

Botanical powdered plant of *Piper nigrum* as bioprotectant to stored rice on *Sitophilus oryzae*

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Abstract: *Piper nigrum* is a promising bioinsecticide for stored-product pest management, yet the insecticidal potential of its different plant parts remains insufficiently studied. This study evaluated the effects of powdered plant parts of *P. nigrum* at different doses on the mortality and the repellency of *Sitophilus oryzae* in stored rice. A completely randomized factorial design was employed, comprising two factors: (1) powder sources of *P. nigrum* (stems, leaves, fruits) and (2) concentrations (2, 3, 4, and 5 g), with three replication for each treatment combination. A dual-choice bio-assay apparatus was designed to measure the mortality and repellency of weevils. A high dose of 5g powdered fruits significantly caused the highest mortality (98.3%) at 9-d exposure, followed by 4g of powdered fruits which resulted in 100% mortality at 12-d. Powdered leaves at 4g and 5g exhibited a significant repellent activity, with repellency rates of 56.7% and 58.8%, respectively, after 12-d. Thereby, powdered fruits and leaves of *P. nigrum* at 4–5 g proved to be the most effective botanical bioinsecticide agents against *S. oryzae*.

Keywords: bioinsecticide, food storage, insecticidal activity, *Piper nigrum*, *Sitophilus oryzae*

Abstrak: *Piper nigrum* merupakan bioinsektisida yang menjanjikan untuk pengendalian hama produk simpanan, namun potensi insektisida dari berbagai bagian tanamannya masih belum banyak diteliti. Penelitian ini mengevaluasi efek serbuk bagian tanaman *P. nigrum* pada berbagai dosis terhadap mortalitas dan daya tolak *Sitophilus oryzae* pada beras simpanan. Rancangan faktorial acak lengkap digunakan, terdiri atas dua faktor: (1) sumber serbuk *P. nigrum* (batang, daun, buah) dan (2) konsentrasi (2, 3, 4, dan 5 g), dengan tiga ulangan untuk setiap kombinasi perlakuan. Instrumen *bio-assay* pilihan ganda dirancang untuk mengukur mortalitas dan daya tolak kumbang bubuk. Dosis tinggi 5 g serbuk buah secara signifikan menyebabkan mortalitas tertinggi (98,3%) pada paparan 9 hari, diikuti oleh 4 g serbuk buah yang menghasilkan 100% mortalitas pada 12 hari. Serbuk daun pada dosis 4 g dan 5 g menunjukkan aktivitas penolak yang signifikan, dengan tingkat penolakan masing-masing 56,7% dan 58,8% setelah 12 hari. Dengan demikian, serbuk buah dan daun *P. nigrum* pada dosis 4–5 g terbukti menjadi agen bioinsektisida nabati paling efektif terhadap *S. oryzae*.

Kata Kunci: bioinsektisida, penyimpanan pangan, aktivitas insektisida, *Piper nigrum*, *Sitophilus oryzae*

INTRODUCTION

Rice, as a staple food, is highly vulnerable to insect pest infestations during storage in granaries or warehouses. Grains infested with devastating *Sitophilus oryzae*, the main internal-feeding insect pest, increased fresh weight loss (Padin et al., 2002). The harmful weevils destroy the seed vitality, are severely toxic to non-target organisms, and threaten the quality and safety of rice storage. Infested rice may experience altered the glycemic indices, posing the health risk due to imbalanced and

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unhygienic nutrition if consumed by humans (Kumar et al., 2020). Rice weevils contribute to 9.36 million tonnes of losses, accounting for nearly 20 percent of the total rice yield in Indonesia (Phillips & Throne, 2010).

To mitigate these losses, chemical insecticides are commonly applied. However, their extensive use results in significant risks to industry workers, consumers, and the environment (Hayat et al., 2019). In Indonesia, pesticide usages ranges widely, from 1000 to 4000 tonnes of active ingredients annually (FAO, 2019). Although synthetic insecticide effectively eliminates pests, its overuse can trigger pest resurgence, highlighting the urgent need for safe mode and long-term control alternatives in integrated pest management strategies (Thorburn, 2015).

