

Efficacy of different extracts of *Allium chinense* bulb against *Sitotroga cerealella* infestation in stored paddy grain, *Oryza sativa*



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Abstract

Stored grain pest *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) is considered as one of the most destructive pests of cereal grains. The study aim was to develop methods to suppress moth emergence and reduce the weight loss of paddy grain (*Oryza sativa* L.) in storage using bulb extracts of *Allium chinense* G.Don from solvents of varying polarity (hexane, petroleum ether, chloroform, methanol, ethanol, and water). The results showed that all of the tested extracts had significant effect on the developmental period and moth emergence. The petroleum ether extract with LC₅₀ value of 0.39% (0.16 to 0.62%) and ethanol extract with LC₅₀ of 0.45% (0.21 to 0.68%) exhibited remarkable activity against *S. cerealella*, as no moth emergence was observed when applied at 2% concentration. For the chloroform extract, 2% concentration showed the highest inhibition on the developmental stage, where moths emerged at 42.50 days, compared to 28.75 days in the control. The weight loss of paddy was best controlled with 2% ethanol extract (2.55%); the highest loss was recorded in paddy treated with water extract. The present study concludes that petroleum ether and ethanol extract of bulbs of *A. chinense* have potential use in integrated pest management of *S. cerealella* for post-harvest protection of paddy, considering availability and easy propagation of the plant, and environmental friendliness.

Key words: *Allium chinense*, bulb extract, mortality, paddy (*Oryza sativa*), *Sitotroga cerealella*.

Abbreviations: LCL, lower confidence limit; UCL, upper confidence limit.

Introduction

Food security has been defined as the ability of food deficit countries or regions within states to meet the target consumption level on a year-to-year basis (Valdes, Siamwalla 1981). Control of post-harvest loss would surge food availability, eliminate hunger and improve farmer's livelihoods. The management of pests in the field and storage is vital for food security. The stored grain pest, *Sitotroga cerealella* (Olivier), is also known as Angoumois grain moth and is considered one of the main storage grain pests in the Nagaland state of North-East India, where farmers usually use traditional storage structures (Jamir et al. 2013). Plant infestation by *S. cerealella* begins in the field before harvest, proceeds up to grain storage and increases with increasing moisture content (Bushra et al. 2013). The pest feeds on the superficial layers of the grains, decreasing its nutritional value and weight of the grain (Sedlacek et al. 2001). The loss of stored grain caused by *S. cerealella* in certain regions accounts for over 40% (Boshra 2007). The heavy rainfall

in Nagaland also provides a moist environment; moisture content increases the risk of infestation by *S. cerealella* (Butron et al. 2008) causing extensive damage to grains by larval feeding, which completes its developmental stages within the grain; the grain acts as additional protection from direct contact with insecticides (Weston, Rattlingourd 2000). Hence, *S. cerealella* is best controlled in the egg and larval stage immediately after hatching, before penetrating the grain.

Plant extracts can be used as a beneficial pest control substance. These extracts are natural means that have the advantage of delivering a novel mode of action, decreasing the risk of cross-resistance (Liu et al. 2014). Biological methods are safe, environment friendly and cost-effective (Mossa et al 2016). Screening of locally available plants with repellent, antifeedant and insecticidal properties as potential alternatives to synthetic or chemical insecticides are gaining importance (Shankar et al. 2013). Species of the genus *Allium* are known for their sulphur-containing secondary metabolites, synthesised following external

attacks by pathogens and pests (Nwachukwu et al. 2012).

Allium chinense G.Don is a medicinal and aromatic plant cultivated in the North-Eastern part of India. The juice of this plant has moth repelling properties (Brewster 2008) and the essential oil extract of the bulb has fumigant and contact toxicity against *Liposcelis bostrychophila* (Liu et al. 2014). Thirty-four organo-sulphur containing compounds account for 94% of the amount of total volatiles present in essential oil from the leaf (Pino et al. 2001). In particular, allyl methyl disulphide is toxic to *Tribolium castaneum* adults by significantly inhibiting growth (Huang et al. 2000), and dimethyl disulphide possesses contact and fumigant toxicity against booklice (Liu et al. 2014). Sulphide containing compounds like diallyl trisulphide and diallyl disulphide extracted from the essential oil of *Allium sativum* causes behavioural deterrence, oviposition inhibition and fumigant toxicity in *S. cerealella* (Yang et al. 2012). Diallyl trisulphide controls the transcriptional expression of *ScerCHSA*, gene, causing alteration at a histological and morphological level in *S. cerealella*, inhibiting the oviposition of the moth (Nwachukwu, Asawalam 2014). In addition, the plant powder from *Aristolochia ringens* has high insecticidal activity against *S. cerealella* (Ashamo, Akinawonu 2012).

