi	83.3d
	300	69.0h	88.0d
Malathion	100	66.0a	100b
	20 0	77.0a	100b
	30	82.0a	100b
Control	0	0.00L	0.00a

Letters in columns indicate separation of means according to the Duncan's multiple range test ($p<0.05$)

Table 3: The main constituents of *Caesalpinia gilliesii* plant extract under GC-MS analysis

Name	Retention time (min)	Area (%)
Cyclohexanone dimethyl acetal	4.93	4.65
Methoxy propoxy 2 propanol	50.30	5.17
Cyclohexasiloxane	7.05	1.19
1,6,10 dodecatriene 7,11-dimethyl-3- methylene	7.96	0.19
Cycloheptasiloxane tetradecamethyl	9.47	0.59
Diethyl phthalate	10.50	0.28
Benzene 1 -butylheptyl	11.87	2.91
Benzene 1- methyldecyl	13.35	1.06
Tetradecanoic acid methyl ester	12.90	5.84
2,6,10 dodecatrien-1- ol,3,7,11 trimethyl	13.15	0.64
Benzene -1- butyl octyl	13.08	5.05
Tetradecanoic acid	13.14	4.65
Cyclononasiloxane	13.88	5.48
Isopropyl myristate	13.99	3.91
Neophytodiene	15.45	0.23
Loliolide	15.72	3.51
Pentadecanoic acid	16.29	4.52
N- hexadecanoic acid	16.45	1.39
9,12,15 octadecanoic methyl ester	19.02	2.33
Phytol	19.04	1.03
9,12,15 octadecatrien-1-ol	20.44	0.13
9,12,15 octadecanoic acid	21.67	1.14
Octadecanoic acid	22.19	2.17
Di-n-octyl phthalate	24.01	0.56
Vitamin E	24.61	1.49
Cyclohexanone dimethyl acetal	29.17	0.57

Composition of the most effective botanical extract: The identified chemical components of the most effective botanical extract against *S. oryzae* (*C. gilliesii*) presented in Table 3. Twenty six compounds were identified from *C. gilliesii* extract separately with different percentages as shown in Table 3. The identified compounds belonged to different fatty acids and it derivatives (aldeyhydes, esters and alcohols). Some compounds such as tetradecanoic acid (4.65%); pentadecanoic acid (4.62%); loliolide (3.51%); octadecanoic acid (2.17%); n-hexadecanoic acid (1.39%) and phytol (1.03%) were detected with high percentages relative to other detected compounds (Table 3).

Toxicity evaluation

Effect of the most effective plant extract on liver enzymes: The ALP and GPT activities are known as cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. In the present study, therefore, both enzyme activities were used as indicators of hepatic damage. The obtained data in Table 4 show that, there were no significant differences in the activity of ALT and GPT after 21 days of rats administrated with the tested plant extract at dose level of 500 mg kg^{-1} body weight relative control treatment. The normal ALP and GPT level in rats treated with the most effective plant extract relative to control treatment assumed to be the normal liver functions.

Effect of the most effective plant extract on kidney function: Regarding to the kidney function, there were no significant differences in creatinine level in rats administrated with the

most effective plant extract at dose level of 500 mg kg^{-1} relative to control one (Table 4). The normal creatinine in rats treated with the most effective plant extract relative to control treatment assumed to be the normal kidney function. Moreover, the histology of kidney tissue treated with the most effective plant extracts relative to control confirms this explanation. The normal creatinine level in rats treated with the most effective plant extract relative to control treatment assumed to be the normal kidney functions.

The histopathological changes in the kidney: The normal structure of kidney tissue was shown in Fig. 1a. For the rats treated with *C. gilliesii* extract at dose level of 500 mg kg^{-1}, the tissue was some what like control with a small vacuolation in renal tubules (Fig. 1b). These results were in agreement with the creatinine level in treated rats mentioned before in this study (Table 4).

The histopathological changes in the liver: The normal structure of liver tissue was shown in Fig. 2a. For the rats treated with *C. gilliesii* at dose level of 500 mg kg^{-1}, blood vessels were observed to be engorged with blood and hepatocyte contained vacuolated cytoplasm (Fig. 2b). Moreover, there was a necrotic area recorded (Fig. 2b). These results were in agreement with the GPT and ALT levels in treated rats in this study (Table 4).

Table 4: Effect of the most effective plant extract (*Caesalpinia gilliesii*) on serum GPT, ALT and creatinine activities of rats at dose level of 500 mg kg^{-1} body weight

Treatments	SPGT (Iu L^{-1})±SE	ALP (Iu L^{-1})±SE	Creatinine (mg dL^{-1})±SE
Control	1.20±45	135.0±5.57	0.07±0.405
C. gilliesii	3.97±47	5.1±141	0.05±0.395

*SE: Standard Error, *IU L^{-1}: International Unit per Liter, mg dL^{-1}: Milligram per deciliter*

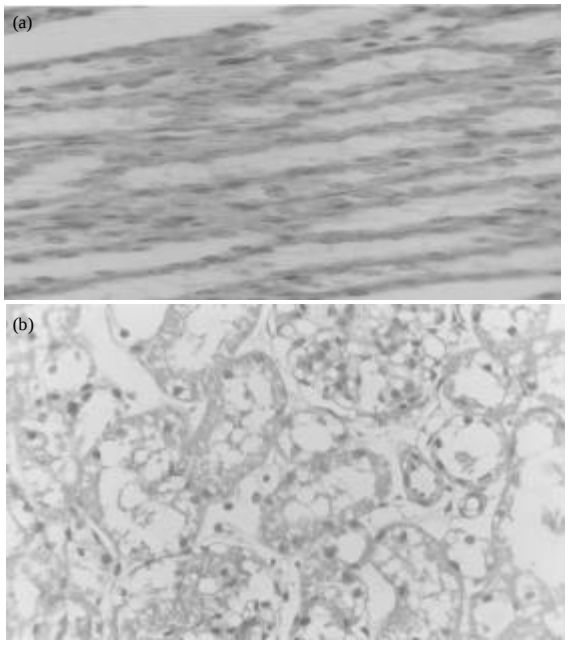

Fig. 1(a-b): Sections from kidney of rats after 21 days of treatment with *Caesalpinia gilliesii* (b) November 2, 2011at dose level of 500 mg kg^{-1} relative to control (a)

