Heliyon 8 (2022) e12589

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Insecticidal activities of long pepper (*Piper retrofractum* Vahl) fruit extracts against seed beetles (*Callosobruchus maculatus* Fabricius, *Callosobruchus chinensis* Linnaeus, and *Sitophilus zeamais* Motschulsky) and their effects on seed germination

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ARTICLE INFO

Keywords:
Long pepper
Bruchid beetles
Maize weevil
Film seeds coating method
Hexane extract
Botanical insecticide

ABSTRACT

Bruchid beetles (*Callosobruchus maculatus* and *Callosobruchus chinensis*), and maize weevil (*Sitophilus zeamais*) are important insect pests during the postharvest period. Botanical insecticide is an alternative solution for controlling these insects, and long pepper (*Piper retrofractum*) has been reported as having insecticidal potential against general insect pests. Film seed coatings with various concentrations of hexane extracts were made for mung bean (*Vigna radiata*) and corn (*Zea mays*) seeds. Insecticidal activities of these treatments were assessed at before and after storage period of six months, and seed germination was also evaluated. The hexane extract was subjected to analysis of the bioactive components by using Gas Chromatography-Mass Spectrometry (GC-MS). Results revealed that the hexane extract presented extreme toxicity to both bruchid beetles higher compared to maize weevil at 24 h with LC_{50} values of 5.57–6.75 and 58.04 μg·cm^{-2}, respectively. Bruchid beetles presented significant response to ethanol, acetone and hexane extracts, whereas maize weevil showed relatively low responsibility. Film seed coating with hexane extract at 1% and 3% concentrations with six-month storage presented high insecticidal activity against bruchid beetles by more than 88% mortality but had low kill rates against maize weevil. The coated mung bean seeds presented non-seed germination effect, whereas high effect was observed on coated corn. Isolation of bioactive components demonstrated that there were 74 compounds, where pentadecane was the main compound. Film seed coating technology for mung bean seed preservation by using 1% hexane extract from long pepper fruit presented to be an extremely effective method to control bruchid beetles without any seed germination effect. It could serve as one of the green insecticides of the future.

1. Introduction

The importance of seed storage is to secure the supply of good quality seeds for planting in future seasons. Farmers need to maintain viable seeds from one growing season to the next. Seed quality has an important potential for increasing productivity and marketing, and also can result in up to a 30% increase in crop yields (Afzal et al., 2016). Stored seeds are vulnerable to pest attacks because of their prolonged period of storage. Stored product insect pests damage to the seed germ and internally feeding, effect on germination (Barbercheck, 2020; Mehta et al., 2021). These insect pests can cause loss of grains to dry powder and hulls (Barbercheck, 2020). *Sitophilus zeamais* is one of the most critical, internal feeding pests of maize and one of the most important stored product pests, infesting grains both in the field, before harvesting, and in storage (Santana et al., 2022). López-Castillo et al. (2018) reported that weevils cause food-grain losses during storage up to 40% of total production, especially in developing countries. Adult female weevils cause damage by penetrating into kernels. The larvae and pupae eat the inner parts of the kernel, resulting in a damaged kernel with reduced grain weight (Ojo and Omoloye, 2012), and reducing germination that affects

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https://doi.org/10.1016/j.heliyon.2022.e12589
Received 4 July 2022; Received in revised form 26 October 2022; Accepted 15 December 2022

the seed physiological quality (Caneppele et al., 2003). In addition, bruchid beetles *Callosobruchus maculatus* and *Callosobruchus chinensis* are the most dangerous insect pests of *Vigna* genus crops including mung bean (*V. radiata*), black gram (*V. mungo*), azuki bean (*V. angularis*) and cowpea (*V. unguiculata*), which are considered as a major agricultural and economical crops in Asia and Africa (Srinives et al., 2007). They penetrate into the fully matured pods, grains in fields, and also during post-harvest storage (Kalpna and Kumar, 2022). Adult beetles mate and oviposit on seed plants but did not eat the seeds. The larva bores into the seed, feeding on its contents until the whole endosperm is eaten up (Ahmed et al., 2018). The insect population occurs in storage and seed damage can be raised by double within six months (Seram et al., 2016), damage to the extent up to 90% (Ahmed et al., 2018). Infestation of this beetle resulted in germination effect of chickpea lost to 100% damage, which is less suitable for consumption (Ahmed et al., 2018). Therefore, those seed insect pests cause substantial quantitative and qualitative losses due to seed perforation and reductions in weight, seed quality, and germination ability of seeds (Kalpna and Kumar, 2022).

Seed treatment is an application of physical agents including chemical, biological, or botanical pesticides to the seed prior to sowing in order to control pests that attack seeds, seedlings, or plants. The application of seed treatment can range from a basic dressing to coating and pelleting (Sharma et al., 2015), a routine practice that helps control initial field pests (De Moraes Dan et al., 2012). The technology for coatings produced included dry coating, seed dressing, film coating, entrustments, and seed pelleting (Afzal et al., 2020). Currently, chemical seed treatment is a common worldwide practice due to its wide spectrum ability to control pests, consume less time and labor, and demonstrate ease of use (Sharma et al., 2015). TNAU Agritech Portal (2016) recommended insecticides such as imidacloprid, chlorpyriphos, endosulfan, and carbosulfan to control insect pests for seed treatment of different crops. Vojvodić and Bažok (2021) indicated good efficacy of active ingredients belonging to the group of anthranilic diamides, cyantraniliprole, and chlorantraniliprole, in the treatment of maize, soybean, sugar beet, and rice seeds on pests. In Thailand, the Department of Agriculture recommended pirimiphos-methyl, chlorpyrifos, fenitrothion, permethrin, and fipronil insecticides for seed treatment of corn, mung bean, and soybean seeds (EZAT, 2010). However, the problems due to insecticide application include insecticide resistance in stored grains, as well as toxic residues that are dangerous to humans, livestock, and the environment (Campos et al., 2019; Kolupaeva et al., 2019). For this reason, the European Union has permanently banned the most used active ingredients for seed treatment, particularly imidacloprid, thiamethoxam, and clothianidin (Vojvodić and Bažok, 2021). Another factor against the use of insecticides for seed treatment is the effects on physiological quality of seeds. De Moraes Dan et al. (2012) reported that treatment with some insecticides affected the germination and vigor of seeds. Green insecticides, especially medicinal plant extract, are of alternative interest for seed treatment to control stored product insect pests. The application of film seed coating technology with plant extracts in coating substances was a promising way for insect protection to replace the use of chemical insecticides (Pumnuan et al., 2021). The research of Yaman and Şimşek (2021) showed that *Hypericum* species extracts had insecticidal potential against various important stored grain insect pests. Gariba et al. (2021) presented that the phytochemical in plant extracts may affect progeny emergence that causes inhibitory effect, repellent action, and antifeedant effect in insect pests for grains. In addition, Bush mint (*Hyptis suaveolens*) extracts have the potential to enhance quality seed production, thereby boosting growth of maize (Gariba et al., 2021).

Long pepper, or *Piper retrofractum* Vahl, is a medicinal herb with mass cultivation in the central part of Thailand, especially the Kanchanaburi, Ratchaburi, Phetchaburi, and Chanthaburi provinces. It has been recognized that the phytochemical content in plants is one factor responsible for pharmacology activities (Wardani and Leliqia, 2021). Long pepper were the common ingredients in various recipes of traditional medicine and Thai foods, whereas no report of toxicity effects to Human (Panphut et al., 2020). The main chemical constituents identified from long pepper are alkaloids, saponins, tannins, flavonoids, steroids, triterpenoid, and glycosides (Salleh and Ahmad, 2020; Wardani and Leliqia, 2021). These compounds are major constituents of well-known substances including botanical insecticides, deterrents, and repellents that may be useful for controlling a wide range of pests (Isman, 2006). Research has been performed regarding the effectiveness of hexane extract from long pepper fruit in controlling different insect pests such as cabbage head caterpillar larvae (Prijono et al., 2020), tobacco cutworm larvae (Ratwatthananon et al., 2020), and southern house mosquito larvae (Wiwattanawanichakun et al., 2018). These reports also showed that by using hexane solvent, the long pepper fruit extract presented higher effectiveness against insects than other solvents such as dichloromethane, ethyl acetate, methanol, and ethanol. Thus, hexane extract of long pepper fruit is recommended to be developed as an effective botanical insecticide against some stored product insect pests.

The study on the effective control of grain cereal seed insect pests, apart from the potential to kill insects, has considered the effort to maintain unaltered quality of the stored seed lots in terms of physical integrity, longevity, seed germination, and potential productivity (Halmer, 2004; Dumitriu et al., 2020). Nevertheless, seed treatment with some dressing products could result in phytotoxic effect, causing reduction in germination and seedling survival (De Moraes Dan et al., 2012). Corn seeds after treatment with deltametrine and pirimiphos-methyl insecticides had lowered longevity, vigor, and emerging speed of seedlings (Fessel et al., 2003). Additionally, soybean seeds after treatment with imidacloprid/thiodicarb, acephate, and carbofuran insecticides affected the germination and vigor of seeds. On the other hand, treating seeds with some insecticides has resulted in increasing productivity or greater percentage on seed germination (De Moraes Dan et al., 2012). Therefore, with regard to their insecticidal activity and phytotoxic effects on these seeds, mention should be made of the importance of alternative technologies for controlling pests of stored products by means of plant extract film seed coating.

This research aimed to evaluate the effectiveness of crude extracts from long pepper (*P. retrofractum*) fruit against seed beetles (*C. maculatus*, *C. chinensis*, and *S. zeamais*) by the film seed coating method for corn and mung bean seeds, as well as their effect on seed germination after coating.

