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# Evaluation of *Piper sarmentosum* Leaf Powders as Seed Protectant against *Sitophilus oryzae* (Coleoptera: Curculionidae) in Stored Rice

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## Abstract

The effects of *Piper sarmentosum* leaf powders were evaluated against adults of the rice weevil, *Sitophilus oryzae*, for their effects on mortality and progeny production. The effects of the plant powders on rice seed germination and seed damage after 3 months of storage were also investigated. The plant powders were applied at 0.25, 0.5, 1, 2, and 4 % (w/w) for the contact/feeding toxicity test, and 0.05, 0.1, 0.2, 0.4, and 0.8 g/L air for the fumigant toxicity test. In the contact/feeding toxicity test, the plant powders at all concentrations except 0.25 % showed higher toxicity against the insect, with mortality ranging from 95.00 - 100.00 % at 5 d. The concentration of 4 % achieved 100.00 % mortality within a short period of 3 d, while the lowest toxicity (10.00 %) was obtained from the concentration of 0.25 % at 5 d. In the fumigant toxicity test, the highest concentration (0.8 g/L air) was the most toxic to *S. oryzae*, with mortality of 95.00 % whereas the lowest concentration (0.05 g/L air) was the least toxic (32.50 %) at 3 d. Additionally, *P. sarmentosum* leaf powders at all concentrations significantly reduced progeny production and weight loss. Complete protection of rice seeds and inhibition of progeny production were observed from the concentrations of 2 and 4 %. Also, all concentrations did not affect seed germination relative to the control, and completely protected seeds from insect infestation after 3 months of storage. The results suggested that *P. sarmentosum* leaf powders can be promoted for controlling *S. oryzae* in stored rice seeds.

**Keywords:** *Piper sarmentosum*, *Sitophilus oryzae*, seed protectant, stored rice, botanical insecticides

## Introduction

Rice (*Oryza sativa* L.) is the most important staple food for a large part of the world's human population, especially in Asia and the West Indies. Thailand is one of the world's largest exporters of rice. In 2014, Thailand exported 10.9 million tons of rice, valued at 5.34 billion USD [1]. Among all Thai rice varieties, Sangyod is a red pigmented variety local to Phatthalung, a province in southern Thailand. It is the first rice variety to be registered for geographical indication (GI) as a product of Thailand [2]. Currently, Sangyod has become one of the most popular rice varieties in the functional food market because of its high nutritional value content, such as iron, vitamin B complex, niacin, carbohydrate, protein, and fibers. Additionally, it contains bioactive components, including γ-aminobutyric acid, γ-oryzanol, flavonoids, ferulic acid, and phylate [3]. These components have played an important role in the prevention and treatment of chronic diseases, such as diabetes mellitus, cardiovascular disease, and cancer [4,5]. However, one of the main problems that occur during Sangyod rice storage is the attack of insect pests. The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the most serious insect pests of stored rice. It usually attacks cereal grains and seeds, including rice, wheat, barley, rye, oats, sorghum, and maize, both before and after harvest throughout the world [6]. The life cycle of *S. oryzae* consists of 4 stages; egg, larva, pupa, and adult. The adult of *S. oryzae* is dull reddish-brown in color, with 4 light red or yellow spots on the elytra. It is 2 - 3 mm in length and has a long snout of about 1 mm. The female adult propagates by laying eggs inside rice kernels. The larva goes through 4 stages, then pupates inside the kernel and later emerges as an adult [7]. The complete life cycle of *S. oryzae* from an egg to an adult takes 28 to 32 days under optimum conditions [8]. The damage to the rice grains is caused by both larva and adult rice weevils. The larva feeds within the kernel and consumes the endosperm. The adult leaves a large, ragged exit hole in the kernel, and feeds on damaged kernels [9]. Feeding by the *S. oryzae* larva and adult causes weight losses of up to 75 % [10] and decreases the nutritional value of the grains. Moreover, *S. oryzae* reduces germination, resulting in lower prices for seed grains [11].

For the past several decades, the control of *S. oryzae* has depended mainly on applications of synthetic insecticides, such as chlorpyrifos-methyl, pirimiphos-methyl, deltamethrin, methyl bromide, and phosphine [12,13]. The indiscriminate use of synthetic insecticides has led to a rapid development of resistance in this insect. For example, *S. oryzae* has developed resistance to deltamethrin, fenitrothion, chlorpyrifos-methyl, pirimiphos-methyl, methyl bromide, and phosphine [14,15]. In addition to resistance, the extensive and long-term use of synthetic insecticides causes many ecological problems, including toxic residues in the environment, lethal effects on non-target organisms, and toxicity to users and consumers [16]. Therefore, the identification and development of effective, anti-resistant, safe, and eco-friendly chemical control alternatives for *S. oryzae* is needed.

