REPRODUCTIVE INHIBITION OF RICE WEEVIL, *Sitophilus oryzae* (L.) INDUCED BY VAPOR OF FORMULATED NEEM OIL-BASED PELLETS

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Abstract

Fumigant toxicity of different neem oil-based pellets formulated with some essential oils or volatile substances : clove oil, cinnamon oil, citronella oil, camphor, borneol and menthol, was investigated under artificial infestation at 24 $\pm$ 4 ^{o}C and 60 $\pm$ 15% r.h against *Sitophilus oryzae* L. Impregnation of different pellet bags along a 48-week storage period was used for testing the fumigant actions. Their biological activities such as feeding deterrence, reproductive inhibition and effect on progeny development were determined. The mortality and lethal time (LT_{50} and LT_{95} –value) were calculated.

All the treatments except for clove oil indicated a more or less fumigant toxicities and inhibited the behavioral responses and development of rice weevil. Mixture of neem oil with volatile substances: camphor, borneol or menthol were the most effective formulations. They acted not only as oviposition deterrents but also adversely influenced fecundity. Besides these effects, the antifeedant and inhibition of progeny development were observed. The LT_{50}, LT_{95} –value and life spans of rice weevil treated with neem oil formulated with camphor or menthol were generally shorter than those found for untreated control, neem oil and neem oil blended with essential oils.

Key words : neem oil, camphor, borneol, menthol, *Sitophilus oryzae* (L.)

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1. Introduction

It is well known that neem extract has proved to be one of the promising plant extract for insect control at the present time. Interest in neem-based insecticide has grown over the past decade as "environmental friendly botanical pesticide". The efficacy of neem-based extracts on various insect species were noted such as repellent, antifeedant, growth-retardant, molt disrupting, progeny development disrupting and oviposition deterrent (Saxena *et al.* , 1989; National Research Center, 1992; Sanguanpong and Schmutterer, 1992; Mordue and Blackwell, 1993; Saxena, 1995; Schmutterer, 1995). In a warehouse trial, neem could be of economic significance and practical application to rural area in developing countries (Jilani and Amir, 1987; Saxena, 1995). It shows considerable potential for controlling various post harvest insect pest species of store products -in particular, weevils (*Sitophilus* sp.), flour beetles (*Tribolium* sp.) and pulse beetles (*Callosobruchus* sp.) (Ketkar, 1987 ; Tanzubil, 1987; Saxena, 1995; Sanguanpong, 1996; Shaya *et al*., 1997). Furthermore 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 the most practical use of these oils is to mix grains or seeds with oil or substances to provide the physical contact of oil with insect cuticle and resulting in behavioral responses. Even the practice of mixing neem materials especially neem oil with store products, food grain and other commodities showed an effective protection against the insect pests. This method is impractical for storaging food grains, as characteristic garlicky odor of neem material can influence on taste, quality and acceptability.

In this work, the application of sub-lethal dose of neem oil-based pellets (Sanguanpong,1996) formulated with various essential oils and volatile substances were explored based on insecticidal properties and non-direct contamination method for protecting rice grain damage caused by rice weevil . Their biological activities against *Sitophilus oryzae* such as feeding deterrence, reproductive inhibition and progeny development disrupting were determined.

2. Materials and Methods

2.1. Mass rearing of test insect

The test insect *S. oryzae* was reared in plastic container, under the laboratory conditions at 24 $\pm$ 4 ^{o}C and 60 $\pm$ 15% r.h. Thai jasmine rice type KDML 105 (*Oryzae sativa* L.) was used to culture the weevil. Only 7-10 day-old adults of *S. oryzae* were used for the experiments.

2.2. Extraction and preparation of test materials

Thai neem seed kernel (*Azadirachta indica* var. *siamensis*) was collected and dried by exposure in an electric hot air oven at 75 ^{o}C for 9 hours from an original moisture content of 60% to about 14% m.c. Dried neem seed kernel was extracted in soxhlett's apparatus with hexane for 8 hours to obtain the Thai neem oil. The solvent was evaporated under a vacuum rotary evaporator. Various essential oils from clove, cinnamon and citronella grass were obtained from different parts of test plants (Tab. 1) by hydrodistillation. Whereas camphor, borneol and menthol were purchased from a local market.

