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Insecticidal Eficacy of Clove (*Syzygium aromaticum*) (L) (Merr. & L. M. Perry) against Rice Weevils (*Sitophilus oryzae*) (L) (Curculionidae, Coleoptera)

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ABSTRACT

A wide range of postharvest pests, including rice weevils (*Sitophilus oryzae*), threatens wheat production in Nepal. This study was conducted to assess the efficacy of clove (*Syzygium aromaticum*) against rice weevils in Nepal under laboratory conditions. Seven amounts of clove (4, 2, 1, 0.5, 0.25, 0.12, and 0 g of clove bud/kg of wheat seed) were tested on three varieties of wheat (Bhrikuti, Vijaya, and NL971), and the dosage-mortality effect of clove bud was investigated. The mortality of rice weevils was indirectly correlated with the amount of clove bud added to all three varieties of wheat. The results suggest that clove buds have considerable pesticidal effects on rice weevils and can be considered as an alternative tool to control rice weevils in wheat during postharvest storage.

Key words: clove, LD_{50}, LT_{50}, rice weevil, wheat

Introduction

Wheat (*Triticum aestivum* Lamk) is a major cereal crop, ranking third after rice and maize in terms of cropping area and production in Nepal. It occupies approximately 23% of the total area of cereal cultivation and contributes to approximately 23% of the total cereal production in Nepal (Anonymous, 2017a). In Nepal, wheat is grown from the tropical plain regions to subtropical mountainous regions (Anonymous, 2017b). After introducing semi-dwarf high-yielding varieties from Mexico, the area of wheat cropping has increased drastically and is currently a considerable contribution to the national food supply (NARC, 2007). Approximately one-third of the food produced is lost globally during the postharvest operations every year (Gustavsson *et al.,* 2011). Insects cause the loss of a high proportion of stored wheat (Kumar and Kalita, 2017). Storage grain losses of major cereal crops, mainly wheat, are due to insect pests, diseases, and rodents (Benhalima *et al.,* 2004; Subedi *et al.,* 2009). *Sitophilus oryzae* is the most cosmopolitan pest, and it causes severe losses in rice, maize, barley, wheat, and other crops (Neupane, 2002). In addition, it is the most destructive insect pest for the stored raw cereal grains in Nepal. High populations of this pest can build up easily (Aitken, 1975). Both larvae and adults bore into the grain kernel, causing considerable damage in a short period. High grain moisture content (> 12%), high relative humidity (> 70%), and high temperature (> 27ºC) favor the growth and development of weevils (Astuti *et al.,* 2013).

For the control of insects in stored grains, various synthetic chemical products belonging to different toxicological classes are used in Nepal. Despite the relative efficiency of synthetic chemicals, their intensive use can cause numerous problems, such as the development of resistance in the insects, the accumulation of residues in foods, damage to human health, and environmental contamination, and increased production costs (Campos *et al*., 2013). An alternative to conventional insect pest control is the use of plants with insecticidal properties. Plant parts can be used as powders, extracts, or oils to control stored-grain pests. These products are cheap, locally available, easy to use, and friendly to humans and the environment (Hernández and Vendramim, 1997; Mazzonetto and Vendramim, 2003). Numerous plant species with pesticidal properties have been reported globally, and 324 plant species with pesticidal properties, including clove, have been reported in Nepal (Neupane, 2000). The major bioactive compound in clove is eugenol, which is toxic to rice weevils in stored grain and to other insects (*Anopheles dirus*, *Culex pipiens*, *Dermatophagoides farina*, *Dermatophagoides pteronyssinus*, *Pediculus capitis*, *Psoroptes cuniculi*, *Sitophilus zeamais,* and *Tribolium castaneum*) (Brogdon and McAllister, 1998; Kafle and Shih, 2013). Accordingly, this study examined the effective amount of clove bud required to control rice weevils on three different varieties of wheat under laboratory conditions.

Materials and Methods

Collection and rearing of weevil

This study was conducted at the entomology laboratory of the Trivuwan University in Paklihawa, Nepal (27°30'0" North, 83°27'0" East), approximately 300 km southwest of the capital city Kathmandu.