Use of natural compounds from plants has been suggested as a viable source of alternative treatments for insect control, because many of such compounds have novel modes of action, no or low toxicity to non-target organisms and mammals, and are less harmful to the environment [17,18]. In addition, plant based insecticides are cheaper than synthetic insecticides. Numerous plants have been reported to have a variety of biological activities against insects, including insecticidal, repellent, antifeedant, fumigant, growth regulatory, and anti-oviposition activities [19,20]. Moreover, plant based insecticides often contain a mixture of active substances, which can delay or prevent resistance development [21]. Plant products can be used for insect pest control in the forms of powders, extracts, or essential oils. However, plant powders are more easily processed, and more often used by small-scale farmers, compared to plant extracts or essential oils.

*Piper sarmentosum* Roxb. is a member of the Piperaceae family and is a well-known medicinal plant in Southeast Asian countries including Thailand. It possesses a range of pharmacological activities, such as antioxidant [22], antibacterial [23], antifungal [24], anti-tuberculosis, antiplasmodial [25], anti-inflammatory, anti-nociceptive and antipyretic activities [26]. The insecticidal activity of the essential oils of *P. sarmentosu*m leaves against *Callosobruchus maculatus*, an economically damaging insect pest of stored legumes [27], has been reported previously; however, there has been no report on the bioactivity of *P. sarmentosum* leaf powders against *S. oryzae* in stored rice seeds. Thus, the objectives of this work were to evaluate the contact/feeding and fumigant toxicity of *P. sarmentosum* leaf powders against *S. oryzae*, as well as their effects on seed germination and seed damage after 3 months of rice seed storage.

## Materials and methods

### Collection of rice seeds

Sangyod rice seeds were purchased from the local market of Khao Chaison District, Phatthalung Province. They were thoroughly cleaned and oven dried at 60 °C for 4 h. The rice seeds were then stored at room temperature in big air tight plastic boxes for further use.

### Insect culture

*S. oryzae* adults were collected from naturally infested rice seeds. Insects were reared in the laboratory on disinfested Sangyod rice seeds. Fifty adult insects were released in 200 g of rice seeds in a 1000 ml plastic container capped with cotton cloth for ventilation. All the containers were maintained at room temperature (26 ± 1 °C) and 75 % RH under 14: 10 (L: D). Two weeks after oviposition, the insect parents were removed. The medium containing the eggs was kept in the same condition until adult emergence. 7 to 10 day old adults of *S. oryzae* were used in all the experiments.

**Preparation of plant powders**

The mature fresh leaves of *P. sarmentosum* were collected from the surroundings of the Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat Campus, in October 2014. They were washed with distilled water, cut into small pieces, and air-dried until crispy. The air-dried leaves were then ground into fine powder using an electric grinder and screened through an 80-mesh screen. The leaf powders were packed in plastic containers with tight lids and stored in a refrigerator at 4 °C prior to use.

**Contact/Feeding toxicity assay**

The contact/feeding toxicity of *P. sarmentosum* leaf powders against *S. oryzae* was conducted according to the method of [28] with some modifications. *P. sarmentosum* leaf powders at the rates of 0.05, 0.1, 0.2, 0.4, and 0.8 g were admixed with 20 g of sterilized rice seeds in 250 ml plastic containers corresponding to 0.25, 0.5, 1.0, 2.0, and 4.0 % w/w, respectively. The containers with their contents were gently shaken to ensure thorough admixture of the rice seeds and treatment powders. The rice seeds in the controls contained no plant powders. Ten unsexed adults of *S. oryzae* were released into each experimental container. The container was then covered with cotton cloth and secured with a rubber band. Adult mortality was monitored daily for 5 d. Adults were considered dead when probed with sharp objects to no response.

**Fumigant toxicity assay**

*P. sarmentosum* leaf powders were evaluated for fumigant toxicity against *S. oryzae* in a space trial test [29]. The plant powders, weighing 0.05, 0.1, 0.2, 0.4, and 0.8 g in cotton cloth bags, were placed at the bottom of a 1 l plastic container. Ten unsexed adults of *S. oryzae* were released into each container by placing them in a separate glass vial containing food materials for the tested insects. Both ends of the vial were covered with stainless wire mesh. The vial was then suspended in each plastic container, the cap of which was tightly screwed on. The control treatment had no plant powders. Insect mortality was recorded for 3 d.

