INSECTICIDAL TOXICITY OF FORMULATED NEEM OIL-BASED PELLETS AGAINST POSTHARVEST DAMAGE BY RICE WEEVIL, *Sitophilus oryzae* L. (FAMILY CURCULIONIDAE) *

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Abstract

Insecticidal toxicity of different neem oil-based pellets formulated with various essential oils and volatile substances: clove oil, cinnamon oil, citronella oil, camphor, borneol and menthol, was tested against rice weevil *Sitophilus oryzae* (L.). Impregnation of different pellet bags for 3 days was used for testing the fumigant actions. Their biological activities such as feeding deterrence, reproductive inhibition and progeny development disrupting were determined.

The results showed that neem oil formulated with menthol, cinnamon oil or citronella oil were identified as the most effective formulas in all respects. In particular, the formulation of neem oil mixed with menthol strongly reduced feeding damage and suppressed the number of eggs laid and F-1 progeny. In contrast, the formulated neem oil with clove oil, camphor or borneol did not have any feeding deterrence or inhibitory effect on egg laying and progeny production. Furthermore, the difference between various treatments in mortality was also not significant.

Keywords : neem oil, citronella oil, clove oil, cinnamon oil, *Sitophilus oryzae* (L.)

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1. Introduction

Neem-based extracts have been found to be a promising environmental friendly botanical insecticide. They were reported to deter feeding activity and other behavioral responses of various species of insects. The application of neem-based extracts affected the insect oviposition, reproduction, longevity and post-embryonic development (Saxena *et al*., 1989; Sanguanpong, 1989; Schmutterer, 1990; National Research Center, 1992; Sanguanpong and Schmutterer, 1992; Mordue and Blackwell, 1993; Sanguanpong, 1994; Saxena, 1995; Schmutterer, 1995). Many investigations have been undertaken with neem seed oil against damage by store insect pests in laboratory bioassays and warehouse trials such as *Callosobruchus chinenensis* and *C. maculatus* (Tanzubil, 1987; Makanjuola, 1989; Yadav, 1993), *Corcyra cephalonica* Stainton (Senguvattana *et al*., 1995), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) (National Research Center, 1992), *Sitophilus granarias* (L.) (Jilani and Su, 1983) and *S. oryzae* (Makanjuola, 1989; Saxena, 1995; Sanguanpong,1996).

Besides the application of neem-based extracts, many authors demonstrated the effectiveness of such plant oils in protecting grains from store insects i.e. sesame oil (Senguttavan *et al.*, 1995); soybean and castor oil (Pacheco *et al*., 1995; Senguttavan *et al.*, 1995); sunflower and rape seed oil (Tembo and Murfitt, 1995). Various essential oils are among the most widely used materials in store pest control, particularly *Acorus calamus* oil (El-Nahal *et al*.; 1989; Schmidt and Streloke, 1994*); Lantana camara* oil (Saxena *et al*., 1992) including some volatile substances as camphor, α-pinene, linalool and eugenol (Regnault-Roger and Hamraoui, 1995)

However no similar work has been carried out with the application of neem oil formulated with essential oils and volatile substances. Certain essential oils and volatile substances such as clove oil, cinnamon oil, citronella oil, camphor, borneol and menthol were selected on the basis of being safe and easily available purchased.

2. Materials and Methods

2.1. Cultures

Colonies of rice weevil (*Sitophilus oryzae* (L.)) has been reared on whole rice grains (Thai jasmine rice type KDML 105) in plastic container under the laboratory conditions at 24 $\pm$ 4 ^{o}C and 60 $\pm$ 15% r.h. The sub-cultures and the tests were carried out under the same conditions. Only 7-10 day-old adults of *S. oryzae* were used for the experiments.

2.2. Extraction and formulation procedure

Thai neem seed kernel(*Azadirachta indica* var. *siamensis*) was extracted in soxhlett's apparatus with hexane for 8 hours to obtain the Thai neem oil. Different plant parts of three plant species : bark of cinnamon (*Cinnamomum aromaticum* Nees , Family Lauraceae) ; stem of citronella grass (*Cymbopogon nadus* Linn., Family Gramineae); flower of clove (*Syzigium aromaticum* (L.) Merr., Family Myrtaceae) were extracted by hydrodistillation to obtain various essential oils. Whereas camphor, borneol and menthol were purchased from a local market. Tween 80 was added to 1% neem oil as emulsifier in O/W emulsion system. Acetone was used as co-solvent for addition of 1% of each essential oils or volatile substances with neem oil-based emulsion. Non-aromatized talcum was mixed with each of neem oil-based emulsions and then extruded to a pellet. After drying, each pellet had a diameter ca. 0.70 cm and 0.50 cm height. Each 50 g of different aromatized pellets was packed in cloth bag and kept in a container to exclude moisture.