In this context, botanical pesticides have been expected as promising alternatives, offering benefits such as restoring soil biodiversity, increasing crop yields and food security and decreasing pest resistance to reduce destructive effects caused by toxic chemical compounds from synthetic pesticides (Campos et al., 2019). To date, numerous studies have demonstrated that plant-based extracts possessed strong insecticidal activity against *S. oryzae* (Jayakumar et al., 2017; Kłys et al., 2017; Amini et al., 2019), as well as other rice pests (Xu et al., 2015), and diverse insect species (Nguemtchouin et al., 2010; Pavela, 2011; Singha and Chandra, 2011; Ribeiro et al., 2015). Biological active compounds from plants exhibit feeding deterrents to protect grain storage from insect infestation (Kedia et al., 2015; Pungitore et al., 2005) and reduce grain damage (Paz et al., 2018). For example, leaf extracts of *Cleistanthus collinus* had high mortality and repellent index against rice weevils (Guru-Pirasanna-Pandi et al., 2018).

Among botanical candidates, the genus *Piper* has shown notable insecticidal activity. Studies have highlighted the potential of nine *Piper* species, including *P. nigrum*, which contained essential oil of α -pinene with insecticidal properties (Purnomo & Asmarayani, 2005; Zoubiri & Baaliouamer, 2014). Other studies have reported seed extraction of *P. guineense* to against *S. zeamais* (Akinbuluma et al., 2017) and toxicity of *P. longum* to disease insect vectors (Dey et al., 2020). Despite its widespread acknowledgment, the insecticidal potency of *P. nigrum* particularly of its different plant parts remains underexplored. Understanding its efficacy could provide an ecofriendly, direct application, and low-cost alternative methods in stored-product protection.

The present study was designed to assess the effects of different powdered stems, leaves, and fruits of *P. nigrum* at different doses on the mortality and repellency of *Sitophilus oryzae* in stored rice. The underlying goal of our study is to derive a new dosage of an alternative bioinsecticide from utilized piper, providing a safer and sustainable alternative to synthetic chemicals. This study aimed to evaluate the mortality and repellency effects of powdered plant parts of *P. nigrum* at different doses against *S. oryzae* in stored rice.

RESEARCH METHODS

The experiment was carried out in Laboratorium of Botany and Zoology, Faculty of Science and Technology, Universitas Islam Negeri (UIN) Alauddin Makassar. A completely randomized design with two independent factors was used in this study. The variables were (1) powder sources (stems, leaves, fruits) of *P. nigrum* and (2) its concentrations (2g, 3g, 4g, and 5g). There were 12 treatment combinations with 3 replications with a total of 36 experimental units. The dependent variables were mortality and repellency in percentage.

1. Propagation of rice weevil (*Sitophilus oryzae*)

In total, 150 adult rice weevils were collected from granaries. All weevils represent similar morphology in size. The samples were propagated for a month in 3L of rice then reared to produce ± 900 weevils.

2. Collection and preparation of plant powders from different sources of *Piper nigrum*

The plant materials of *P. nigrum* were collected from Tambangan Village, Kajang District, Bulukumba Regency, South Sulawesi – Indonesia. Samples of leaves were determined by 3 – 5 young leaves from apical shoots, stems were 30 cm in length from apical twigs, then fruits were dark green to reddish with hard texture. All collected samples were assured phytopathogens-free symptoms and getting rid of herbivore bites. Each sample of 1 kg were dried at room temperature and macerated into a small piece then grounded to produce a powdery texture. Each powder was wrapped in sterile filter paper.