2. Materials and methods

2.1. Plant material and preparation of plant extract

The fresh fruit of long pepper, *P. retrofractum,* was collected from a plantation without pesticides located in Kanchanaburi province, Thailand. Plant materials were cut into small pieces and dried in a hot air oven at 45 °C for three days. The dried long pepper fruits were separately ground into powders (1 kg) and extracted by maceration with 4 L of three different solvents, hexane, acetone, and ethanol, according to increasing polarity. The plant extracts were extracted by the maceration method with solvent extraction modified as followed by Saenmanot et al. (2018). The maceration occurred at room temperature (30 ± 5 °C) for three days, agitated twice per day for comprehensive compatibility. The first extraction was performed using hexane as a solvent, after filtering through a Buchner funnel and Whatman No. 1 filter paper. Next, the extracts were concentrated under low pressure using a rotary evaporator at 40 °C to obtain the hexane crude extract that resulted in volume equal to 24.2 g. In addition, the remains from hexane extraction were then immersed with acetone and ethanol, respectively, by the same continued immersion method to obtain acetone and ethanol crude extracts, respectively. The extract volume of acetone and ethanol crude extracts were equal to 35.7 g and 30.0 g, respectively. This crude extract was stored at 4 °C in a refrigerator for future experiments, including insecticidal activity test, seed germination evaluation after coating, and isolation of bioactive components.

2.2. Insect rearing

Three species of seed beetles are as follows: Bruchid beetles (*C. maculatus* and *C. chinensis*) and maize weevil (*S. zeamais*) were collected from Hua-Ta-khe Old Market, Bangkok, Thailand. These seed beetle pests were screened and cultured according to the method adapted from Pumnuan et al. (2021) at room temperature (30 ± 5 °C). Bruchid beetles were fed mung bean (*Vigna radiata*), while maize weevil were fed sweet corn seed (*Zea mays*). Adults of each beetle species were added to each square plastic box cultured with their food seeds. Then, 15−day−old adults from the second generation were used for the tests.

2.3. Insecticidal activity test

2.3.1. Paper residue contact method

2.3.1.1. Toxicity test. In the first assay, the toxicity test of different extracts was examined, and the extract that presented with the high insecticidal property against seed beetles was selected for further experiments. Efficacy of crude extract from long pepper fruit using hexane, acetone, and ethanol as solvents against three species of seed beetles, *C. maculatus*, *C. chinensis*, and *S. zeamais*, were evaluated by paper residue contact method (Pumnuan et al., 2020). The 20% stock solution of various extracts was prepared from 20 g of each crude extract and mixed with 20 g of Tween-20, a homogeneous mixture, and distilled water was gradually added while stirring constantly until a total solution of 100 ml was obtained. Preparation of the working solution was diluted with the distilled water. The preliminary study found that bruchid beetles (*C. maculatus* and *C. chinensis*) were more susceptible to extracts than maize weevil (*S. zeamais*), achieving complete mortality at 0.1 and 1.0% concentration of some extract for bruchid beetles and maize weevil, respectively. The various concentrations (0.02, 0.04, 0.06, 0.08, and 0.10%) of the working solution were used to proceed with a toxicity test on bruchid beetles, while 0.2, 0.4, 0.6, 0.8, and 1.0% were used for maize weevil, compared with the control groups (0.1 and 1.0% Tween-20 in water). About 1 ml of the working solution was dropped onto each Whatman No. 1 filter paper and put in a 9 cm diameter glass Petri dish. It was air dried until damp for 1 min and then 20 insect adults were released on the treated filter paper and the Petri dish was covered. The working solution at various concentrations as 0.02–0.10% and 0.2–1.0% were calculated as concentration per area equaling to 3.15–15.75 and 31.5–157.5 μg·cm^{-2}. Mortality rates were observed at 24 h and the LC_{50} and LC_{90} values (lethal concentration of extract required to kill 50% and 90% of insects, respectively) were calculated via probit analysis, and the regression equipment as reported by Pumnuan et al. (2021). This first results showed that hexane extract from long pepper fruit presented the highest insecticidal activity, were selected for repellency and attraction test, and effect on seed germination in further experiments.

2.3.1.2. Repellency and attraction test. The response of the three species of seed beetles to hexane extract obtained from long pepper fruit was evaluated for repellency and attraction activities using a similar toxicity test modified from the experimental method according to Doungnapa et al. (2021). The paper filter were cut in half and equally divided, and the first half was dipped in the hexane extract solution, while the other half was dipped in the control solution (0.1% Tween-20 in water). In this study, the concentrations of hexane extract solution used were an estimate of the range 1/10 times the LC_{50} and LC_{90} values. Hereby, the concentration at 0.315, 0.945, and 1.575 μg·cm^{-2} were used for response test on bruchid beetles, while the concentration at 3.15, 9.45, and 15.75 μg·cm^{-2} were used to test on maize weevil. Both halves were removed for air-drying at room temperature until damp for 1 min. Twenty adult insects were transferred, and the percentages of repellency and attractive response were determined after 24 h.

2.3.2. Film seed coating method

The mung bean and corn seeds coated with hexane extracts were prepared by the modified experimental method according to Pumnuan et al. (2021). The hexane extract was mixed with a coating agent in the same ratio, and dissolved in distilled water until it reached concentration at 1%, 3%, and 5%. Each coating solvent with 300 and 150 ml for 1 kg of mung bean and corn seeds, respectively, was employed for pellet processing by using the rotary-coater (Center Oceania, Australia). The positive control, fipronil insecticide at recommendation and double rates (0.1% and 0.2%, respectively) were also mixed with this coating agent for comparing the activities, and compared with the negative control (3% coating agent in water). After coated for 24 h, those coated seeds (10 g) were placed in a 55 ml glass bottle. Twenty adults of each insect were placed in each glass bottle, then, covered with net on the top glass and maintained at room temperature (30 ± 5 °C). Both bruchid beetles and maize weevil were maintained in coated mung bean and corn seeds, respectively. Mortality rate of each insect was assessed at 24 h after treatment. For the next bioassay, all treatments kept under 4 °C were individually evaluated for insecticidal property after periods of two, four, and six months.

2.4. Evaluation of seed qualities

Seed quality evaluation of mung bean and corn seeds coated with hexane extracts from long pepper fruit at 1% and 3% concentrations were conducted before and after the stored periods of two, four, and six months and compared with negative control (3% coating agent in water), positive control (fipronil insecticide group of 0.1% and 1.0%), and the blank group (untreated). The seed qualities were each classified as percentage of moisture content (%MC) and germination percentage in the laboratory (%GL) as well as germination index (GI) of seed were evaluated. The evaluation of seed qualities were performed by following the method adapted from Pumnuan et al. (2021).

A high constant temperature oven-dry method was used to gauge the %MC of the seeds. A 5–6 g of each seed-coated treatment were broken up and dried at 105 °C for 17 h in a hot air oven. %MC is calculated as $[(W1–W)/(W2–W1)] \times 100$ where W is the weight of empty aluminum can, W1 is the weight of the seed-filled aluminum can before drying, and W2 is the weight of the seed-filled aluminum can after drying.

Three replicates of the paper culture were used for the germination test. A 50 seeds were randomly chosen from each treatment, and they were incubated on moist paper and placed inside the plastic container at ambient temperature (25–30 °C). After seven days of incubation, the %GL of seedlings was evaluated. The predicted GI was derived as follows: GI is calculated as $\Sigma(Gt/Dt)$, where Gt is the total number of seeds that germinated on day t and Dt is the amount of time in days that corresponds to Gt.

2.5. Isolation of bioactive components

The hexane crude extract (8.04 g) was subjected to quick column chromatography over silica gel G60 using gradient elution by gradually increasing the polarity (beginning with 0–100% hexane in petroleum ether and then 0–50% dichloromethane in hexane). The eluents were collected in nice fractions (A-I) according to similar TLC pattern at 254 and 365 nm, and fractions with the similar TLC pattern were combined. The purified fractions (PF) were stored and future analyzed for chemical components, and the PF process has been shown as a flowchart (Figure 1). The seven purified fractions (PF1 to 7) of hexane extract from dried long pepper fruit were obtained and subjected to analysis of their chemical components by Gas Chromatography-Mass Spectrometry (GC-MS), following the same method by Pumnuan et al. (2021) as mentioned above. The GC-MS was performed on an Agilent technology (Agilent Technologies Inc., USA) equipped with capillary column HP5MS. Direct injection as split mode was completed. Helium was used as a carrier gas.

