Evaluation on Efficacy of *Piper nigrum* as a bio-pesticide against *Sitophilus zeamais*

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Abstract

Piper nigrum L. seed powder and oil extract was evaluated for its insecticidal property against *Sitophilus zeamais* in the laboratory. The motality count was recorded after every 24 hours and every after an hour for repellency test. For antifeedent, the grains were weighed after 15 days of introducing the pest. The bioassays for oil extract and powder of piper seed tested separately in a completely randomized design (CRD) by incorporating different concentrations into the maize with the pest. Analysis of variance (ANOVA) and means of different treatments compared at significance level ($p \leq .05$) using Bonferroni pair wise Post Hoc Test for data analysis. Different concentrations showed significantly different effect in all the bioassay tests. Mortality percentage of *S. zeamais* using oil extract at 96 hours of exposure time was 100±.0% for 5% (w/v) concentrations. The mean weight loss percent of maize was minimum (0.13±.12%) when treated with 10% (w/v) oil extract. Maximum (84.0±17.4%) mean repellency percent at concentration 10% (w/v) indicating high repellency and minimum (20.4±17.3%) at concentration 2.5% (w/v) indicating low repellency. Powder of *P. nigrum* seed exhibits 100% mortality of *S. zeamais* after 192 hours when treated with concentration 1.5 g/kg of maize. In case of antifeedant property, significantly lower weight loss of 0.91±.25% was recorded in maize treated with 1 g/kg maize compared to 2.92±.38% weight loss in control. Therefore, efficiency of pesticide properties in oil extract of *Piper nigrum* L performed better than the powder form.

Keywords: antifeedant, maize, *Piper nigrum*, repellency, *Sitophilus zeamais*, toxicity

Introduction

Maize (*Zea mays* L.) belongs to the Gramineae family and is one of the most important cereals in the world in terms of total production (Food and Agriculture Organization of United Nations, 2011), cultivated for both human consumption and animal feed. It is also an important food crop in Bhutan and one of the major components of dru-na-gu (the nine basic crops) of the country (National Biodiversity Centre, 2015).

Storage is an important aspect of food security in the developing countries (Jacob, 2014). A considerable amount of cereal grain is lost during the storage. People store their grains using traditional method where insects' infestation is prominent. Insects develop inside kernel and feeds on embryo, reducing the protein content and lowering the germination percentage of the seed. One of the most destructive insect pests is the maize storage weevil, *Sitophilus zeamais* (Motschulsky).

To control pest, application of synthetic pesticides are adopted. Use of chemical insecticides and fumigants have caused toxicity to humans and domestic animals, pesticide residues in food, environmental contamination and development of insecticide resistance (Zhu et al., 2016). Thus, it is necessary to develop alternatives for a safe and natural management, which might have no adverse effect on the environment and non-targeted animals.

Plant products and their secondary metabolites are receiving increased attention in stored product pest management (Adeyemi, 2011; Salunke, Prakash, Vishwakarma, & Maheshwari, 2009). Botanical insecticides are safer for the environment, usually can be easily processed and used by farmers and small-scale industries. Black pepper, *Piper nigrum* revealed its great potency as an effective insecticide against wide ranges of pest. The unique secondary plant compounds of *Piper* have several modes of action including contact toxicity, synergism, repellent and anti-feedant properties (Scott, Jensen, Philoge`ne, & Arnason, 2008). Thus, objective of this study was to evaluate insecticidal property of *Piper nigrum* seed powder and oil extract against *Sitophilus zeamais* in the laboratory.

Methods and Materials

Site/ study area

The study was conducted in the laboratory of College of Natural Resources, Lobesa Punakha from December 2018 to March 2019. It is located at an elevation of 1,450 masl. The area experiences an annual rainfall of about 500 mm to 1,500 mm with the temperature ranging from 5°C to 30°C.

Materials used

Dried seed of *Piper nigrum* (1 kg), maize (10 kg), maize weevils (*Sitophilus zeamais*), petroleum ether as a solvent for extraction, petri-dishes (12.5 cm diameter), conical flasks (1000 ml), B.O.D (Biological Oxygen Demand) incubator, Soxhlet extractor, electric weighing balance, muslin clothes and elastic bands were used for the experiment.

