Agricultural Sci. J. 37 : 5 (Suppl.) : 232-235 (2006) ว. วิทย. กษ. 37 : 5 (พิเศษ) : 232-235 (2549)

Biological Activity of *Cleome* spp. Extracts Against the Rice Weevil, *Sitophilus oryzae* L.

Sungwarl Somboon[1] and Supanee Pimsamarn[2]

Abstract

The methanolic extracts of *Cleome gynandra* (L.) Briq., *Cleome chelidonii* L., and *Cleome viscosa* L. were tested against the rice weevil, *Sitophilus oryzae* L. under laboratory conditions. Impregnated-paper test with the extracts (10μl/cm^2) were conducted on early emerged adults. The extracts of *C. viscosa* and *C. gynandra* showed higher toxic than *C. chelidonii* extract . The LC_{50} values were 198, 988 and 1111 ppm, respectively. Moreover, *C. viscosa* extract was highly inhibited the oviposition of these insects. The present result is an indicative of the potential of using *Cleome* extracts for *S. oryzae* management.

Key words: *Cleome* spp., plant extracts, *Sitophilus oryzae* L.

Introduction

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is one of the most widespread pests and causes heavy losses of stored grain both quantitatively and qualitatively throughout the world (Madrid *et al*., 1990; Lucas and Riudavets, 2000; Park *et al*., 2003). Control of these insect populations around the world is primarily dependent upon continued applications of organophosphorus and pyrethroid insecticides and the fumigants methyl bromide and phosphine. These are still the most effective treatments for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation. Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of resistance. It has had undesirable effects on non-target organisms, and fostered environmental and human health concerns (Subramanyam and Hagstrum, 1995; White and Leesch, 1995). The use of methyl bromide will be prohibited in the near future because of its ozone depletion potential and high toxicity (Anonymous, 1993). These problems have highlighted the need for the development of selective insect-control alternatives.

The use of plant materials in pest control could become an important alternative to the use of synthetic insecticides. Plant secondary metabolites are known to have several biological activities against different insect species (Gonzalez-Coloma *et al*., 1998; Huang and Ho, 1998). Many plant extracts and essential oils may be an alternative source of insect control agents (Hill and Schoonhoven, 1981; Konstantopoulou *et al*., 1992; Desmarchelier, 1994; Shaaya *et al*., 1997) because they constitute a rich source of bioactive chemicals. Much effort has therefore been focused on plant-derived materials as potential sources of commercial insect control agents.

Plants from the Capparaceae, etc. *Cleome gynandra* (L.) Briq., *Cleome chelidonii* L., *Cleome viscosa* L. have widespread medicinal use, both in Thailand and elsewhere in South East Asia. They have many medicinal applications, often in rubifacient and counter irritant preparations, used for rheumatism and even headaches. *C. viscosa* is a subtropical bushy aromatic herb with secretory glandular trichomes. The plant is not eaten by herbivores in the wild and its leaves and stems are covered with a waxy aromatic odorous chemical mixture which may have derived from the glandular trichomes. Leaves, leaf juice, and seeds of *C. viscosa* are used in a range of medicines due to the presence of glucosinolates, and *C. chelidonii* is also used to a lesser extent. *C. chelidonii* and *C. viscosa* contain glucocapparin and glucocleomin. (Songsak and Lockwood, 2002). Glucosinolates are the major organoleptic and bioactive constituents of the Capparaceae and a few other related plant families. The extracts of *Cleome* spp. have demonstrated contact insecticidal, repellent, antifeedant and nematicidal properties

[1] Ph.D Student of Graduate School, Khon Kaen University 40002
[2] Insecticide Toxicology and Environment Lab., Department of Entomology, Faculty of Agriculture, Khon Kaen University 40002

in several publication (Fletcher *et al*., 1999; Ndungua *et al*., 1999; Williams *et al*., 2003; Lazzeri *et al*., 2004). The findings of the present study indicate the possibility of utilization of indigenous plants such as *Cleome* spp., as insect control agents for safe use in stored grain protection. Based on this observation, the leaves and stems of this plant were extracted and evaluated for biological activities for insect pest control.

Materials and Methods

Insect.