The rice weevils were collected from a local farmer's wheat storage house. Weevils were reared on whole, undamaged, and untreated wheat grains in the laboratory. A plastic container (5 L) was used to maintain the stock of weevil and for mass rearing. Five hundred pairs of weevils were released into the preconditioned wheat grain kept in the plastic container for mass rearing. Adult weevils were removed from the container after 12 days and kept in another plastic container (600 mL) for further study. The weevil rearing and experiments were conducted under laboratory conditions (33°C ± 3°C, RH 70% ± 5%, and 14 h light and 10 h dark conditions).

Mortality tests

To determine the efficacy of clove bud against rice weevil on the three varieties of wheat (Vijay, Bhrikuti, and NL 971), 250 g of wheat grain of each variety was placed in a plastic container (600 mL). Subsequently, 1, 0.5, 0.25, 0.125, 0.063, 0.031, and 0 g of whole clove bud were placed atop the grain's surface in each container. After 2 weeks, 50 pairs (50 males and 50 females) of weevils were released into each container, and the top of the containers was covered with a fine mess plastic net to prevent the weevils from escaping.

The number of deaths among weevils was counted on a daily basis from day 1 until day 16 after treatment (DAT). The dead weevils were removed from the containers during the daily observations. The number of weevils that died was recorded as a percentage to compare the means. The three varieties of wheat seeds used in this study were received from the National Wheat Research Station of Bhairahawa in Nepal, and the clove buds were purchased from a local market.

The lethal time (LT_{50}) and time (days) required to realize the death of 50% of the weevils were determined through a probit

Table 1. Mortality of rice weevils in three varieties of wheat after exposure to different amounts of clove buds at 16 days after treatment

Amount of clove bud application	% weevils died (Mean ± SD)*		
	Brikuti	Vijaya	NL971
4 g/kg	97.33 ± 0.58aA	97.33 ± 1.53aA	98.33 ± 1.15aA
2 g/kg	76.33 ± 1.53bA	78.67 ± 2.31bA	78.67 ± 3.51bA
1 g/kg	59 ± 6.08cA	59 ± 4.58cA	60.67 ± 4.51cA
0.5 g/kg	47 ± 3.61dA	47.67 ± 3.21dA	47.33 ± 4.51dA
0.25 g/kg	33.33 ± 7.23eA	33.67 ± 4.16eA	32.33 ± 4.51eA
0.0125 g/kg	20.33 ± 4.16fA	23.33 ± 2.52fA	19.67 ± 3.51fA
0 g (Control)	3 ± 1.00gA	3 ± 0.00gA	3.33 ± 0.58gA

* Means within the same column followed by the same letters (lower case) and the same row (upper case) are not significantly different ($P < 0.05$) (SAS, 2017).

analysis using StatPlus (2017) (StatPlus, AnalystSoft Inc. Version v6.); means were compared using the SNK of SAS (2017).

Results

Mortality

When the different rates of clove bud per container were applied to determine the mortality of weevils in var. Bhrikuti, at 16 DAT, all seven treatments killed a significantly higher number of weevils compared with the control. The highest and lowest weevil mortality was caused by 4 and 0.125 g clove buds per kg of wheat, respectively ($F = 180.60$, $P < 0.0001$) (Table 1).

The same trend was observed when different amounts of clove bud per container were used to determine the mortality of weevils in var. Vijaya and var. NL971 at 16 DAT (Vijaya: $F = 353.42$, $P < 0.0001$; NL971: $F = 267.63$, $P < 0.0001$) (Table 1).

When the weevil's mortality was compared after treating the three varieties of wheat with the same amount of clove bud, none of the treatments exhibited a significant difference on the weevil's mortality among the three wheat varieties (4 g/kg: $F = 0.75$, $P < 0.51$; 2 g/kg: $F = 0.82$, $P = 0.49$; 1 g/kg: $F = 0.11$, $P = 0.90$; 0.5 g/kg: $F = 0.05$, $P = 0.95$; 0.25 g/kg: $F = 0.05$, $P = 0.95$; 0.125 g/kg: $F = 0.95$, $P = 0.43$; 0 g/kg: $F = 0.25$, $P = 0.79$) (Table 1).