Tab. 1 List of plant species and the parts used in the evaluation

Common name	Plant species	Family	Plant parts
Cinnamon	*Cinnamomum aromaticum* Nees	Lauraceae	bark
Citronella grass	*Cymbopogon nadus* Linn.	Gramineae	stem
Clove	*Syzigium aromaticum* (L.) Merr.	Myrtaceae	flower
Neem	*Azadirachta indica* var. *siamensis*	Meliaceae	seed

2.3 Formulation of various formulas of neem oil-based pellet

Stock solution of 1% neem oil-based emulsion was prepared by using Tween 80 (Polyoxyl Oleyl Ether-(20)-sorbitan monooleate) 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. All formulas were homogenized at 10,000 rpm for 1 minute. Non-aromatized talcum powder was crushed and sifted through a mesh size 300 μ. The powder was mixed with each formula 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.

2.4 Experimental techniques and design

As exposure chambers, plastic jars with 1,550 ml capacity covered with fine nylon mesh were used. The rice grains in each jar were artificially infested with 50 adults of 7-10 days-old of *S. oryzae*. Each pellet bag of different neem oil-based formulas was impregnated into the middle of rice container over a 48-week storage period. The mortality of adult weevils was assessed at 1-7 days after treatment. Each 7 days after infestation the test insects were screen out, the number of seed damage, egg-laids, new emerged adults and total population were monitored. The grains were further stored under ambient conditions. 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 Longevity effect

The toxic action of neem oil-based pellets varied with the additive volatile substances and essential oils. The data have been expressed as lethal time (LT). The LT_{50}, LT_{95} and life spans of treated adult *S. oryzae* were given in Tab.2. The most toxic compound was neem oil formulated with menthol and the least toxic clove oil. Differences in sensitivity could be observed for both of LT_{50} and LT_{95} - value. These responses affected also the longevity of tested insects. It should be noted that the life span of rice weevil treated with neem oil plus volatile substances were generally shorter than those found for untreated control, neem oil and neem oil blended with essential oils. However, there was no significant differences between the treatments and the respective control.

3.2 Antifeedant effect

Fig.1 demonstrated the toxic effect of various formulas of neem oil-based pellet against *S. oryzae* feeding activity during different periods of treatment. Along a 48 week storage period, no depression of stored weight of treated grains was observed in neem oil formulated with cinnamon oil. Similar effect was found in each application of the neem oil-based formulated with volatile substances, in particular grains treated with neem oil formulated with menthol was less damaged than

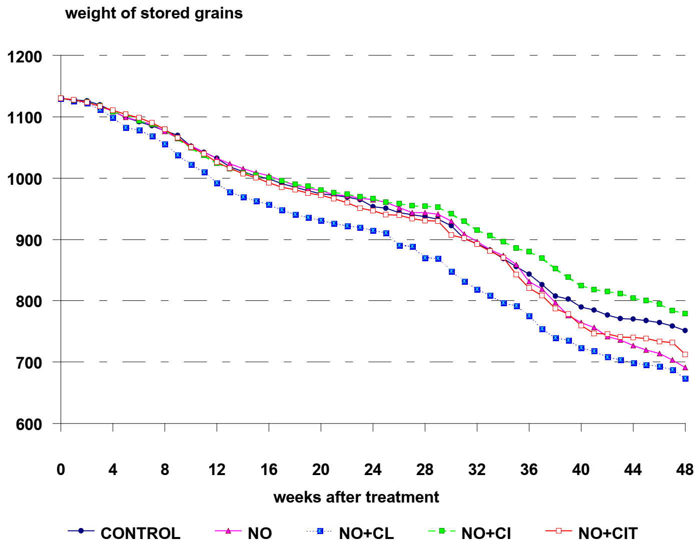
Tab. 2 LT_{50} , LT_{95} and life span (week) of adults *S. oryzae* L. treated with different formulated neem oil-based pellets

Treatment	LT_{50}	LT_{95}	Life span
control	3.61	6.89	7.25
neem oil	2.97	7.06	7.52
neem oil + clove oil	4.43	9.25	9.79
neem oil + cinnamon oil	3.72	8.51	9.04
neem oil + citronella oil	3.11	7.10	7.54
neem oil + camphor	1.93	5.88	6.32
neem oil + borneol	2.27	7.46	8.04
neem oil + menthol	1.96	5.12	5.47

untreated grains over a 44-week storage period. The treatment with neem oil formulated with camphor or borneol could maintain the weight of stored grains along 36 weeks after treatment. Generally, untreated seeds were more damaged than treated seeds with neem oil-based formulated with volatile substances. On the other hand, greater feeding by *S. oryzae* adults occurred on the neem oil formulated with clove oil than untreated control. The amount of stored weight of treated grains was lowered compared to the control. The statistical analysis (F-test) of weight of stored grains indictated that the differences in weight loss between control and treatments were significant (α=0.05) within 10 weeks. After 11 weeks there was no statistical evidence in preventing stored grains damage by *S. oryzae*.