2.3 Bioassy procedure

Plastic jars with 1,550 ml capacity were used for the bioassays. Fifty adults of 7-10 days-old of *S. oryzae* were transferred to the plastic jar containing untreated rice grains. Each pellet bag of different neem oil-based formulas was impregnated into the middle of rice container. The jars were covered with fine nylon mesh such that the oil vapors saturated the atmosphere of the container. The control consisted of a similar set up but with non-aromatized pellets.

2.4 Data determination and analysis

Three days after infestation the test insects were screen out. Mortality counts were made each 24 hours up to 7 days after treatment. The grains were further stored under ambient conditions in the laboratory. The number of seed damage, egg-laids, new emerged adults and total population were monitored each 7 days over a 24-week storage period. All bioassays were carried out under the laboratory conditions at 24 $\pm$ 4 ^{o}C and 60 $\pm$ 15% r.h. All treatments were replicated at 3 times in completely randomized design (CRD). All datas were statistically analyzed. Analysis of variance was performed on the data with different neem oil–based formulas as main variable. Significant differences were determined at $p < 0.05$ level by Duncan's New Multiple Range test (DNMRT).

3. Results

3.1 Antifeedant effect

Feeding deterrent affected by various formulated neem oil-based pellets was given in Fig. 1. It showed that the weight of treated grains in neem oil (NO), neem oil formulated neem oil with cinnamon oil (NO+CI) or citronella oil (NO+CIT) in each period of monitoring was higher than untreated control (CONTROL). They gave no significant effect on feeding activity compared to the neem oil. Furthermore it was found that the formulated neem oil with menthol (NO+ME) provided satisfactory protection to the stored grains against seed damage due to rice weevil. On the other hand, significantly fewer amounts of treated grains were remained on neem oil formulated with clove oil (NO+CL), camphor (NO+CA) or borneol (NO+BO). No feeding deterrence was observed. In addition, it provoked the insects feeding activities by reducing the treated grains. During a storage period, however, no direct mortality of tested insects in all the treatments were noted.

3.2 Antioviposition effect

The effectiveness of different formulas on the oviposition of *S. oryzae* is expressed in Fig. 2. It showed that the cumulative number of eggs laid were higher in the control than in neem oil, neem oil formulated with cinnamon oil, citronella oil or menthol. In the case of neem oil formulated with menthol, particularly, we found that the presence of the substances strongly reduced number of eggs laid. In contrast, no pronounce inhibition of oviposition was noticed in neem oil formulated with clove oil, camphor or borneol. Besides, the number of eggs laid provoked by camphor, borneol and clove oil were also observed. However, the analyses of variance indicated that the cumulative number of eggs laid were not significant difference.

3.3 Effect on adult emergence

By comparing the reduction in cumulative number of progeny emerging with untreated control, it appeared that the decrease in progeny resulting from eggs laid on grains was much more affected by neem oil, neem oil formulated with cinnamon oil, citronella oil or menthol (Fig.3). Compare to the control, the strong inhibition of emergence seen with neem oil formulated with menthol. On the other hand, cumulative number of offspring emerging from eggs laid during treatment with neem oil formulated with clove oil, camphor or borneol was considerably higher than in the respective control.