3. Adult weevil mortality and repellency bioassay

Total of 20 rice weevils were acclimated in storage with 500g pest-free rice for two days without treatment. Method of dual-choice bio-assay apparatus was used in this study (Kłys *et al.*, 2017). Experimental container was designed according to using 2 jars with a diameter of 46 cm and a height of 15 cm that connected by a 30 cm tube. Each jar contained 500g of rice, covered with sterile filter paper to prevent escaping of weevils, and then kept in a store room. One jar (A) was a treatment group excluding for another jar (B) and tube was installed to connect between two jars. Observation was carried out at 14 days storage period and parameters were recorded at 3, 6, 9, and 12 days after treatment (DAT). The number mortality (Equation 1) and repellency (Equation 2) were counted by these formulas:

$$\text{Mortality} = \frac{\text{Total of dead weevils}}{\text{Total of weevils}} \times 100\% \quad (1)$$

$$\text{Repellency} = \frac{\text{Total of weevils moving from jar A to jar B}}{\text{Total of weevils}} \times 100\% \quad (2)$$

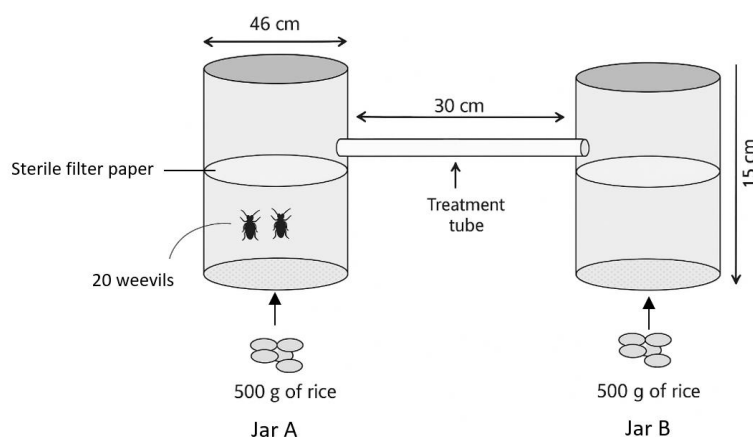


Figure 1. The bioassay apparatus for the observation of weevil mortality and repellency

The data was analysed using ANOVA test within statistical package SPSS Ver.25. The data were transformed to arcsin scale before analysis. Transformation was used to homogenise variances and standardise mean values. The comparison of means was tested using Tukey HSD at $p > 0.05$.

RESULTS AND DISCUSSION

1. Effect of *P. nigrum* on the mortality of *S. oryzae*

Mortality of *S. oryzae* was significantly affected by powdered plant parts of *P. nigrum*, concentrations, and interaction factor (Table 1). The powdered fruits represent the highest mortality, followed by leaves, whereas stems showed the lowest mortality. The concentration of 5g produced the highest mortality among the concentration levels.

Table 1. Effect of *P. nigrum* powders and its concentration on mortality and repellency of *S. oryzae*

Treatment	Mortality (%)	Repellency (%)
Powdered plant parts of <i>P. nigrum</i>:		
Stems	13.3 (20.48 ^a)	40.0 (38.20 ^a)
Leaves	57.1 (53.08 ^b)	48.8 (44.22 ^b)
Fruits	91.7 (80.42 ^c)	50.4 (45.26 ^b)
Concentrations:		
2 gram	31.1 (31.91 ^a)	42.2 (40.46 ^{ns})
3 gram	44.4 (45.38 ^b)	44.4 (41.76 ^{ns})
4 gram	66.1 (59.52 ^c)	48.9 (44.35 ^{ns})
5 gram	74.4 (68.50 ^d)	50.0 (45.00 ^{ns})
Source: Mean Square		
Powdered plant parts of <i>P. nigrum</i>	10805.88*	125.89 *
Concentrations	2322.85 *	41.39 ns
Powdered plant parts of <i>P. nigrum</i> *concentration	758.56 *	70.56 *

Values in parentheses show arcsin transformation data; The numbers followed by the same letter in the same columns are not significant based on Tukey HSD test at $p > 0.05$. (*) indicates significant level at $p > 0.05$, (ns) = not significant.

