

## Effect of *Artemisia vulgaris* isolated extract and determination of AD<sub>50</sub> and LD<sub>50</sub> for antifertility and mortality of *Callosobruchus chinensis* (L.)

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**Abstract :** Antifertility, AD<sub>50</sub> with effect of *Artemisia vulgaris* isolated leaf extract on hatchability was noted. These median antifertility doses 0.6529 $\mu$ g, 0.4753 percent and 0.5198 percent per weevil were obtained in topical, dipping and injection (12 $\mu$ l/weevil) methods respectively. Probit analysis for dose response data for *Artemisia vulgaris* leaf extract showed that linear relationship existed in between the concentration (Log x) and percentage antifertility induced (Probit Y). Mortality, LD<sub>50</sub> was observed with effect of *Artemisia vulgaris*, isolated leaf extract during hatchability were 0.7589 $\mu$ g; 0.4743 percent and 0.5139 percent per weevil were obtained in topical, dipping and injection (12 $\mu$ l/weevil) methods respectively. Probit analysis showed that a linear relationship existed in between the concentration (Log x) and percent mortality induced (Probit Y).

**Key words :** *Artemisia vulgaris*, *Callosobruchus chinensis*, Alcoholic extract

### Introduction

The autocidal method of insect control has attracted much attention of the entomologists during the past decade and a large number of chemicals liable to induce sexual sterility in weevils have been evaluated Nagasawa *et al.* (1967). Doses have been similarly studied on adult Mediterranean flour moth (Tan and Mordue 1977). Presumption of doses respond curve was also studied in different other biopesticide agents on *Callosobruchus chinensis* (L.) (Khalequzzaman *et al.*, 2007) in *Tribolium castaneum* (Herbst), (Zahara *et al.*, 2008). Biopesticide of plant origin had been used for centuries because they were non hazardous to non target organisms. Many workers had reported that mixing of various plant products with grains repel insects. (Anonymous, 1983; Dakshinamurthy, 1988, 1992). Effectiveness of

citrus clean against *Callosobruchus chinensis* (L.) that egg laying was significantly reduced.

In this paper authors wish to report the result of comparative studies on method of application for *Artemisia vulgaris* leaf extract to find out AD<sub>50</sub> and LD<sub>50</sub> of *Callosobruchus chinensis* (L) in which *Artemisia vulgaris* in logarithim and average number of the net data were analysed by standard statistical treatment of quantities reported.

### Material and Methods

Three methods used for the screening of weevils i.e., topical application, dipping and injection method. All experiments were performed in thermohumidity in cubator maintained at 30 $\pm$ 2°C and 70 $\pm$ 5% of R.H.

**a- Topical Application.** Newly emerged 24 adult of azuki bean weevil taken for the experimental purposes and treated topically (micropipette technique). They have been divided in fourteen groups and were treated with some grades of antifertility agent *Artemisia vulgaris* leaf extract which were dissolved in 1litre. of distilled water and made into the ropes that were 0.25, 0.3, 0.412, 0.5, 0.615, 0.79, 1.0, 1.25, 1.49, 2.0, 2.5, 3.2, 3.52, 4.8 µg. per weevil and were applied with a micropipette to the dorsal side of the weevil, one group was not treated with *Artemisia vulgaris* leaf extract and was used as control.

**b- Dipping Method.** Adult azuki bean weevil obtained from the laboratory culture were divided into eleven groups of 24 and were treated with the aqueous solution of *Artemisia vulgaris* leaf extract i.e. 0.0548, 0.8621, 0.127, 0.164, 0.27, 0.37, 0.5, 0.69, 0.8, 1.0, 1.5 percent. One group was not treated with *Artemisia vulgaris* extract and was used as control, oviposition in each group was fully recorded.