A literature survey showed that previous work on *A. chinense* was mostly focused on essential oil activity, while the crude extracts, which are cost-effective compared to essential oil, have been overlooked. To the best of our knowledge, there are no reports on the insecticidal activity of bulb extracts of *A. chinense*. In this context, the present study was undertaken to have a better understanding of the bio-efficacy of *A. chinense* bulb extracts as an insecticide to inhibit egg hatchability, inhibition of larvae in the developmental stages, and prevention of moth emergence.

Materials and methods

Collection of plant material

Fresh bulbs of *A. chinense* were collected in July 2018 from agriculture fields in Viswema village (25.5615°N, 94.1450°E) Kohima district, Nagaland, India. The plant was identified by Dr. V. Rama Rao, Regional Ayurvedic Research Institute for Metabolic Disorders (Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India). A specimen was submitted in the Medicinal Plant Herbarium at the Botany Department, Bangalore University (voucher No. RRCBI-mus 244). The bulbs were washed with distilled water and dried in the shade until constant weight. Plant material was further ground to a fine powder by using an electric grinder and sieved using a 200-mesh sieve.

Extraction

Twenty grams of powdered *A. chinense* bulb was extracted separately using a Soxhlet extractor with 200 mL of either hexane, petroleum ether, chloroform, methanol, ethanol,

or distilled water (Rhetso et al. 2020). The extract was separated from the solvent using a rotary evaporator at 30 to 40 °C for non-polar solvents and 40 to 50 °C for polar solvents. After extraction, stock solutions of 0.5, 1 and 2% concentration of the extracts were prepared using the same solvent used for extraction, for further experimental use.

Insect culture and collection of eggs

Eggs of *S. cerealella* were obtained from the Division of Entomology, Indian Agricultural Research Institute, New Delhi in July 2018. An incubator was used to maintain the insect culture at 25 ± 2 °C temperature and $70 \pm 5\%$ relative humidity. The insects were reared in 1 L bottles covered with muslin cloth with 250 g of disinfected unbroken paddy (crude rice grain). The oviposition substrate consisted of a smooth black cardboard folded at intervals and inserted into the culture bottle; the eggs of *S. cerealella* were collected using a brush for further experimentation (Joshi et al. 2013).

Effect of bulb extract on the developmental stage and moth emergence of *S. cerealella*

Twenty grams of paddy grain were weighed and placed in a 250 mL bottle and further mixed with 5 mL of the extract at each concentration (0.5, 1 and 2%), using a glass rod to ensure equal spread on the grain. The bottles were left in an oven at 60 °C for solvent evaporation for 12 h. Paddy grain (20 g) treated with 5 mL of the solvent (water, ethanol, methanol, chloroform, petroleum ether, and hexane) and 20 g of untreated paddy grain were used as controls.

Twenty eggs (0 to 24 h old) of *S. cerealella* were introduced into each bottle. The bottles were covered with a muslin cloth, and left for 20 days for moth emergence. The day of moth emergence and the total number of moths emerged were recorded, and the percentage of mortality was calculated. From the 20th day onwards, the bottles were checked daily for moth emergence until no moth emergence was observed for 10 consecutive days. The experiment was then ceased. Finally, the grains were weighed for each treatment and percentage weight loss was calculated with the following formula:

$$[(W_1 - W_2) \div W_1] \times 100,$$

where W_1 and W_2 represent weight of paddy seed before and after the experiment, respectively. All experiments were performed in four replicates.