Table 2. Effect of *P. nigrum* powders on the mortality of *S. oryzae*

Powder (concentration)	Mortality (%)			
	3 DAT	6 DAT	9 DAT	12 DAT
Stems (2 gram)	1.0 (0.00 ^{ns})	1.0 (0.00 ^{ns})	1.5 (4.31 ^{ns})	2.7 (14.76 ^{ns})
Stems (3 gram)	1.0 (0.00 ^{ns})	1.0 (0.00 ^{ns})	2.3 (10.45 ^{ns})	2.7 (14.76 ^{ns})
Stems (4 gram)	1.0 (0.00 ^{ns})	1.0 (0.00 ^{ns})	3.0 (16.59 ^{ns})	3.3 (18.05 ^{ns})
Stems (5 gram)	1.0 (0.00 ^{ns})	1.5 (4.31 ^{ns})	3.6 (19.88 ^{ns})	4.3 (24.81 ^{ns})
Leaves (2 gram)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	6.7 (14.76 ^a)	11.7 (19.5 ^a)
Leaves (3 gram)	0.0 (0.00 ^a)	1.7 (4.31 ^a)	8.3 (16.21 ^a)	15.0 (22.60 ^a)
Leaves (4 gram)	0.0 (0.00 ^a)	20.0 (22.14 ^{ab})	45.0 (42.12 ^b)	65.0 (53.87 ^b)
Leaves (5 gram)	6.7 (14.76 ^b)	30.0 (33.00 ^b)	70.0 (57.24 ^c)	88.3 (73.55 ^c)
Fruits (2 gram)	1.0 (0.00 ^{ns})	11.7 (19.31 ^a)	28.33 (32.09 ^a)	43.3 (41.10 ^a)
Fruits (3 gram)	1.0 (0.00 ^{ns})	23.3 (28.27 ^{ab})	48.3 (44.04 ^a)	78.3 (62.79 ^b)
Fruits (4 gram)	1.0 (0.00 ^{ns})	23.3 (28.27 ^{ab})	56.7 (48.44 ^a)	100.0 (85.69 ^c)
Fruits (5 gram)	3.3 (8.62 ^{ns})	46.7 (42.99 ^b)	98.3 (85.69 ^b)	98.3 (90.00 ^c)

DAT – Days After Treatment; Values in parentheses show arcsin transformation data; The numbers followed by the same letter in the same columns are not significant based on Tukey HSD test at $p > 0.05$. (*) indicates significant level at $p > 0.05$, (ns) = not significant.

After 6 d after exposure, powdered fruits at 5g demonstrated higher mortality than lower doses, although mortality did not exceed 50% (Table 2). Mortality increased markedly with exposure time. At 9-d exposure, the same treatment exceeded 80% mortality. By 12-d exposure, 5g of powdered fruits caused the high mortality that was significantly similar to that at 4g, which was 98% and 100% respectively (Table 2 and Figure 2). At 14-d exposure, powdered fruits (3g, 4g, and 5g) significantly caused lethal of adult *S. oryzae* exceeding 98.3% (Figure 2).

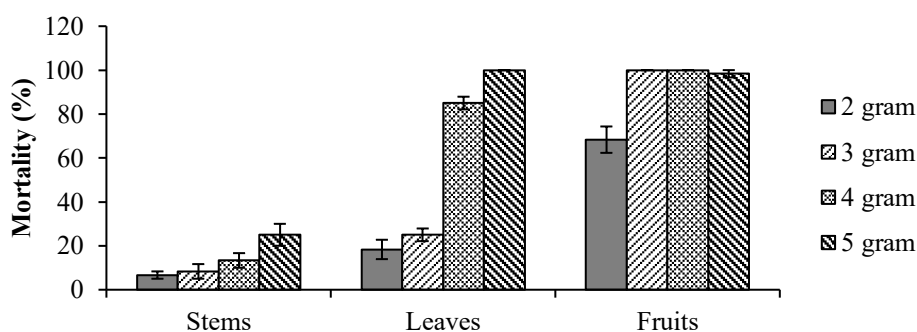


Figure 2. Effect of *P. nigrum* powders on the mortality of *S. oryzae* at 14 DAT (Days After Treatment). Values represent Mean $\pm$ Standard Error (SE) at $p > 0.05$.