Rearing of pest Sitophilus zeamais

Three numbers of five-liter jars were filled with mixture of non-infested maize grains and flour, in which the infested maize with weevils was also mixed. All the jars were covered with muslin clothes held with the rubber band to prevent the escape of the weevils. Then they were kept in B.O.D incubator at temperature of 30 ± 3°C and relative humidity of 70 ± 5%. The weevils could multiply for one month before the experiment.

Extraction of *Piper nigrum* oil

Preparation of powder

One kg of dry seed of *P. nigrum* was dried in an oven dryer overnight (for 12 hours) and grinded to a fine powder using an electric grinder. 500 grams of powder stored in an airtight container to evaluate the efficacy of *Piper nigrum* powder as a bio-insecticide. Rest 500 grams used to extract essential oil.

Oil extraction procedure

Soxhlet extractor apparatus maintained at a temperature of 70°C for 30 minutes and 140°C for 40 minutes used to extract essential oil from powder. Petroleum ether (boiling point 60 - 80°C) was used as a solvent during the extraction. Fat percentage formula to calculate the fat/oil content of the *Piper*.

$\%\ \text{Fat} = \frac{W2-W1}{W} \times 100$, where W1 is the empty beaker weight, W2 is final weight of the beaker containing oil extract and W is the weight of the sample (*Piper nigrum*). The extracted oil had been stored in an airtight container in the refrigerator at 4°C until use. For an experimental purpose, a stock solution of 10% [w/v] was prepared by dissolving 10 grams of oil extract in 100 ml of petroleum ether.

Evaluation of toxicity and antifeedant activity of *Piper nigrum* seed oil extracts

For toxicity evaluation of *Piper nigrum* oil extract, Khani, Awang, Omar, Rahmani & Rezazadeh (2012) method was used and antifeedant activity was studied by following the method of Gomathi and Rathinam (2017) with minor modification. Laboratory bioassay conducted to evaluate the toxicity and antifeedant property of petroleum ether oil extracts of *P. nigrum* at three different concentrations. The 10% (w/v) stock solution was diluted in petroleum ether to three different concentrations.

Efficacy of oil extract evaluated at concentration 2.5%, 5%, and 10% (Hussein, Abd, Mohamed, Abdel, & Abou, 2017). For positive control group, only petroleum ether (solvent) was mixed into the maize. This was to determine whether the solvent used for extraction influences bioassay or not. The negative control group was prepared with the untreated media (maize kernel) only. All the experiments were carried out in completely randomized design (CRD).

Treatment 1 (T1): Positive control

Treatment 2 (T2): Negative control

Treatment 3 (T3): 2.5% oil extract

Treatment 4 (T4): 5% oil extract

Treatment 5 (T5): 10% oil extract

3 ml of each concentration of oil extract and petroleum ether was mixed with 120 grams of maize grain put in each of first four conical flasks. The fifth flask was not treated (negative control). To ensure uniform coverage of treatments shook the flasks well and air-dried to let the solvent evaporate completely. Then the treated maize kernels in each conical flask divided into three equal parts (40 grams each) and put in different petri dish making three replications for each treatment. Then 10 adult weevils introduced into all petri dish. All the petri dishes covered with muslin cloth held with rubber band. Then the set ups were placed in B.O.D incubator at the temperature of 28°C.

Repellency evaluation of *Piper nigrum* oil extract

An area preference test described by Khani et al. (2012) Gomathi and Rathinam (2017) was applied to evaluate repellent action of *S. zeamais* against *P. nigrum* oil extract. Whatman No. 1 filter papers (12.5 cm diameter) were cut into two parts from the centre. 1 ml of 2.5%, 5% and 10% *P. nigrum* oil extract was uniformly applied on the first part of filter paper with a micropipette. The other part (control) was treated with 1 ml of petroleum ether. Both the treated and the control part were air dried to evaporate the solvent completely. A full disc carefully remade by attaching the treated and the control part with an adhesive paper tape. Each filter paper placed in a petri dish and 10 adult weevils released in the center of each filter paper disc and covered with a lid.