**Effect on progeny production and weight loss**

Twenty grams of rice seeds were placed in a 250 ml plastic container. Five pairs of a day old adults of *S. oryzae* were introduced into each container. The weevils were cultured, allowed to lay eggs, and removed by sieving after 2 weeks. The rice seeds were then thoroughly mixed with *P. sarmentosum* leaf powders at the rates of 0.05, 0.1, 0.2, 0.4, and 0.8 g, corresponding to 0.25, 0.5, 1.0, 2.0, and 4.0 % w/w, respectively. The control had no plant powders. The culture was continued until adult emergence. The number of emerged adult insects was then recorded at 49 d after treatment. The percentage reduction in adult emergence or reproduction inhibition rate (IR) was calculated by the method of [30], as follows;

$$\text{Inhibition rate (\%)} = \frac{(\mathrm{Cn}-\mathrm{Tn})}{\mathrm{Cn}} \times 100 \qquad (1)$$

where Cn is the number of emerged adults in the untreated seeds and
Tn is the number of emerged adults in the treated seeds.

After counting the number of emerged adults, the rice seeds were then reweighed and the percentage of weight loss was computed according to [31], as follows;

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100 \quad (2)$$

**Effect on seed germination and seed damage**

*P. sarmentosum* leaf powders at 2.5, 5, 10, 20, and 40 g were admixed with 1 kg of rice seeds in the trays, corresponding to 0.25, 0.5, 1.0, 2.0, and 4.0 % w/w, respectively. The seeds in the control contained no plant powders. After thorough mixing, the treated and control seeds were then stored in 5 kg jute bags and kept at room temperature for 3 months. Seed germination tests were carried out using the top of paper method after 3 months of storage [32]. One hundred seeds were randomly selected from each treatment and planted on moist germination papers in plastic containers. The germination results were recorded after 14 d of growth by counting the number of normal seedlings. Germination rate was expressed as the percentage of germinated seeds over the total seeds planted. For the seed damage bioassay, another one hundred seeds were randomly selected and assessed for seed damage after 3 months of storage. Percentage seed damage was determined by the formula;

$$\text{Seed damage (\%)} = \frac{\text{No.of seeds damaged}}{\text{Total no.of seeds}} \times 100 \quad (3)$$

**Statistical analysis**

All experiments were arranged in a completely randomized design with 4 replications. All data were analyzed with one-way ANOVA, followed by Duncan's multiple range test at $P < 0.05$. All values were represented as mean ± S.E.

**Results and discussion**

**Contact/Feeding toxicity**

Significant differences ($P < 0.01$) among different concentrations of *P. sarmentosum* leaf powders were observed at 1, 2, 3, 4, and 5 d after treatment (**Table 1**). The mortality of *S. oryzae* increased with increase of concentration of the plant powders and exposure time. The highest concentration (4 %) showed significantly higher toxicity against *S. oryzae* than the other concentrations, with mortalities of 25.00 and 97.50 % at 1 and 2 d after treatment, respectively. By 3 d, the concentration of 4 % achieved 100 % mortality, followed by the concentration of 2 % (90.00 %) whereas the lower concentrations could not achieve up to 50 % insect mortality. The concentration of 2 % caused 100 % mortality in *S. oryzae* at 4 d. All concentrations except the concentration of 0.25 % showed strong toxicity, with mortalities ranging from 95.00 to 100.00 % at 5 d after treatment. No mortality was observed in the control (**Table 1**).

**Table 1** Contact/feeding toxicity of *P. sarmentosum* leaf powders against adults of *S. oryzae*.

| Concentration | Mortality (mean ± S.E., %)[a] | | | | |
|---|---|---|---|---|---|
| (%) | 1 d | 2 d | 3 d | 4 d | 5 d |
| 4 | 25.00 ± 6.46[a] | 97.50 ± 2.50[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] |
| 2 | 7.50 ± 2.50[b] | 65.00 ± 11.90[b] | 90.00 ± 7.07[a] | 100.00 ± 0.00[a] | 100.00 ± 0.00[a] |
| 1 | 2.50 ± 2.50[b] | 7.50 ± 4.79[c] | 42.50 ± 11.09[b] | 77.50 ± 4.79[b] | 95.00 ± 2.89[a] |
| 0.5 | 0.00 ± 0.00[b] | 5.00 ± 5.00[c] | 27.50 ± 2.50[bc] | 82.50 ± 6.29[b] | 95.00 ± 5.00[a] |
| 0.25 | 0.00 ± 0.00[b] | 0.00 ± 0.00[c] | 7.50 ± 2.50[cd] | 10.00 ± 4.08[c] | 10.00 ± 4.08[b] |
| Control | 0.00 ± 0.00[b] | 0.00 ± 0.00[c] | 0.00 ± 0.00[d] | 0.00 ± 0.00[c] | 0.00 ± 0.00[b] |

[a] Means within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT

**Fumigant toxicity**

The fumigant toxicity of *P. sarmentosum* leaf powders at different concentrations against *S. oryzae* is presented in **Table 2**. There was no insect mortality in the control and treated treatments within 2 d after treatment. Significant differences ($P < 0.01$) among treatments were observed at 3 d after treatment. Insect mortality increased proportionally with increase of concentration of the plant powders and exposure time. The highest concentration (0.8 g/L air) showed the strongest fumigant toxicity with mortality of 95.00 %, while the lowest fumigant toxicity was obtained from the lowest concentration (0.05 g/L air) with mortality of 32.50 %. There was no insect mortality in the control.