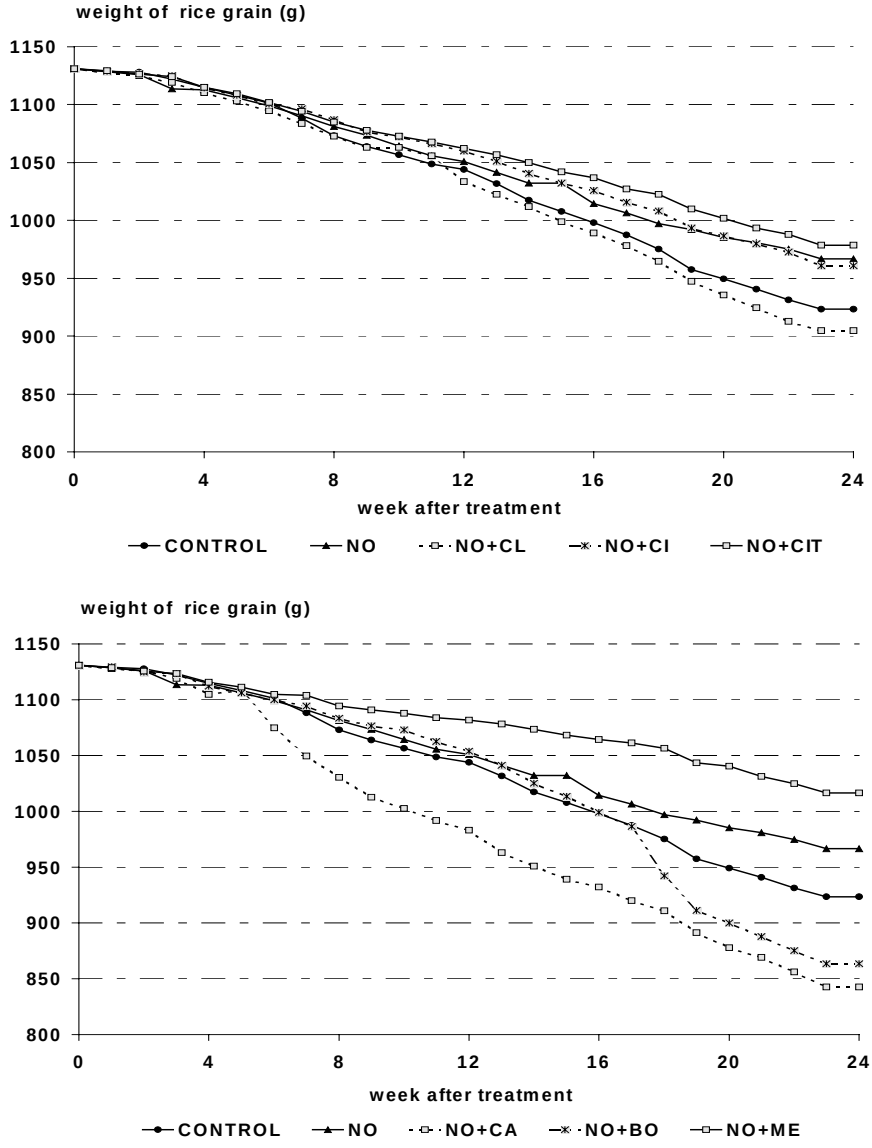

Fig. 1 Weight of stored rice grains affected by different formulas of neem oil-based pellets during a 24-weeks observation period
(Top) neem oil formulated with various essential oils
(Down) neem oil formulated with various volatile substances