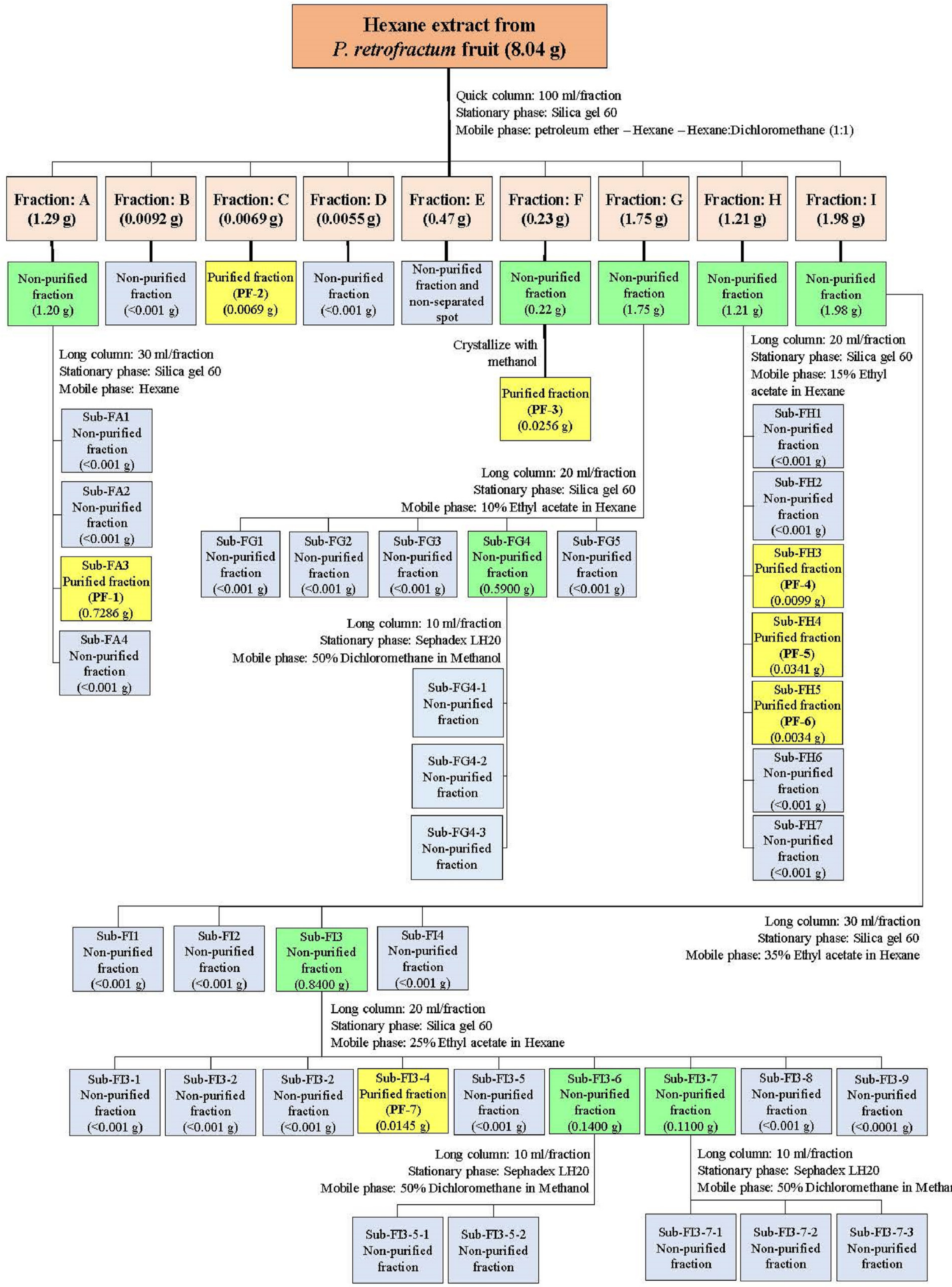

Figure 1. Purification flowchart of hexane extract from the long pepper (*Piper retrofractum* Vahl) fruit containing bioactive compounds.

The oven program began with a 40 °C beginning temperature kept for 3 min, then induced the oven temperature by heating at a rate of 10 °C/min to 280 °C, which was then held isothermally for 20 min. Both the injector and the detector were kept at 250 °C. The identification of purified fraction bioactive compounds was compared with the spectra of standard compounds in the spectral library (Wiley7n).

2.6. Data analysis

The mortality rates according to Abbott's (1987) formula was applied to obtain the actual insect death rate. The experiment of insecticidal activities was designed in five completely randomized replicates; similarly, the experiment of seed qualities was designed in three completely randomized replicates. The data obtained were statistically analyzed by applying analysis of variance (ANOVA), whereas the differences among treatments were tested by Duncan's multiple range test (DMRT). The insecticidal toxicity in forms of LC_{50} and LC_{90} were calculated by the probit method, report according to the method of Pumnuan et al. (2021). As for the percentage response in terms of the repellency and attractive test, frequencies of insects in the selected test were analyzed by the χ^2 test, report according to the method of Doungnapa et al. (2021).

3. Results

The effectiveness of various extracts from long pepper *P. retrofractum* fruit, including ethanol, acetone, and hexane extracts at different concentrations, against bruchid beetles *C. maculatus* and *C. chinensis* and corn weevil *S. zeamais* by contact method. All extracts were several times more effective at killing bruchid beetles than corn weevil-a significant 10-fold increase was observed with the hexane extract. Toxicity level of hexane extract had greatest efficacy against all studied seed beetles, bruchid beetles at 24 h post-treatment with LC_{50} and LC_{90} values of 5.57–6.75 and 9.30–11.56 $\mu g \cdot cm^{-2}$, respectively, and for maize weevil of 58.04 and 101.03 $\mu g \cdot cm^{-2}$, respectively. Interesting results appeared when the crude hexane extract presented the higher toxicity to kill all beetles compared to acetone and ethanol extracts. The LC_{50} values of acetone and ethanol extracts at 24 h after treatment were 11.23–15.49 and 21.77–22.47 $\mu g \cdot cm^{-2}$, respectively, for bruchid beetles, as well as 92.59 and 102.14 $\mu g \cdot cm^{-2}$, respectively for maize weevil (Table 1). In this study, the high effective insecticidal property of hexane extract was subjected for further insecticidal activities, including their effect on seed germination.

For bruchid beetles and corn weevil, the insect responses to the hexane extract were approximately 10-fold lower than the LC_{50} concentrations of 0.315–1.575 and 3.15–15.75 $\mu g\ cm^{-2}$ per insect, respectively, indicating that bruchid beetles exhibited high repellent responses to the hexane extract, but in the case of the corn weevil, showed no significant difference in responses compared to the high-concentration control group. Impressively, the hexane extract had potential to kill both bruchid beetles and also expressed effective repellency response. Especially, the extract at 15.75 $\mu g \cdot cm^{-2}$ concentration showed the most repellency response with more than 70% response and significant difference compared with the control group at 12 h post-treatment. However, less response was found at lower concentrations. The *C. chinensis* had higher repellency response to the extract than *C. maculatus*. Additionally, *C. chinensis* expressed more than 70% repellency response to the extract at 0.315 $\mu g \cdot cm^{-2}$ concentration after treatment, while *C. maculatus* expressed more than 70% at 15.75 $\mu g \cdot cm^{-2}$ concentration after treatment. Furthermore, maize weevil showed response to the extract at the high concentration as a non-significant difference compared with the control group. The low concentration of extract presented high repellency response with more than 70% repellency, a significant difference compared with the control group at 6 h after treatment (Table 2).

Film seeds coating with hexane extracts from long pepper demonstrated high effectiveness in controlling bruchid beetles, but low activity with maize weevil. Mung bean coated with 1% extract concentration and stored for six months post-treatment was able to kill *C. maculatus* and *C. chinensis* at more than 90% and 80%, respectively, with non-significant difference compared to the fipronil insecticide group at recommendation rate. In addition, the extracts at 2% and 3% concentrations showed toxicity effect against both bruchid beetles with non-significant difference compared to the fipronil insecticide group at double recommendation rate. For the corn seeds coated with the extract to control maize weevil, 1% and 3% extract concentrations and the fipronil insecticide group at recommendation rate showed low capacity in killing maize weevil after storage period of six months at approximately 50% mortality with non-significant difference. Additionally, 5% extract concentration showed some toxicity effect to maize weevil with non-significant difference compared to the fipronil insecticide group at double recommendation rate (Table 3).

Germinations of mung bean and corn seeds coated with hexane extracts from long pepper indicated that coated mung bean seed showed lower seed germination effect than corn seeds. The mung bean seed coated at 1% and 3% hexane extract concentrations after a six-month period showed %MC, %GL, and GI with no significant difference compared to the fipronil insecticide, control, and blank groups. There was a significant difference compared with a 5% extract concentration. The non-affected group of coated mung bean seeds presented %MC, %GL, and GI of 9.57–9.73%, 91.3–96.8%, and 13.04–13.82, respectively, as well as 9.27%, 88.3%, and 12.61, respectively, for the affected group. In addition, all coated corn seeds got higher effect on seed germination than the blank group with significant difference. Corn seeds coated with 5% hexane extract showed %MC %GL, and GI of 9.36%, 6.0%, and 0.86,

Table 1. Toxicity of different extracts from long pepper, *Piper retrofractum* Vahl fruit at various concentrations against bruchid beetles, *Callosobruchus maculatus* Fabricius and *Callosobruchus chinensis* Linnaeus and maize weevil, *Sitophilus zeamais* Motschulsky by contact method.

Treatments	Toxicity[1/]			SE	χ^2	P
	Regression[2/]	LC_{50} ($\mu g \cdot cm^{-2}$) (range)	LC_{90} ($\mu g \cdot cm^{-2}$) (range)			
C. maculatus						
Ethanol	Y = −2.328 + 0.104x	22.47 (19.38–28.23)	34.84 (28.87–46.36)	0.017	2.002	0.735^{ns}
Acetone	Y = −1.410 + 0.126x	11.23 (8.50–15.89)	21.43 (16.49–37.34)	0.012	18.787	0.001**
Hexane	Y = −1.913 + 0.343x	5.57 (4.03–7.00)	9.30 (7.73–12.39)	0.025	18.056	0.001**
C. chinensis						
Ethanol	Y = −2.188 + 0.100x	21.77 (18.87–27.05)	34.53 (28.34–45.40)	0.016	5.236	0.264^{ns}
Acetone	Y = −2.308 + 0.149x	15.49 (14.36–17.00)	24.09 (21.74–27.55)	0.015	2.746	0.601^{ns}
Hexane	Y = −1.796 + 0.266x	6.75 (5.42–8.00)	11.56 (10.00–14.14)	0.018	11.60	0.021*
S. zeamais						
Ethanol	Y = −1.775 + 0.017x	102.14 (76.73–134.56)	175.89 (141.08–273.25)	0.001	25.038	<0.001**
Acetone	Y = −1.708 + 0.018x	92.59 (69.01–118.38)	162.05 (132.19–234.18)	0.001	22.697	<0.001**
Hexane	Y = −1.731 + 0.030x	58.043 (46.73–68.75)	101.03 (87.84–122.14)	0.002	9.524	0.049*

[1/] Data were based on adult, n = 20 adult insects/replicate of five replications, lethal concentrations of plant extract were needed to kill 50% and 90% of the insects (LC_{50} and LC_{90}, respectively) at 24 h after treatment. [2/] Probit (Y) = Intercept + Slope × (Concentration: x). *, **: Significant difference at P < 0.05 and P < 0.01, respectively, ns: nonsignificant difference. SE: standard error, χ^2: chi-square value.