Treatment 1 (T1): 2.5% oil extract

Treatment 2 (T2): 5% oil extract

Treatment 3 (T3): 10% oil extract

Evaluation of toxicity and antifeedant activity of *Piper nigrum* powder

Antifeedant activity was carried out following the method of Gomathi and Rathinam (2017) with some modification. To evaluate the efficacy of *Piper nigrum* powder as a toxic and an antifeedant agent and to determine the minimum concentration to control *weevil,* three concentrations of 0.5 gram, 1.0 gram and 1.5 gram of *P. nigrum* in 1 kg of maize grain (Issa, Afun, Mochiah, Owusuakyaw & Braimah, 2011) were evaluated. Each concentration mixed with 1 kg of maize grains in a beaker and tumbled for two minutes for uniform distribution of the powder. For control, maize was not treated. From the stock treated maize, weighted 40 grams, and put in a petri dish. Then each petri dish introduced with ten adult weevils and allowed to feed there. The petri dishes were covered with muslin cloth held with rubber band. All the set ups were placed in a B.O.D incubator at the temperature 28°C.

Treatment 1 (T1): control

Treatment 2 (T2): 0.5 g/1 kg maize

Treatment 3 (T3): 1.0 g/ 1 kg maize

Treatment 4 (T4): 1.5 g/ 1 kg maize

The toxicity, antifeedant and repellent experiments for oil extract and powder had three replications for every treatment. To ensure accuracy of the data, experiments repeated for three times consecutively. However, due to unavailability of kaolin powder (white clay) to aromatize the Piper nigrum extract in the given duration of the study. The repellency test of powdered form was excluded from the experiment.

Data collection

Toxicity and antifeedant properties of *Piper nigrum* seed oil extract

Weighted maize in all petri dishes before introducing pest and recorded as an initial weight. The mortality count recorded after every 24 hours exposure of weevils to the treatments until all the treatments reached 100% mortality except for control groups. After 15 days of introducing weevils to the treatments, weighed again to measure weight loss of maize due to feeding of weevils. The percentage of weight loss calculated using the formula:

$\% \text{ WL} = \frac{\text{IW}-\text{FW}}{\text{IW}} \times 100$, where the IW is the initial weight and FW is the final weight of maize.

Repellency of *Piper nigrum* seed oil extract

Number of insect present on the control (NC) and the treated (NT) strips recorded after 1, 2, 3, 4 and 12 hours of set up. Percent repellency (PR) values then computed using Uzakah formula:

$\text{PR} = \frac{\text{NC}-\text{NT}}{\text{NC}+\text{NT}} \times 100$. The strength of different concentration of oil extract was interpreted by following the scale given by Talukder & Howse (1994) as shown in Table 1.

Table 1 Interpretation of PRs (Talukder & Howse, 1994)

Class	Repellence rate (%)	Interpretation
0	> 0.01 to < 0.1	Non repellent
I	0.1 to 20	Very low repellence
II	20.1 to 40	Low repellence
III	40.1 to 60	Moderately repellent
IV	60.1 to 80	Repellent
V	80.1 to 100	Very repellent

Toxicity and antifeedant property of *Piper nigrum* powder

Initial weight of maize in all petri dishes weighted. Mortality count recorded every after 24 hours exposure of weevils to the treatments until 100% mortality reached by all treatments except control groups. After 15 days of feeding period, grain in each petri dish weighed to compute the weight loss. The percentage of weight loss calculated by the formula: $\%WL = \frac{IW-FW}{IW} \times 100$, where the IW is the initial weight and FW is the final weight.

Data analysis

Data were entered into Microsoft Excel spreadsheet. The analysis was carried using R Statistical Software. Compared the means of different treatments in antifeedent test with One-way Analysis of Variance. Two-way ANOVA performed to study interaction between the treatment and time on mortality. We also determined the effect size of independent variables on dependent variables. Bonferroni pair wise post hoc test used to evaluate means of different treatments at .05 significance level ($p \leq .05$).

Results

Toxicity of *Piper nigrum* seed oil extract

Mortality of *Sitophilus zeamais* depends on the concentration of *Piper nigrum* oil and exposure time of *S. zeamais* to the treatments. 10% (w/v) was significantly more effective with mortality rate of 58.9±15.4% compared to positive and negative control having mortality rate of .0±0% and 1.1±3.3% respectively after 24 hours of treatment (Table 2). However, there was no significant difference between postitive and negative control (p = 1). This indicates that solvent (petroleum ether) used in extraction of oil does not have any effect on mortality of the pest. It was noted that there was not much difference between 5% (w/v) and 10% (w/v). Both the treatments achieved 100.0±.0% mortality at 96 hours of exposure to the treatments. Therefore, 100% mortality of *S. zeamais* can be achieved by 5% (w/v) concentration of *P. nigrum* oil extract at 96 hours. Increasing the concentration beyond 5% indicates wastage of resources.