Statistical analysis

The data obtained in this study were analysed by one-way analysis of variance (ANOVA) at 5% probability. The significant difference between different concentrations of extracts was evaluated using Tukey's multiple comparisons test in Prism V. 5.00 (Graphpad Inc. USA). Probit analysis was used for calculating the LC_{50} , 95% lower and upper confidence limits (LCL – UCL) (Finney 1971). Corrected mortality was calculated using the Abbott (1925) method.

Table 1. Time of moth emergence, moth mortality and weight loss of paddy seed infested with *Sitotroga cerealella* and treated with ethanol extract of *Allium chinense* bulbs. Data are means \pm SE. Means with identical letters within the column are not statistically significantly different. LCL, lower confidence limit; UCL, upper confidence limit

Treatment, concentration (%)	Time of moth emergence (days)	Mortality (%)	Efficacy (corrected mortality)	Weight loss (%)
Control	28.75 \pm 0.47 a	18.75 \pm 0.47 a		36.65 \pm 0.13 a
Ethanol control	29.75 \pm 1.03 a	20.00 \pm 0.40 a	1.54	36.32 \pm 0.13 a
0.5	33.50 \pm 0.64 b	62.50 \pm 0.64 b	53.85	17.42 \pm 0.56b
1.0	33.75 \pm 0.47 b	81.25 \pm 0.47 c	76.92	6.24 \pm 0.41 c
2.0	0.00 \pm 0.00 c	100.00 \pm 0.00 d	100.00	2.55 \pm 0.39 d
F	522.80	254.30		1910.00
P	< 0.0001	< 0.0001		< 0.0001
LC ₅₀ (LCL – UCL)		0.45 (0.21 – 0.68)		

Results

Effect of bulb extracts on the moth emergence of *S. cerealella*

The efficacy of the six extracts with different solvents in inhibiting the developmental stages of *S. cerealella* was evaluated by recording the emergence from egg to adult stage in terms of number of days. The larval stage and pupal stages occurred within the grains. Effects of treatment with solvent alone and control were similar without any significant difference (Tables 1 to 6). The greatest effect was obtained using 2% ethanol and 2% petroleum ether extracts. These treatments showed 100% moth control and no moth emergence (Tables 1 and 2). Chloroform extract at 2% concentration was found to be effective in prolonging the developmental stage of moth emergence (day 42.50 \pm 0.28), in comparison with emergence in the control (day 28.75 \pm 0.47) (Table 3). The methanol and hexane extracts showed inhibition, but did not cause complete mortality (Tables 4 and 5). There was no significant difference between effects of the aqueous extract on moth emergence, compared to the control (Table 6).

Effect of bulb extracts on the mortality of *S. cerealella*

The analysis of mean mortality using one-way ANOVA

showed that effect of all treatments significantly differed from control and solvent alone. Mortality increased with higher extract concentration. Among the extracts, petroleum ether showed the highest potency to kill 50% of *S. cerealella*, with LC₅₀ value of 0.395% (0.16 to 0.62%), followed by ethanol extract with LC₅₀ 0.45% (0.21 to 0.68%) (Tables 1 and 2). In treatments with ethanol and petroleum ether extracts, complete moth control was observed at 2% concentration. In chloroform and hexane extract (Table 3 and 5), 2% concentration was effective in decreasing number of moths. In methanol and aqueous extract, 1% concentration was equally effective as 2% concentration on moth mortality (Table 4 and 6). However, the lowest mortality was observed in the extract treatment.

Effect of bulb extracts on the weight loss of infested paddy

There was no significant difference in weight loss of infested paddy between control and solvent treatments. The maximum mean weight loss of paddy was recorded for the aqueous treatment, as the mortality of *S. cerealella* was very poor (Table 6); ethanol extract treatment showed highest effect, with minimal weight loss of paddy seed (Table 1). Weight loss was best controlled in 2% ethanol extract, with 2.55 \pm 0.39% loss, while maximum loss was observed in the control, with 36.65 \pm 0.13% due to high infestation.

