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Towards Sustainable Green Pesticides for stored grain insects

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Abstract

Rice is one of the major cereal crops and staple food for large part of the world, especially Egypt. Unfortunately, huge quantities of it are lost because of being infected with insect pests during storage. *Sitophilus oryzae* is one of these pests. These insect pests are controlled by using highly toxic chemicals that threaten human health and negatively affect the environment. The trend has been to search for an eco-friendly insecticide instead of these dangerous chemicals. *Moringa oleifera* has insecticidal properties against wide variety of insect pests. It can be grown easily, and the studies have proven its many nutritional and medical benefits for humans. So, the present research tried to find its effectiveness against *Sitophilus oryzae*. The powders of leaves, roots, and seeds of the *Moringa* plant were evaluated against this insect at 0.5, 1, 3 and 5 g/100 of flour. The results indicated that the *Moringa* showed clear effects compared to the control, and the most effective part was the seeds powder. Therefore, *M. oleifera* can be a sustainable and safe natural pesticide for stored grain insects.

Key Words:

Sustainable pesticides, Stored grain insects, *Moringa oleifera*, *Sitophilus oryzae*, Susceptibility.

1. Introduction

It is recognized that insect pests represent a major problem in fields and warehouses. Insect pests destroy more than 35 percent of crops globally (Okwor *et al.*,2021-a, 666). We lose a lot of cereal grains via insect infestation during storage, particularly in the developing countries such as Egypt, where damage to stored grains and their products by insects may amount to 5 - 10% in the temperate countries and 20 - 30% in the tropical zones (Dubey *et al*., 2008,182; Ileke and Ogungbite,2014,57).

Rice, being one of the staple foods mostly consumed in our daily life all over the world, has been infested by many important insect pests such as *Sitotroga cerealella*, *Sitophilus oryzae*, *Sitophilus granarius*. (Sarwar, 2011,10 and Ileke and Ogungbite,2014,57).

The rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae), is one of these insects, it is considered a serious pest for stored grains. The rice weevil is small, its body is very strong, reddish-brown to black in colour with four light yellow or reddish spots on the corners of the elytra (the hard protective forewings). It feeds on wheat, corn, oats, rye, barley, sorghum, buckwheat, dried beans, cashew nuts, wild bird seed, and cereal products, especially macaroni, the adult rice weevil can fly and is attracted to lights (Koehler,2022,2 and Almobidoon®, 2024,1).

The adult female *S. oryzae*_lays an average of 4 eggs per day may live for 4 to 5 months. The full life cycle may take only 26 to 32 days during hot summer months but requires a much longer period during cooler weather. The eggs hatch in about 3 days. The larvae feed inside the stored grains for an average of 18 days. The pupa is naked and pupal stage lasts an average of 6 days. The new adult will remain in the seed for 3 to 4 days while it matures (Koehler,2022,1).

One of the serious damages caused by the tested insect is the larva feeds within the kernel and consumes the endosperm till it becomes pupa, and the adult emerges. The adult leaves a large, ragged exit hole in the kernel and feeds on damaged kernels. The rice weevil adult gathers and reproduces in stored grains (Kemabonta and Falodu, 2013, 259).

Traditional control methods of stored grains insects are chemical insecticides, such as methyl bromide and phosphine and fumigation (Rajashekar and Shivanandappa, 2010, 910). But these methods are very hazards to the environment and human health. Due to such risks well-known for the traditional control methods, it has become necessary to search and evaluate safe and environmentally friendly control methods. The best of these methods are green pesticides, which originated from plants. (Sutherland *et al*., 2002,97; Ileke and Ogungbite, 2014,57). Thus, we must turn to the use of safe and natural plant products against the stored grain insects.

Moringa oleifera (Brassicales: Moringaceae) can be grown easily, and its growth is rapid as compared to other trees. Meanwhile, it has insecticidal properties against a wide variety of insect pests:

-*Moringa oleifera*, at highest concentrations, is reported to reduce adult appearance of *S. Oryzae* in stored grains. (Mbaiguinam *et al.*,2006,421 and Negbenebor and Salisu,2020,130).