A 5g dose of powdered leaves showed a significant higher mortality than lower concentrations at 6, 9, and 12-d after treatment. Mortality reached 88% at 12 d and 100% at 14 d after exposure (Table 2 and Figure 2). At the final observation (14 d), powdered leaves at 5 g caused the highest mortality (100%) (Figure 2). It is likely showed that increased mortality was correlated with the increased concentration. In contrast, the lowest mortality was showed by powdered stems that did not different statistically across all concentrations and exposure periods (Figure 2).

The data of our study suggest the highest dose of powdered fruit and leaves as biopesticide which exceeded 95% lethal effect. The present results revealed that all plant powders tested were highly toxic to *S. oryzae* varied with the plant sources and periods. The present study has shown that the efficacy of powdered fruits and leaves of *P. nigrum* with increased concentrations has high mortality on *S. oryzae*. This level of effectiveness was affected by the doses and periods of exposure. This divergence in effectiveness could be due to the fact that the bioactive contents of plant sources have been related to the amount of concentrated secondary metabolites released that interferes with the physiological death of insect pest.

These findings are consistent with Sahoo et al. (2018) who reported the high lethal effects of piper fruit after two weeks of exposure. In accordance with Kim et al. (2003), the first lethal effect which is $>20\%$ was in agreement with applying the highest dose of powdered fruits. Moreover, plant powders of *P. nigrum* in our study showed highly effective to mortality weevils than other plant used in Mehta & Kumar (2020) excluding *Ageratum conyzoides* tested to *S. oryzae*. To compare with insecticide of pyrethroids, the weevil mortality was notably higher and faster in our study than Athanassiou et al. (2004) that showed 89% after one month exposure. The recent studies on zeolites reported by Eroglu et al. (2019) showed 87% mortality after 21-d after treatment in which is lower and slower lethal than our study. The insecticidal powdered-plants has been studied recently by Ileke et al. (2020) who exposed powders of plant leaves on *S. zeamais* to prevent oviposition, adult emergence, grain damage and loss of stored rice weight. Although some plant powders, such as *Acanthus montanus* and *Argyreia nervosa*, caused 100% mortality within four days, the differences may attributed to variation in plant species and target insect pests (Ileke et al., 2020). Nevertheless, the present results indicate that *P. nigrum* powders can provide effective and economically viable protection of stored rice within a relatively short period at 9-d and 12-d application.

The strong insecticidal activity of powdered fruits and leaves could be linked to the high content of bioactive compounds. Piperine, which constitute up to 63.9% of *P. nigrum* fruits (Kapoor et al., 2009), is known to possess potent insecticidal properties.

The potential of the monoterpenes as a major constituent has been reported to act as toxicants on *S. oryzae* in stored-product (Saad & Abdelgaleil, 2018). Alkaloids such as pellitorine, guineensine, piperidine, dan retrofractamide also contribute to mortality by disrupting insect nervous systems (Park et al., 2002). Secondary metabolites from this plant has been reported increased mortality and repellency on other insects such as *Musca domestica*, *Anopheles stephensi* and *Culex quinquefasciatus* (Haselton et al., 2015; Kamaraj et al., 2011; Khan, 2020). The bioactive compounds consisted in *P. nigrum*, particularly monoterpenes, have been shown to inhibit enzymes of acetylcholinesterase (AChE) and adenosine triphosphatases (ATPases), leading to impaired neurotransmission (Saad et al., 2018). Inhibition of AChE restricts neuromodulator octopamine (Maazoun et al., 2017; Trivedi et al., 2018) and blocks protein receptor of GABA to the active site confined to neurotransmission (Priestley et al., 2003), resulting in cell destruction and mortality.