Table 2. Time of moth emergence, moth mortality and weight loss of paddy seed infested with *Sitotroga cerealella* and treated with petroleum ether extract of *Allium chinense* bulbs. Data are means \pm SE. Means with identical letters within the column are not statistically significantly different. LCL, lower confidence limit; UCL, upper confidence limit

Treatment, concentration (%)	Time of moth emergence (days)	Mortality (%)	Efficacy (corrected mortality)	Weight loss (%)
Control	28.75 \pm 0.47 a	18.75 \pm 0.47 a		36.65 \pm 0.13 a
Petroleum ether control	29.75 \pm 0.47 a	20.00 \pm 0.37 a	1.54	36.11 \pm 0.93 a
0.5	40.00 \pm 0.40 b	65.00 \pm 0.40 b	56.92	11.10 \pm 0.35 b
1.0	41.25 \pm 0.47 b	80.00 \pm 0.40 c	75.38	5.50 \pm 0.25 c
2.0	0.00 \pm 0.00 c	100.00 \pm 0.00 d	100.00	4.02 \pm 0.25 c
F	1620.00	361.60		1235.00
P	< 0.0001	< 0.0001		< 0.0001
LC ₅₀ (LCL – UCL)		0.39 (0.16 – 0.62)		

Table 3. Time of moth emergence, moth mortality and weight loss of paddy seed infested with *Sitotroga cerealella* and treated with chloroform extract of *Allium chinense* bulbs. Data are means \pm SE. Means with identical letters within the column are not statistically significantly different. LCL, lower confidence limit; UCL, upper confidence limit

Treatment, concentration (%)	Time of moth emergence (days)	Mortality (%)	Efficacy (corrected mortality)	Weight loss (%)
Control	28.75 \pm 0.47 a	18.75 \pm 0.47 a		36.65 \pm 0.13 a
Chloroform control	29.50 \pm 0.64 a	25.00 \pm 0.40 a	7.69	36.36 \pm 0.42 a
0.5	40.00 \pm 0.40 b	55.00 \pm 0.40 b	44.62	21.16 \pm 0.10 b
1.0	40.25 \pm 0.75 bc	71.25 \pm 0.47 c	64.62	15.77 \pm 0.50 c
2.0	42.50 \pm 0.28 c	87.50 \pm 0.28 d	84.62	7.10 \pm 0.85 d
F	146.50	198.50		710.80
P	< 0.0001	< 0.0001		< 0.0001
LC ₅₀ (LCL – UCL)		0.60 (0.28– 0.92)		

Table 4. Time of moth emergence, moth mortality and weight loss of paddy seed infested with *Sitotroga cerealella* and treated with methanol extract of *Allium chinense* bulbs. Data are means \pm SE. Means with identical letters within the column are not statistically significantly different. LCL, lower confidence limit; UCL, upper confidence limit

Treatment, concentration (%)	Time of moth emergence (days)	Mortality (%)	Efficacy (corrected mortality)	Weight loss (%)
Control	28.75 \pm 0.47 a	18.75 \pm 0.47 a		36.65 \pm 0.13 a
Methanol control	29.00 \pm 0.40 ab	23.75 \pm 0.25 a	6.15	36.45 \pm 0.07 a
0.5	29.75 \pm 0.75 ab	48.75 \pm 0.62 b	36.92	28.58 \pm 0.90 b
1.0	31.25 \pm 0.62 b	60.00 \pm 0.40 c	50.77	22.87 \pm 0.54 c
2.0	33.75 \pm 0.47 c	68.75 \pm 0.47 c	61.54	14.88 \pm 0.46 d
F	13.42	89.48		305.00
P	< 0.0001	< 0.0001		< 0.0001
LC ₅₀ (LCL – UCL)		0.62 (0.16– 1.08)		

Comparison of the effect of 2% concentration extract on *S. cerealella*

The days taken for moth emergence at 2% concentration of the six extracts were compared (Fig. 1). Ethanol extract was as effective as petroleum ether extract in controlling *S. cerealella*, with zero moth emergence. Hence, the highest mortality, with complete control, was observed in ethanol and petroleum ether extracts.