Table 2 Mean mortality (%) of *S. zeamais* exposed to *P. nigrum* oil extract

Treatment	Mortality (%) after indicated hours				
	24	48	72	96	120
Positive control (T1)	.0^{c}±0	3.33^{c}±5.0	6.7^{c}±7.1	7.8^{b}±6.7	7.8^{b}±6.7
Negative control (T2)	1.1^{c}±3.3	2.2^{c}±4.4	6.7^{c}±5.0	7.8^{b}±4.4	7.8^{b}±4.4
2.5% (T3)	20.0^{b}±13.2	46.7^{b}±21.2	70.0^{b}±18.7	92.2^{a}±8.3	100.0^{a}±.0
5% (T4)	30.0^{b}±15.0	66.7^{ab}±18.7	84.4^{b}±15.1	100.0^{a}±.0	100.0^{a}±.0
10% (T5)	58.9^{a}±15.4	88.9^{a}±10.5	98.9^{a}±3.3	100.0^{a}±.0	100.0^{a}±.0

* Each value is a mean ± standard deviation of nine replicates. Mean followed by the same letter in the column are not significantly different ($p \leq .05$) from each other using Bonferroni pair wise comparison test.

Toxicity of *Piper nigrum* seed powder against *Sitophilus zeamais*

Piper nigrum powder also exhibited the same effect on mortality like the oil extract. As shown in Table 3 that 0.5 g, 1 g and 1.5 g of *Piper nigrum* powder in 1 kg of maize are significantly different from 0.5g/kg but there is no significant difference among them. At 192 hours of exposure, the mortality was only 6.7±7.1% for control whereas it was 91.1±9.3%, 95.6±7.3% and 100.0±.0% for 0.5 g, 1 g and 1.5 g/kg maize respectively. Exposure time had a significant effect on the mortality. Mortality rate increases with the increase in exposure time to the treatments (Table 3). Interaction of concentration and exposure time had significant effect on the mortality rate. The result shows that 1.5 g/kg maize is suitable concentration to achieve 100 % mortality at 192 hours. The experiment also observed death of few pests in the control treatment over period of time. This could be due to natural death from injuries. Mortality percentage (7.8±6.7) at 216 hours in 0.5 g/kg can be used as a baseline to indicate that mortality in all treatment for powder form is due to natural death and remaining from insectidal effect of *Piper nigrum*.

Table 3 Mean mortality (%) of *S. zeamais* exposed to diff. conc. of *P. nigrum* powder

Treatment (g/kg maize)	Mortality (%) after indicated hours								
	24	48	72	96	120	144	168	192	216
Control (T1)	$.0^{a}$±.0	2.2^{b}±4.4	2.2^{b}±4.4	2.2^{b}±4.4	3.3^{c}±5.0	4.5^{c}±5.3	5.6^{b}±5.3	6.7^{b}± 7.1	7.8^{b}±6.7
0.5 (T2)	4.4^{a}± 5.3	16.7^{ab} ±14.1	31.1^{a} ±10.5	46.7^{a} ±10.1	55.6^{b} ±11.3	68.9^{b} ±15.4	78.9^{a} ±16.9	91.1^{a} ±9.3	97.8^{a} ±4.4
1 (T3)	10.0^{a} ±14.1	21.1^{a} ±10.5	35.6^{a} ±12.4	48.9^{a} ±11.7	66.7^{ab} ±16.6	78.9^{ab} ±15.4	88.9^{a} ±12.7	95.6^{a} ±7.3	100.0^{a}±.0
1.5 (T4)	10.0^{a} ±15.8	21.1^{a} ±16.9	37.8^{a} ±25.9	58.9^{a} ±19.7	76.7^{a} ±14.1	88.9^{a} ±10.5	94.4^{a} ±8.8	100.0^{a}±.0	100.0^{a}±.0

* Each value is a mean ± standard deviation of nine replicates. Mean followed by the same letter in the column are not significantly different ($p \leq .05$) from each other using Bonferroni pair wise comparison test.