Table 2. Percentage response of bruchid beetles, *Callosobruchus maculatus* Fabricius and *Callosobruchus chinensis* Linnaeus and maize weevil, *Sitophilus zeamais* Motschulsky to different concentrations of hexane extracts from long pepper, *Piper retrofractum* Vahl fruit by contact method after various time treatments.

Concentrations/Times	*C. maculatus*				*C. chinensis*				*S. zeamais*			
	Response		χ^2	P	Response		χ^2	P	Response		χ^2	P
	%R	%A			%R	%A			%R	%A		
0.315 μg·cm^{-2}												
1 h	56	44	0.723	0.3953^{ns}	54	46	0.321	0.571^{ns}	–	–	–	–
3 h	47	53	0.180	0.671^{ns}	55	45	0.501	0.479^{ns}	–	–	–	–
6 h	56	44	0.723	0.3953^{ns}	63	37	3.438	0.063^{ns}	–	–	–	–
12 h	53	47	0.180	0.671^{ns}	64	36	3.998	0.046*	–	–	–	–
24 h	58	42	1.288	0.2564^{ns}	70	30	8.333	0.004**	–	–	–	–
0.945 μg·cm^{-2}												
1 h	48	52	0.080	0.7773^{ns}	48	52	0.080	0.777^{ns}	–	–	–	–
3 h	49	51	0.020	0.888^{ns}	65	35	4.634	0.032*	–	–	–	–
6 h	53	47	0.180	0.671^{ns}	71	29	9.227	0.002**	–	–	–	–
12 h	55	45	0.501	0.479^{ns}	72	28	10.172	0.001**	–	–	–	–
24 h	56	44	0.723	0.3953^{ns}	70	30	8.333	0.004**	–	–	–	–
1.575 μg·cm^{-2}												
1 h	48	52	0.080	0.7773^{ns}	51	49	0.020	0.888^{ns}	–	–	–	–
3 h	62	38	2.922	0.0874^{ns}	66	34	5.255	0.022*	–	–	–	–
6 h	67	33	5.952	0.015*	71	29	9.227	0.002**	–	–	–	–
12 h	73	27	11.171	<0.001**	77	23	15.727	<0.001**	–	–	–	–
24 h	81	19	21.263	<0.001**	82	18	22.816	<0.001**	–	–	–	–
3.15 μg·cm^{-2}												
1 h	–	–	–	–	–	–	–	–	35	65	4.634	0.032*
3 h	–	–	–	–	–	–	–	–	33	67	5.952	0.015*
6 h	–	–	–	–	–	–	–	–	26	74	12.224	<0.001**
12 h	–	–	–	–	–	–	–	–	27	73	11.171	<0.001**
24 h	–	–	–	–	–	–	–	–	27	73	11.171	<0.001**
9.45 μg·cm^{-2}												
1 h	–	–	–	–	–	–	–	–	46	54	0.321	0.571^{ns}
3 h	–	–	–	–	–	–	–	–	45	55	0.501	0.479^{ns}
6 h	–	–	–	–	–	–	–	–	52	48	0.080	0.777^{ns}
12 h	–	–	–	–	–	–	–	–	45	55	0.501	0.479^{ns}
24 h	–	–	–	–	–	–	–	–	48	52	0.080	0.777^{ns}
15.75 μg·cm^{-2}	–	–	–	–	–	–	–	–				
1 h	–	–	–	–	–	–	–	–	45	55	0.501	0.479^{ns}
3 h	–	–	–	–	–	–	–	–	52	48	0.080	0.777^{ns}
6 h	–	–	–	–	–	–	–	–	49	51	0.020	0.888^{ns}
12 h	–	–	–	–	–	–	–	–	54	46	0.321	0.571^{ns}
24 h	–	–	–	–	–	–	–	–	53	47	0.180	0.671^{ns}
Control	50	50			50	50			50	50		

*, **: Significant difference at $P < 0.05$ and $P < 0.01$, respectively, ns: nonsignificant difference. %R: indicates the percentage response to the treatment (repellency), %A: indicates the percentage response to the control (attraction). SE: standard error, χ^2: chi-square value.

respectively, which was considered as receiving the most effect on seed quality. Interestingly, film corn seed coating with fipronil insecticide presented getting lower quality effect than that of seed coated extracts, whereas coated mung bean seed tended to be without any effect (Table 4).

The 1 kg dried long pepper fruit was prepared to obtain hexane extract equal to 24.2 g (2.42%). This extract (8.04 g) was subjected for solation of bioactive components by quick column chromatography that could be collected in nine fractions. Then, seven purified fractions (PF) were stored for future analysis to chemical component. Isolation of bioactive components analysis by using GC-MS exhibited 74 compounds in all PF. Interestingly, PF1 presented high content at 88.56% of the total PF or 30.11 mg·g^{-1} of hexane extract. Pentadecane was a major compound (7.081 mg·g^{-1} of hexane extract), followed by heptadecane, 1−heptadecene, 8−heptadecene, 1−tetradecanol, 1−tridecene, and tridecane of 5.337, 5.266, 2.867, 2.785, 1.512, and 1.087 mg·g^{-1} of hexane extract, respectively. All major chemical compounds are presented in the PF1. Tridecane, 1−tridecene, pentadecane, 8−heptadecene, 1−heptadecene, and heptadecane showed their chemical structure as hydrocarbon structure, while 1−tetradecanol showed chemical structure as alcohol structures (Table 5).

4. Discussion

The various solvent extracts from long pepper expressed wide range of effectiveness against tested insect species. In this research, the hexane extract had greatest efficacy on bruchid beetles compared to maize weevil, and also presented having more insecticidal activity in controlling all studied seed beetles compared to acetone and ethanol extracts, relatively consistent with the polarity characteristics of the extract solvents. Surprisingly, this extract could kill bruchid beetles approximately 10 times more than maize weevil, which may be influenced by the proportion of insect body size. Insecticidal activities of plant extracts depended on plant material, solvent extraction, insect species. and exposure time (Kim et al., 2003; Thein et al., 2013; Ahmad et al., 2019).

Table 3. Percentage mortality of bruchid beetles, *Callosobruchus maculatus* Fabricius and *Callosobruchus chinensis* Linnaeus and maize weevil, *Sitophilus zeamais* Motschulsky caused by hexane extract from long pepper, *Piper retrofractum* Vahl fruit at various concentrations compared with the insecticide group by film seed coating method and stored at 4 °C for 6 months.

Insects	Storage time (months)	Means[1/]						
		Control (Coating agent, 3%)	Insecticide (Fipronil)		Hexane extract from long pepper			
			0.1%	0.2%	1%	3%	5%	
C. maculatus								
	0	0.0^{C}	98.3^{AB}	100.0^{A}	95.5^{B}	97.1^{AB}	100^{A}	**
	2	0.0^{C}	91.6^{AB}	99.0^{A}	90.4^{B}	98.2^{AB}	100^{A}	**
	4	0.0^{C}	93.5^{B}	99.0^{A}	95.2^{B}	99.0^{A}	100^{A}	**
	6	0.0^{D}	90.2^{C}	99.0^{AB}	92.0^{BC}	97.0^{ABC}	100^{A}	**
		ns	ns	ns	ns	ns	ns	
C. chinensis								
	0	0.0^{C}	98.1^{B}	100.0^{A}	100.0^{A}	100.0^{A}	100^{A}	**
	2	0.0^{D}	83.9^{C}	95.0^{AB}	90.8^{B}	99.1^{A}	100^{A}	**
	4	0.0^{C}	83.0^{B}	95.0^{A}	87.0^{B}	98.0^{A}	100^{A}	**
	6	0.0^{C}	86.0^{B}	95.0^{A}	88.2^{B}	99.0^{A}	100^{A}	**
		ns	**	ns	**	ns	ns	
S. zeamais								
	0	0.0^{D}	61.8^{C}	90.1^{Aa}	65.0^{BC}	78.5^{ABa}	90.1^{A}	**
	2	0.0^{D}	60.3^{C}	89.2^{Aa}	63.8^{C}	77.7^{Ba}	88.4^{A}	**
	4	0.0^{E}	55.9^{D}	79.6^{ABb}	59.9^{CD}	69.7^{BCab}	88.0^{A}	**
	6	0.0^{C}	54.3^{B}	81.7^{Ab}	53.8^{B}	64.0^{Bb}	86.1^{A}	**
		ns	ns	*	ns	*	ns	

[1/] Data were based on adult, n = 20 adult insects/replicate of five replications. Means in the same column of each insect followed by the same common letter, and the means in the same row followed by the same capital letter are not significantly different as determined by DMRT. *, **: Significant difference at $P < 0.05$ and $P < 0.01$, respectively, and ns: nonsignificant difference.

Kim et al. (2003) reported that *Cinnamonum* sp. extract (0.7 mg·cm^{-2}) had different potent insecticidal activity on *C. chinensis* and *Sitophilus zeamais,* which gave 100% mortality at one and two days, respectively, after treatment by using contact method. In addition, the other extract from plants including *Acorus gramineus*, *Agastache rugosa,* and *Foeniculum vulgare* killed those insects after three days of post-treatment. However, citronella, langkauas, and patchouli crude extracts showed slight contact toxicity against *Sitophilus* spp., whereas langkauas crude extract was the most toxic (LD_{50} = 0.0854 μg·mg^{-1} body weight) against *C. chinensis* at 48-hour exposure (Thein et al., 2013). The overall results in this study implied that bruchid beetles were much more susceptible than maize weevil. The result was similar to Ogunsina et al. (2011), who reported that African nutmeg extract had greater mortality action against *C. maculatus* (100%) and *S. zeamais* (96%) after 24 h of treatment with extract concentration of 10 g/100 ml (extract/solvent) for lantana extract of 93% and 73%, respectively.