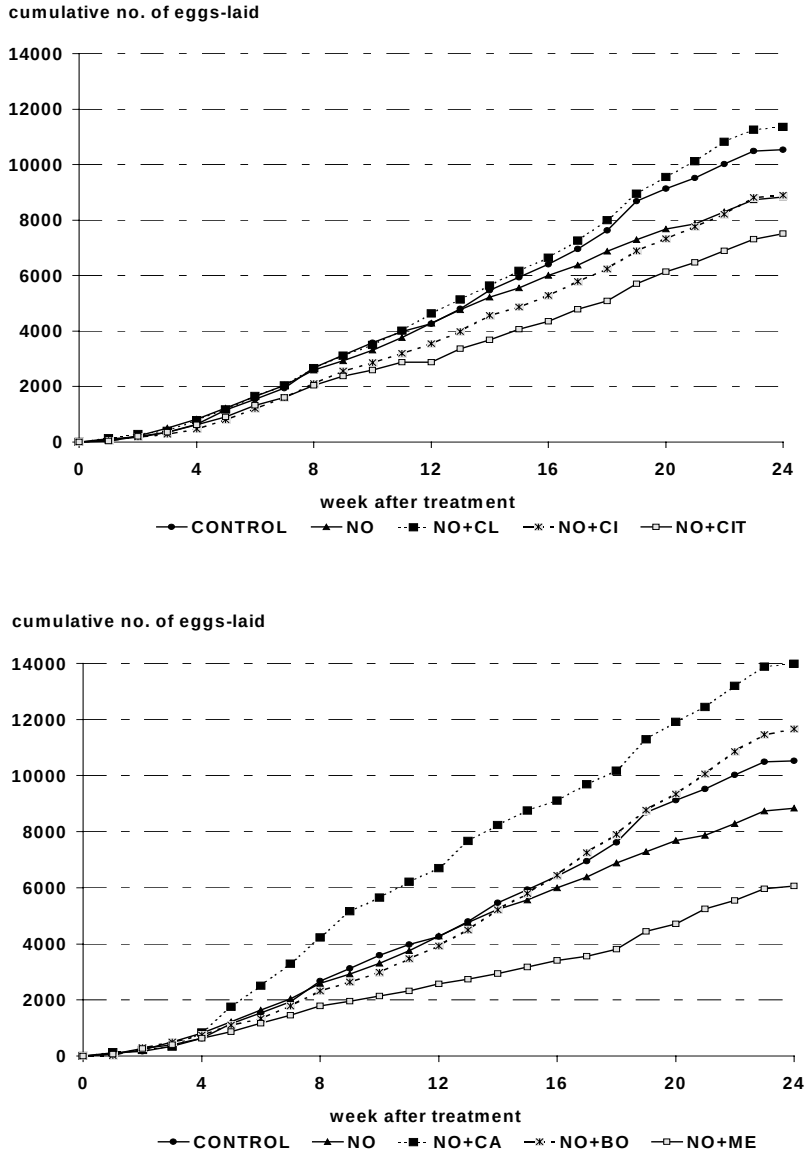

Fig. 2 Cumulative number of eggs laid by *S. oryzae* exposed to rice grains treated with different formulas of neem oil-based pellets
(Top) neem oil formulated with various essential oils
(Down) neem oil formulated with various volatile substances