Antifeedant property of *Piper nigrum* seed oil extract

This study revealed that *P. nigrum* oil extract is effective as an antifeedant agent against *S. zeamais.* There was no significant difference between positive and negative control (p = 1). This shows that solvent (petroleum ether) used in positive control does not have any effect on deterring the pest feeding. The highest weight loss of 2.58±.14% was noted in negative control (untreated maize) (Figure 1). This indicates that in the absence of any control measures, post-harvest losses due to *S. zeamais* during storage can be severe. Weight loss in maize was significantly lower (0.13±.12%) when the maize was treated with 10 % (w/v) followed by 5 % (w/v) concentration of oil extract with 1.12±.45 % mean weight loss (Figure 1). There was no significant difference between treatment with 10 % and 5 %. Therefore, treatment with 5 % concentration is effective as antifeedent to protect *S. zeamais* in the storage.

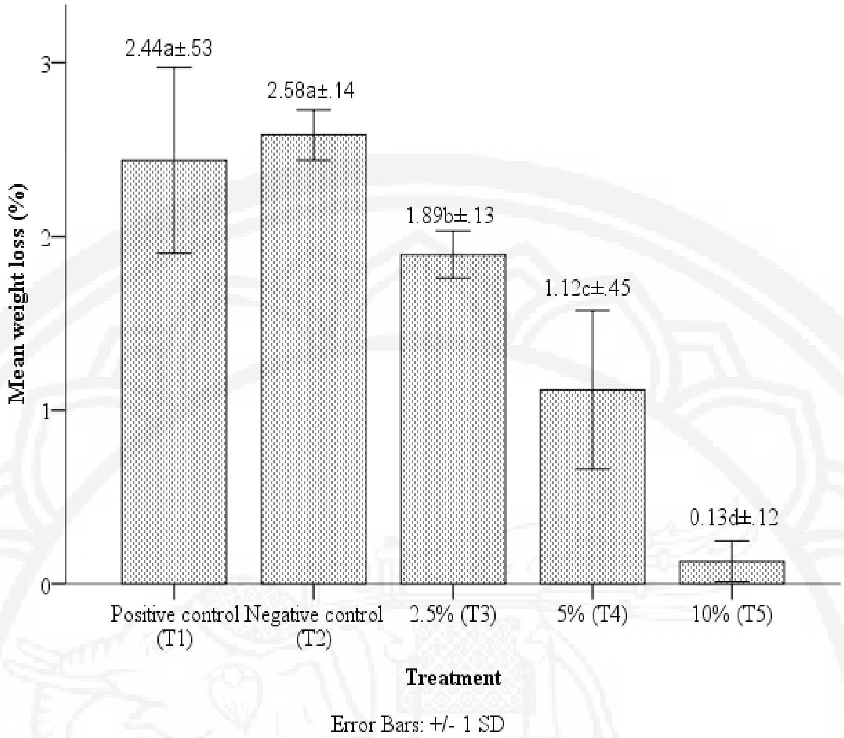

Figure 1 Mean weight loss (%) of maize treated with different concentration of oil extract. Different lowercase letters indicate that the treatments are significantly different by Bonferroni pair-wise comparison test at $p \leq .05$

Antifeedant property of *Piper nigrum* seed powder

There was significant difference in the weight loss of maize among the treatments. Higher the concentration of powder, lower is the reduction in weight loss of maize (Figure 2). The lowest weight loss of 0.46±.30% was recorded in 1.5 g/kg maize. This is because when the maize grains are treated with *P. nigrum* seed powder, the pest could not feed on the grain. Within 15 days of pest feeding, maximum of 2.92±.38% weight loss of maize was recorded in control where no powder was used. The result indicates that, huge grain loss is caused when no protectants are used. Since there is no significant difference between 1 g and 1.5 g of *P. nigrum* seed powder in 1 kg of maize. For the antifeedent test 1 g/kg maize of *P. nigrum* seed powder is adequate to protect maize grains from *S. zeamais.*