**Table 2** Fumigant toxicity of *P. sarmentosum* leaf powders against adults of *S. oryzae*.

| Concentration (g/L air) | Mortality (mean ± S.E., %)[a] | | |
|---|---|---|---|
| | 1 d | 2 d | 3 d |
| 0.8 | 0.00 ± 0.00 | 0.00 ± 0.00 | 95.00 ± 2.89[a] |
| 0.4 | 0.00 ± 0.00 | 0.00 ± 0.00 | 80.00 ± 4.08[a] |
| 0.2 | 0.00 ± 0.00 | 0.00 ± 0.00 | 60.00 ± 4.08[b] |
| 0.1 | 0.00 ± 0.00 | 0.00 ± 0.00 | 45.00 ± 5.00[bc] |
| 0.05 | 0.00 ± 0.00 | 0.00 ± 0.00 | 32.50 ± 4.79[c] |
| Control | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00[d] |

[a] Means within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT

**Effect on progeny production and weight loss**

*P. sarmentosum* leaf powders at all concentrations significantly ($P < 0.01$) reduced progeny production and weight loss caused by *S. oryzae* compared to the control (**Table 3**). Completely inhibited progeny production was observed from the higher concentrations (2 and 4 %). The percentage inhibition rates at the concentrations of 0.25, 0.5 and 1 % were 47.47, 75.41, and 83.78 %, respectively. There was no weight loss recorded in rice seeds treated with *P. sarmentosum* leaf powders at the concentrations of 2 and 4 %. The weight losses at concentrations of 0.25, 0.5, and 1 % were 5.91, 2.25, and 1.25 %, respectively. The highest weight loss was observed in the control (17.25 %).

**Table 3** Effects of *P. sarmentosum* leaf powders on inhibition rate of progeny production and weight loss caused by *S. oryzae*.

| Concentration (%) | Inhibition rate (mean ± S.E., %)[a] | Weight loss (mean ± S.E., %)[a] |
|---|---|---|
| 4 | 100.00 ± 0.00[a] | 0.00 ± 0.00[d] |
| 2 | 100.00 ± 0.00[a] | 0.00 ± 0.00[d] |
| 1 | 83.78 ± 3.51[b] | 1.25 ± 0.14[cd] |
| 0.5 | 75.41 ± 4.36[b] | 2.25 ± 0.14[c] |
| 0.25 | 47.47 ± 1.47[c] | 5.91 ± 0.56[b] |
| Control | 00.00 ± 0.00[d] | 17.25 ± 0.78[a] |

[a] Means within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT

**Effect on seed germination and seed damage**

After 3 months of storage, there was no significant difference in seed germination between the treated and control seeds. The germination percentages at the concentrations of 0.25, 0.5, 1, 2, and 4 % were 72.25, 74.25, 69.75, 73.00, and 73.75 %, respectively, as compared to the control (72.00 %). There was a significant difference ($P < 0.01$) in seed damage between the treated and control seeds. No seed damage was observed from the seeds treated with *P. sarmentosum* leaf powders at all concentrations. On the other hand, the control seeds showed the highest damage of 19.00 % (**Table 4**).

**Table 4** Effects of *P. sarmentosum* leaf powders on rice seed germination and seed damage after 3 months of storage.

| Concentration (%) | Seed germination (mean ± S.E., %)[a] | Seed damage (mean ± S.E., %)[a] |
|---|---|---|
| 4 | 73.75 ± 1.18 | 0.00 ± 0.00[b] |
| 2 | 73.00 ± 1.58 | 0.00 ± 0.00[b] |
| 1 | 69.75 ± 2.75 | 0.00 ± 0.00[b] |
| 0.5 | 74.25 ± 2.25 | 0.00 ± 0.00[b] |
| 0.25 | 72.25 ± 1.89 | 0.00 ± 0.00[b] |
| Control | 72.00 ± 0.82 | 19.00 ± 2.68[a] |

[a] Means within a column followed by the same letter are not significantly different at $P < 0.01$ by DMRT

Plants have been used as sources of medicine in virtually all cultures. In recent years, interest in screening medicinal plants for insect control has increased significantly. The advantage of using medicinal plants is that they are easily available, and they have been used extensively for medicinal purposes, implying that they have low or no toxicity to humans. In addition, they can be applied to insect pests in the same way as conventional insecticides. However, numerous plant species remain unexploited and underutilized in insecticidal activity against *S. oryzae*. The search for new botanical insecticides is still necessary for *S. oryzae* control [17,18].