Many reports demonstrated that the hexane solvent used for plant extraction had more effectiveness in controlling some stored product insect pests than other solvents. For example, Paventi et al. (2021) reported that wild hop extracted from hexane application against granary weevil (*Sitophilus granaries*) found insect mortalities were significantly faster than acetone extract. Mokhtar et al. (2021) indicated that hexane extract from desert date seed was more toxic for red flour beetle (*Tribolium castaneum*) compared with the ethanol extract with LD_{50} value of 0.327 and 3.392 mg·cm^{-2}, respectively, at 24 h after treatment by contact toxicity method. The hexane extract from goat weed (*Ageratum conyzoides*) showed insecticide activity against coleoptera pests of stored products with LD_{50} of 2.72–39.71 mg·g^{-1}, but ethanol extract (Moreira et al., 2007). Fotso et al. (2020) revealed that all the tested *Hemizygia welwitschii* leaf extracts exhibited insecticidal action against maize weevil, which was more susceptible to the hexane extract than acetone extract. The solvents for plant extraction could be used to obtain different compounds depending on their polarity. Therefore, if a rational management of natural products is desired, it is recommended the use of the more polar extracts (Castillo-Sánchez et al., 2010), the hexane considered as high polar solvent. In this study, the excellent insecticidal property of hexane extract from long pepper against all tested insects was found with more than that of the other extracts. Therefore, it was suitable for further study on using it for film seed coating as well as the evaluation of its insect toxicity and seed germination.

Secondary substances of medicinal plants have been reported as having high toxicity effect to insect pests, and the repellency potential of this plant extract was also important for field condition application. In this study, the extract presented repellency response significantly to bruchid beetles but slightly to maize weevil. Many reports have been done regarding the repellency capacity of plant extracts against various insect species. Khan and Shahjahan (1998) informed that *S. oryzae* was repelled and *C. chinensis* was attracted by eucalyptus leaf extracts. Thein et al. (2013) found that the variance of repellent effects such as citronella crude extract provided the highest repellency against *Sitophilus* spp., while langkauas provided 100% reduction in F1 emergence. In contrast, langkauas, citronella, and patchouli provided similar level of repellency against *C. chinensis*. Paventi et al. (2021) reported that the wild hop extracts showed short-range repellent effects. More importantly, they reduced the attractiveness of stored food. The hexane extract of wild hop showed high repellency response by higher concentration, but lower response when the exposure time was longer. The various reports regarding response effect of different insect species emphasized that the repellency and attraction test was always necessary with toxicity studies. The insecticidal properties of botanical insecticides both in forms of toxicity and repellent effects might show positive results, suggesting that it could be an alternative application for field conditions.

Film seed coating by hexane extract from long pepper was evaluated for its insecticidal activity and effect on seed germination before and after storage. The result presented that it had the most effectiveness to control bruchid beetles, but low toxicity to maize weevil. The result was in accordance with the residue contact test in a previous experiment. However, the hexane extract at 3% concentration had more efficacy to kill both bruchid beetles than the 1% concentration with significant difference but it showed toxicity of non-significant difference compared

Table 4. Percentages of moisture content, germination in the laboratory, and germination index of mung bean and corn seeds coated with hexane extract from long pepper, *Piper retrofractum* Vahl fruit at various concentrations compared with the insecticide group by film seed coating method and stored at 4 °C for 6 months.

Seed germination after coating	Storage time (months)		Control (Coating agent, 3%)		Means[1/]			Storage time (months)	
				Insecticide (Fipronil)		Hexane extract from long pepper			
				0.1%	0.2%	1%	3%	5%	
Mung bean									
%Moisture content (%MC)									
	0	9.66^{A}	9.47^{AB}	9.68^{A}	9.54^{A}	9.65^{A}	9.63^{A}	9.26^{B}	**
	2	9.79^{A}	9.67^{A}	9.86^{A}	9.77^{A}	9.81^{A}	9.68^{A}	9.26^{B}	**
	4	9.65^{AB}	9.59^{B}	9.82^{A}	9.65^{AB}	9.77^{AB}	9.48^{C}	9.24^{D}	**
	6	9.68^{A}	9.59^{A}	9.66^{A}	9.57^{A}	9.73^{A}	9.59^{A}	9.27^{B}	**
		ns	ns	ns	ns	ns	ns	ns	
%Germination in the laboratory (%GL)									
	0	99.0	95.3	96.3	99.0	95.3	96.5	99.8^{a}	ns
	2	97.3^{A}	95.3^{A}	93.3^{AB}	93.3^{AB}	92.3^{AB}	89.3^{B}	89.0^{Bb}	*
	4	98.8	98.3	93.0	91.3	91.8	93.8	89.0^{b}	ns
	6	96.8^{A}	96.5^{A}	91.3^{AB}	92.5^{AB}	93.3^{AB}	91.5^{AB}	88.3^{Bb}	*
		ns	ns	ns	ns	ns	ns	**	
Germination index (GI) at 7 days									
	0	14.14	13.61	13.75	14.14	13.61	13.79	14.25^{a}	ns
	2	13.89	13.61	13.32	13.32	13.18	12.75	12.71^{b}	ns
	4	14.11^{A}	14.04^{AB}	13.29^{ABC}	13.04^{BC}	13.11^{ABC}	13.39^{AB}	12.71^{Cb}	*
	6	13.82^{A}	13.79^{AB}	13.04^{ABC}	13.21^{ABC}	13.32^{ABC}	13.07^{ABC}	12.61^{Cb}	*
		ns	ns	ns	ns	ns	ns	**	
Corn kernel									
%Moisture content (%MC)									
	0	8.11^{B}	9.41^{A}	9.25^{A}	9.39^{A}	9.36^{A}	9.38^{A}	9.30^{A}	**
	2	8.22^{C}	9.45^{AB}	9.34^{B}	9.48^{AB}	9.51^{A}	9.47^{AB}	9.33^{B}	**
	4	8.26^{B}	9.39^{A}	9.47^{A}	9.52^{A}	9.50^{A}	9.40^{A}	9.28^{A}	**
	6	8.24^{B}	9.39^{A}	9.38^{A}	9.55^{A}	9.55^{A}	9.36^{A}	9.36^{A}	**
		ns	ns	ns	ns	ns	ns	ns	
%Germination in the laboratory (%GL)									
	0	91.8^{A}	72.0^{BC}	74.8^{B}	66.8^{C}	60.0^{Da}	54.0^{Ea}	8.0^{E}	**
	2	91.8^{A}	65.5^{C}	75.8^{B}	67.9^{C}	40.1^{Db}	45.8^{Db}	8.8^{E}	**
	4	88.0^{A}	64.0^{C}	74.3^{B}	65.5^{C}	38.3^{Db}	44.3^{Db}	6.5^{E}	**
	6	92.0^{A}	77.3^{B}	76.8^{B}	70.5^{C}	44.5^{Db}	45.3^{Db}	6.0^{E}	**
		ns	ns	ns	ns	**	*	ns	
Germination index (GI) at 7 days									
	0	13.11^{A}	10.29^{B}	10.68^{B}	9.54^{C}	6.36^{D}	6.57^{D}	1.14^{E}	**
	2	13.11^{A}	9.36^{C}	10.82^{B}	9.57^{C}	5.79^{D}	6.54^{D}	1.25^{E}	**
	4	12.57^{A}	9.14^{C}	10.61^{B}	9.36^{C}	5.46^{D}	6.32^{D}	0.93^{E}	**
	6	13.14^{A}	11.04^{B}	10.92^{B}	10.00^{C}	6.36^{D}	6.46^{D}	0.86^{E}	**
		ns	ns	ns	ns	ns	ns	ns	

[1/] Means in the same column of each parameter followed by the same common letter, and the means in the same row followed by the same capital letter are not significantly different as determined by DMRT. *, **: Significant difference at $P < 0.05$ and $P < 0.01$, respectively, and ns: nonsignificant difference.

with the insecticide (recommended rate) group. Both concentrations expressed non-effect on seed germination. In contrast, 5% extract concentration presented to control both bruchid beetles, but also showed toxic symptom on mung bean with significant difference compared with the other treatments. Therefore, result of this study aligned with the study of Pumnuan et al. (2021), who found that mung bean seed coated hexane extract of clove at 1% concentration showed no effect on seed germination, but 5% of them presented high effect.

The mung bean seed coated with the extract showed lower germination effect than corn seed. The consequences of corn seed coated with hexane extracts were very unsatisfactory, and it showed seed germination effect higher than 50% at 1% and 3% extract concentrations, and >90% for 5% extract concentration. The high concentration of hexane extract from long pepper therefore should not be used for film seed coating method. Many reports indicated fluctuation effects of seed coated herb extract on seed germination. Ahmed et al. (2013) and Mahal (2014) found that *Azadirachta indica* leaf extract treated rice and lentil seeds increased germination percentage over the control group, increasing germination 16% and 17.5%, respectively. This agrees with the findings of Girase et al. (2019), who concluded that okra seed treated with *Albizia amara* leaf extract at 3% concentration increased the seed germination and vigor. In addition, Mbega et al. (2012) found treatment

Table 5. Chemical compounds were identified in purified fraction samples of hexane extract from long pepper (*Piper retrofractum* Vahl) fruit using GC-MS.

