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**NM Eesa**
Department of Entomology, Faculty of Science, Cairo University, Giza-12613-Egypt

**H El-Sherif**
Department of Entomology, Faculty of Science, Cairo University, Giza-12613-Egypt

**WM ElSayed**
(A). Department of Entomology, Faculty of Science, Cairo University, Giza-12613-Egypt
(B). Laboratory of Systematic Entomology, Division of Environmental Resources, Department of Agrobiology and Bioresources, Hokkaido University, Japan

**DH Abd El-Monim**
Department of Entomology, Faculty of Science, Cairo University, Giza-12613-Egypt

**Correspondence**
**NM Eesa**
Department of Entomology, Faculty of Science, Cairo University, Giza-12613-Egypt

# Bio-efficacy of *Catharanthus roseus* and *Artemisia herba alba* against *Spodoptera littoralis* (Boisduval, 1833)

**NM Eesa, H El-Sherif, WM ElSayed and DH Abd El-Monim**

**Abstract**
The second and fourth instars of the cotton leaf worm *Spodoptera littoralis* (Boisduval, 1833) were reared on castor bean leaves *Ricinus communis* (L.) treated with aqueous extracts of two doses, $LC_{25}$ and $LC_{50}$, of *Catharansus roseus* (L.) and *Artemisia herba alba* (L.), using the dipping method, to evaluate the effect of these botanical extracts on nutritional and percentage antifeeding indices. It was observed that the consumption index was significantly decreased as the dose of the botanical extract from the second to the fourth instar. The approximate digestibility, was significantly decreased at the fourth instar. The food utilization efficiencies, in terms of the conversion of ingested and digested food (ECD) to biomass, were significantly decreased at both botanical extracts. Moreover, the least values of ECD were confined to *C. roseus* (L.) when compared with *A. herba alba* (L.). The values of growth rate (GR) were observed to decrease drastically at the two studied doses for both the second and fourth instars.

**Keywords:** *Spodoptera littoralis, Catharansus roseus, Artemisia herba alba,* percentage Antifeeding, Nutritional indices

## 1. Introduction
The cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae), is characterized by high mobility and reproductive potential causing great economic loss [17]. It is considered as one of the most destructive sporadic agricultural lepidopteran pests in Egypt as well as Mediterranean and Middle East countries [5, 36, 2-4].
Feeding deterrents or antifeedants are considered as chemicals which inhibit feeding of an insect species on a treated food material without the necessity to kill or repel the insect [34]. In this sense, different plant species were evaluated for their antifeeding properties against spodopteran species and their antifeeding properties are increasingly being used against phytophagous insect pests for protection of crops [32].
Hence, the aim of this research is to investigate the effect of botanical extracts of *Catharanthus roseus* (L.) (Gentianales: Apocynaceae) and *Artemisia herba alba* (L.) (Lamiales: Lamiaceae) on food utilization efficiencies such as approximate digestibility (AD), the efficiency of conversion of ingested food (ECI), the efficiency of conversion of digested food (ECD), growth rate (GR) and consumption index (CI) together with the percentage antifeeding activity of the second and fourth instars of *S. littoralis*.

## 2. Materials and Methods
### 2.1 Insect rearing
The colony of *S. littoralis* was started with egg patches obtained from a standard laboratory colony maintained in the Department of Entomology, Faculty of Science, Cairo University. Newly hatched larvae were collected from rearing stock and were kept in ventilated plastic containers (12 × 6 × 18 cm). Larvae were fed fresh castor bean leaves, *Ricinus communis* (L.), until pupation. The culture was kept at 25 ± 2 °C, 65 ± 5% R. H. and 12:12 hours (D:L) photoperiod.

### 2.2 Botanical extracts
Aqueous extracts were prepared from plant species collected from the experimental plantations at the Faculty of Science, Cairo University. We used the leaves and flowers of *Catharanthus roseus* (L.) and *Artemisia herba alba* (L.). These plant parts were washed thoroughly with distilled water and dried at 40 °C for 48 h then ground in an electric mill to obtain a fine

powder. Crude extracts were obtained by mixing 10g of fine powder per 100 ml of distilled water and storing them for 48 h in hermetically sealed containers. The suspensions were filtered using filter paper (No. 1 Watman™ filter paper) to obtain 10% aqueous extract. Distilled water was used as negative control and considered to be 0%.
The mortality of the 2nd and 4th instars in each test was corrected using Abbots equation [1]. The regression equations for $LC_{25}$ and/or $LC_{50}$ and confidence limits of 95% were calculated by probit analysis [13] using SAS Proc PROBIT [31].