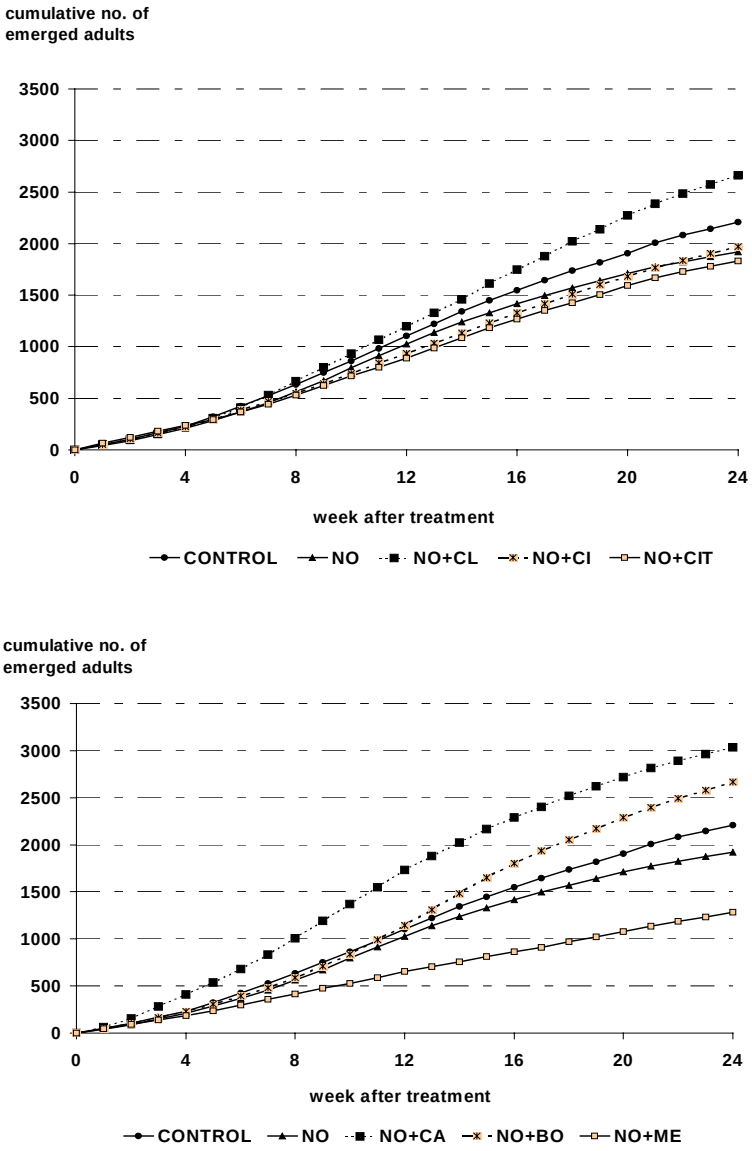

Fig. 3 Cumulative number of adult emergence after fumigation with different formulas of neem oil-based pellets
(Top) neem oil formulated with various essential oils
(Down) neem oil formulated with various volatile substances

4. Discussion

In laboratory experiments, neem oil-based pellet provided protection to rice grains against *Sitophilus oryzae* as shown by the level of adult feeding, the number of egg laying and adult emergence. In earlier studies, neem based- extracts and powder formulation have been reported to provide satisfactory protection of stored grains. For example, Ivbijaro (1983) reported that neem seed powder protected maize grains from *S. oryzae* damage for 6 months and the post-embryonic development were completely halted at all doses. Similarly, Makanjuola(1989) reported a significant reduction in egg laying of *S. oryzae* on maize seeds treated with neem based-extracts. Neem oil has been shown to be effective in reducing egg laying and adult emergence of *Callosobruchus maculatus* than neem powder (Lale and Abdulrah-man, 1999; Lale and Mustapha,2000). These studies suggested that the method of extraction and formulation seem to play an important role in pest control.

With regard to formulation, it appeared that neem oil-based extract formulated with different essential oils or volatile substances had a significant interaction effect on the behaviors of *S. oryzae*. With the exception of neem oil formulated with clove oil, camphor or borneol, there was a toxic effect on *S. oryzae*. The formulated neem oil with cinnamon oil, citronella oil or menthol displayed several different modes of action by reducing feeding ability, oviposiiton and adult emergence. Similar to our results, Huang and Ho (1998) demonstrated that cinnamaldehyde significantly reduced food consumption of *S. Zeamais* at a concentration range of 6.8 to 13.6 mg g^{-1} , but had no significant effects on food utilization and growth. Compared with the investigation of Sighamony *et al.(*1986), clove oil can reduce the infestation of stored grains insects without any adverse effect. Non-polar extracts of clove is insecticidal to *S. zeamais* Motsch., and suppress the progeny production (Ho *et al.*, 1994; 1995). Regnault- Roger and Hamraoui(1995) reported that eugenol, the main constituents of the essential oil of clove, also produced a strong inhibition of larval penetration of *Acanthoscelides obtectus* (Say) and finally a complete inhibition of emergence. In contrast, no pronounced inhibition of emergence was noticed with cinnamaldehyde and camphor.

In view of the variation in the toxicity of essential oils and volatile substances, Phillips *et al.* (1995) reported that the toxicity of each terpene alcohol, when presented separately, against the *T. castaneum* was up to 10 times more toxic than either the mixture. On the other hand , the mixture of terpene alcohol was as toxic to *Oryzaephilus surinamensis* as single monoterpenoid. These studies suggested that the formulation of neem oil with differrent essential oils and volatile substances could be a potential grain protectant by fumigant actions. However, the variation in the toxicity regarding the species of the insects is to be considered. Further research needs to be carried out to find a suitable formulation, as well as to clarify the mode

of action of these substances. Further isolation of effective compounds will be also determined.

5. Summary

The effectiveness of formulated neem oil-based pellets in feeding deterrent of *S. oryzae* showed that the weight of stored grains in neem oil and neem oil formulated with cinnamon, citronella oil or menthol were less damaged than untreated control. The formulated neem oil with menthol proved to be the best in protection grains damage, whereas neem oil formulated wit cinnamon or citronella oil gave no significant effect compared to neem oil. Similar to feeding deterrent effect, the formulated neem oil with menthol also inhibited reproduction and emergence of new progeny. In contrast, the cumulative number of eggs laid and offspring emergence in neem oil formulated with clove oil, camphor or borneol were considerably higher than untreated control and neem oil singly.

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