-High concentrations of *M. oleifera* powder significantly reduced the longevity of adult *Callosobruchus maculatus* on cowpea seeds and *S. Zeamais* adults on wheat grains (Ileke and Oni 2011,3047).

One of the most advantages of *Moringa* as a useful plant that it is friendly to environment and human is that it has many benefits for humans, and therefore its use is safe when mixed with edible grains to protect them from the bad effects of stored grain insects. Many benefits of *Moringa* have been proven by scientific research:

- It can prevent or treat diseases such as lung cancer and breast cancer. Diabetes, gingivitis, and acute pancreatitis (Su *et al*., 2023, 24358)

-*M. oleifera* seeds are wealthy in glucosinolates, for example, Niazimicin (NZ), is the vitally bioactive substance. It can assume as neuroprotective agent by influencing oxidative pressure, and have antitumor activity (Abdelsayed *et al*., 2021, 128,129).

- Moringa leaves have several health benefits, such as antioxidant, antimicrobial, anti-tumor, and anti-inflammatory properties (Marref *et al*.,2024,404)

- In addition, it is used to treat anaemia, also fresh moringa leaves increase the amount of milk produced by expectant and nursing women. Besides, it is reported to have medicinal value and are not toxic to vertebrates (Derbio and Debelew 2023, 1133).

Based on the aforementioned harmful effects of the insect being tested, as well as the potential effectiveness of *Moringa* plant, as shown in previous research, at the same time its many benefits to humans, so, the current research aims to survey the susceptibility of *S. oryzae* to some products of *M. oleifera* as a potential sustainable green pesticide.

2. Materials and Methods

2.1 Insect breeding:

Adults of *S. oryzae* were collected, in October 2023, from infected stored rice. Stock culture was established in the laboratory under the same normal storage conditions in homes. Rice weevils were transferred and raised on wheat flour in Zoology Lab, Biological and Geological Department, Faculty of Education, Ain shams University. Clean wheat flour was used instead of rice, during laboratory experiments, for ease of handling and separating weevils from the flour using a sieve.

2.2 Tested plant parts:

The plant materials used were powders of Moringa *oleifera* leaves, roots, and seeds, which were purchased from the *Moringa* products sales outlet of the Egyptian Moringa Scientific Society at the National Research Centre (NRS), Dokki, Egypt. Both the leaves and roots were obtained in powder form, but the seeds were ground to obtain them in a powder form.

2.3 Susceptibility tests:

Three experiments were conducted for the study of the susceptibility of *S. oryzae* adults towards *Moringa* leaves, roots, and seeds powders, according to the method described in (WHO, 2022, 7-13) with some modification made to suit the nature of the present tested insect.

For each part of the tested plant, 4 concentrations were prepared: 0.5, 1, 3 and 5g / 100g flour, then mixed well with clean wheat flour. The weights used of plant powders and flour were weighed using a digital sensitive balance in the Central Lab in the same scientific department.

In parallel, the control experiment was conducted under the same laboratory conditions except for the addition of the tested plant.

In each above-mentioned concentrations and the control, there were four replicates, in each replicate 50g flour and 10 adult weevils, randomly selected and counted. The containers were covered with cotton cloth and firmly tied with rubber band to allow air circulation and prevent the insect from escaping.

The setups were monitored daily and counted every 3 days for 15 days to observe and record the number of weevils' deaths.

2.4 Data Analysis:

The recorded mortality percentages were calculated:

Mortality%= [(Number of dead weevils/ numbers of tested weevils) *100].

We found that no need for Mortalities' corrections because the control mortality was less than 5%, according to Abbott's formula criteria (Abbott, 1925, 302; and WHO, 2022, 7-13). Then, results were presented graphically using Excel program (Office 365). Statistical analysis to estimate LC_{50} were calculated using straight line equation (which calculated using excel graphs). The confidence limits of each LC (at 95% probability) were computed using the method of Litchfield and Wilcoxon (1949, 254).