Chemical compounds	Molecular formula	Purified fraction (mg)								$mg \cdot g^{-1}$ of hexane extract
		PF1	PF2	PF3	PF4	PF5	PF6	PF7	Total	
6-Tridecene	$C_{13}H_{26}$	2.26	–	–	–	–	–	–	2.26	0.093
Tridecane	$C_{13}H_{28}$	26.30	–	–	–	–	–	–	26.30	1.087
β-Elemene	$C_{15}H_{24}$	10.78	–	–	–	–	–	–	10.78	0.445
Zingiberene	$C_{15}H_{24}$	1.97	–	–	–	–	–	–	1.97	0.081
α-Santalene	$C_{15}H_{24}$	2.33	–	–	–	–	–	–	2.33	0.096
α-Bergamotene	$C_{15}H_{24}$	3.42	–	–	–	–	–	–	3.42	0.141
1-Tridecene	$C_{13}H_{26}$	36.58	–	–	–	–	–	–	36.58	1.512
1-Tetradecanol	$C_{14}H_{30}O$	67.40	–	–	–	–	–	–	67.40	2.785
Pentadecane	$C_{15}H_{32}$	171.37	–	–	–	–	–	–	171.37	7.081
β-Bisabolene	$C_{15}H_{24}$	20.47	–	–	–	–	–	–	20.47	0.846
(−)-α-Panasinsen	$C_{15}H_{24}$	3.28	–	–	–	–	–	–	3.28	0.136
Hexadecane	$C_{16}H_{34}$	4.30	–	–	–	–	–	–	4.30	0.178
8-Heptadecene	$C_{17}H_{34}$	69.07	0.30	–	–	–	–	–	69.37	2.867
1-Heptadecene	$C_{17}H_{34}$	127.14	0.29	–	–	–	–	–	127.43	5.266
Heptadecane	$C_{17}H_{36}$	128.67	0.49	–	–	–	–	–	129.16	5.337
Octadecane	$C_{18}H_{38}$	3.79	-	–	–	–	–	–	3.79	0.157
1-Nonadecene	$C_{19}H_{38}$	10.42	-	–	–	–	–	–	10.42	0.431
Z-5-Nonadecene	$C_{19}H_{38}$	22.22	0.11	–	–	–	–	–	22.33	0.923
Nonadecane	$C_{19}H_{40}$	12.17	0.14	–	–	–	–	–	12.31	0.509
Eicosane	$C_{20}H_{42}$	2.77	–	–	–	–	–	–	2.77	0.114
Docosane	$C_{22}H_{46}$	1.89	–	–	–	–	–	–	1.89	0.078
Piperonal	$C_8H_6O_3$	–	0.12	–	–	–	–	–	0.12	0.005
3−Heptadecene	$C_{17}H_{34}$	–	0.10						0.10	0.004
Cinnamoylglycine, methyl ester	$C_{12}H_{13}NO_3$	–	0.10	–	–	–	–	–	0.10	0.004
Cinnamic acid methyl ester	$C_{10}H_{10}O_2$	–	4.19	–	–	–	–	–	4.19	0.173
Cinnamyl cinnamate	$C_{18}H_{16}O_2$	–	0.69	–	–	–	–	–	0.69	0.029
Spongia-13 (16), 14-dien-19-oic acid	$C_{20}H_{28}O_3$	–	0.36	–	–	–	–	–	0.36	0.015
Caryophyllene oxide	$C_{15}H_{24}O$	–	–	10.23	–	–	–	–	10.23	0.423
2−Cyclohexen-1-one, 4-(1-methylethyl)	$C_9H_{14}O$	–	–	6.44	–	–	–	–	6.44	0.266
Methyl 5-benzylthiophen-2-carboxylate	$C_{13}H_{12}O_2S$	–	-	8.92	–	–	–	–	8.92	0.369
Cyclopentanol, 1-methyl	$C_6H_{12}O$	–	–	–	0.61	–	0.07	–	0.68	0.028
1-Thiacyclohept-2-ene	$C_6H_{10}S$	–	–	–	0.42	–	–	–	0.42	0.017
7−Heptadecyne, 17-chloro	$C_{17}H_{31}Cl$	–	–	–	0.37	–	–	–	0.37	0.015
β−Bisabolene	$C_{15}H_{24}$	–	–	–	0.51	–	–	–	0.51	0.021
13−Octadecenal	$C_{18}H_{34}O$	–	–	–	0.49	10.91	–	–	11.40	0.471
Octadecenal	$C_{18}H_{36}O$	–	–	–	0.34	–	–	–	0.34	0.014
Isolongifolene, 9,10−dehydro	$C_{15}H_{22}$	–	–	–	0.51	–	–	–	0.51	0.021
Longifolenaldehyde	$C_{15}H_{24}O$	–	–	–	0.49	–	–	–	0.49	0.020
Dibenzylidene-d-glucose	$C_{20}H_{20}O_6$	–	–	–	0.42	–	–	–	0.42	0.017
Spiro (4,5)decane	$C_{11}H_{18}$	–	–	–	0.68	–	–	–	0.68	0.028
Ledol	$C_{15}H_{26}O$	–	–	–	2.35	–	–	–	2.35	0.097
Limonene dioxine 1	$C_{10}H_{16}O_2$	–	–	–	1.23	–	–	–	1.23	0.051
(E)-13-methyl-11-tetradecen-1-olacetate	$C_{17}H_{32}O_2$	–	–	–	0.37	–	–	–	0.37	0.015
9−Octadecenal	$C_{18}H_{34}O$	–	-	-	0.46		–	–	0.46	0.019
Cyclotetradecane	$C_{14}H_{28}$	–	–	–	0.64	–	–	–	0.64	0.026
Methone	$C_8H_{12}O_2$	–	–	–	–	8.22	–	–	8.22	0.340
Tetradecanal	$C_{14}H_{28}O$	–	–	–	–	11.06	–	–	11.06	0.457
Octadecanal	$C_{18}H_{34}O$	–	–	–	–	3.90	–	–	3.90	0.161
Hexanal	$C_6H_{12}O$	–	–	–	–	–	0.10	–	0.10	0.004
Cyclopropane, 1,1,2,2-tetramethyl	C_7H_{14}	–	–	–	–	–	0.12	–	0.12	0.005
Bicyclo [4.1.0] heptan−2−one	$C_7H_{10}O$	–	–	–	–	–	0.06	–	0.06	0.002
5-Dodecen-1-al	$C_{12}H_{22}O$	–	–	–	–	–	0.09	–	0.09	0.004
7−Dodecen-1-al	$C_{12}H_{22}O$	–	–	–	–	–	0.06	–	0.06	0.002
Lomustine	$C_9H_{16}ClN_3O_2$	–	–	–	–	–	0.13	–	0.13	0.005
Piperidine, 2-pentyl	$C_{10}H_{21}N$	–	-	-	-	-	0.15	–	0.15	0.006
3,11-Tetradecadien-1-ol	$C_{14}H_{26}O$	–	–	–	–	–	0.09	–	0.09	0.004
E-11-Hexadecenal	$C_{16}H_{30}O$	–	–	–	–	–	0.02	–	0.02	0.001

(*continued on next page*)

Table 5 (*continued*)

Chemical compounds	Molecular formula	Purified fraction (mg)								mg · g^{-1} of hexane extract
		PF1	PF2	PF3	PF4	PF5	PF6	PF7	Total	
Ethamivan	$C_{12}H_{17}NO_3$	–	–	–	–	–	0.38	–	0.38	0.016
Palmitic acid	$C_{16}H_{32}O_2$	–	–	–	–	–	0.42	–	0.42	0.017
Piperidine, 1−cinnamoyl	$C_{14}H_{17}NO$	–	-	-	-	-	0.05	–	0.05	0.002
9−Octadecenoic acid	$C_{18}H_{34}O_2$	–	–	–	–	–	0.17	–	0.17	0.007
Tran−2−(2−hydroxycyclopentyl) furan	$C_9H_{12}O_2$	–	–	–	–	–	0.30	–	0.30	0.012
9−Octadecenoic acid (Z)	$C_{18}H_{34}O_2$	–	–	–	–	–	0.04	–	0.04	0.002
9−Octadecenoic acid (E)	$C_{18}H_{34}O_2$	–	–	–	–	–	0.05	–	0.05	0.002
Hexadecenoic acid, Z−11	$C_{16}H_{30}O_2$	–	–	–	–	–	0.10	–	0.10	0.004
4−Methylurazole	$C_3H_5N_3O_2$	–	–	–	–	–	0.09	–	0.09	0.004
γ−Sitosterol	$C_{29}H_{50}O$	–	–	–	–	–	0.68	–	0.68	0.028
9−Octadecenamide, n−butyl	$C_{22}H_{43}NO$	–	–	–	–	–	0.12	–	0.12	0.005
9-Octadecenamide, N,N-diethyl	$C_{22}H_{43}NO$	–	–	–	–	–	0.12	–	0.12	0.005
Cyclopentane, 1,1,3,4−tetramethyl−, cis	C_9H_{18}	–	–	–	–	–	–	1.99	1.99	0.082
Phenol, 2,4−bis (1,1−dimethylethyl)	$C_{14}H_{22}O$	–	–	–	–	–	–	6.05	6.05	0.250
Docosane	$C_{22}H_{46}$	–	–	–	–	–	–	3.07	3.07	0.127
Isochiapin B	$C_{19}H_{22}O_6$	–	–	–	–	–	–	3.40	3.40	0.140
Total (mg · g^{-1})		**728.60**	**6.89**	**25.59**	**9.89**	**34.09**	**3.41**	**14.51**	**822.98**	**34.005**
Percentages of purified fraction		**88.56**	**0.82**	**3.12**	**1.21**	**4.15**	**0.41**	**1/76**	**100.00**	**-**

of tomato seeds with *Aloe vera*, *Coffea arabica,* and *Yucca schidigera* extracts had no effect on seedling vigor, height, and weight. In contrast, Ismail et al. (2011) presented the potential use of *Juniperus oxycedrus* extract with germination-inhibiting properties and its effectiveness in inhibiting the growth of some weed seedlings. While Kadioglu and Yanar (2004) found that germination of *Amaranthus retroflexus* was strongly inhibited by the extracts of *Galium aparine*, *Lolium temulentum*, *Conium maculatum,* and *Avena sterilis* of 13%, 12.5%, 19.5%, and 26.0%, respectively. Interestingly, some of the plant extracts treated on plant seeds could be used as inhibitor while others could be used as stimulator for the crops, for example, chickpea seed germination was inhibited by extracts of *Solanum nigrum*, *Chenopodium album*, and *Matricaria chamomilla* of 10%, 20%, and 22.5%, respectively. However, *Glycyrrhiza glabra*, *Sorghum halepense*, and *Reseda lutea* extracts stimulated chickpea seed germination with non-difference compared to the control (Kadioglu et al., 2005). This occurrence was also supported by Michelin et al. (2016), who found the extracts of *Callistemon viminalis*, *Tephrosia vogelii*, and *Cupressus lusitanica* exerted the inhibition on seed germination, affected the stem length, and reduced the root length. Additionally, *Oryza sativa* suffered the inhibitory effect of the aqueous extracts from *Lolium multiflorum*, especially inflorescence and stem extracts that were able to reduce both the seed germination and the seedling growth, while the root extracts affected only the seedling growth (Vitalini et al., 2020).