### 2.3 Botanical extracts treatments
The newly hatched 2nd and 4th instars of *S. littoralis* were freely fed on fresh discs (Ø = 6 cm) of *R. communis* leaves dipped in $LC_{25}$ and $LC_{50}$ of aqueous botanical extracts for 30 seconds, then air-dried at room temperature [19, 25, 2-3]. Each experiment was replicated three times of twenty larvae each. The remaining diet was replaced regularly with freshly treated one every 24 hours. The experiment was started with larvae hatched in the same day and were offered daily aqueous botanical extract-treated discs from hatching till pupation.

### 2.4 Effects of botanical extracts on nutritional indices
The fresh weight of larvae, faeces, and of leaf consumed, and weight gain of larvae were recorded regularly. All uneaten food and faeces excreted were collected and immediately frozen; these matters were later dried at 105 ºC in an oven (Thermo Scientific® Compact Oven) and weighed using analytical balance (Mettler® M22) for calculating the food utilization [5-6]. Exuviae were measured with faeces since they were considered as parts of the insect at the end of the experiment [28].
Nutritional indices of the 2nd and 4th instars were calculated using standard gravimetric procedures [40, 35] as follows:
- Consumption index (CI) = F/TA,
- Approximate digestibility (AD) = 100 x (F-E)/F,
- Efficiency of conversion of ingested food into biomass (ECI) = (G/F) x 100,
- Efficiency of conversion of digested food into biomass (ECD) = (P/F- E) x 100,
- Growth rate (GR) = P/TA,

where:
A = fresh weight of larvae during the feeding period, E = dry weight of produced feces
F = dry weight of food eaten, G = weight gain at the end of the feeding period
P = dry weight of the biomass of larvae, T = duration of feeding (days)

### 2.5 Antifeedant activity of the botanical extracts
Antifeedant activity of the botanical extracts of the plants was assayed against the early (2nd) and late (4th) instar larvae of *Spodoptera littoralis* (Boisd.) using the treated-leaf disc bioassay. The experiment was done in sterilized plastic boxes (⌀ = 14 cm and 10 cm height). A moist paper towel was kept at the bottom of each container in order to maintain proper humidity level and to keep the treated discs fresh. Antifeedant activity was assessed by calculating the '*Antifeedant index*' [18].

### 2.6 Statistical analysis
All data were presented as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test [11]. Mann-Whitney test was used to test the significance in the percentage antifeeding index. All statistical computations were carried out using PAST® program [15] and significance was set at $P<0.05$.

## 3. Results and Discussion
The two doses of botanical extract-treatments ($LC_{25}$ and $LC_{50}$) decreased the value of CI compared to the control at the 2nd and 4th instars (Table 1). The CI was steadily decreased through the studied instars, with a significant difference ($P<0.05$) could be observed between control and $LC_{50}$-*Catharanthus roseus* treatment at the 4th instar (Table 1).
Approximate digestibility (AD) was found to decrease steadily due to botanical extract treatments at the 2nd and 4th instars compared to the control at both $LC_{25}$ and $LC_{50}$ treatments (Table 1). With advancing age, 4th instar, significant difference ($P<0.05$) was attained only between the control and the treated 2nd and 4th instars with insignificant difference between the two doses ($LC_{25}$ and $LC_{50}$) of treatments (Table 1).
Treatment with $LC_{25}$ and $LC_{50}$-extracts of *C. roseus* and *A. herba alba* decreased the ECI compared to the control at 2nd and 4th instars (Table 1). Moreover, The ECD at botanical extracts-treatments significantly decreased ($P<0.05$) at both instars (Table 1).
The GR was significantly decreased ($P<0.05$) post-treatment with $LC_{25}$ and $LC_{50}$-extract treatments at the 2nd and 4th instars. Whereas, it was observed that the change in GR for the two botanical extracts treatments was significant compared to the control at the 4th instar (Table 1). It has to be mentioned that high or moderate food consumption by insects is not always associated with high GR, as the latter might be affected by a low ECI value [40][21]. The decrease in GR in the present study may be accounted for by the decrease in CI and food utilization efficiencies expressed in terms of ECI and ECD. This suggestion is confirmed by the findings of Woodring *et al*. [42] who indicated that the amount of growth reduction was proportional in general to the reduced food consumption. In agreement with our results, reduced values of AD, ECI, and ECD were recorded in *Acheta domesticus* (Linnaeus, 1758) exposed to heavy metals [23, 24].
In general, the observed trend in percentage antifeeding activity of *C. roseus* was higher than that of *A. herba alba* at LC-25 and LC-50 treatments for both the 2nd and 4th larval instar (Fig. 1) which could suggest that extracts of *C. roseus* appear to be more potent than that of *A. herba alba*.
Moreover, there was significant difference ($P<0.05$) in the percentage antifeeding activity for the 2nd instar larvae between *C. roseus* and *A. herba alba* at both treatments (LC-25 and LC-50). However, no significant difference could be observed in the percentage antifeeding activity for the 4th instar larvae treated with LC-25 and LC-50 doses of *C. roseus* and *A. herba alba* extracts (Fig. 1). This could suggest that the 2nd instars were more readily sensitive than 4th instar larvae.
From the results it was evident that the tested plant extracts possessed significant larvicidal properties and caused high mortality in larvae of *S. littoralis*. The antifeeding properties for the larvae could be attributed to the general toxicity of the chemical compounds present in the plant extracts [34].
Recent studies on plant protection strategies, particularly of the last decade, revealed the importance of eco-friendly botanical extracts that disrupt the normal insect growth and population dynamics. These extracts are rich sources of natural substances that can be utilized in the development of environmentally safe methods [30].
Different plant species contain secondary metabolites that are deleterious to different insect species and other herbivores in