In previous studies, some plant powders have been reported to be effective against *S. oryzae*. For example, *Azadirachta indica* and *Anacardium occidentale* seed powders at concentrations of 20 % produced 100 % mortality of *S. oryzae* and protected the wheat grains from the insect for up to 12 weeks [9]. *Zanthoxylum zanthoxyloides* root powders at a rate of 1 g/ 50 g of rice grains caused 100 % mortality and completely inhibited progeny production of *S. oryzae* [33]. Rice grains mixed with *Melia azadarach* leaf powders at 25 % were toxic to *S. oryzae* with mortality of 80.54 % at 35 d after treatment and also significantly reduced weight loss caused by the insect compared to the control [34]. *Olax zeylanica* leaf powder has been reported as having contact and fumigant toxicity against *S. oryzae* [28]. Also, *Annona squamosa*, *A. reticulata*, and *A. muricata* leaf powders have been found to possess contact toxicity, repellent activity, and suppression of progeny development in *S. oryzae* [35].

The present investigation showed that *P. sarmentosum* leaf powders were very effective against *S. oryzae*. These findings are similar with the observation of other research that ethanol extract of *P. sarmentosum* leaf had adulticidal effects against *Stegomyia aegypti*, a main vector of dengue and dengue hemorrhagic fever, with an $LD_{50}$ value of 0.14 µg/female [36]. Petroleum ether extract of *P. sarmentosum* leaf has been reported to be toxic to *Spodoptera litura* eggs, with an $LD_{50}$ value of 1.9787 % [37]. In addition, essential oils of *P. sarmentosum* leaf exhibited contact toxicity against *C. maculatus*, with an $LC_{50}$ value of 8864 ppm at 48 h using the residual film assay technique [27]. They also caused 100 % mortality of *Coptotermes* sp. at concentrations of 1 % at 2 d after treatment [38]. Furthermore, the essential oils of *P. sarmentosum* leaf showed a strong fumigation effect on the eggs and pupae and contact toxicity on the larvae and imagoes of *Brontispa longissima* [39].

Our results are the first to demonstrate the strong contact/feeding and fumigant toxicity of *P. sarmentosum* leaf powders against *S. oryzae*. Insect mortality was directly proportional to the concentration of the plant powders and exposure time. It indicated that higher concentrations and longer exposure times are needed to achieve appreciable management of the pest. These findings are in agreement with the toxicity of the powders of *Curcuma longa* rhizome, *Dennettia tripetala* fruit*, Piper guineense* seed, and *Zingiber officinale* rhizome [40]. In addition, wheat grains treated with *A. indica* seed powders at concentrations of 20 % caused *S. oryzae* mortalities of 66.25, 96.25, 100.00, and 100.00 % at 24, 48, 72, and 96 h after treatment, while insect mortality at the concentration of 5 % was 28.75, 58.75, 81.25, and 95.00 % at the same time [9].

The insecticidal activity of *P. sarmentosum* leaf powders against *S. oryzae* may possibly be dependent on different factors, such as the presence of active components with diverse activities. Phytochemically, *P. sarmentosum* leaf has been found to contain various components, including alkaloids (amides), pyrones, flavonoids, sterols, phenylpropanoids, neolignans [22-26], and essential oils [38,39]. Early studies on the essential oils of the *P. sarmentosum* leaf indicated that spathulenol, myristicin, β-caryophyllene, and (*E*,*E*)-farnesol were the major components [38]. The insecticidal activity of myristicin has been reported as contact toxicity against various insect pests, including *Coptotermes* sp. [38], *B. longissima* [39], *Culex pipiens*, and *Aedes aegypti* [41]. Myristicin also showed fumigant toxicity on the eggs and pupae of *B. longissima* [39]. Furthermore, β-caryophellene has been found to have contact and fumigant toxicity against *S. oryzae* [42]. Thus, myristicin and β-caryophyllene may be responsible for insecticidal activity against *S. oryzae*. However, no data on the insecticidal activity of the other components (alkaloids, pyrones, flavonoids, sterols, phenylpropanoids, and neolignans) of *P. sarmentosum* leaf was available. The isolation and identification of the bioactive compounds in *P. sarmentosum* leaf are of utmost importance, so that their potential application in controlling *S. oryzae* can be fully exploited. Also, the plant powders may act as a physical barrier, blocking the spiracles of the insect and thus impairing respiration, leading to suffocation [43]. In addition, the high mortality effect of the plant powders could be due to the inability of the insects to feed on the rice seeds that have been coated with them, thereby leading to their starvation. Furthermore, the plant powders may cause abrasion of the insect cuticle and lead to water loss, stress, and eventual death.