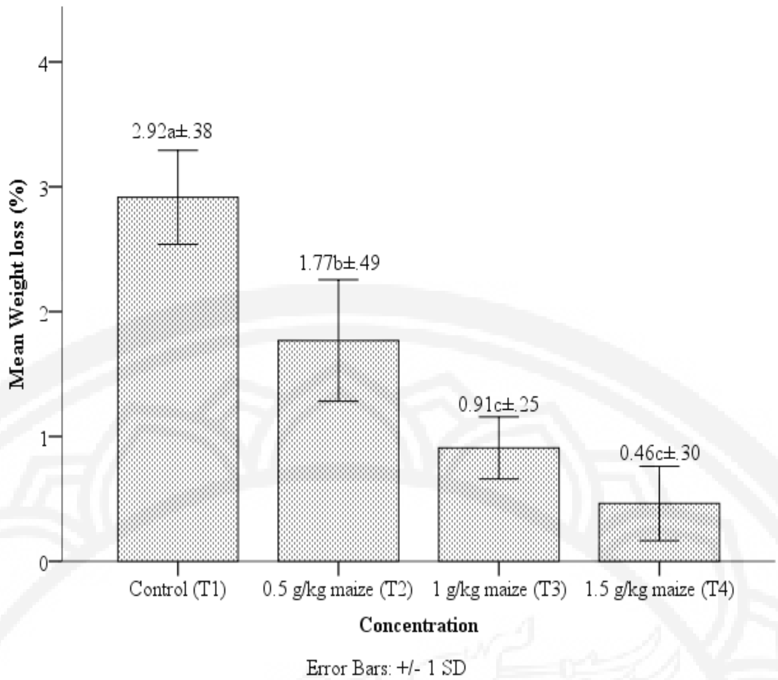

Figure 2 Mean weight loss (%) of maize treated with different conc. of *P. nigrum* powder. Different lowercase letters indicated that the treatments are significantly different by Bonferroni pair-wise comparison test at $p \leq .05$

Repellent property of *Piper nigrum* seed oil extract

There was a significant difference in the repellency percent among different concentrations. Interpretation of repellency percent (Table 1) by Talukder and Howse was followed to classify the repellency of different concentrations. Overall mean repellency rate for 10 % (w/v) was 84.0±17.4 %, which indicates that *S. zeamais* is very repellent to this concentration. Whereas the mean repellency percentage of 2.5 % and 5 % was 20.4±17.3 % and 39.1±27.0 % respectively, indicating that they had low repellent effects on *S. zeamais* (Table 4). Therefore, 10 % (w/v) concentration of *P. nigrum* seed oil is required to be effective against *S. zeamais.*

Table 4 Mean repellency (%) after indicated exposure time

Concentration (w/v)	Average repellency rate at indicated hours					Mean repellency rate (%)
	1	2	3	4	12	
2.50% (T1)	8.9^{b}±10.5	13.3^{b}±17.3	15.6^{b}±13.3	22.2^{b}±12.0	42.2^{c}±12.0	20.4^{c}±17.3
5% (T2)	15.6^{b}±19.4	33.3^{b}±17.3	40.0^{b}±31.6	42.2^{b}±21.1	64.4^{b}±21.9	39.1^{b}±27.0
10% (T3)	75.6^{a}±21.9	80.0^{a}±20.0	88.9^{a}±14.5	84.4^{a}±16.7	91.1^{a}±10.5	84.0^{a}±17.4

* Each value is a mean ± standard deviation of nine replicates. Mean followed by the same letter in a column are not significantly different ($p \leq .05$) from each other using Bonferroni pair wise comparison test.

Discussion

The findings on 100% mortality of *S. zeamais* to exposure at 5% and 10% (w/v) concentrations of *P. nigrum* seed oil extract at 96 hours found to be similar to Hussein et al. (2017). They found that the maximum mortality of 95.5% and 100% at concentration of 1.25% and 2.5% (w/w) respectively after 21 days of treatment, whereas 100% mortality at 5% (w/w) concentration was observed after 14 days and 100% mortality at 10% (w/w) after 10 days of treatment. However, finding from this study was comparatively less effective than the result of Awoyinka, Oyewole, Amos, and Onasoga (2006). Their result revealed that when 0.298 mg/ml (0.0298%) of the extract applied on the *S. zeamias*, 100% mortality was achieved at 135 minutes after exposure. Different solvent used and extraction duration had influenced the properties of active components in the oil that alters its efficacy.

Toxicity effect of *P. nigrum* powder showed same effect on mortality of pest but took longer duration. Increase in the concentration and exposure time, increased the mortality of the pest. Concentration of powder form at 1g/kg maize and 1.5g/kg maize killed 100% of the pest introduced in the maize at 192 hours of exposure. Similar observation on efficacy of *P. nigrum* powder on *S. granarius* and *R. dominica* was also reported by Ashouri and Shayesteh in 2010. It was reported that, *P. nigrum* at 0.5% (0.5 g/100 g) concentration caused 100% mortality of *S. granarius* in the first five days and *R. dominica* showed complete mortality at 5% (5 g/100 g) level after 14 days. Mahdi and Rahman (2008) also reported comparatively similar findings that at the dose rate of 25 g of *P. nigrum* per kg of maize and 30 g/kg, *Callosobruchus maculates* takes 3 and 2.33 days respectively to reach 100% mortality.