**c- Injection Method.** In this experiment also freshly emerged 24 weevils were taken and were injected with different concentrations of *Artemisia vulgaris* extract i.e., 0.12, 0.22, 0.34, 0.56, 0.73, 1.0, 1.2, 1.5 percent. To each weevil 12µl. solution of *Artemisia vulgaris* extract was injected. After time interval the weevils were allowed for mating and then they were transferred to the experimental area to lay eggs. One group was not treated with extract and used as control.

After conducting above methods, the most effective dose and median antifertility dose of *Artemisia vulgaris* isolated leaf extract as well as  $AD_{50}$  and  $LD_{50}$  was calculated by Golden method (1956). From the above method it is concluded that which is the most effective one for creating antifertility in *Callosobruchus chinensis* (L).

## Result and Discussions

(i) Determination of  $AD_{50}$  and  $LD_{50}$  of Naturally Isolated product of *Artemisia vulgaris* for Antifertility and Mortality of *Callosobruchus chinensis* (L.) by Topical, Dipping and Injection Method.

The relationship between dose of *Artemisia vulgaris* leaf extract, hatchability of eggs and methods of application in each series of determination are arranged in figure (1 to 6). The resulting regression equation median antifertility dose, fiducial limits and variance were find out by statistical calculation by Golden estimation method of parameter tolerance distribution based on probit transformation which were given (Fig 1 to 6).

(ii) **Determination of  $AD_{50}$**  Figs (1 - 3) summarizes the effect of *Artemisia vulgaris* isolated extract by three methods that concentrations were in between 2.0 µg to 3.5 µg. in topical application, 0.5 to 1.5 percent in dipping method and 0.5 to 1.5 percent (12 µl./ weevil) of *Artemisia vulgaris* leaf extract per weevil in injection method. The relation between the concentration of chemicals in logarithms and the average number of unhatched eggs per pair of weevils in probits could be fitted satisfactorily by three probit regression lines Figs. 1-3 as well as in equation :-

$$Y = 2.5476 + 2.1935 x$$

$$Y = 4.6341 + 2.5639 x$$

$$Y = 4.8618 + 2.4854 x$$

for topical, dipping and injection methods respectively.

The median antifertility, hatchability inhibition dosages of *Artemisia vulgaris* leaf extract (Isolated chemical) at 5.0 percent fiducial limits were estimated as for topical application 0.6529 µg./weevil with fiducial limit 0.4027 ~ 0.4178 x  $10^{-1}$  µg. For dipping method 0.4753 percent per

weevil with fiducial limit 0.3452 ~ 0.5318. For injection method 0.5198 percent (12 µl/weevil) with fiducial limit 0.4342 ~ 0.6463. Less than 0.971µg. per weevil in topical application, 0.451 percent per weevil in dipping application and 0.483 percent per weevil in injection method of *Artemisia vulgaris* leaf extract (naturally isolated chemical) had negligible effect on hatchability.

Variance was also calculated out at AD<sub>50</sub> that were 0.02486ig, 0.1876 percent and 0.1679 percent per weevil in topical, dipping and injection method respectively. It was significant at 5.0 percent level (n-2) degree of freedom.

**(iii) Determination of LD<sub>50</sub>** Figs. 4-6 shows the toxicity of *Artemisia vulgaris* leaf extract (naturally isolated product) to hatchability of weevils of both the sexes. They also summeries the effect of *Artemisia vulgaris* leaf extract concentration by three methods. Those concentrations were in between 2.0 to 3.5 µg, 1.5 percent and 0.5 to extract, concentration per weevil in topical, dipping and injection method respectively. The statistical analysis was given (Figs. 4 to 6). The experimental data have been analysed statistically by for tolerance distribution (Golden, 1956). The relation between concentration of *Artemisia vulgaris* leaf extract in logarithms and average number of net mortality per pair of weevils in probit could be fitted by three probit regression lines shown in figure (4 to 6) in the following equation :