3. Results:

Susceptibility of *S. oryzae* to *Moringa* products:

In the present study, Susceptibility tests were conducted with leaves, roots, and seeds powders of M. *oleifera* against *S. oryzae*. Results of the Susceptibility tests are tabulated in Tables 1- 4 and represented graphically in Figs. 1- 4.

3.1 Susceptibility of *S. oryzae* to *Moringa* leaves:

Table 1 and Fig. 1 represent the data of susceptibility tests of *Moringa* leaves powder. The estimated value of LC_{50} with confidence limits were 4.73 < **7.32** < 11.35 g/100 g flour.

3.2 Susceptibility of *S. oryzae* to *Moringa* roots:

Table 2 and Fig. 2 represent the data of susceptibility tests of *Moringa* roots powder. The estimated value of LC_{50} with confidence limits were 11.07 < **17.04** < 26.24 g/100 g flour.

3.3 Susceptibility of *S. oryzae* to *Moringa* seeds:

Table 3 and Fig. 3 represent the data of susceptibility tests of *Moringa* seeds powder. The estimated value of LC_{50} with confidence limits were 2.20 < **3.90** < 6.90 g/100 g flour.

3.4 The comparison between the effects of the four tested products of *M. Oleifera* against *S. oryzae* adults:

The obtained results showed that the percentage mortality of *S. oryzae* adults depended on the kinds of *Moringa* products and their concentrations. Generally, for the tested products used, percentage mortality increased directly with concentration.

The comparison between the effect of different *Moringa* products was shown in Table 4 and Fig. 4. The highest effective product was seeds, followed by leaves, and the roots was the least effective ones.

The slopes of the regression lines were calculated at 95% confidence limits. They were 5.6897, 2.6847 and 9.8522 for leaves, roots, and seeds, respectively (Table 4).

Table (1): Susceptibility of *S. oryzae* to *Moringa* leaves.

leaves Concentration (g/100g flour)	Dead insects [1]	Observed mortality %
0.5	2	5
1	8	20
3	11	27.5
5	14	35
Control	1	2.5
Slope of the regression line		5.6897
LC_{50} with confidence limits [2]		4.73 < 7.32 < 11.35
Number of tested insects: 40 insects/concentration, [1] sum of 4 replicates, [2] confidence limits at 95% probability. No correction for observed mortality because control mortality was less than 5%.		