diverse ways; through acute toxicity, enzymatic inhibition and direct interference with the consumption and/or utilization of food [41]. These eco-friendly botanicals are highly effective, safe and ecologically acceptable [33].

Previous studies have proved that *C. roseus possesses* insecticidal properties [10]. These properties of *C.roseus* have been previously reported against related spodopteran species; *S. litura* [27] and our experimental insect; *S. littoralis* [22]. It is suggested that extracts from the leaves and flowers of *C.roseus* may cause imbalance in enzymatic activity which could have collapsed the digestive system of the tested larvae [26]. Moreover, larval and pupal mortality could be attributed to direct insecticidal action or due to feeding inhibition and disruption of food assimilation [26].

Indeed, previous pharmacological studies have revealed that *C. roseus*, formerly known as *Vinca rosea* (L.), has great medicinal value and contain more than 70 different types of alkaloids [34]. In addition, *in vitro* studies have shown that this plant produces a large number of alkaloids upon elicitation [39].

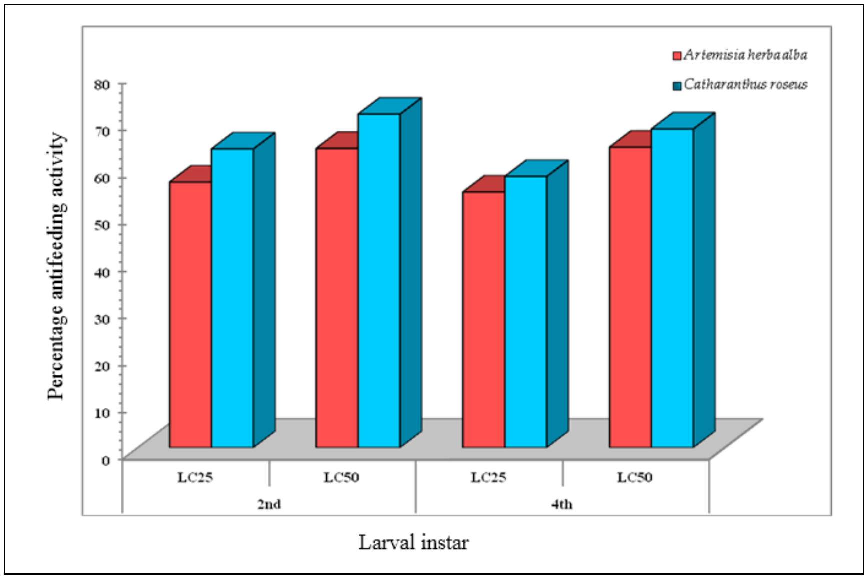


**Fig 1:** Percentage antifeeding activity of the 2nd and 4th instar larvae of *Spodoptera littoralis* (Boisd.) fed castor bean leaves, *Ricinus communis* (L.), treated with $LC_{25}$ and $LC_{50}$ *Catharanthus roseus* (L.) and *Artemisia herba alba* (L.) aqueous extracts