Plant extracts obtained from various medicinal plant varieties show different insecticidal properties (Degu et al., 2020) and effects on seed germination (Fessel et al., 2003; De Moraes Dan et al., 2012; Jarecki and Wietecha 2021). A variety of extracts and compounds from different plant families have been evaluated to show a new and promising insecticides and larvicides (Degu et al., 2020). Several classes of plant-derived chemicals can be distinguished: alkaloids, rotenoids, phenolic compounds, pyrethrins, oils, and saponins, among others. Some of them are already widely used for their toxic activity against various insects (Spochacz et al., 2018). Kubo et al. (2013) reported that the chemical compound of long pepper fruit has been researched as alkaloids, terpenoids, lignans, flavones, propenylphenols, kawapyrones, and dihydrochalcones. The result of the present study found that isolation of bioactive components as 74 compounds in purified fraction including pentadecane, which was a major compound, followed by heptadecane, 1−heptadecene, 8−heptadecene, 1−tetradecanol, 1−tridecene, and tridecane. These compounds presented high volume at 21.639 mg·g^{-1} or 71.87% of purified fraction from hexane extract. It was surprising that the chemical compounds of this study showed some differentiations with other previous reports. Ratwatthananon et al. (2020) reported that the hexane extract of dried long pepper fruit showed the isolated compounds were alkaloids including (2E,4E,14Z)-N-isobutylicosa-2,4,14-trienamide, guineensine, retrofractamide D, pipernonaline, piperine, and piperanine. Musthapa et al. (2018) reported that methyl piperate was the main compound isolated from this extract. Piperine and piperanine are alkaloid compounds found from the crude hexane extract (Wiwattanawanichakun et al., 2018). In this study, found that the major chemical compounds were hydrocarbons or terpenes in aroma hexane extract, which are the main active components in pepper essential oil (Wang et al., 2022). Terpenes and terpenoids are the main bioactive compounds of plant essential oils, a wide range of biological activities (Masyita et al., 2022). The chemical compound certainty in plant extracts is the main factor for considering insecticidal activities against the various insects.

5. Conclusion

The hexane extract from long pepper (*P. retrofractum*) fruit had efficacy to kill and repel both bruchid beetles (*C. maculatus* and *C. chinensis*), and it showed non-effect on mung bean seed germination. However, this extract was inappropriate to be used for controlling maize weevil (*S. zeamais*) due to the visibility of low insecticidal properties and high effect on corn seed germination. Mung bean seed coated with the extract at 1% concentration and stored for six months post-treatment presented high mortality of both bruchid beetles with more than 88% mortality and non-significant difference compared to the fipronil insecticide group. The mung bean seed coated extract presented seed qualities of no significant difference compared to the positive and negative controls. This film seed coating technology of using hexane extract from long pepper fruit for controlling some stored product insect pests could be applied as a green insecticide and alternative substitute for insecticides in the future. However, further studies on its effect against other stored product insect pests, as well as its effects on non-target organisms and to seed qualities, are necessary.

Declarations

Author contribution statement

Jarongsak Pumnuan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Duangkamon Namee: Performed the experiments; Analyzed and interpreted the data.

Kritima Sarapothong: Performed the experiments.

Thanaporn Doungnapa: Performed the experiments.

Sudjai Phutphat: Contributed reagents, materials, analysis tools or data.

Chutichot Pattamadilok: Contributed reagents, materials, analysis tools or data.

Kamronwit Thipmanee: Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

Acknowledgements

This work is supported by King Mongkut's Institute of Technology Ladkrabang (grant number 2562-02-04-003) and the National Research Council of Thailand. We also thank the Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL) for encouragement.

References

Abbott, W.S., 1987. A method of computing the effectiveness of an insecticide. J. Am. Mosq. Control Assoc. 3, 302–303.
Afzal, I., Javed, T., Amirkhani, M., Taylor, A.G., 2020. Modern seed technology: seed coating delivery systems for enhancing seed and crop performance. Agriculture 10, 526.
Afzal, I., Rehman, H.U., Naveed, M., Basra, S.M.A., 2016. Recent advances in seed enhancements. In: Araujo, S., Balestrazzi, A. (Eds.), New Challenges in Seed Biology-Basic and Translational Research Driving Seed Technology. InTechOpen, London, pp. 47–74.
Ahmad, F., Iqbal, N., Zaka, S.M., Qureshi, M., Saeed, Q., Khan, K., Ansari, M., Awar, M.B., 2019. Comparative insecticidal activity of different plant materials from six common plant species against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Saudi J. Biol. Sci. 26, 1804–1808.
Ahmed, M., Hossain, M., Hassan, K., Dash, C.K., 2013. Efficacy of different plant extract on reducing seed borne infection and increasing germination of collected rice seed sample. Univ. J. Plant Sci. 1, 66–73.
Ahmed, S., Haque, M.A., Mahmud, H., 2018. Effect of pulse beetle, *Callosobruchus chinensis* L on oviposition and damage in some important genotypes of pulse crops in Bangladesh. Biomed. J. Sci. Technol. Res. 2, 2544–2548.
Barbercheck, M., 2020. Management of stored grain pests in organic systems. In: Organic Grain Production Resource Book 182−202.
Campos, E.V.R., Proença, L.F., Oliveira, J.L., Bakshic, M., Abhilashc, P.C., Fraceto, L.F., 2019. Use of botanical insecticides for sustainable agriculture: future perspectives. Ecol. Indicat. 105, 483–495.
Caneppele, M.A.B., Caneppele, C., Lazzari, F.A., Lazzari, S.M.N., 2003. Correlation between the infestation level of *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera, Curculionidae) and quality factors of stored corn, *Zea mays* L. (Poaceae). Rev. Bras. Entomol. 47, 625–630.
Castillo-Sánchez, L.E., Jiménez-Osornio, J.J., Delgado-Herrera, M.A., 2010. Secondary metabolites of the annonaceae, Solanaceae and meliaceae families used as biological control of insects. Trop Subtrop 12, 445–462.
De Moraes Dan, L.G., De Almeida Dan, H., De Lucca e Braccini, A., Leão de Lemos Barroso, A., Ricci, T.T., Piccinin, G.G., Scapim, C.A., 2012. Insecticide treatment and physiological quality of seeds. In: Perveen, F.K. (Ed.), Insecticides: Advances in Integrated Pest Management. InTech. Croatia 327−342.
Degu, S., Berihun, A., Muluye, R., Gemeda, H., Debebe, E., Amano, A., Abebe, A., Woldkidan, S., Tadele, A., 2020. Medicinal plants that used as repellent, insecticide and larvicide in Ethiopia. Pharm. Pharmacol. Int. J. 8, 274–283.
Doungnapa, T., Pumnuan, J., Insung, A., 2021. Acaricidal activity of essential oil nanoemulsion against the African red mite (*Eutetranychus africanus*). Chil. J. Agric. Res. 81, 228–236.
Dumitriu, I.M., Stoica, C., Munteanu Pila, M., Florea, A.M., Stanciu, S., 2020. Management and quality control of grain seed. Research on the influence of storage conditions. In: Soliman, K.S. (Ed.), Education Excellence and Innovation Management: a 2025 Vision to Sustain Economic Development during Global Challenges. International Business Information Management Association (IBIMA), USA, p. 8836, 8344.
Entomology and Zoology Association of Thailand (EZAT), 2010. A Guidance for the Control of Insect and Other Animal Pests 2010, 17th Printing. Division of Entomology and Zoology, Department of Agriculture, Thailand (Thai language).
Fessel, S.A., Mendonca, E.A.F., Carvalho, R.V., 2003. Effect of chemical treatment on corn seeds conservation during storage. Rev. Bras. Sementes 25, 25–28.
Fotso, T.G., Gangue, T., Langsi, D.J., Lakane, M.N., Zourmba, M.C., Tchao, N., Kapche, W.F.G.D., Nukenine, E.N., 2020. Potentiality of fractionated extracts from *Hemizygia welwitschii* Rolfe-Ashby (Lamiaceae) leaf to protect maize against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in storage. Eur. J. Nutr. Food Saf. 12 (10), 68–80.
Gariba, S.Y., Dzidzienyo, D.K., Eziah, V.Y., Moral, M.T., 2021. Assessment of four plant extracts as maize seed protectants against *Sitophilus zeamais* and *Prostephanus truncatus* in Ghana. Cogent. Food Agric. 7, 1918426.
Girase, I.P., Rai, P.K., Bara, B.M., Singh, B.A., 2019. Effect of plant extracts on seed germination behaviour and vigour of okra [*Abelmoschus esculentus* (L.) Moench]. Int. J. Curr. Microbiol. Appl. Sci. 8, 830–835.
Halmer, P., 2004. Methods to improve seed performance in the field. In: Benech-Arnold, R.L., Sanchez, R. (Eds.), Handbook of Seed Physiology. Food Product Press, New York, pp. 125–156.
Ismail, A., Lamia, H., Mohsen, H., Bassem, J., 2011. Chemical composition of *Juniperus oxycedrus* L. subsp macrocarpa essential oil and study of their herbicidal effects on germination and seedling growth of weeds. Asian J. Appl. Sci. 4, 771–779.
Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51, 45–66.
Jarecki, W., Wietecha, J., 2021. Effect of seed coating on the yield of soybean *Glycine max* (L.) Merr. Plant Soil Environ. 67, 468–473.
Kadioglu, I., Yanar, Y., 2004. Allelopathic effects of plant extracts against seed germination of some weeds. Asian J. Plant Sci. 3, 472–475.
Kadioglu, I., Yanar, Y., Asav, U., 2005. Allelopathic effects of weeds extracts against seed germination of some plants. J. Environ. Biol. 26, 169–173.
Kalpna, Hajam YA., Kumar, R., 2022. Management of stored grain pest with special reference to *Callosobruchus maculatus*, a major pest of cowpea: a review. Heliyon 1 (8), e08703.
Khan, M., Shahjahan, R.M., 1998. Evaluation of eucalyptus leaf extracts as repellent for adult *Sitophilus oryzae* (L.) (Col.: Curculionidae) and attractant for *Callosobruchus chinensis* (L.) (Col.: Bruchidae). Bangladesh. J. Entomol. 8, 81–88.
Kim, S.I., Roh, J.Y., Kim, D.H., Lee, H.S., Ahn, Y.J., 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. J. Stored Prod. Res. 39, 293–303.
Kolupaeva, V.N., Kokoreva, A.A., Belik, A.A., Pletenev, P.A., 2019. Study of the behavior of the new insecticide cyantraniliprole in large lysimeters of the Moscow State University. Open Agric. 4, 599–607.
Kubo, M., Ishii, R., Ishino, Y., Harada, K., Matsui, N., Akagi, M., Kato, E., Hosoda, S., Fukuyama, Y., 2013. Evaluation of constituents of *Piper Retrofractum* fruits on neurotrophic activity. J. Nat. Prod. 76, 769–773.
López-Castillo, L.M., Silva-Fernández, S.E., Winkler, R., Bergvinson, D.J., Arnason, J.T., García-Lara, S., 2018. Postharvest insect resistance in maize. J. Stored Prod. Res. 77, 66–76.
Mahal, M.F., 2014. Effects of fungicides and plant extracts on seed germination and seed associated mycoflora of *Lens arietinum* L. and *Lathyrus sativus*. J. Biosci. 22, 101–110.
Masyita, A., Sari, R.M., Astuti, A.D., Yasir, B., Rumata, N.R., Emran, T.B., Nainu, F., Simal-Gandara, J., 2022. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem. X 13, 100217.
Mbega, E.R., Mortensen, C.N., Mabagala, R.B., Wulff, E.G., 2012. The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania. J. Gen. Plant Pathol. 78, 277–286.
Mehta, V., Kumar, S., Jayaram, C.S., 2021. Damage potential, effect on germination, and development of *Sitophilus oryzae* (Coleoptera: Curculionidae) on wheat grains in Northwestern Himalayas. J. Insect Sci. 21, 1–7.
Michelin, K.T.P., Jean, A.-N., Donatien, G., Keilah, L.P., Stephen, L.T., Jules-Roger, K., 2016. *In vitro* allelopathic effects of extracts and fractions of five plants on tomato seed germination and vigor index. Cogent Biol. 2, 1220661.
Mokhtar, M.M., Li, J., Du, Z., Cheng, F., 2021. Insecticidal efficacy and chemical composition of *Balanites aegyptiaca* (L.) Delile seed oils against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Chil. J. Agric. Res. 81, 102–108.
Moreira, M.D., Picanço, M.C., Barbosa, L.C.D.A., Guedes, R.N.C., Campos, M.R.D., Silva, G.A., Martins, J.C., 2007. Plant compounds insecticide activity against Coleoptera pests of stored products. Pesqui. Agropecu. Bras. 42, 909–915.
Musthapa, I., Gumilar, G.G., Dara, F., 2018. Isolation of metyhl-piperate from n-hexane extract of fruit of cabe jawa (*Piper retrofractum* Vahl.). Pertanika J. Trop. Agric. Sci. 41, 1489–1495.
Ogunsina, O.O., Oladimeji, M.O., Lajide, L., 2011. Insecticidal action of hexane extracts of three plants against bean weevil, *Callosobruchus maculatus* (F.) and maize weevil, *Sitophilus zeamais* Motsch. J. Ecol. Nat. Environ. 3, 23–28.
Ojo, J.A., Omoloye, A.A., 2012. Rearing of maize weevil, *Sitophilus zeamais* on an artificial maize-cassava diet. J. Insect Sci. 12, 1–2.