Discussion

Plant extracts have been shown to reduce and prevent hatching of eggs and development into larva and adult stages, which are the most damaging stages of insects (Soujanya et al. 2016). If egg hatching can be controlled, then the other destructive stages can be eliminated. According to our findings, petroleum ether and ethanol extract showed higher ovicidal activity compared to methanol, hexane

Table 5. Time of moth emergence, moth mortality and weight loss of paddy seed infested with *Sitotroga cerealella* and treated with hexane extract of *Allium chinense* bulbs. Data are means \pm SE. Means with identical letters within the column are not statistically significantly different. LCL, lower confidence limit; UCL, upper confidence limit

Treatment, concentration (%)	Time of moth emergence (days)	Mortality (%)	Efficacy (corrected mortality)	Weight loss (%)
Control	28.75 \pm 0.47 a	18.75 \pm 0.47 a		36.65 \pm 0.13 a
Hexane control	29.00 \pm 0.40 a	21.25 \pm 0.47 a	3.08	36.31 \pm 0.25 a
0.5	35.75 \pm 0.47 b	48.75 \pm 0.25 b	36.92	27.15 \pm 0.51 b
1.0	37.5 \pm 0.28 bc	56.25 \pm 0.75 b	46.15	14.99 \pm 0.43 c
2.0	39.5 \pm 0.64 c	75.00 \pm 0.40 c	69.23	10.41 \pm 0.25 d
F	109.00	91.55		1218.00
P	< 0.0001	< 0.0001		< 0.0001
LC ₅₀ (LCL – UCL)		0.82 (0.46 – 1.19)		

Table 6. Time of moth emergence, moth mortality and weight loss of paddy seed infested with *Sitotroga cerealella* and treated with water extract of *Allium chinense* bulbs. Data are means \pm SE. Means with identical letters within the column are not statistically significantly different. LCL, lower confidence limit; UCL, upper confidence limit

Treatment, concentration (%)	Time of moth emergence (days)	Mortality (%)	Efficacy (corrected mortality)	Weight loss (%)
Control	28.75 \pm 0.47 a	18.75 \pm 0.47 a		36.65 \pm 0.13 a
Water control	30.00 \pm 0.40 ab	16.25 \pm 0.25 a	-3.08	37.03 \pm 0.42 a
0.5	31.50 \pm 0.64 cb	50.00 \pm 0.40 b	38.46	29.85 \pm 0.82 b
1.0	32.00 \pm 0.47 c	55.00 \pm 0.40 bc	44.62	30.45 \pm 0.47 b
2.0	33.00 \pm 0.28 c	60.00 \pm 0.40 c	50.77	23.78 \pm 1.46 c
F	14.43	109.90		46.61
P	< 0.0001	< 0.0001		< 0.0001
LC ₅₀ (LCL – UCL)		1.83 (1.27 – 2.39)		

and aqueous extract. The higher activity of ethanol and petroleum ether extracts against moth emergence may be due to the polarity of the solvent used for extraction, as polarity plays a significant role in completely extracting all of the active components. The insecticidal potential of ethanol and petroleum ether extract in controlling stored grain pests was shown by 100% control of *S. cerealella* emergence at 2% concentration. Extracts of *A. chinense* are known for their organo-sulphur (Pino et al. 2001; Liu et al. 2014) and saponin content (Wang et al. 2016; Yao et al. 2016). The juice of *A. chinense* also contains moth-repelling properties (Brewster 2008). Saponins have an insecticidal property, which can inhibit egg hatching, cause moth mortality and interfere with feeding behaviour and growth regulation (Chaieb 2010). The lower larval or pupal survival rate in grains treated with ethanol and petroleum ether extract may be due to a broad spectrum of effects on different developmental stages of *S. cerealella*. The deterrent effect of *A. chinense* extracts was probably due to the presence of saponin compounds, which caused antifeeding activity (Faizal, Geelen 2013).

In the present study, the highest inhibition on the developmental period was observed in chloroform extract

– moth emergence occurred on day 42.50 when 2% concentration was used, while at 1% concentration, moth emergence was observed on day 40.25, and on day 40 in 0.5%, whereas in the control emergence was observed on day 28.75. Saponins can regulate the growth of numerous insect species by increasing the time period of larval stages and also delay the time needed to reach maximum size (Adel et al. 2000). The 2% chloroform extract was effective in causing mortality (87.50%) and reducing weight loss. Significant mortality was also observed in 2% hexane (75.00%), methanol (68.75%) and water (60.00%) extracts, with significant reduction of moth emergence, lower larval survival capacity. This shows the efficacy of *A. chinense* extract as a potential insecticide. With application of higher dosage, moth emergence can be completely controlled, as shown earlier (Soujanya et al. 2016). All extracts showed a significant reduction of moth emergence, compared to the control; the time taken for moth emergence increased as concentration increased. A similar result was reported in experiments on *Callosobruchus chinensis* where saponin reduced the rate of adult emergence (Weissenberg et al. 1998).