Adults of *S. oryzae* were cultured on a diet of insecticide-free milled rice under controlled condition (25±2 °C, 75%RH and photoperiod 12 h light:12 h dark). The culture population density was approximately 500 pairs per 0.5L jar half filled with food. After 1 week they were removed by sieve. The jar were returned to the room for 1 month to allow time for the new adults to emerge. Insects use were generally 3–5 days post adult eclosion.

Plant extraction.

The fresh aerial part (flowering stage) of the cultivated *C. gynandra*, *C. chelidonii* and *C. viscosa* were collected, dried under shade, and ground in a mixer. Each plant sample (500 g) was extracted with organic solvents of increasing polarity. Hexane, dichloromethane, and methanol were sequentially used to extract with Soxhlet apparatus for a period of 12 h each. Each extract was evaporated to dryness under reduced pressure.

Bioassay.

Female adults of *S. oryzae* were used as experimental insects in a Completely Randomized Design (CRD) experiment. The experiment was performed using the impregnated filter paper method with crude extracts dissolved in acetone as serial dilutions (10, 50, 250, 1250, 6250 ppm). The dissolved solutions were dropped onto filter paper of diameter 9 cm (Whatman® No. 2) placed in petri dishes at a rate of 0.5ml per sheet. They were left for 3 minutes and 20 insects were released in each dish. The top was placed on. The experiment had 5 replications for each concentration, and plain acetone was used as the control. Deaths were counted every 24 hours. Analysis was performed by finding the Median Lethal Concentration (LC_{50}) using Probit analysis with the program POLO-PC.

The LC_{20} of each plant extract was treated on adults by Potter's spray (2 ml/100 insects). Controls were treated with the solvent alone. After treatment, five females and two males (3-day old) were then introduced into each vial (2 cm diameter, 10 cm high) with food (5 g/vial) and allowed to lay eggs for 5 days. Ten replicates were set up for each compound and control. On the 5th day, all insects were removed and the eggs on grain in each vial were counted. Thereafter, all emerged adults in each replicate were counted. Number of eggs and emerged adults were then subjected to two-way ANOVA. Means were compared using the least significant difference statistic ($P \leq 0.05$) (SAS, 1988).

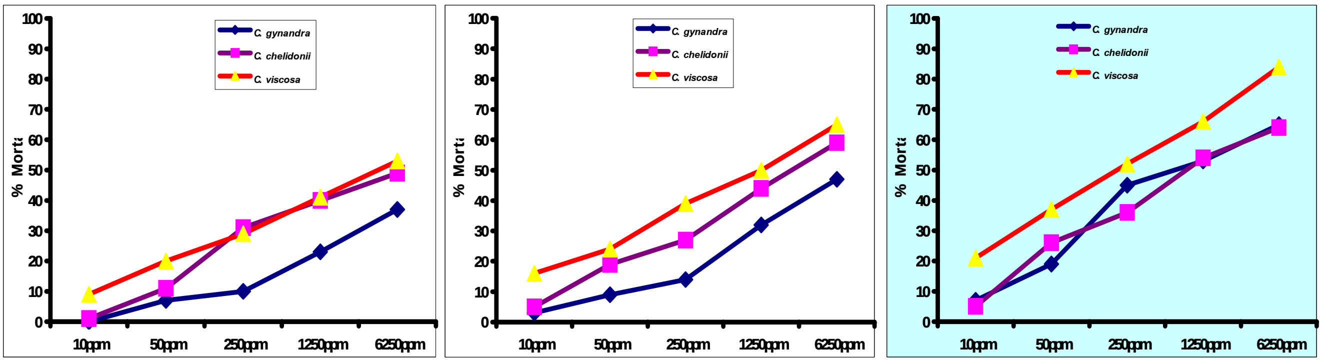
Results and Discussion

All *Cleome* spp. methanolic extracts were toxic to adults of *S. oryzae*. The test insects were sensitive to the toxicity of *C. viscosa*, followed by *C. gynandra* and *C. chelidonii*. Moreover, *C. viscosa* extract also increased the mortality of insects (Fig. 1).

