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Duration of Developmental Stages of *Callosobruchus chinensis* L. (Coleoptera:Bruchidae) on Azuki Bean and the Effects of Neem and Sesame Oils at Different Stages of Their Development

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Abstract: The present study was conducted to determine the duration of developmental stages of *Callosobruchus chinensis* and to identify the susceptible stages of azuki bean weevil to neem and sesame oils. The results of the study showed that the duration for the 1st to 4th instar larva and pupal stages of *C. chinensis* were 7-12, 12-16, 16-19, 19-22 and 22-27 days, respectively. The result showed that both the oils can control the larvae inside the bean cotyledons, indicating that the oils might penetrate into the cotyledons and chemically inhibit or kill the larvae inside the bean. It is concluded that earlier developmental stages were more susceptible than the later ones and the depth of the larval position in the bean and its tolerance might be responsible for the age-dependant effects of the oils.

Key words: *C. chinensis*, larval duration, age-dependant effect, survivability, neem, sesame oils

Introduction

Azuki bean weevil, *C. chinensis* L. is a cosmopolitan insect and attacks the seeds of legumes such as azuki bean, *Vigna angularis* (Wild.) Okwi and Ohashi; cowpea, *Vigna unguiculata* (L.), Walpers; mung bean, *Vigna radiata* (L.) Wilczek. (Arora and Singh, 1970). Population dynamics of azuki bean weevil has been intensively studied and is considered to be a biological model for theoretical population biology (Fujii *et al*., 1990). It is known that both larva and pupa of azuki bean weevil remain completely inside the bean (Bhuiyan and Peyara, 1978). However, there are no reports about the duration of larval and pupal stages of *C. chinensis.*

Uses of various plant materials and oils for the control of stored grain pests are being implemented in many parts of the world. The effectiveness of neem and other oils in controlling insect pests has been well documented (Singh *et al*., 1978; Messina and Renwick, 1983; Ali *et al*., 1983; Ahmed *et al*., 1993; Isman *et al*., 1990). Ahmed *et al*. (1999) showed that both neem and sesame oils are very effective for the control of azuki bean weevil and suggested that the oils may protect beans physically or chemically. Lale and Abdulrahman (1999) reported that the effect of plant oils differ between developmental stages. The reasons for these age-dependent effects have not been investigated and there has been no report on the most sensitive developmental stages for controlling *C. chinensis* using plant oils.

Thus the objective of this study was performed to determine the duration of developmental stages of *C. chinensis* and to determine which developmental stages are susceptible to oils. Further experiments were conducted to delineate the mechanism of oil action in controlling azuki bean weevil.

Materials and Methods

Collection of oils and rearing of insects: Cold-pressed 100% pure neem and sesame oils were purchased from The Original Neem Company, USA and Musou Co. Ltd., Japan, respectively. The oils were initially dissolved in a small amount of acetone, then 5% (v/v) oil suspensions were made separately by adding distilled water. After that the oil suspensions were shaken and allowed to stand for a few min for the complete volatilization of acetone. The beetles were collected from Miki-cho, Kagawa, Japan. To obtain a steady supply, the beetles were cultured on azuki beans in a control chamber at 25°C under 70±5% r.h. and 14L: 10 D regime. Azuki beans grown and harvested in Hokkaido, Japan were used for the rearing materials. To prevent pre-infestation, the beans were kept in an oven at 50°C overnight, then placed in a large glass jar covered with netted cloth for 24 h to let them acquire normal seed moisture without further infestation.

Duration of developmental stages: Ten, one-day-old beetles were released in a jar (12 cm in diameter and 6.5 cm in depth) containing fresh azuki beans. The beetles were allowed to lay eggs on the beans for 1 h and then the beetles were removed. It was replicated 20 times to obtain a steady supply. Single egg containing beans were selected and kept in the control chamber. Every day 100 single-egg containing beans were randomly taken from the chamber and put in water overnight. On the next day, each bean was cut very carefully and the development (stage, position and mortality) of immature inside the bean was observed under the microscope. This was continued up to 31 days. The larval instars were confirmed by the count of the shed skin and a frequency curve was constructed from the data.

The effects of neem and sesame oils on survivability of *C. chinensis*: The effect of oil treatments was examined at 3 different times. Group-1: Oil was applied before oviposition. 20 g of fresh azuki beans were taken in a petri-dish (5.5 cm in diameter) and 5% neem or sesame oil was sprayed uniformly on beans with a fixed number of sprays with a hand sprayer. Solvent was sprayed in the control group. In oil-treated petri dishes 150 pairs of day-old beetles were allowed to lay eggs for one day, whereas only 5 pairs of beetles were allowed to lay eggs in solvent-treated petri dishes. Group-2: Oil was applied after one day of oviposition. 5 pairs of one-day-old beetles were taken into each petri dish contain 20 g of fresh azuki bean. The beetles were allowed to lay eggs for one day and then were removed. One day after removal, the beans with eggs were sprayed with 5% neem or sesame oil thoroughly with a fixed number of sprays, while in control, only solvent was sprayed. Group-3: Oil was applied as in treatment 2 but after seven days of oviposition. Three replications were made for each treatment. After oil application, the petri dishes were kept in a control chamber at 25°C, 70±5% r.h. and 14 L: 10D regime for normal growth and development. After 31 days of oviposition, the numbers of eggs or eggshells on each bean were counted and the beans were put separately in water for overnight. On the next day, the soaked beans were cut very carefully under a binocular microscope and the larval/pupal conditions (stage and mortality) inside the bean were checked. Numbers of adults emerged were also counted from the hole of the seed coat.