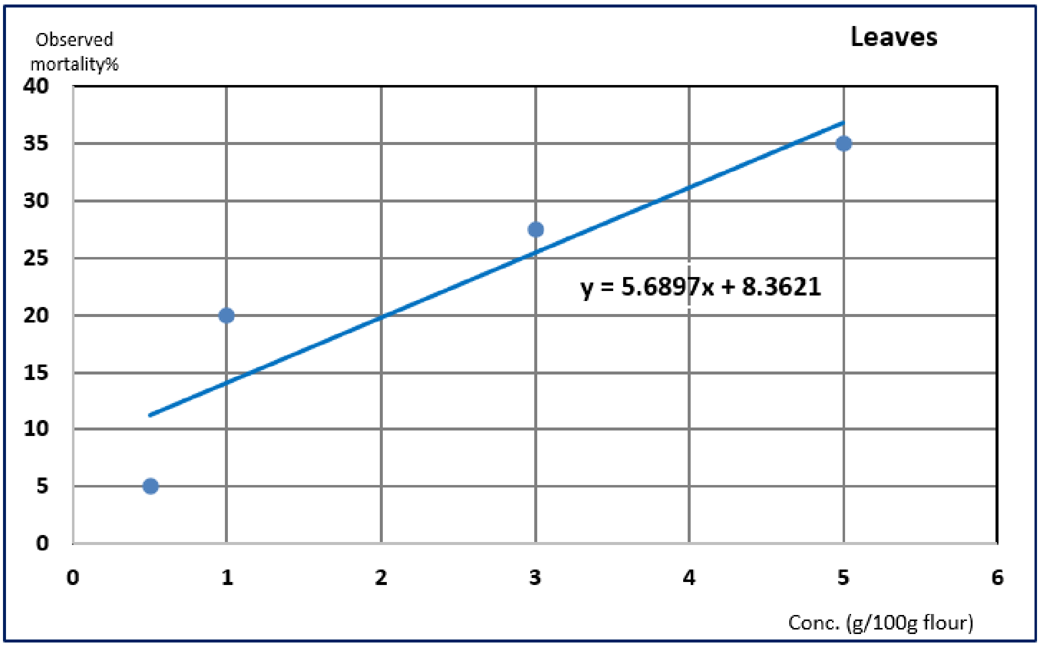

Figure (1): Susceptibility line of *S. Oryzae* adults to *Moringa* leaves treatments.

Table (2): Susceptibility of *S. oryzae* to *Moringa* roots.

Roots Concentration (g/100g flour)	Dead insects [1]	Observed mortality %
0.5	2	5
1	4	10
3	3	7.5
5	8	20
Control	1	2.5
Slope of the regression line		2.6847
LC_{50} with confidence limits [2]		11.07 < 17.04 < 26.24

Number of tested insects: 40 insects/concentration,

[1] sum of 4 replicates, [2] confidence limits at 95% probability.

No correction for observed mortality because control mortality was less than 5%.

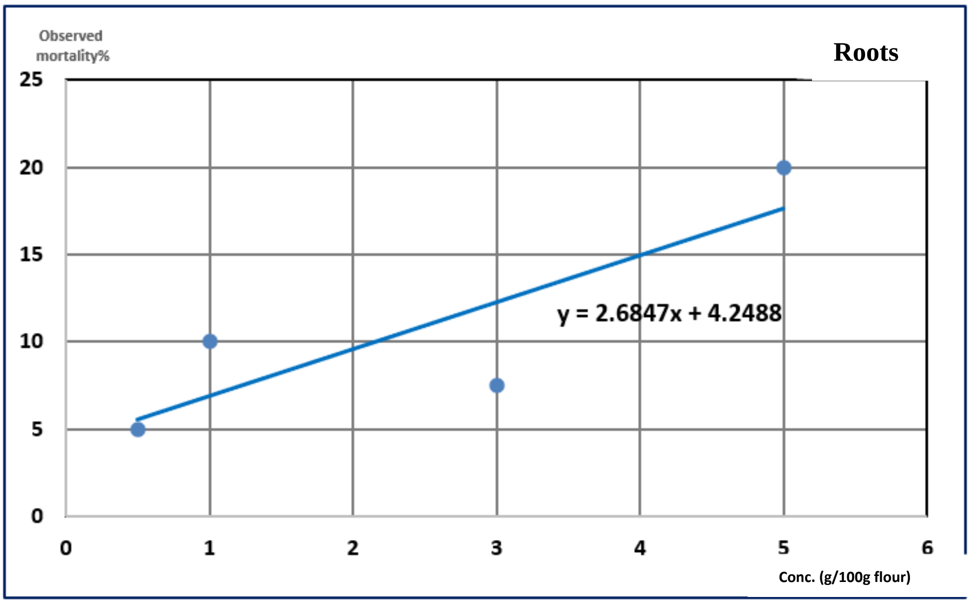

Figure (2): Susceptibility line of *S. Oryzae* adults to *Moringa* roots treatments.

Table (3): Susceptibility of *S. oryzae* to *Moringa* seeds

Seeds Concentration (g/100g flour)	Dead insects [1]	Observed mortality %
0.5	6	15
1	9	22.5
3	17	42.5
5	24	60
Control	1	2.5
Slope of the regression line		9.8522
LC_{50} with confidence limits [2]		2.20 < 3.90 < 6.90

Number of tested insects: 40 insects/concentration,
[1] sum of 4 replicates, [2] confidence limits at 95% probability.
No correction for observed mortality because control mortality was less than 5%.