3.3 Antiovipositional effect

The antiovipositional effect is demonstrated in Fig.2. It is evidenced by the reduced number of egg deposition. Cumulative number of egg laying by the females was seriously inhibited by neem oil, formulated neem oil with camphor, menthol or cinnamon oil. It appeared from these results that neem oil formulated with camphor, menthol or cinnamon oil significantly depressed the egg deposition along a 48-week storage period. Similar effect was found in the application of neem oil singly and neem oil formulated with citronella oil or borneol, which could reduced the egg-laying up to 36 weeks after treatment. After these critical periods, the increase number of eggs laid was noted. It may be suggested that there was a tendency for the *S. oryzae* populations to become more tolerant to neem oil, citronella oil and borneol when the fumigation was allowed over a long period. However, the increase in the



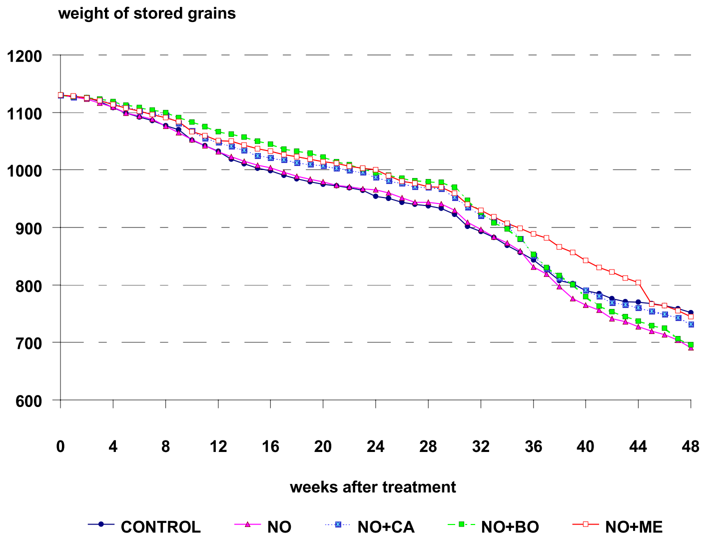
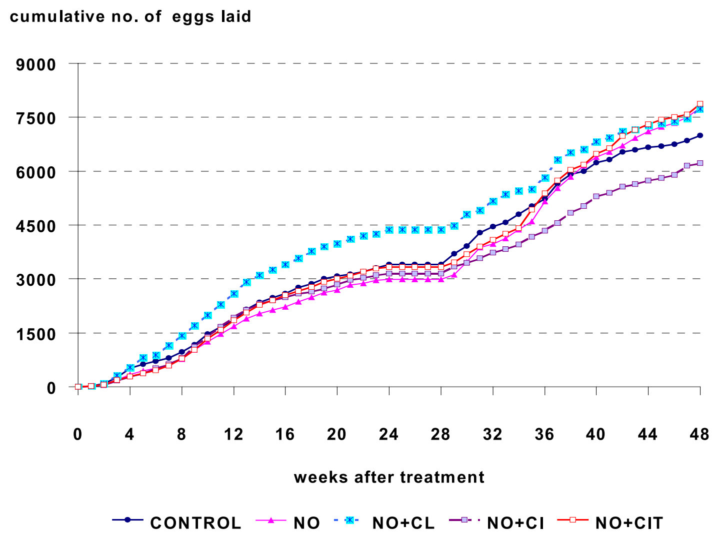


Fig. 1 Weight loss of stored rice grains treated with various formulas of neem oil-based pellets during a 48-week storage period
(Top) neem oil formulated with various essential oils
(Down) neem oil formulated with various volatile substances