Similarly, antifeedant test showed significantly lower weight loss (0.13±.12%) when the maize was treated with 10% concentration of oil extract. Tadele, Stephen and Paddy (2011) with similar results, supported the findings. Their research reported mean weight loss of 6.9% in maize after 90 days of feeding by *S. zeamais* without any treatment. Similar effect reported in case of *S. oryzae.* Minimum weight loss of 0.1% after 7 days of feeding by *S. oryzae* was reported in rice when 8 μl of petroleum ether extract of *P. nigrum* per gram of rice was used (Khani et al., 2012).

Even the powder form of *P. nigrum* showed effectiveness with minimum (0.46±.30%) weight loss when treated with 1.5g/kg maize. Maximum (2.92±.38%) weight loss of maize recorded in treatment with no powder. Tadele et al. (2011) also found out that 4.2±0.2% of grain damage was caused when *S. zeamais* feed on 200 g of maize for 30 days. Mahdi and Rahman (2008) also reported a similar finding that *C. maculates* causes only 2.2% weight loss of black gram seed when 30 g of P. nigrum powder per kg of black gram seed was used. The pests die due to starvation as well as because of the toxicity of the *P. nigrum* seed powder on the *S. zeamais.*

The repellency activities were more dependent on the concentration than on exposure time. Repellency increases with the increase in the concentration of oil extract. The overall mean repellency rate (84.0±17.4%) for 10% concentration showed very repellent property of *P. nigrum* oil extract. While 2.5% showed low (20.4±17.3%) repellency effect on *S. zeamais.* Khani et al. (2012) reported that the mean repellency percent of an acetone extract of *P. nigrum* at the concentration of 5% was 92% against *S. oryzae.* The highest repellency percent (PR) of 95.40±1.84% was recorded at 0.8% of *P. nigrum* essential oil after four hours when acetone is used as a solvent

(Chaubey, 2017). Comparison of results noted that their test was significantly effective. Therefore, the acetone used as a solvent in the extraction is having a synergism effect on the insecticidal property of *P. nigrum* oil.

Conclusion and Suggestions

Results of this study have shown that *Piper nigrum* is found to control *S. zeamais*. In this study, the toxicity was evaluated in terms of the mortality rate at increasing exposure time using different concentration of powder and oil extract separately. The mortality increased with increasing concentration and exposure time. Though the oil extract was found to be more effective in controlling the pest, yet at farmer's field, application of powder is more affordable and easy at all level.

There are some species of Piper growing wild in Bhutan. Other species of Piper could be tested for their insecticidal property against different pest of stored grains. Due to variation in the bio-chemical composition, different results will be obtained. Therefore, there is a need to study the composition of the plant. With time both the internal and external property of plants and their derivatives deteriorates. So there is a chance that the pesticidal property of *P. nigrum* may decrease with increasing storage duration. Hence, change in the property and strength needs to be studied.

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References

Adeyemi, M. M. H. (2011). A review of secondary metabolites from plant materials for post-harvest storage. *International Journal of Pure and Applied Science Technology*, *6(2)*, 94-102. Retrieved from https://www.ijsr.net/

Ashouri, S., & Shayesteh, N. (2010). Insecticidal activities of two powdered spices, black pepper, and red pepper on adults of *Rhyzopertha dominica* (F.) and *Aitophilus granarius* (L.). *Munis Entomology and Zoology*, 5*(2)*, 600-607. Retrieved from https://www.munisentzool.org/

Awoyinka, O. A., Oyewole, I. O., Amos, B. M. W., & Onasoga, O. F. (2006). Comparative pesticidal activity of dichloromethane extracts of *Piper nigrum* against *Sitophilus zeamais* and *Callosobruchus maculates. African Journal of Biotechnology, 5(24)*, 2446-2449. Retrieved from https://www.ajol.info/index.php/ajb/index