**Table 1:** The mean values of nutritional indices of the 2nd and 4th instars of *Spodoptera littoralis* (Boisd.) fed castor bean leaves, *Ricinus communis* (L.), treated with $LC_{25}$ and $LC_{50}$ *Catharanthus roseus* (L.) and *Artemisia herba alba* (L.) aqueous extracts.

| | | **Control** | ***Catharanthus roseus* (L.)** | | ***Artemisia herba alba* (L.)** | |
|---|---|---|---|---|---|---|
| | | | **$LC_{25}$** | **$LC_{50}$** | **$LC_{25}$** | **$LC_{50}$** |
| **2nd instar** Mean ± SD | | | | | | |
| **CI** | | 2.025$^{a}$ ± 0.365 | 1.222$^{a,b}$ ± 0.638 | 0.983$^{b}$± 0.251 | 1.339 $^{a,b}$ ± 0.714 | 1.208$^{a,b}$ ± 0.416 |
| AD | | 77.12$^{a}$ ± 2.77 | 76.701$^{a}$ ± 2.28 | 71.669$^{b,c}$ ± 1.867 | 75.397 $^{b,c}$ ± 3.429 | 67.749 $^{b,c}$ ± 1.212 |
| ECI | (%) | 28.902$^{a}$ ± 3.272 | 21.721$^{a,b}$ ± 2.637 | 17.958$^{b,c}$ ± 6.049 | 24.891 $^{b,c}$ ± 2.408 | 20.06 $^{b,c}$ ± 5.128 |
| ECD | | 37.513$^{a}$ ± 4.459 | 28.269$^{a,b}$ ± 2.671 | 25.088$^{b,c}$ ± 8.567 | 32.959 $^{b,c}$ ± 1.908 | 29.581 $^{b,c}$ ± 4.409 |
| GR | | 0.589$^{a}$ ± 0.154 | 0.275$^{b}$ ± 0.167 | 0.185$^{b,c}$ ± 0.101 | 0.345 $^{b,c}$ ± 0.202 | 0.252 $^{b,c}$ ± 0.12 |
| 4th instar Mean ± SD | | | | | | |
| CI | | 1.01$^{a}$ ± 0.365 | 0.856$^{a,b}$ ± 0.127 | 0.712$^{b}$ ± 0.019 | 0.904$^{a,b}$ ± 0.308 | 0.727$^{a,b}$ ± 0.065 |
| AD | | 77.405$^{a}$ ± 3.805 | 66.311$^{b}$ ± 5.05 | 64.697$^{b,c}$ ± 3.986 | 67.175 $^{b,c}$ ± 2.5 | 66.396 $^{b,c}$ ± 3.148 |
| ECI | (%) | 39.53$^{a}$ ± 3.165 | 24.801$^{b}$ ± 5.912 | 21.537$^{b,c}$ ± 3.098 | 27.97 $^{b,c}$ ± 6.649 | 23.217 $^{b,c}$ ± 5.128 |
| ECD | | 52.422$^{a}$ ± 3.035 | 37.039$^{b}$ ± 6.484 | 33.412$^{b,c}$ ± 5.272 | 41.85 $^{b,c}$ ± 11.242 | 35.207 $^{b,c}$ ± 8.898 |
| GR | | 0.402$^{a}$ ± 0.089 | 0.218$^{b}$ ± 0.077 | 0.154$^{b,c}$ ± 0.024 | 0.249 $^{b,c}$ ± 0.081 | 0.167 $^{b,c}$ ± 0.025 |

Means in rows followed by the different letters are significantly different ($P<0.05$)

## 4. Conclusion and Recommendations

In conclusion, the study revealed that the aqueous extracts of *C. roseus* and *A. herba alba* possess remarkable inhibition activity in the nutritional indices of *S. littoralis* (Boisduval). The exposure of the early and late *S. littoralis* larvae to botanical extracts would reduce the feeding pattern, food consumption and utilization and increase the deterrence. From practical standpoint, this finding may have negative impacts on the population parameters and dynamics of the proceeding generations of this insect.

Further investigations are in need to elucidate this activity against a wide range of insect pests. The active ingredients and biomolecule characterization of these botanical extracts need more research studies for better understanding their action against the pest under field conditions. It is possible to extract these substances if they show environmental stability and are eco-friendly safe and it is possible to use these extracts in plant protection. For this point, these botanicals could be recommended as suitable as eco-friendly regulators against common phytophagous pests in crop systems.

## 5. Conflict of Interest

The authors declare that there are no conflicts of interest associated with this article.

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