At 16 DAT, when the treatment with 4 g clove bud per kg of wheat was applied, the cumulative morality of weevils exceeded 97% for all three wheat varieties. Similarly, when the amount of clove bud applied decreased to 2 g/kg, the mortality of weevils exceeded 75% for all three wheat varieties. When the amount of clove bud applied was decreased to 1 g/kg, the mortality of weevils reached approximately 60% for all three wheat varieties. The cumulative mortality of weevils observed increased from approximately 20% to more than 97% when the amount of clove bud applied was increased from 0.0125 to 4 g/kg of wheat during the study period (Table 1).

The percentage of weevils killed increased with the increase of clove bud amounts in all treatments. In other words, a dose effect of clove buds on the mortality of weevils was clearly observed during the study: a higher amount of clove bud caused a higher percentage of mortality in weevils (Figure 1).

Discussion

Clove, clove powder, and clove oil have been studied as solutions against urban and agricultural pests, and they have been found to possess pesticidal properties (Kafle and Shih, 2013). The mortality of weevils observed in this study increased with the amount of clove bud applied, and this increase was due to secondary metabolites, which exert strong toxic effects on insects (Forim *et al.*, 2012). The essential oil of clove contains 85~92% eugenol (Dorman and Deans, 2000), which is known to completely inhibit the development of the eggs, larvae, and pupae of *S. granaries*, *S. zeamais, T. castaneum,* and *Prostephanus truncatus* inside grains (Obeng-Ofori and Reichmuth, 1997). Adedire *et*