Chaubey, M. K. (2017). Evaluation of insecticidal properties of *Cuminum cyminum* and *Piper nigrum* essential oils against *Sitophilus zeamais. Journal of Entomology, 14*, 148-154. http://dx.doi.org/10.3923/je.2017.148.154

Food and Agriculture Organization of United Nations. (2011). *Crop information: Maize.* Retrieved from http://www.fao.org/land-water/databases-and-software/crop-information/maize/en/

Gomathi, S., & Rathinam, K. M. S. (2017). Evaluation of repellency and antifeedant activities of *Saraca asoca* and *Terminalia arjuna* Bark extracts against *Sitophilus oryzae. International Journal of Zoology Studies, 2(6)*, 282-285. Retrieved from http://www.zoologyjournals.com/

Mahdi, S. H. A., & Rahman, M. K. (2008). Insecticidal effect of some spices on *Callosobruchus maculatus* (Fabricius) in black gram seeds. *University Journal of zoology, 27*, 47-50. https://doi.org/10.3329/ujzru.v27i0.1953

Hussein, A. E., Abd, E. H., Mohamed, R. A., Abdel, M. M., & Abou, E. Z. (2017). Toxicity of three chemical extracts of black pepper fruits against two stored grain insect pests. *International Journal of Pharmaceutical Science Invention, 6* (10), 20-29. Retrieved from http://www.ijpsi.org/

Issa, U. S., Afun, J. V. K., Mochiah, M. B., Owusuakyaw, M., & Braimah, H. (2011). Effect of some local botanical materials for the suppression of the maize weevil S*itophilus zeamais* (motschulsky) (coleoptera: Curculionidae) populations. *International Journal of Plant, Animal, and Environmental Sciences, 1*(3), 270-275. Retrieved from www.ijpaes.com

Jacob, L.D. (2014). *Potentials of Fractionated Extracts of Ocimum canum (Lamiaceae) and Laggera pterodonta (Compositae) for the Protection of Maize against the Infestation of Sitophilus zeamais (Coleoptera: Curculionidae).* Retrieved from https://www.researchgate.net/publication/319289140_Potentials_of_Fractionated_Extracts_of_Ocimum_canum_Lamiaceae_and_Laggera_pterodonta_Compositae_for_the_Protection_of_Maize_against_the_Infestation_of_Sitophilus_zeamais_Coleoptera_Curculionidae

Khani, M., Awang, R. M., Omar, D., Rahmani, M., & Rezazadeh, S. (2012). Bioactivity effect of *Piper nigrum* L. and *Jatropha curcas* L. extracts against *Corcyra cephalonica* [Stainton]. *Agrotechnology, 2,* 1-6. http://dx.doi.org/10.4172/2168-9881.1000105

National Biodiversity Centre. (2015). *The history of the introduction and adoption of important food crops in Bhutan: Rice, maize, potato, and chili.* Retrieved from http://nbc.gov.bt/wp-content/uploads/2010/06/History-of-the-introduction-adoption.pdf

Salunke, B. K., Prakash, K., Vishwakarma, K. S., & Maheshwari, V. L. (2009). Plant metabolites: an alternative and sustainable approach towards post-harvest pest management in pulses. *Physiology and Molecular Biology of Plants, 15,* 185-197. https://doi.org/10.1007/s12298-009-0023-9

Scott, I. M., Jensen, H. R., Philoge`ne, B. J. R., & Arnason, J. T. (2008). A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. *Journal of Phytochemistry Reviews, 7*, 65-75. DOI: 10.1007/s11101-006-9058-5.

Talukder, F. A., & Howse, P. E. (1994). Laboratory evaluation of toxic and repellent properties of the pithraj tree, *Aphanamixis polystachya* Wall & Parker, against *Sitophilus oryzae* (L.). *International Journal of Pest Management, 40*, 274-279. https://doi.org/10.1080/09670879409371897.

Tadele, T., Stephen, M., & Paddy L. (2011). Effects of insect population density and storage time on grain damage and weight loss in maize due to the maize weevil *Sitophilus zeamais* and the larger grain borer *Prostephanus truncates. African Journal of Agricultural Research, 6*, 2249-2254. https://doi.org/10.5897/AJAR11.179

Zhu, F., Lavine, L. C., O'Neal, S., Lavine, M., Foss, C., & Walsh, D. (2016). Insecticide resistance and management strategies in urban ecosystems. *Insects, 7*, 1-26. DOI: 10.3390/insects7010002