$$Y = 1.529 + 4.3658 x$$

$$Y = 4.8621 + 0.97532 x$$

$$Y = 3.6539 + 0.1585 x$$

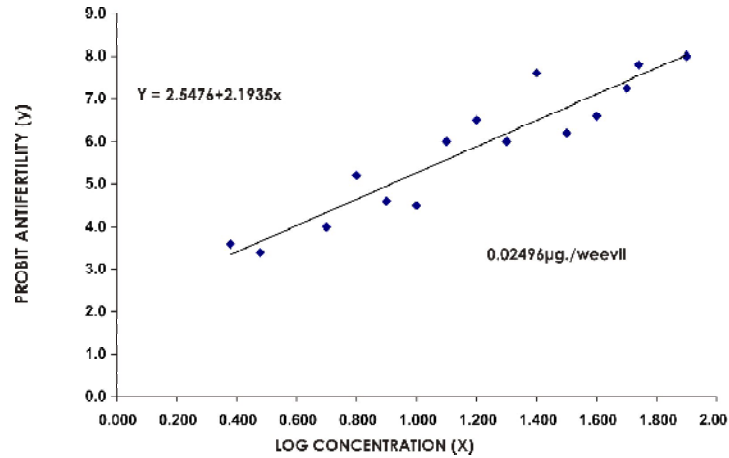
in topical, dipping and injection method respectively.

The median mortality hatchability inhibition concentration of *Artemisia vulgaris* chemical at 5.0 percent level of fiducial limits were estimated as for topical method 0.7589 µg per weevil were fiducial limit 0.4275~0.8693 for

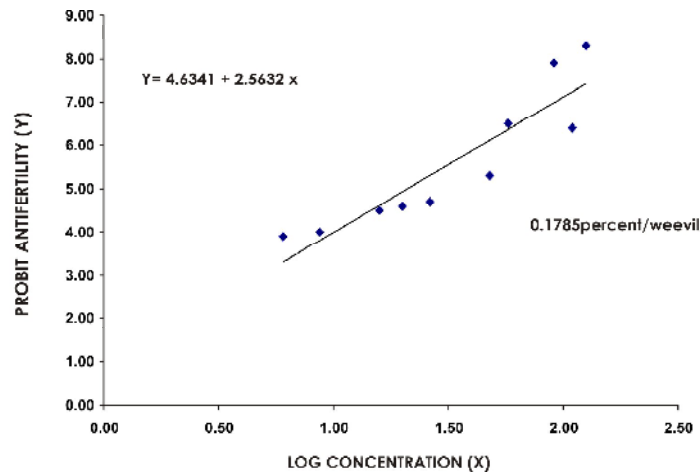
dipping 0.4793 percent weevil with fiducial limit 0.4747~0.5539. Less than 0.89µg. 0.049 percent and 0.059 percent per weevil in topical, dipping and injection methods respectively with *Artemisia vulgaris* has negligible effect on mortality.

At LD<sub>50</sub> the variance calculated was 0.0363 µg 0.1573 percent and 0.1569 percent of *Artemisia vulgaris* chemical per weevil in topical, dipping and injection methods respectively, it is significant at 5.0 percent level of (n-2) degree of freedom.