2. Repellent activity of *P. nigrum* powders on *S. oryzae*

Repellency of *S. oryzae* was significantly affected by powdered plant parts and the interaction (Table 1). Among the tested materials, powdered leaves and fruits of *P. nigrum* showed the strongest repellent activity.

Table 3. Effect of *P. nigrum* powders on the repellency of *S. oryzae*

Powder (concentration)	Repellency (%)			
	3 DAT	6 DAT	9 DAT	12 DAT
Stems (2 gram)	3.3 (6.14 ^{ns})	18.3 (11.90 ^{ns})	26.7 (24.05 ^{ns})	33.3 (29.93 ^{ns})
Stems (3 gram)	3.3 (6.14 ^{ns})	6.7 (19.88 ^{ns})	20.0 (26.07 ^{ns})	33.3 (35.22 ^{ns})
Stems (4 gram)	3.3 (6.14 ^{ns})	11.7 (20.76 ^{ns})	16.7 (27.59 ^{ns})	25.0 (35.25 ^{ns})
Stems (5 gram)	3.3 (6.14 ^{ns})	13.3 (25.31 ^{ns})	21.7 (31.07 ^{ns})	38.3 (38.08 ^{ns})
Leaves (2 gram)	0.0 (0.00 ^a)	10.0 (15.00 ^{ns})	21.7 (25.31 ^a)	26.7 (29.80 ^a)
Leaves (3 gram)	0.0 (0.00 ^a)	10.0 (18.05 ^{ns})	18.3 (27.59 ^a)	25.0 (30.94 ^a)
Leaves (4 gram)	8.3 (16.59 ^b)	20.0 (26.14 ^{ns})	36.7 (37.26 ^b)	56.7 (48.84 ^b)
Leaves (5 gram)	11.7 (19.88 ^b)	20.0 (26.45 ^{ns})	43.3 (41.16 ^b)	58.8 (49.83 ^b)
Fruits (2 gram)	0.0 (0.00 ^{ns})	15.0 (21.90 ^{ns})	31.7 (34.15 ^{ns})	50.0 (44.04 ^{ns})
Fruits (3 gram)	3.3 (6.14 ^{ns})	21.7 (27.71 ^{ns})	41.7 (39.21 ^{ns})	50.0 (44.04 ^{ns})
Fruits (4 gram)	3.3 (6.14 ^{ns})	21.7 (27.71 ^{ns})	40.0 (40.18 ^{ns})	48.3 (44.99 ^{ns})
Fruits (5 gram)	10.0 (18.43 ^{ns})	36.7 (37.12 ^{ns})	48.3 (44.04 ^{ns})	48.3 (45.00 ^{ns})

DAT – Days After Treatment; Values in parentheses show arcsin transformation data; The numbers followed by the same letter in the same columns are not significant based on Tukey HSD test at $p > 0.05$. (*) indicates significant level at $p > 0.05$, (ns) = not significant.

At 9-d and 12-d exposure, the highest significant repellency values (56.7% and 58.8%) were recorded for powdered leaves at 4g and 5g, respectively (Table 3). In contrast, powdered fruits and stems did not show significant differences among concentrations. However, powdered fruits showed relatively high repellency of 50% and 48.3% at 12-d after exposure (Table 2). Subsequently, powdered fruits produced higher mean repellency at earlier exposure periods (6-d and 9-d), whereas powdered leaves showed stronger repellency at later periods. At 14-d exposure, the highest repellency was represented for powdered leaves at 4g and 5g (Figure 3).

The present results demonstrate that powdered leaves are highly effective against *S. oryzae*, producing moderate to high repellency. The findings is similar with Sahoo et al. (2018) who also reported high repellent effects after two weeks of exposure. Compared with Ileke et al., (2020), the repellent effect would probably exceed than that presented in these recorded periods. Entire control of rice beetles might be more effective in long exposure and treatment, suggested by Kavallieratos et al. (2015).