In addition to causing adult mortality, *P. sarmentosum* leaf powders at all concentrations were effective in reducing the emergence of *S. oryzae*. The concentrations of 2 and 4 % showed a 100 % inhibition rate of progeny production, and other concentrations caused significant suppression of progeny development of *S. oryzae* relative to the control. Our results showed that the percentage reduction in adult emergence from the treated seeds after 49 d of storage was dose-dependent. The reduction in adult emergence could be due either to egg mortality, larval mortality, pupal mortality, or even reduction in egg hatching [9]. Seed weight loss indicated a quantitative loss in stored seeds due to the insects, showing a direct relationship between insect population and weight loss. The reduction in seed weight loss could be as a result of the reductive effect recorded in adult emergence.

The present study also indicated that *P. sarmentosum* leaf powders at all concentrations did not show any adverse effects on the germination of rice seeds when applied as a seed protectant. However, the rice seeds in all treatments had rather low germination rates, ranging from 69.75 - 74.25 %. These values may have been affected by field and storage fungi detected on the seeds. Seed quality is a prerequisite condition that affects the germination and the yield of the crops [44]. Hence, *P. sarmentosum* leaf powders can be used as an alternative in rice seed protection with no adverse effects on germination, especially by smallholder farmers.

Furthermore, *P. sarmentosum* leaf powders at all concentrations completely protected rice seeds from the infestation of *S. oryzae* after 3 months of storage. The possible reason for this could be that the active components might act as a repellent against the pest. An insect repellent is a chemical stimulus, which causes the insect to make oriented movements away from the source of the stimulus [45]. Repellence is a method of preventing insect attack. Therefore, repellence is an important method for maintaining good quality stored products. In previous studies, the various components of essential oils from plants were found to possess repellent activity against insects. For example, β-caryophellene showed repellent activity against stored product insects, including *S. oryzae* [46], *Tribolium castaneum*, and *Liposcelis bostrychophilai* [47]. Limonene and α-pinene have been reported as repellents against *S. oryzae* [48]. These components are also found in the essential oils of *P. sarmentosum* leaf [38,39]. Hence, they might be responsible for the repellent activity of *P. sarmemtosum* leaf against *S. oryzae*. However, the repellent activity of *P. sarmemtosum* leaf against *S. oryzae* should be subjected to further study.

## Conclusions

*P. sarmentosum* leaf powders demonstrated strong contact/feeding and fumigant toxicity against *S. oryzae*. They also significantly inhibited progeny production and reduced weight loss in rice seeds caused by the insect. Additionally, they completely protected the rice seeds from insect infestation after 3 months of storage, with no adverse effects on seed germination. *P. sarmentosum* is an easily available medicinal plant throughout Thailand. Thus, the admixing of *P. sarmentosum* leaf powders may be recommended as a cheap, usable, eco-friendly, and non-toxic method of seed protectant, and may provide a suitable alternative to the synthetic insecticides for small-scale farmers in the management of *S. oryzae*. More developmental research is necessary on the feasibility of introducing plant powders at the farm level. Also, isolation of the insecticidal components of *P. sarmentosum* leaf powders should be further investigated.

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## References

[1] Office of Agricultural Economics. *Thailand Foreign Agricultural Trade Statistics 2014*. Ministry of Agricultural and Cooperatives, Bangkok, 2015, p. 19-20.

[2] T Chaichana, S Phethuayluk, T Tepnual and T Yaibok. Energy consumption analysis for Sangyod rice production. *Energ. Proc*. 2014; **52**, 126-30.

[3] J Banchuen, P Thammarutwasik, B Ooraikul, P Wuttijumnong and P Sirivongpaisal. Effect of germinating processes on bioactive component of Sangyod Muang Phatthalung rice. *Thai J. Agric. Sci.* 2009; **42**, 191-9.

[4] M Wojcik, I Burzynska-Pedziwiatr and LA Wozniak. A review of natural and synthetic antioxidants important for health and longevity. *Curr. Med. Chem*. 2010; **17**, 3262-88.

[5] T Ratanavalachai, S Thitiorul, S Tanuchit, C Jansom, S Uttama and A Itharat. Antigenotoxic activity of Thai Sangyod red rice extracts against a chemotherapeutic agent, doxorubicin, in human lymphocytes by sister chromatid exchange (SCE) assay *in vitro*. *J. Med. Assoc. Thai* 2012; **95**, S109-S114.

[6] SR Yankanchi and AH Gadache. Grain protectants efficacy of certain plant extracts against rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *J. Biopest*. 2010; **3**, 511-3.

[7] KC Narayana, GP Mutthuraju, E Jagadeesh and GT Thirumalaraju. Biology of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) on stored maize grains. *Curr. Biotica* 2014; **8**, 76-81.

[8] BC Longstaff. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): A critical review. *Prot. Ecol*. 1981; **2**, 83-130.

[9] KA Kemabonta and BB Falodu. Bioefficacy of three plant products as post-harvest grain protectants against *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) on stored wheat (*Triticum aestivum*). *Int. J. Sci. Nat*. 2013; **4**, 259-64.