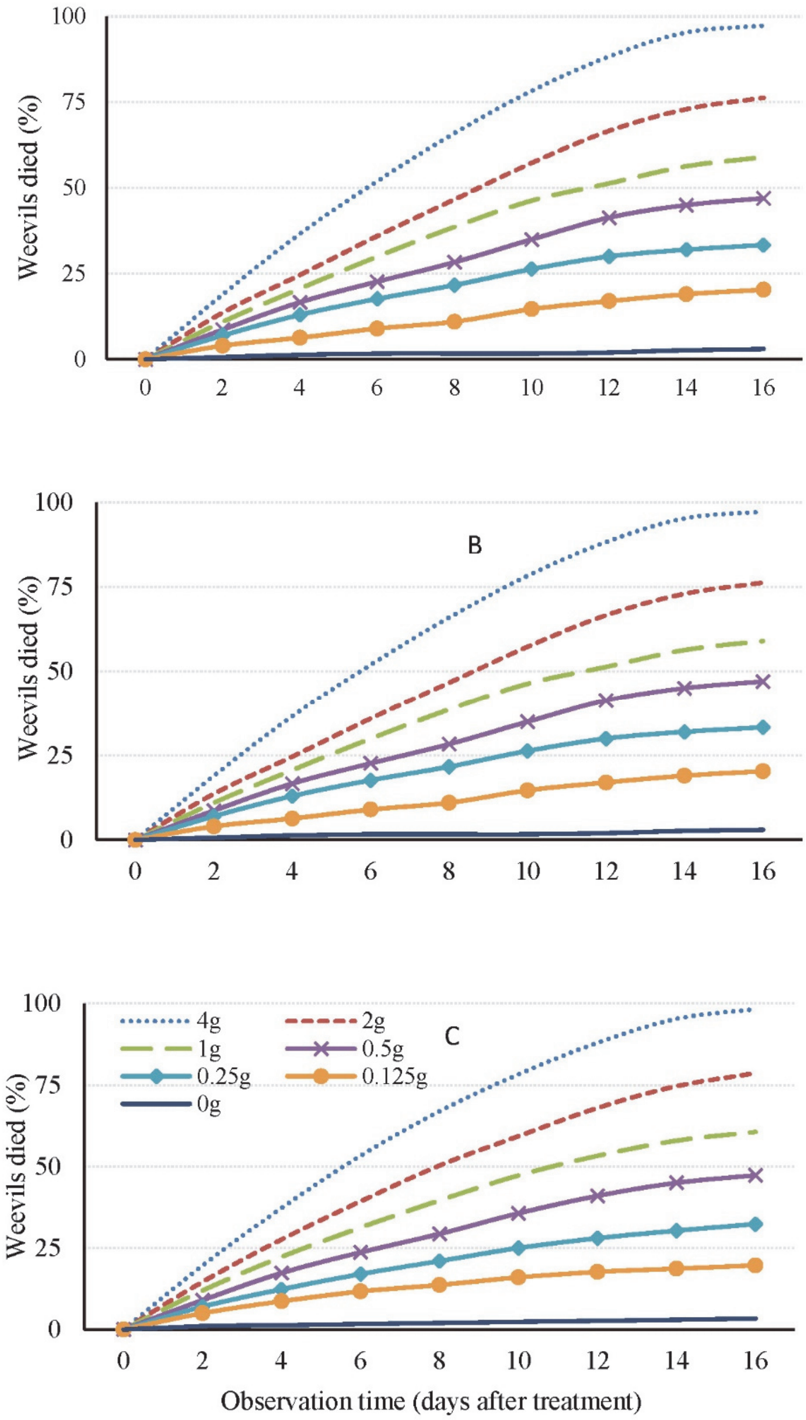

Fig. 1. Mortality of rice weevils in three varieties of wheat (A = Brikuti, B = Vijaya, and C = NL) after exposure to different amounts of clove buds.

al. (2011) and Ileke *et al.* (2013) have stated that clove powders and oils inhibit the locomotion of adult insects or cause their death. This phenomenon may have affected the mating activities and sexual communication of rice weevils (Ileke and Olotuah, 2012). The reduction in the progeny growth of rice weevils may be due to early mortality and partial or complete retardation of the embryonic development in adult female weevils (Dike and Mbah, 1992). Moreover, the physiological changes induced by the secondary metabolites of clove may cause poor egg laying capacity in the weevils. Oigiangbe *et al.* (2010) reported that insects at the larval or pupal stage died after the application of clove because they were unable to fully cast off their exoskeleton.

The main mechanism of the plant extracts action is their ability to penetrate the chitin of insects (Ntonifor *et al.,* 2010). Eugenol acetate, eugenol, and beta-caryophyllene are the major bioactive compounds of clove, and they are lipophilic in nature (Kafle and Shih, 2013). Studies have reported that these compounds can be absorbed by the cuticular lipids of the insects and slowly moved into their hemocoel and nervous system. Another mode of toxicity of those bioactive compounds of clove is when these compounds are absorbed into the tracheal system (Appel *et al*., 2004; Cheng *et al*., 2008), followed by their slow movement into the hemocoel and nervous system of the insects. The bioactive compounds of clove have delayed toxicity in fire ants (Kafle and Shih, 2013). In their study, fire ants became paralyzed, twitched, and died. Eugenol was also reported as a neuroinsecticide against carpenter ants (*Camponotus pennsylvanicus*). The octpaminergic system mediates the insecticidal activity of eugenol (Enan, 2001). These mechanisms of killing insects through the bioactive compounds of clove also apply to rice weevils. Consequently, we obtained a high mortality rate of rice weevils in all three wheat varieties in this study.

The results of this study suggest that the use of clove bud in rice weevil control is a potential alternative tool in postharvest storages. Additional studies are necessary to determine the efficacy of bioactive compounds from clove oils against rice weevils under laboratory and field conditions.

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丁香 (*Syzygium aromaticum*) (L) (Merr. & L. M. Perry) 對水稻象鼻蟲 (*Sitophilus oryzae*) (L) (Curculionidae, Coleoptera)之殺蟲效力

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摘　　要

尼泊爾的小麥生產受到包括水稻象鼻蟲(*Sitophilus oryzae*)在內的各種採後害蟲的威脅。本研究旨在評估丁香(*Syzygium aromaticum*)在實驗室條件下對尼泊爾水稻象鼻蟲的致死功效。以 7 個不同量的丁香芽(4、2、0.5、0.25、0.12、和 0 g/kg 小麥種子)及在三種品種(Bhrikuti，Vijaya 和 NL971)小麥上測試結果，三種小麥品種的水稻象鼻蟲的死亡率均與丁香芽數量呈間接正相關，顯示丁香芽對水稻象鼻蟲有顯著的殺蟲作用，可以作為採後貯藏期間防治小麥水稻象鼻蟲的替代方法。

關鍵詞： 丁香、LD_{50}、LT_{50}、水稻象鼻蟲、小麥