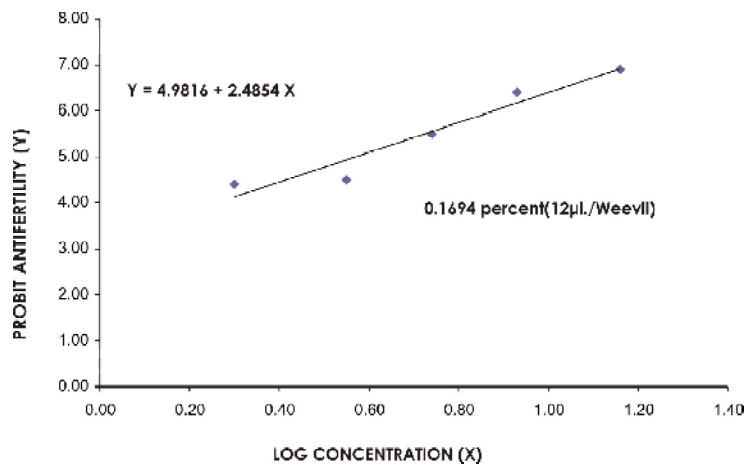
The median antifertility doses with the effect of *Artemisia vulgaris* isolated leaf extract during hatchability are 0.6529 µg./weevil with 0.4027 ~ 0.4178 x 10 fiducial limits; 0.4753 percent with .3452 ~ 0.5318 fiducial limits; 0.5198 percent (12 µl./weevil) with 0.4349 ~ 0.6463 fiducial limits in topical, dipping and injection methods respectively. These results obtained by probit analysis of the dose response data for *Artemisia vulgaris* extract applied to *Callosobruchus chinensis* showed that a linear relationship existed between the concentration (Log x) and the percentage anterfertility induced and mortality (Probit Y). With this method the relation between antifertility and toxic doses of the applied *Artemisia vulgaris* extract have been calculated. In support of the above fact *Callosobruchus maculatus* were showed antifertility with metapa by topical application and median antifertility dose (AD<sub>50</sub>) was 1.2 µg./ weevil, Rahim and Wahab, (1974). Similarly, Nagasawa *et.al.* (1967) found that most probable median hatchability inhabiting concentration of apholate was estimated as 0.128 percent with fiducial limits of 0.086 ~ 0.520 percent for 95 percent probability and that of hempa was 6.236 percent with fiducial limit of 4.242 to 9.167 in azuki bean weevil, *Callosobruchus chinensis* by dipping method. Tan and Mordue (1977) reported that at 95 percent fiducial limits (0.85 ~ 1.67). The AD<sub>50</sub> was 1.38 µg./female and mortality LD<sub>50</sub> was



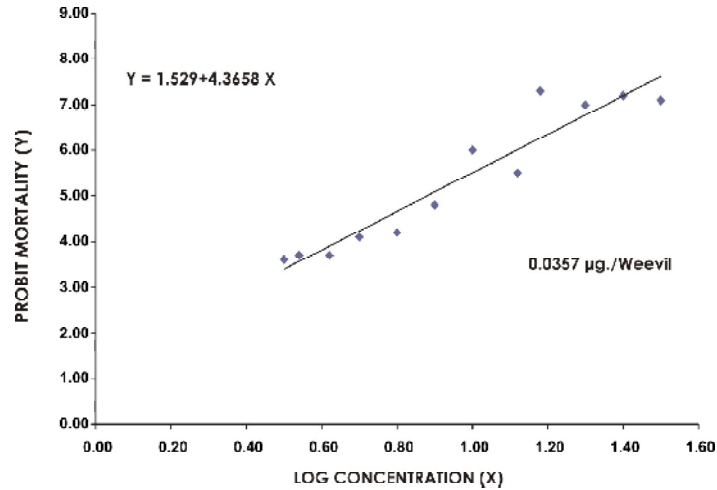
**Fig. 1.** Probit regression line for the antifertility of *Artemisia vulagris* (naturally isolated product) through topical application to the *Callosobruchus chinensis* (L.) just after their emergence.



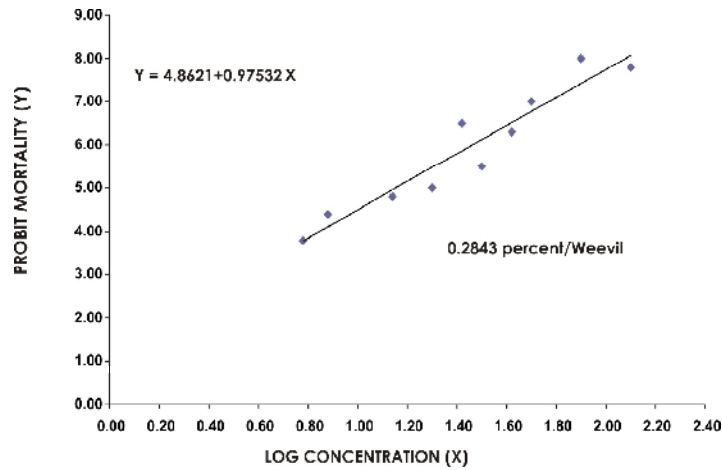
**Fig. 2.** Probit regression line for the antifertility of *Artemisia vulagris* (naturally isolated product) through dipping method to the *Callosobruchus chinensis* (L.) just after their emergence.