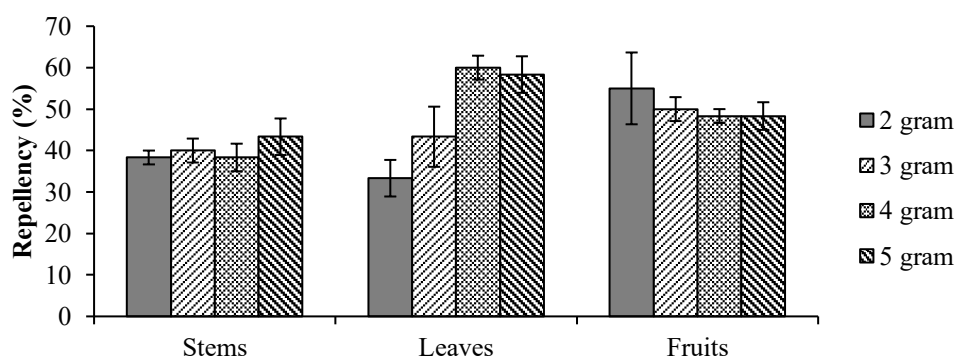


Figure 3. Effect of *P. nigrum* powder on the repellency of *S. oryzae* at 14 DAT (Days After Treatment). Values represent Mean $\pm$ Standard Error (SE) at $p > 0.05$.

Regarding the repellent effect, essential oil of caryophyllene oxide (6%) and spathulenol (6.22%) excreted from leaves might have been primarily repelled the rice weevils (Xiang et al., 2017). In addition, the fruits contain various monoterpenes of α -pinene (30%), caryophyllene (30%), limonene (10%), and ϵ -nerolidol (6%) (Parreira et al., 2019) as natural repellent (Nerio et al., 2010). Moreover, repellent effect from fruits of *P. nigrum* is due to constituents of higher sesquiterpene (90%) and total of essential secondary metabolites (95.3%) than components in their stems and leaves. Limonene contained in powdered fruits of *P. nigrum* has been reported to suppress population of *S. oryzae* caused by high feeding-deterrence (Tripathi et al., 2003) and claimed to toxic action in AChE activity of *S. oryzae* (Abdelgaleil et al., 2009). Plata-rueda et al. (2018), using essential oils of cinnamon and clove, have shown that terpenoid constituents of black piper in this study (caryophyllene oxide, α -pinene, α -humulene) supported by Xiang et al. (2017) reduced respiratory rates and avoided exposure of terpenoids. The derivatives of monoterpenes and sesquiterpenes have been claimed to enhance repellent activity to insects and exhibited neurotoxicity (García et al., 2005). Therefore, powdered fruits and leaves of *P. nigrum* could be an alternative strategy that environmental friendly to reduce use of highly damage chemical pesticides which induces high resistance on *S. oryzae* (Attia et al., 2020).

Overall, the present data provide strong evidence that powdered fruits and leaves of *P. nigrum* are effective botanical insecticides against *S. oryzae*. The lethal and repellent effects, supported by statistically significant differences among treatments suggest that these plant powders could serve as environmentally alternatives to synthetic pesticides. This use may help reduce chemical pesticides and mitigate resistance development in stored-product insects.

CONCLUSION

Powdered fruits of *P. nigrum* at 4-5 g and powdered leaves at 5 g displayed a significant high mortality against *S. oryzae*. For repellency, the use of 4-5 g of leaf powders showed the best results. Bioinsecticides derived from *P. nigrum* leaves and fruits could be definitely applied by resource farmers considering the low-cost, accessible, and ecofriendly to cope extremely high risk of chemical insecticides. Future research should therefore focus on field validation trials, optimization of dose combinations, and evaluation of long-term exposure.

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