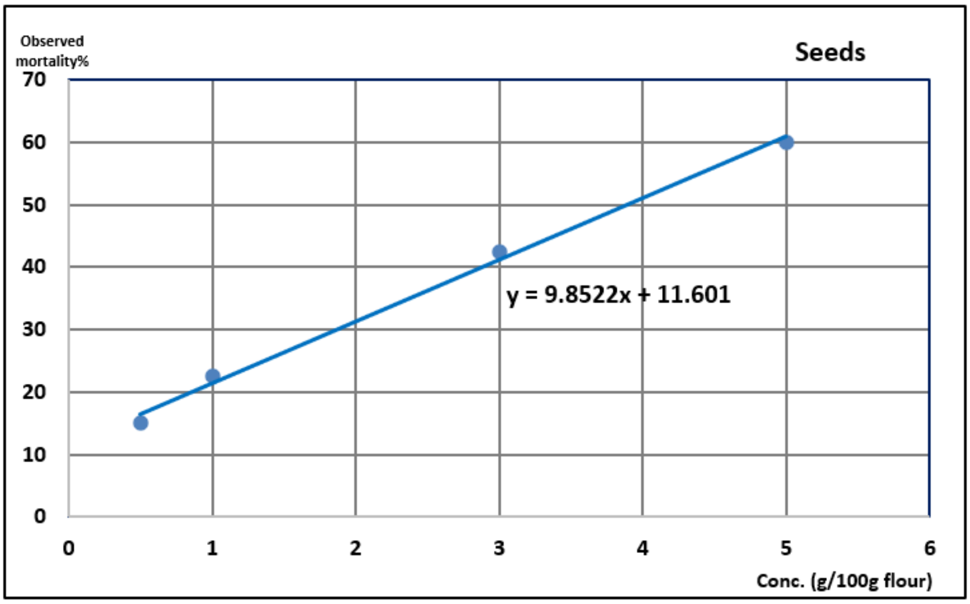

Figure (3): Susceptibility line of *S. Oryzae* adults to *Moringa* seeds treatments.

Table (4): The median lethal concentrations (LC_{50}s) of the tested *Moringa* products:

Moringa product	Slope	LC_{50} with confidence limits*
Leaves	5.6897	4.73 < 7.32 < 11.35
Roots	2.6847	11.07 < 17.04 < 26.24
Seeds	9.8522	2.20 < 3.90 < 6.90

* Confidence limits at 95% probability.

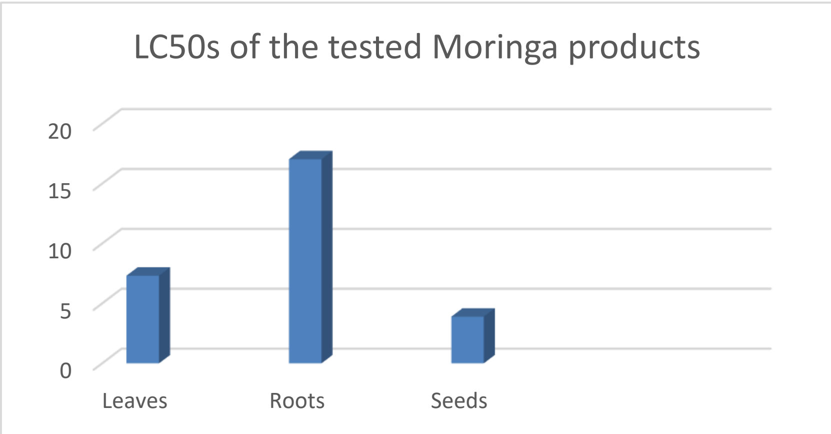

Fig. (4): The median lethal concentrations (LC_{50}s) of the tested *Moringa* products.

4. Discussion

Most of insecticides used around the world are synthetic chemicals due to their high toxicity against these pests and their rapid results. But the excessive use of these insecticides creates development of pesticide-resistant population, environmental pollution, and human health problems. Recently the use of botanical-source insecticides as alternatives to popularly used synthetic chemical insecticides has increased (Ileke and Ogungbite 2014, 57 and Nisar, *et al.* ,2021,4-5).

Numerous studies have been conducted on plants that may have lethal properties against stored insect pests to verify its effectiveness. *Moringa oleifera* was discovered as one of them, as *Moringa oleifera* is widely used in the treatment of various diseases. It is believed that its active medicinal properties may have played major roles in killing pests. (Ileke and Ogungbite,2014,61).