Efficacy of neem and sesame oils at different stages of their development: The same procedure was followed as in Group-2 except that the oils were applied after 1, 7, 12, 16, 19 and 22 days (which are considered as egg, 1st to 4th instar larva and pupal stages, respectively), after oviposition (Fig. 1). Three replications were made for each treatment. The eggs laid in each petri-dish were counted and kept in a control chamber at 25°C, 70±5% r.h. and 14 L: 10D regime for normal growth and development of the beetle. Adults that emerged were counted daily following the initial emergence until no further adults emerged for 5 consecutive days.

Experimental design and statistical analysis: The experiments were carried out using a complete randomized design. The number of adults that emerged in each replicate was converted into proportions of the total number of eggs laid and expressed as percentages. Percentage data were transformed to arcsine values and subjected to analysis of variance (MSTAT). Significant differences between means were determined by Tukey's test ($p<0.05$).

Results

Duration of developmental stages: The larvae started hatching after 6 days of oviposition. From the frequency curve, it was found that the maximum numbers of 1st to 4th instar larvae and pupae were found on the 8th, 13th, 17th, 21st and 26th day of oviposition, respectively (Fig. 1). Most of the adults emerged by 31st day.

Table 1: Percentage of adult emergence of *C. chinensis* grown in azuki bean treated with neem and sesame oils at different application times

	Time of oil application		
	Before oviposition	1 day after oviposition	7 days after oviposition
Neem	0.0±0.0 d (0.0)	0.31±0.06 cd (2.3)	9.40±2.37 b (17.6)
Sesame	0.0±0.0 d (0.0)	2.19±0.46 c (8.4)	8.88±1.99 b (17.0)
Control	83.80±1.98 a (66.2)	79.68±1.39 a (63.2)	75.07±2.04 a (60.17)

Data are mean ± SE of emergence rate (%). Figures in parentheses are arcsine values to which Tukey's test was applied. Means followed by same letter(s) did not differ significantly at $P<0.05$.

Table 2: Position (depth in mm) of the immature inside the soaked bean

Stage (N=20 each)	Position from seed coat (mm) Mean ± SE
Instar 1	0.4 ± 0.10
Instar 2	1.0 ± 0.04
Instar 3	2.0 ± 0.05
Instar 4	2.2 ± 0.10
Pupa	Just below the seed coat

Survivability of *C. chinensis* to oils at different times of oil application: In Group-1, where eggs were laid on oil-coated beans and the eggs had direct contact to the oil layer, egg-respiration was not in question. In Group-2, the oils were coated on the eggs and the beans where eggs had direct contact to the oil layer, egg-respiration was in question. In Group-3, the oils were coated only on the beans, the hatched larvae had already bored into the

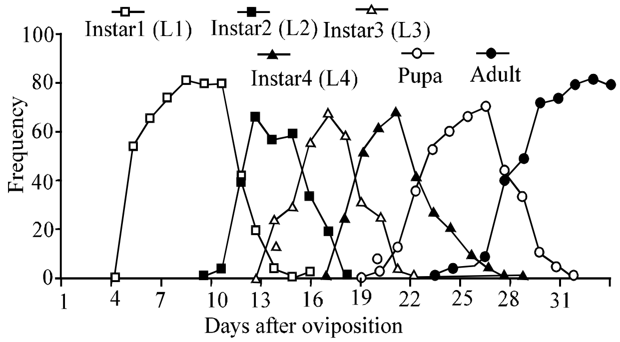

Fig. 1: Frequency distribution of *C. chinensis* at each developmental stage when grown on azuki bean in control conditions (25°C under 70±5% r.h. and 14L: 10D).

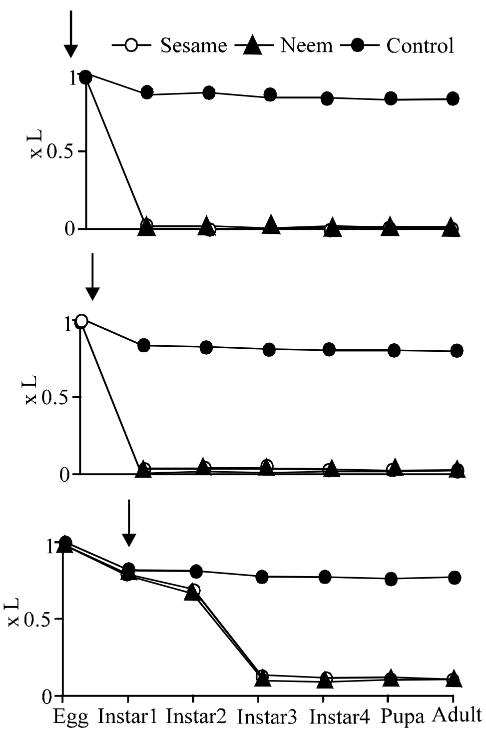

Fig. 2 Survivorship curves of *C. chinensis* in azuki beans treated with 5% neem and sesame oils Group-1: A, Group-2: B and Group-3: C. Arrows indicate the timing of oil application. L_x is the survival rate at the beginning of each stage