[10] G Dal Bello, S Padin, CL Lastra and M Fabrizio. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. *J. Stored Prod. Res.* 2001; **37**, 77-84.

[11] W Auamcharoen, A Chandrapatya, A Kijjoa and Y Kainoh. Toxicity and repellent activities of the crude methanol extract of *Duabanga grandiflora* (Lythraceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae). *Pak. J. Zool*. 2012; **44**, 227-32.

[12] M Khani, RM Awang, D Omar, M Rahmani and S Rezazadeh. Tropical medicinal plant extracts against rice weevil, *Sitophilus oryzae* L. *J. Med. Plants Res*. 2011; **5**, 259-65.

[13] RK Upadhyay. Insecticidal and oviposition inhibition efficacy of *Capparis decidua* to *Sitophilus oryzae* Linn. *Int. J. Chem. Biochem. Sci*. 2012; **2**, 14-23.

[14] FC Ceruti and SMN Lazzari. Use of bioassays and molecular markers to detect insecticide resistance in stored products beetles. *Rev. Brazil Entomol*. 2003; **47**, 447-53.

[15] OO Odeyemi, MO Ashamo, RO Akinkurolere and AA Olatunji. Resistance of strains of rice weevil, *Sitophilus* oryzae (Coleoptera: Curculionidae) to pirimiphos methyl. *Julius Kühn Archiv* 2010; **425**, 167-72.

[16] P Vanichpakorn, W Ding and XX Cen. Insecticidal activity of five Chinese medicinal plants against *Plutella xylostella* L. larvae. *J. Asia Pac. Entomol.* 2010; **13**, 169-73.

[17] GM Liang, W Chen and TX Liu. Effect of three neem-based insecticides on diamondback moth (Lepidoptera: Plutellidae). *Crop. Prot.* 2003; **22**, 333-40.

[18] DS Charleston, R Kfir, M Dicke and LEM Vet. Impact of botanical pesticide derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth. *Biol. Contr*. 2005; **33**, 131-42.

[19] MB Isman. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 2006; **51**, 45-66.

[20] DA Ukeh, MA Birkett, TJA Bruce, EJ Allan, JA Pickett and AJM Luntz. Behavioral responses of the maize weevil, *Sitophilus zeamais*, to host (maize grain) and non-host plant volatiles. *Pest. Manag. Sci.* 2010; **66**, 44-50.

[21] YN Wang, GL Shi, LL Zhao, SQ Liu, TQ Yu, SR Clarke and JH Sun. Acaricidal activity of *Juglans regia* leaf extracts on *Tetranychus viennensis* and *Tetranychus cinnabarinus* (Acari: Tetranychidae). *J. Econ. Entomol*. 2007; **100**, 1298-303.

[22] A Chanwitheesuk, A Teerawutgulrag and N Rakariyatham. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem*. 2005; **92**, 491-7.

[23] S Kondo, C Sattaponpan, S Phongpaichit, A Srijanand and A Itharat. Antibacterial activity of Thai medicinal plants Pikutbenjakul. *J. Med. Assoc. Thai* 2010; **93**, S131-S135.

[24] MHM Nazmul, I Salmah, A Syahid and AA Mahmood. *In-vitro* screening of antifungal activity of plants in Malaysia. *Biomed. Res*. 2011; **22**, 28-30.

[25] T Rukachaisirikul, P Siriwattanakit, K Sukcharoenphol, C Wongvein, P Ruttanaweang, P Wongwattanavuch and A Suksamrarn. Chemical constituents and bioactivity of *Piper sarmentosum*. *J. Ethnopharmacol.* 2004; **93**, 173-6.

[26] S Sireeratawong, S Vannasiri, S Sritiwong, A Itharat and K Jaijoy. Anti-inflammatory, antinociceptive and antipyretic effects of the ethanol extract from root of *Piper Sarmentosum* Roxb. *J. Med. Assoc. Thai* 2010; **93**, S1-S6.

[27] N Chaona and S Pimsamarn. Contact toxicity of indigenous edible aromatic plants against the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *Agric. Sci. J.* 2003; **34**, 180-2.

[28] HSD Fernando and MMSC Karunaratne. Ethnobotanicals for storage insect pest management: effect of powdered leaves of *Olax zeylanica* in suppressing infestations of rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *J. Trop. Forest. Environ.* 2012; **2**, 20-5.

[29] AK Tripathi, AK Singh and S Upadhyay. Contact and fumigant toxicity of some common spices against the storage insects *Callosobruchus maculatus* (Coleoptera: Bruchidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Int. J. Trop. Insect Sci*. 2009; **3**, 151-7.

[30] LA Tapondjou, C Adler, H Bouda and DA Fontem. Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored products beetles. *J. Stored Prod. Res.* 2002; **38**, 395-402.