The contact toxicity of plant extracts were observed by median lethal concentration (LC_{50}) at 72 h after treatment. The results for methanolic extracts of *C. gynandra*, *C. chelidonii* and *C. viscosa* are shown in Table 1. The LC_{50} values were 988, 1111 and 198 ppm, respectively. The LC_{50} values *C. viscosa* showed higher toxicity for *S. oryzae* than *C. gynandra* and *C. chelidonii*. William *et al.* (2003) have reported that the hexane extract of *C. viscosa* demonstrated a pyrethroid type of contact insecticidal activity on adult *Cylas formicarius elegantulus* Summer (Coleoptera: Curculionidae). Moreover, the extract also had high nematicidal activity with a percentage Abbott's value of 72.69 for the plant parasitic nematode *Meloidogyne incognita* Chitwood; however, the extract lost its potency upon subfractionation. These results indicate that the polar extract (methanol) caused higher toxicity by contact application.

Table 1 Toxicity of *Cleome* spp. methanolic extracts on *Sitophilus oryzae* L. at 72 h after treatment

Species	LC_{50} (ppm)	95% Fiducial limit		Slope±SE
		Lower	Upper	
Cleome gynandra (L.) Briq.	988	633	1,688	0.65±0.06
Cleome chelidonii L.	1,111	713	1,894	0.67±0.06
Cleome viscosa L.	198	125	307	0.62±0.06



24h 48h 72h

Fig. 1 Mortality of *Sitophilus oryzae* L. females after treated with *Cleome* spp. extracts at 24, 48 and 72 h

Effects of *Cleome* spp.extracts on *S. oryzae* reproduction were evaluated from the number of eggs laid after treatment and the number of emerged adults (F1). The overall ANOVA indicated significance that the extracts affected the number of eggs and emerged adults from untreated insects ($P \leq 0.04$). *C. viscosa* extract showed higher effect on *S. oryzae* than *C. gynandra* and *C. chelidonii* extracts (Table 2). However, *C. viscosa* extract caused a reduced percentage of egg laying and emerging adults. The percent inhibitions is shown in Fig 2. Ndungu *et al*. (1999) used Y-tube olfactometer bioassay to evaluate repellent activity, and the oil of *C. hirta* showed higher or comparable repellency against *S. zeamais* relative to DEET at all the doses tested.

The chemistry of natural compounds is a very complex subject and screening for activity will have to face, among other factors, isolation and identification of the products, variability due to the plants or the environment, and synergism due to mixtures of compounds in crude extracts. Our next approach will be to concentrate efforts in a few more promising extracts to fractionate and isolate active compounds. According to Cole (1994), the choice of insect and bioassay can greatly influence the outcome of a screening. However, to develop a useful commercial product, testing against agricultural pests is important.

Table 2 Means of egg number and newly emerged adults of *Sitophilus oryzae* L. after treatment with LC_{20} concentrations

Treatment	No. of eggs (mean±SE)	No. of emerged adult (mean±SE)
Cleome gynandra (L.) Briq.(LC_{20} = 50 ppm)	254.0±53.5 b	178.9±33.1 ab
Cleome chelidonii L. (LC_{20} = 60 ppm)	225.7±33.0 b	127.4±41.3 c
Cleome viscosa L. (LC_{20} = 10 ppm)	169.5±58.4 c	100.9±16.8 c
Control (acetone)	368.2±86.4 a	214.4±26.2 a

Means within columns followed by the same letter are not significantly different ($P > 0:05$).

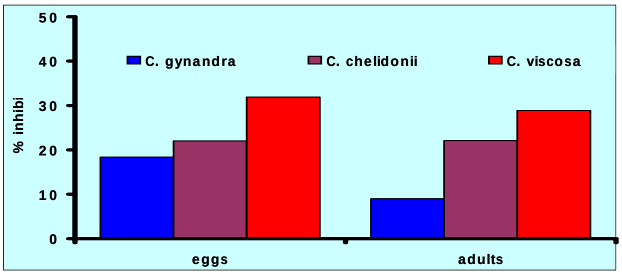


Figure 2 Effects of *Cleome* spp. extracts on egg laying and emergence adults of *Sitophilus oryzae* L. (% inhibition = (C-T / C+T)x100), when C; untreated insects and T; treated insects)

Acknowledgement

The authors acknowledge the Graduate School, Khon Kaen University for the provision financial support to attend and present this work in the 4th National Seminar on Postharvest & Post Production and the Postgraduate Education & Research Development Project in Postharvest Technology, Khon Kaen University for financial support for this work.