The percentage weight loss of paddy was controlled best by ethanol extract (2.55%) and petroleum ether extract (4.02%) treatment; this loss in paddy weight may have been due to infestation of the grain by larvae, which would have penetrated the grain but died in the developmental stages due to the growth-inhibiting activity of the extracts. Similar results were observed in sweet flag, turmeric and neem extract (Iqbal et al. 2010). The number of insects emerging is a principal factor determining the efficacy of the extract, as it directly affects weight loss of the grain in storage.

The potential of *A. chinense* bulb extracts as an insecticide for control of stored grains pests was shown, as *S. cerealella* adult emergence was fully controlled by using 2% ethanol and 2% petroleum ether bulb extract. The study showed the insecticidal potential of the six extracts of *A. chinense* bulb against *S. cerealella*. Similar observations were made by Ashamo and Akinnawonu (2012) in experiments

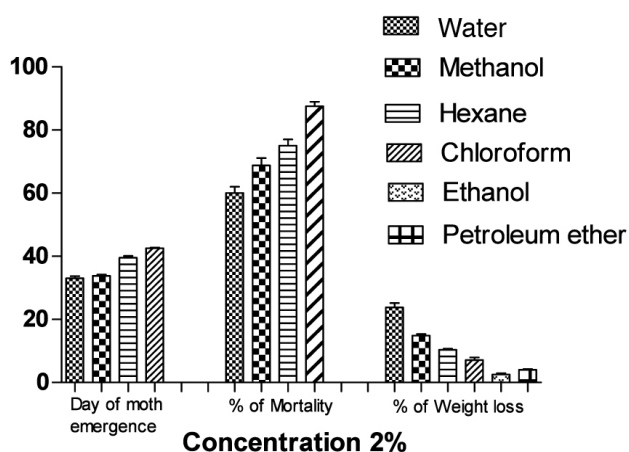


Fig. 1. Insecticidal activity of the six extracts of *Allium chinense* bulb at 2% concentration.

using stem bark ethanol extract of *Aristolochia ringens*, *Khaya ivorensis*, *Strophanthus hispidus*, and root extract of *Zanthoxylum zanthoxyloide*, where 100% moth mortality was recorded. The flower extract of *Tithonia diversifolia* and *Dimorphandra mollis* has ovicidal effect on *S. cerealella*; *T. diversifolia* was also reported to have larvicidal activity and reduced the egg hatching (Fouad et al. 2014). Stem bark powder of *Alstonia boonei* and leaf powder of *Acalypha godseffiana* showed control of *S. cerealella* moth, but could not elicit 100% mortality (Oyeniyi 2018). The root bark powder of *Cleistopholis patens* also showed high mortality of *S. cerealella* moth (Akinneye, Oyeniyi. 2016).

However, the effectiveness of the six extracts against *S. cerealella* differed depending on concentration used. Extracts and essential oil from garlic (*Allium sativum*) have been reported and products have been marketed for pest control (Kimparris et al. 2009). Still, there is no previous report on the insecticidal activity of solvent extracts of *A. chinense* bulbs.

Conclusions

The present study concludes that ethanol and petroleum ether bulb extracts of *A. chinense* have promising active bio-constituents for controlling the emergence of *S. cerealella* adults from eggs. The bulb extract can be used as a potential bioinsecticide for pest control in post-harvest protection, considering its easy availability, and mass production of the extract is possible. Further, the plant is edible, has therapeutic value and it is non-toxic. For the first time, this work was carried out using the crude extract of *A. chinense* bulb to control storage pest *S. cerealella*. Further evaluation leading to separation, screening of bioactive compounds and enhancing their activity is recommended.

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