[31] OO Odeyemi and AM Daramola. *Storage Practices in the Tropics.* Vol I. Food Storage and Pest Problems. 1st ed. Dave Collins Publications, Nigeria, 2000, p. 235.

[32] NK Rao, J Hanson, ME Dulloo, K Ghosh, D Nowel and M Larinde. *Manual of Seed Handling in Genebanks.* Handbooks for Genebanks No. 8. Bioversity International, Rome, 2006, p. 50-85.

[33] KD Ileke and OC Ogungbite. Entomocidal activity of powders and extracts of four medicinal plants against *Sitophilus oryzae* (L.), *Oryzaephilus mercator* (Faur.) and *Ryzopertha dominica* (Fabr.). *Jordan J. Biol. Sci.* 2014; **7**, 57-62.

[34] MB Devi, NV Devi and SN Singh. Effect of six medicinal plant powder extracts on the control of rice weevil, *Sitophilus oryzae* L. in stored rice grains. *Int. J. Agric. Innovat. Res*. 2014; **2**, 683-6.

[35] P Vanichpakorn and Y Vanichpakorn. Bioactivities of leaf powders of sugar apple (*Annona squamosa* L.), custard apple (*A. reticulata* L.) and soursop (*A. muricata* L.) against rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Khon Kaen Agr. J.* 2014; **42**, 249-54.

[36] W Choochote, U Chaithong, K Kamsuk, E Rattanachanpichai, A Jitpakdi, P Tippawangkosol, D Chaiyasit, D Chamoakaew, B Tuetun and B Pitasawat. Adulticidal activity against *Stegomyia aegypti* (Diptera: Culicidae) of three Piper spp. *Rev. Inst. Med. Trop. Sao Paulo* 2006; **48**, 33-7.

[37] HF Liu, SZ Di and YG Fu. Effects of different extracts from polarity positions of *Piper sarmentosum* on biological activity of *Spodoptera litura*. *J. South. Agric.* 2014; **45**, 995-9.

[38] TC Chieng, ZB Assim and BA Fasihuddin. Toxicity and antitermite activities of the essential oils from *Piper sarmentosum*. *Malays. J. Anal. Sci.* 2008; **12**, 234-9.

[39] W Qin, C Huang, C Li, S Chen and Z Peng. Biological activity of the essential oil from the leaves of *Piper sarmentosum* Roxb. (Piperaceae) and its chemical constituents on *Brontispa longissima* (Gestro) (Coleoptera: Hispidae). *Pest. Biochem. Physiol*. 2010; **96**, 130-9.

[40] EF Asawalam, UE Ebere and KC Emeasor. Effect of some plant products on the control of rice weevil *Sitophilus oryzae* (L.) Coleoptera: Curculionidae. *J. Med. Plants Res.* 2012; **6**, 4811-4.

[41] ZS Abou-Elnaga. Insecticidal bioactivity of eco-friendly plant origin chemicals against *Culex pipiens* and *Aedes aegypti* (Diptera: Culicidae). *J. Entomol. Zool. Stud*. 2014; **2**, 340-7.

[42] MK Chaubey. Responses of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) against essential oils and pure compounds. *Herba Polonica* 2012; **58**, 33-45.

[43] CO Adedire, OO Obembe, RO Akinkurolele and O Oduleye. Response of *Callosobruchus maculatus* (Coleoptera: Chysomelidae: Bruchidae) to extracts of cashew kernels. *J. Plant Dis. Protect.* 2011; **118**, 75-9.

[44] R Musundire, F Mazoaze, L Macheka, R Ngadze, J Mubaiwa and F Manditsera. *Eucalyptus grandis* and *Tagetes minuta* leaf powders effectively protect stored maize against *Sitophilus zeamais* without affecting grain organoleptic properties. *Afr. J. Agric*. 2015; **10**, 49-57.

[45] VG Dethier, LB Browne and CN Smith. The designation of chemicals in terms of the responses they elicit from insects. *J. Econ. Entomol*. 1960; **35**, 134-6.

[46] MK Chaubey. Fumigant toxicity of essential oils and pure compounds against *Sitophilus oryzae* L. (Coleoptera: Curculionidae). *Biol. Agric. Hortic.* 2012; **28**, 111-9.

[47] SS Guo, CX You, JY Liang, WJ Zhang, ZF Geng, CF Wang, SS Du and N Lei. Chemical composition and bioactivities of the essential oil from *Etlingera yunnanensis* against two stored product insects. *Molecules* 2015; **20**, 15735-47.

[48] C Yoon, SH Kang, SA Jang, YJ Kim and GH Kim. Repellent efficacy of caraway and grapefruit oils for *Sitophilus oryzae* (Coleoptera: Curculionidae). *J. Asia. Pac. Entomol.* 2007; **10**, 263-7.