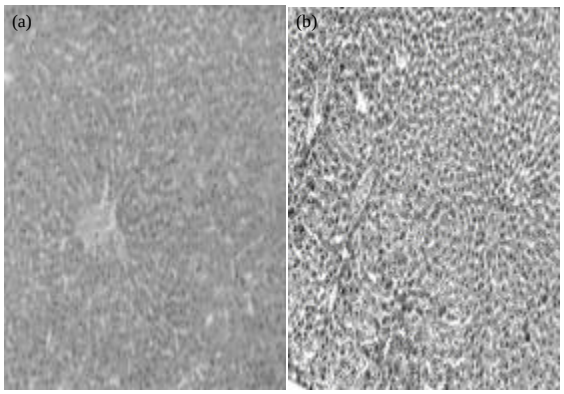

Fig. 2(a-b): Sections from liver of rats after 21 days of treatment with *Caesalpinia gilliesii* (b) at dose level of 500 mg kg^{-1} relative to control (a)

DISCUSSION

The results of the present study implied that, the tested plant extracts were effective against *S. oryzae* in stored rice with the respect to progeny and adults mortality. The efficacy of plant extracts against *S. oryzae* insect in stored rice with the respect to progeny and adults morality have been reported by many researchers (Tapondjou *et al.*, 2002; Ketoh *et al.*, 2005; Kestenholz *et al.*, 2007; Iboudo *et al.*, 2010; Derbalah and Ahmed, 2011). However, the efficacy of the tested plant extracts especially the most effective ones, has not been reported against *S. oryzae* and is considered first report.

Among the identified compounds from *C. gilliesii* extract, some compounds such as tetradecanoic acid; pentadecanoic acid; n-hexadecanoic acid; phytol; loliolide and octadecanoic were detected with high percentages relative to other detected compounds. The antifungal activity of *C. gilliesii* extract against *S. oryzae* may be due to the presence of the previous these fatty acids and its derivatives (Negahban *et al.*, 2006; Rozman *et al.*, 2007; Lopez *et al.*, 2008; Ogendo *et al.*, 2008; Derbalah and Ahmed, 2011).

Although, the insecticidal activity of tested plant extract is attributed mainly to its major compounds mentioned before, the synergistic or antagonistic effect of some compounds in the mixture has to be considered (Ragasa *et al.*, 2002). Each of the plant extract components has its own contribution on biological activity of the extract against the tested insect. For example loliolide detected with low percentage but it is known to possess diverse biological properties such as insect repellant (Gordon *et al.*, 1982).

The mode of action of the bioactive natural monoterpinoids (hydrocarbons, alcohols and ketones) isolated from plant extracts oils may be due to inhibition of acetylcholinestearase (Miyazawa *et al.*, 1997; Lee *et al.*, 2000; Derbalah and Ahmed, 2011). Since, Lee *et al.* (2000) reported that, 1,8-cineole was the most potent inhibitor of AChE among the monoterpenes tested. This inhibition may be a mode of action for essential oils and monoterpenes against stored grain insect pests as well. Also, the mode of action of the tested botanical extracts may be largely attributable to its fumigant action (Shaaya *et al.*, 1997; Park *et al.*, 2003).

The botanical extracts as pest control agents present two main characters: the first is their safety to the people and the environment and the second is, the less resistance development against it by the tested insect. Regarding to the safety, the toxicity evaluation of the most effective plant

extract revealed that, there were some slight variations occurred sporadically in treated rats relative to control with the respect to enzymes markers and histopathology of treated organs. Moreover, the observed changes in the tissues were mostly uncorrelated with a dosage which potentially indicates the safety of the plant extracts in the context of human health. Also, the rat tests are often more sensitive and may not reflect human sensitivity. Moreover, the exposure levels may be far greater than what would actually be experienced or detected in rice grains after they grow and are processed. With the referring to resistance development, it is believed that, it is difficult for the insect to develop resistance to such a mixture of bioactive components with, apparently, different mechanisms of insecticidal activity (Liu *et al*., 2008).

This study considered the first step toward more investigation and concern about using these effective botanical extracts as alternative for controlling of stored products pests. This will be help to reduce the environmental pollution and the adverse effect on human health resulted from insecticides using. Since, these botanical extract revealed non significant toxicity relative to the high dosage that given orally and will not reached to human by this dose as a residue under any conditions.

CONCLUSIONS

The insecticidal activity of the tested plant extracts against *S. oryzae* indicated the potential of some plant species as a natural source of insecticidal material. Insecticidal activity was confirmed in all the tested plant species, although the results showed variation in their effectiveness against *S. oryzae*. The ability of using botanical products as alternative of chemical control of *S. oryzae* is possible if the problem of cost-effective commercial production can be solved. Moreover, some of these botanical extracts could find a place in IPM strategies, especially where the emphasis is on environmental, food safety and on replacing the more dangerous toxic insecticides. Also, work in this regards should continue to obtain information regarding its practical effectiveness under natural conditions to protect the stored products without any side effects.

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