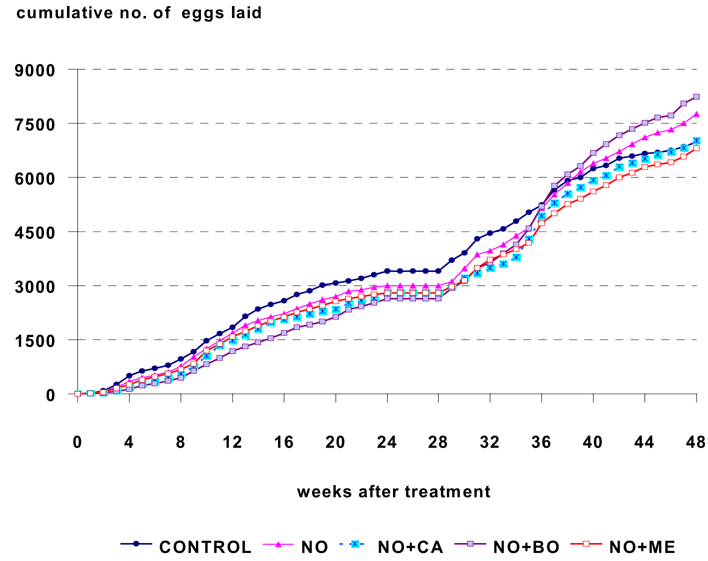


Fig. 2 Cumulative number of eggs laid of *Sitophilus oryzae* L. treated with various formulas of neem oil-based pellets during a 48-week storage period
(Top) neem oil formulated with various essential oils
(Down) neem oil formulated with various volatile substances

cumulative number of egg laying was obviously noted in the treatment of neem oil formulated with clove oil.

3.4 Effect on adult emergence

Adult emergence of *S. oryzae*, resulting from eggs laid was given in Fig.3. Strongly inhibition of adult emergence was observed with neem oil formulated with camphor, borneol, menthol or citronella oil. Furthermore neem oil and neem oil formulated with cinnamon oil also produced a strong inhibitory effect on adult emergence of *S. oryzae* compared to the untreated control. No pronounced inhibition of emergence was noticed with neem oil formulated with clove oil. However, the inhibition of emergence seen with treatments was probably a result of the low number of eggs laid. Emergence compared to oviposition showed that only 26% of eggs laid produced the next progeny in the control, 33% in the treatment of neem oil formulated clove oil and less than 21% in the other treatments. The statistical analysis of adult emergence in each of exposure period (week) showed that all the formulations except for clove oil affected the survival of new emerged weevil , but they did not act with the same toxicity at each developmental stage.

4. Discussion

Except for neem oil formulated with clove oil, all the formulation of neem oil with volatile substances or essential oils exhibited fumigant toxicity against *S. oryzae* adults. Even there was no direct mortality within 7 days at the sub-lethal dose used in the experiments, but they exerted more potent toxicities than untreated control in behavioral responses such as longevity, antifeedant, inhibition of egg laying and reduction of fecundity of *S. oryzae*. This agrees with the earlier finding of Makanjuola(1989) that neem-based extract displayed several different modes of action. They were active as suppressants and resulted in a significant reduction in oviposition, percentage of egg hatch and adult emergence of *S. oryzae*. In addition, Senguttavan *et al*.(1995) demonstrated that with neem oil tested, loss of damaged seeds and pods of stored groundnut due *to Corcyra cephalonica* Stainton was less than in untreated control. The percentage of dry mass losses and damaged kernels exposed to oviposition were lowered and the number of emerged adults was significantly less than control. Lale and Mustapha (2000) also reported the interaction of neem oil application and cow pea varietal resistance. It showed that neem seed oil reduced seed damage from over 25% in the control to less than 5 to 10%. Furthermore, the oviposition and percentage adult emergence of *Callosobruchus maculatus* were also suppressed.