References

Anonymous. 1993. Regulatory Action under the Clean Air Act on Methyl Bromide. United States Environmental Protection Agency, Office of Air Radiation Stratospheric Protection Division, Washington, DC.

Cole, M.D. 1994. Key antifungal, antibacterial and antiinsect assays, a critical review. Biochem. Syst. Ecol. 22: 837–856.

Desmarchelier, J.H. 1994. Grain protectants: trends and developments. In: Highley, E., E.J. Wright, H.J. Banks, and B.R. Champ, (Eds.), Stored Product Protection. CAB International, Wallingford, UK.

Fletchers, R. 1999. *Cleome gynandra* (Cat's whiskers). The Australian New Crops Newsletter, 11.

Gonzalez-Coloma, A., F. Valencia, N. Martin, J.J. Hoffmann, L. Hutter, J.A. Marco, and M. Reina. 2002. Silphinene sesquiterpenes as model insect antifeedants. Journal of Chemical Ecology 28: 117–129.

Hill, J.M., and A.V. Schoonhoven. 1981. The use of vegetable oils in controlling insect infestations in stored grains and pulses. Recent Advances in Food Science and Technology 1: 473–481.

Huang, Y. and S. H. Ho. 1998. Toxicity andantifeedant activities of cinnamaldehydeagainst the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Journal of Stored Products Research 34: 11-17.

Konstantopoulou, L., L. Vassilopoulou, P. Mauragani-Tsipidov, and Z.G. Scouras. 1992. Insecticidal effects of essential oils. A study of the effects of essential oils extracted from eleven Greek aromatic plants on *D. auraria*. Experientia 48: 616–619.

Lazzeri, L., O. Leoni and L.M. Manici. 2004. Biocidal plant dried pellets for biofumigation. Industrial Crops and Products 20: 59–65.

Lucas, E. and J. Riudavets. 1990. Lethal and sublethal effects of rice polishing process on *Sitophilus oryzae* (Coleoptera: Curculionidae). J. Econ. Entomol. 93: 1837-41.

Madrid, F.J., N.D.G. White and S.R. Loschiavo. 1990. Insects in stored cereals and their association with farming practices in southern Manitoba. Canadian Entomologist 122: 515–523.

Ndungua, M.W., S.C. Chhabraa and W. Lwandeb. 1999. *Cleome hirta* essential oil as livestock tick *Rhipicephalus appendiculatus* and maize weevil *Sitophilus zeamais* repellent.Fitoterapia 70: 514-516.

Park, I.K., S.G. Lee, D.H. Choib, J.D. Park and Y.J. Ahna. 2003. Insecticidal activities of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) *and Sitophilus oryzae* (L.) Journal of Stored Products Research 39: 375–384.

SAS Institute. 1988. SAS user's guide : Statistics version 6ed. SAS Institute, Cary, N.C.

Shaaya, E., M. Kostjukovski, J.Eilberg, and C. Sukprakarn. 1997. Plant oils as fumigant and contact insecticides for the control of stored-product insects. Journal of Stored Products Research 33: 7–15.

Songsak, T. and G.B. Lockwood. 2002. Glucosinolates of seven medicinal plants from Thailand. Fitoterapia 73: 209-216.

Subramanyam, B. and D.W. Hagstrum. 1995. Resistance measurement and management. In: Subramanyam, B. and D.W. Hagstrum (Eds.), Integrated Management of Insects in Stored Products. Marcel Dekker, New York, pp. 331–397.

White, N.D.G. and J.G. Leesch. 1995. Chemical control. In: Subramanyam, B. and D.W. Hagstrum, (Eds.), Integrated Management of Insects in Stored Products. Marcel Dekker, New York.

Williams, L.A.D., E. Vaques, W. Reid, R. Porter, and W. Kraus. 2003. Biological activities of anextract from *Cleome viscoza* L. (Capparaceae). Naturwissenschaften 90:468-472.