beans and the larvae had no direct contact with the oil layer. In Group-1 and Group-2, the survival rate (L_x) in neem and sesame oils was almost zero in the first instar larvae (Fig. 2). In contrast with Group-3, the survival rate in both oils was almost similar to the control up to the 2nd instar larvae although from the 3rd instar, it decreased greatly. While any or very few adults emerged in the first

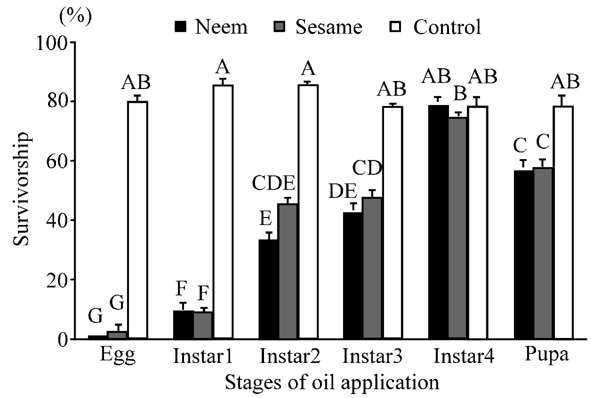

Fig. 3 Efficacy of 5% neem and sesame oils to *C. chinensis* at different stages of_@their development. Data were transformed to arcsine and subjected to Tukey's test. Values with the same letters above bars indicate no significant difference in survivorship (mean ± SE) at p=0.05.

and second treatments, a few adults emerged in the third treatment. The adult emergence ratio in oil treated beans was significantly lower than the controls in all the three treatments (Table 1).

Evaluation of susceptible stages of *C. chinensis* to neem and sesame oil: The survival rate to adulthood was significantly lower in oil-treated beans than in controls except in the 4th instar larvae (Fig. 3). This tendency was more pronounced when oils were sprayed at the earlier larval stage. The efficacy of neem and sesame oils was almost similar in every case.

Discussion

The results show that the beetle needs at least 25 days to complete their life cycle under control condition (Fig. 1). Duration of the developmental period was determined by the points of intersection of the curves of incidence of the previous stage with the next stage (Kiritani and Hokyo, 1962) and our results demonstrate that 6-12, 12-16, 16-19, 19-22 and 22-27 days are needed for 1st, 2nd, 3rd, 4th instar larva and pupal stages, respectively.

The timing of oil application had significant effects on *C. chinensis* survivorship (Table 1 and Fig. 2). Complete inhibition of hatch ability of *C. chinensis* eggs with neem and other oils has been reported by a number of workers (Singh *et al.*, 1978; Messina and Renwick, 1983; Lambert *et al.*, 1985; Ahmed *et al.*, 1999). It has also been suggested that the protective properties of oils involve chemical rather than physical factors (Ahmed *et al.*, 1999). The present results confirmed their findings since in Group-1 and Group-2, the eggs had direct contact with the oil layer but there was no oil layer (which may affect egg-respiration) over the eggs in group-1. Furthermore, from Group-3 the oils still killed most of the second instar larvae (Fig. 2.) Although the oils, in this case, were supposed not to inhibit insect respiration physically or did not have direct contact with the larvae. In this condition, most of the eggs became larvae and entered into the cotyledons. This suggests that the action of oils is not only through direct contact but that they also penetrate into the cotyledons and inhibit larval development. This finding is of great economic significance because *C. chinensis* is a field-to-store pest (Shinoda and Yoshida, 1984); which commences infestation of beans in the field and has grown to larval stage by the time the beans are stored. The oils can adequately protect early larval infestations.

Similar to our result, other researchers reported that one of the important mechanisms of oil action is due to ability of oils to penetrate the chorion through the micropyle and cause the death of the developing embryo (Singh *et al.*, 1978; Don-pedro, 1989a, b; Credland, 1992). This may be one of the reasons why neem and sesame oils completely check the hatch ability of *C. chinensis* when applied at egg stage (Fig. 2).

The survival rate of *C. chinensis* increased when oils were applied later in their developmental stages. As larval development progresses, the larvae enter deeper into the bean and become larger. So, survivability is assumed to depend on the larval position inside the bean and/or the larval tolerance against the oils. On the other hand, however, the application of oils at a pupal stage had less of a killing effect on the insects than when they were applied at the 4th instars. This result indicates that the position of the insects may be more important than their tolerance because the pupa always stay just under the seed coat while the 4th instar larvae enter deeper into the bean (Table 2). Based on these results, we conclude that neem and sesame oils can completely protect beans when applied at egg stage and application of oils up to 3rd instar larvae can suppress the emergence of adults to a great extent.

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