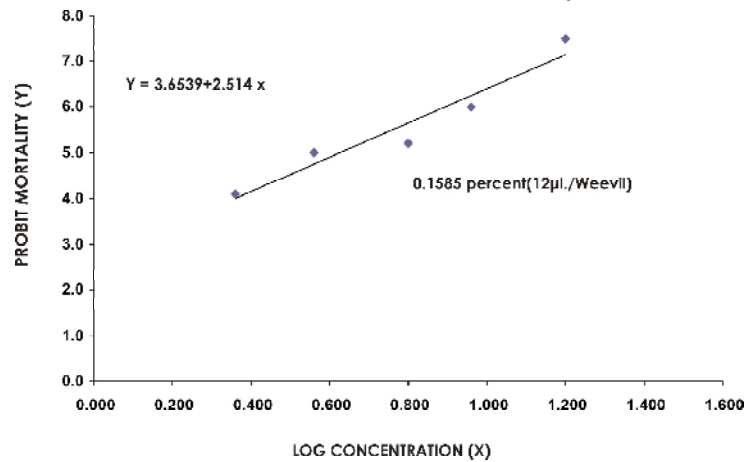
**Fig. 3.** Probit regression line for the antifertility of *Artemisia vulagris* (naturally isolated product) through injection method to the *Callosobruchus chinensis* (L.) just after their emergence.



**Fig. 4.** Probit regression line for the mortality of *Artemisia vulgaris* (naturally isolated product) through topical application to the *Callosobruchus chinensis* (L.) just after their emergence.



**Fig. 5.** Probit regression line for the mortality of *Artemisia vulgaris* (naturally isolated product) through dipping method to the *Callosobruchus chinensis* (L.) just after their emergence.



**Fig. 6.** Probit regression line for the mortality of *Artemisia vulgaris* (naturally isolated product) through Injection method to the *Callosobruchus chinensis* (L.) just after their emergence.

26.87 µg./female at 95 percent fiducial limits (23.34 ~ 29.94) when *Ephestia kuehniella* was treated with tepa by topical application. Similarly Jalaza and Prabhu (1976) recorded LD<sub>50</sub> in *Dysdercus cingulatus* at dose 12.5 µg. and 7.5 µg. injected metapa and apholate per insect respectively.

In the present study of LD<sub>50</sub> also similar results have been noted as given in the preceding para. LD<sub>50</sub> was recorded with *Artemisia vulgaris* in azuki bean weevil at concentration of 0.7589 µg. per weevil with fiducial limit (0.4575 ~ 0.8693); 0.4743 percent with fiducial limit (0.4875 ~ 0.6185 x 10); 0.5139 percent with fiducial limits (0.4747~ 0.5539) in topical, dipping and injection method respectively. Similarly, Khalequzzaman *et. al.* (2007) also observed efficacy of essential oils on *Triboleum castaneum* and *Callosobruchus chinensis* (L.) respectively.

This study has been undertaken to record the percentage of antifertility and toxicity (AD<sub>50</sub> and LD<sub>50</sub>) in *Callosobruchus chinensis* against *Artemisia vulgaris* efficacy and found that pollution – free biological antifertility agent *Artemisia vulgaris* is one of the good control measure for *Callosobruchus chinensis* (L.)

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