Our current research is considered one of the research projects that have proven *Moringa*'s considerable effectiveness against one of the stored-grain insects, the rice weevil *S. oryzae*. The highest effective product was seeds, followed by leaves, and the roots was the least effective one.

The project results agree with the findings of Boraei (2014,172) and Okwor *et al.* (2021-b,1940). Authors found that *M. oleifera* was showed that seed extract was recorded higher mortality effect against S. oryzae than leaves. Also, many researchers agree with the present results. Mbaiguinam *et al.* (2006,423) tested some vegetable powders and oils against *S. oryzae* and found that *Moringa* have an apparent toxic effect. Ileke and Oni (2011, **3045**) reported that the *Moringa* seeds and tasteless oil were toxic to the rice weevil, *S. oryzae*. Besides, the powders and essential oils of *M. oleifera* and some other green plants tested were toxic to *S. oryzae* (Ileke and Ogungbite, 2014, 61). *M. oleifera* at high concentration also contributed to blocking the emergence of *S. oryzae* as reported with Ogban *et al.* (2022,22); Ileke and Oni (2011,2); and Mbaiguinam *et al.* (2006,423).

In the present study, roots powder showed less effectiveness comparing with seeds and leaves, the higher occurrence of *S. oryzae* adults in roots treatments may be a result of the low concentration used or its low effects to protect the flour (Kemabonta and Falodu, 2013,264).

The obtained results showed that the percentage mortality of *S. oryzae* adults depended on the kinds of *Moringa* products and their concentrations. Generally, for the tested products used, percentage mortality increased directly with concentration.

This finding is agreed with the results of Okwor *et al.* (2021, 6), who found that the effects of *Moringa* treatments are dependent on the concentrations, higher concentrations reduced the insect's ability to search for food. In addition, Ogban *et al.* (2022,24) studied the effect of *Moringa* against *S. oryzae* and found that the *Moringa* shown high mortality rate whenever the time of exposure to *Moringa* increased.

Arabi (2008,5-8) also found that *M. oleifera* exhibited significant bioactivity with a gradual increase in mortality and exposure time, and with its insecticidal potential becoming more pronounced from 24 to 72 hours. This may indicate that vegetable oils are slow-acting insecticides and supports the findings of who reported that *S. oryzae* mortality increased with increasing exposure time.

The significant mortality rate of leaves and seeds powder could be due to that *S. oryzae* breathes through its respiratory openings; therefore, it is possible that these openings were blocked by powders *Moringa*, which leads to the insect's suffocation and death (Kemabonta and Falodu, 2013,264 and Ogban *et al*. 2022,22).

It is also possible that the cause of death of the *Sitophilus oryzae* adults, because of *Moringa* different treatments, is the presence of many phytochemicals such as glycosides, steroids, alkaloids. (Okwor *et al*., 2021-b,1940). Ogban *et al*. (2022,24) stated that the biological activity of

M. oleifera is due to the presence of Chavicine and piperine causing the deterrence of weevils.

Another reason for the observed weevils' mortality, results from treatment, is the insects' inability to ingest the wheat particles coated with tested plant powders, which leads to starvation then death (Boraei, 2014, 172 and Ogban *et al.*, 2022,24).

5. Conclusion

From the current results, we can conclude that the *Moringa oleifera* showed clear effects against the rice weevil, *Sitophilus oryzae* adults compared to the control. The most effective tested part was the seeds.

M. oleifera trees are available in Egypt which can provide a cheap and safe natural pesticide methods to control insects. Using this natural method can reduce the use of synthetic chemical insecticides, as they are not harmful to humans.

Moringa oleifera have medicinal value and do not pose any danger to humans or other mammals, so it can be used as a safe biocide against *S. oryzae*.
So, efforts should be intensified towards extraction of its bioactive components, mass production, packaging, and field application.

The present work net results are consistent with Egypt's 2030 plan for sustainable development and going green.

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