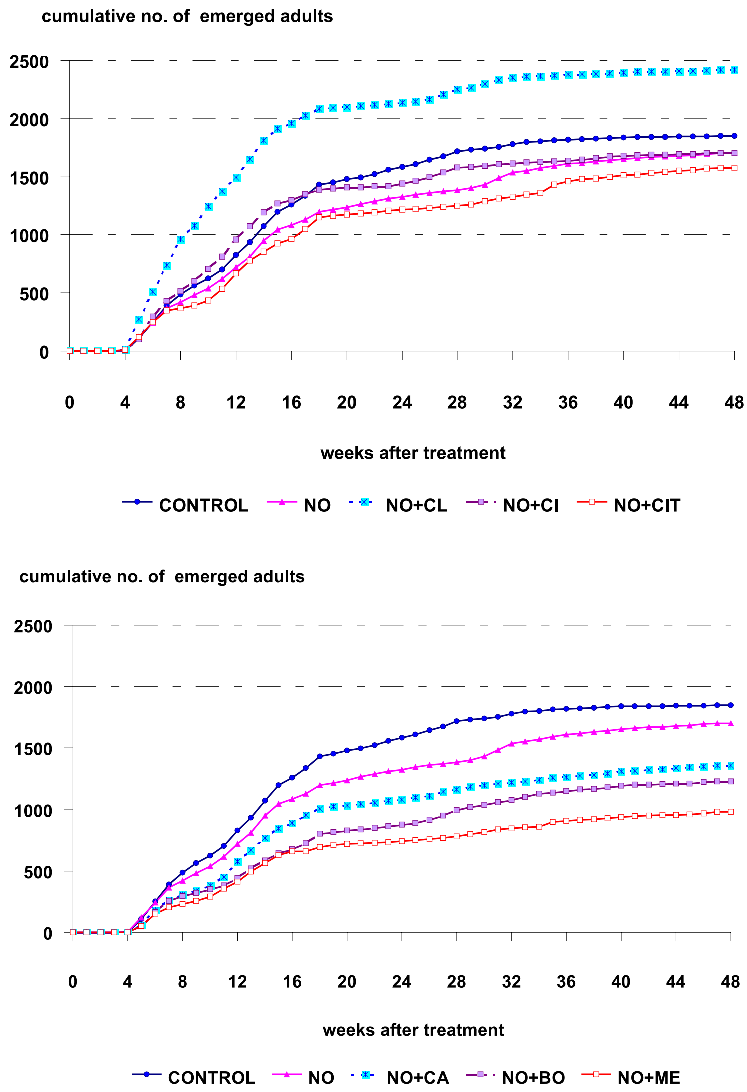


Fig. 3 Cumulative number of adult emergence resulting from eggs laid of *S. oryzae* treated with various formulas of neem oil-based pellets during a 48-week storage period

(Top) neem oil formulated with various essential oils

(Down) neem oil formulated with various volatile substances

The present studies with neem oil blended with volatile substances : camphor, borneol or menthol reduced feeding damage on stored rice grains. All the volatile substances showed satisfactory activities and the addition of menthol or camphor with neem oil proved to be promising as control agents against *S. oryzae*. Similarly, the formulations of neem oil mixed with essential oils as cinnamon oil or citronella oil showed good feeding inhibitors and ovipostion deterrents against *S. oryzae*. Earlier studies indicated that a methylene chloride extract of cinnamon was shown to be insecticides to *S. oryzae* (Huang and Ho,1998). It demonstrated a higher level of fumigant toxicity and reduced food consumption of tested insects. The results on the effect of monoterpenoids : camphor, cinnamaldehyde (main active substances in cinnamon oil) and eugenol (main active ingredient in clove oil) were similar to other store insects as *Acanthoscelides obtectus* (Say), a bruchid pest of kidney bean (Regnault-Roger and Hamraoui,1995). They exhibited some fumigant toxicity and reproductive inhibition, which involved ovipisition, fecundity and the development of larvae.

However, not all the essential oils tested showed satisfactory toxicity, but clove oil formulated with neem oil had no antifeedant action and also possessed lower significant effects on the oviposition and adult emergence resulting from eggs-laid by *S. oryzae* than untreated control. The mixture of clove oil and neem oil considerably elicited a less toxicity to *S. oryzae* than neem oil singly. In contrast to the results obtained in the present study, Sighamony *et al.* (1986) reported that oil of clove at doses of 25-100 ppm provided protection to wheat against *S. oryzae* and *Rhyzopertha dominica* for up to 60 and 30 days of exposure respectively. Phillips *et al.(*1995), however, reported that the mixture of four terpene alcohols, linalool, geraniol, α-terpineol and nerol was several times less toxic to *Tribolium castaneum* (Herbst) than either linalool or α-terpineol singly. Conversly, the mixture of terpene alcohols was as toxic to *Oryzaephilus surinamensis* (L.) as either of the four terpene alcohols presented separately.

Although not all published results are in agreement, our results and those reported earlier clearly indicated the variations in the mode of action and activities of neem oil, essential oils and volatile substances regarding the formulation and insect species. It is recommended that in the future studies the assays on relative toxicities of individual components and natural mixtures are required for various target species. Further research into suitable formulations to improve the toxicity, as well as isolation of chemical analogues of the effective compounds will be the next stage.

5. Summary

The toxicity of formulated neem oil-based pellets against the rice weevil indicated that the addition of volatile substances such as camphor, borneol or menthol with neem oil gave a greater mortality than untreated control. Their LT_{50} and LT_{95} - value were also generally shorter than all respective control, neem oil singly or neem oil formulated with essential oils. Similar to mortality effect, it was noted that neem oil formulated with camphor, borneol or menthol could maintain less damage rice due to rice weevil than other treatments.

It is observed that neem oil formulated with camphor, borneol or menthol significantly depressed the egg deposition along a 48-week storage period. On the other hand, neem oil formulated with clove oil obviously increased the cumulative number of egg laying. Besides, strongly inhibition of adult emergence was observed with neem oil formulated with camphor, borneol, menthol or citronella oil. No pronounced inhibition of emergence was noticed with neem oil formulated with clove oil.

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