Panphut, W., Budsabun, T., Sangsuriya, P., 2020. *In vitro* antimicrobial activity of *Piper retrofractum* Fruit extracts against microbial pathogens causing infections in human and animals. Internet J. Microbiol. 2020, 5638961.

Paventi, G., Rotundo, G., Pistillo, M., D'Isita, I., Germinara, G.S., 2021. Bioactivity of wild hop extracts against the granary weevil, *Sitophilus granarius* (L.). Insects 2, 564.

Prijono, D., Wulan, R.D.R., Ferdi, Saryanah, N.A., 2020. Insecticidal activity of the extracts of *Piper retrofractum* fruit and *Tephrosia vogelii* leaf and their mixtures against *Crocidolomia pavonana*. Cropsaver 3, 68–75.

Pumnuan, J., Insung, A., Klompanya, A., 2020. Effects of seven plant essential oils on mortalities of chicken lice (*Lipeurus caponis* L.) adult. Curr. J. Appl. Sci. 20, 52–58.

Pumnuan, J., Sarapothong, K., Sikhao, P., Pattamadilok, C., Insung, A., 2021. Film seeds coating with hexane extracts from *Illicium verum* Hook.f. and *Syzygium aromaticum* (L.) Merrill & Perry for controlling *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* L. Pest Manag. Sci. 77, 2512–2521.

Ratwatthananon, A., Yooboonb, T., Bullangpotib, V., Pluempanupata, W., 2020. Insecticidal activity of *Piper retrofractum* fruit extracts and isolated compounds against *Spodoptera litura*. Agric. Nat. Resour. 54, 447–452.

Salleh, W.M.N.H.W., Ahmad, F., 2020. Phytopharmacological investigations of *Piper retrofractum* Vahl. A review. Agric. Conspectus Sci. 85, 193–202.

Santana, A.S., Baldin, E.L.L., Santos, T.L.B., Baptista, Y.A., Santos, M.C., Lima, A.P.S., Tanajura, L.S., Vieira, T.M., Crotti, A.E.M., 2022. Synergism between essential oils: a promising alternative to control *Sitophilus zeamais* (Coleoptera: Curculionidae). Crop Protect. 153, 105882.

Saenmanot, S., Insung, A., Pumnuan, J., Tawatsin, A., Thavara, U., Phumee, A., Gay, F., Tachaboonyakiat, W., Siriyasatien, P., 2018. Insecticidal activity of Thai botanical extracts against development stages of German cockroach, *Blattella germanica* (L.) (Orthoptera: Blattellidae). Southeast Asian J. Trop. Med. Publ. Health 49, 46–59.

Seram, D., Mohan, S., Kennedy, J.S., Senthil, N., 2016. Development and damage assessment of the storage beetle, *Callosobruchus maculatus* (Thanjavur and Coimbatore strain) under normal and controlled conditions. In: Navarro, S., Jayas, D.S., Alagusundaram, K. (Eds.), Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), 7-11 November 2016. Hotel The Ashok Chanakyapuri, New Delhi, India, pp. 25–31.

Sharma, K.K., Singh, U.S., Sharma, P., Kumar, A., Sharma, L., 2015. Seed treatments for sustainable agriculture. A review. J. Appl. Nat. Sci. 7, 521–539.

Spochacz, M., Chowański, S., Walkowiak-Nowicka, K., Szymczak, M., Adamski, Z., 2018. Plant-derived substances used against beetles–pests of stored crops and food–and their mode of action: – A review. Compr. Rev. Food Sci. Food Saf. 17, 1339–1366.

Srinives, P., Somta, P., Somta, C., 2007. Genetics and breeding of resistance to bruchids (*Callosobruchus* spp.) in *Vigna* crops: a review. Naresuan Univ. J: Sci. Tech. 4, 1–17.

Thein, W.M., Javier, P.A., Ceballo, F.A., 2013. Insecticidal activity of crude plant extracts against *Sitophilus* spp. (Coleoptera: Curculionidae) and *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). Philippine Agric. Sci. 96, 154–162.

TNAU Agritech Portal, 2016. Seed Treatment: Insecticides & Fungicides. Retrieved: January 2016. Department of Seed Science & Technology, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India.

Vitalini, S., Orlando, F., Vaglia, V., Bocchi, S., Iriti, M., 2020. Potential role of *Lolium multiflorum* Lam. in the management of rice weeds. Plants 9, 324.

Vojvodić, M., Bažok, R., 2021. Future of insecticide seed treatment. Sustainability 13, 8792.

Wardani, N.K.S.L.A., Leliqia, N.P.E., 2021. A review of phytochemical and pharmacological studies of *Piper retrofractum* Vahl. J. Pharmaceut. Sci. Appl. 3, 40–49.

Wiwattanawanichakun, P., Ratwatthananon, A., Poonsri, W., Yooboon, T., Pluempanupat, W., Piyasaengthong, N., Nobsathian, S., Bullangpoti, V., 2018. The possibility of using isolated alkaloid compounds and crude extracts of *Piper retrofractum* (Piperaceae) as larvicidal control agents for *Culex quinquefasciatus* (Diptera: Culicidae) larvae. J. Med. Entomol. 55, 1231–1236.

Wang, J., Fan, R., Zhong, Y., Luo, H., Hao, C., 2022. Effects of Cabya (*Piper retrofractum* Vahl.) fruit developmental stage on VOCs. Foods 11, 2528.

Yaman, C., Şimşek, S., 2021. Insecticidal effect of from three *Hypericum* species extracts against *Rhyzopertha dominica, Sitophilus oryzae* and *Tribolium confusum*. Cienc Agrotecnol. 45, e001921.