Scientific Journal for Damietta Faculty of Science **13**(1) 2023, 39-47
ISSN Print 2314-8594
ISSN Online 2314-8616

*https://sjdfs.journals.ekb.eg/*

# Insecticidal and Phytochemical Investigation of Catharanthus Roseus L. Extracts to Spodoptera Littoralis (Boisd.)

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Received: 23 May 2023 /Accepted: 20 June 2023

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## Abstract

Chromatographic separation of Catharanthus roseus yielded six fractions. Of the sex fractions, the alkaloid and butanol fractions were the most effective fraction against Spodoptera littoralis (Boisd.) larvae in their second instar after seven days of exposure using leaf dipping technique with LC50 value 234.58 and 481.09 ppm, respectively. Toxicological effect guided isolation and elucidation of the most toxic fraction and yielded three monoterpenoid indole alkaloids, vindoline (1), vindorosin (2) and ajmalicine (3) using extensive chromatographic and spectroscopic techniques. The effect of alkaloid and butanol fractions on biochemical aspects including transaminases enzymes (AST and ALT), acelylcolinesterase and chitinase for S. littoralis, 2 nd instar larvae were evaluated. A significant activation on AST and ALT activities were obtained after treatment with LC50 of alkaloid and butanol fractions also, both fractions might be used as AchE inhibitors and suggested an ability in biodegradation of chitin. The sublethal effect of both alkaloid and butanol fractions on the biological aspects of S. littoralis immature and adult stages were studied in details.

***Keywords:*** Catharanthus roseus, Spodoptera littoralis, alkaloids, biological, biochemical responses.

## Introduction

The world tends to consume healthy food with minimal pesticide residues to avoid the risks that threaten human health and the environment. Therefore, the use of botanical pesticides in insect control as safe alternatives to chemical pesticides has become the focus of concern **(Divekar, 2023)**, to reduce the incidence of diseases, like cancer and nervous system diseases caused by various toxic chemicals **(Kaur and Singh, 2023)**. The role of natural products not only in agriculture also in medicine, where the plant's secondary metabolites, such as terpenes, flavonoids, tannins and alkaloids which have antioxidant, antibacterial, antifungal and anticancer effect. For example, indole alkaloids from

*Catharanthus roseus*, which was studied in the present research has promising anticancer properties **(Deep et al. 2023)**.

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), is a one of the lepidopteran family called Noctuidae, is one of the most devastating insects in tropical and subtropical areas of the world and destroys a variety of crops **(Abdou and Zyaan, 2023)**. So the success in controlling such insect will result in improving the quality and the quantity of cotton production. The pesticidal activity of Egyptian plant extracts were investigated throughout several studies **(Soliman, 2001; Mostafa, 2017; Mostafa, 2021)**. Assessment of the toxicity and the activity of latent bio-effects of some conventional and non-conventional insecticides have been extensively studied on *Spodoptera littoralis* **(Kandil et al. 2023)**.

*Catharanthus roseus* (Madagasker periwinkle) (Apocynaceae) is consider a popular medicinal plant, so it widely distributed and cultivated in North, South America, Australia, China, India and Indonesia **(Nataraj, 2023)**. It is a white latex-producing subshrub species that may grow to heights of between thirty and one hundred cm, may be either decumbent or straight, and lends itself to an unpleasant odor. Its trunk can expand to a diameter of one centimetre and is a light gray color. It has a pilose, or translucent, dark crimson, terete, broadly winged stem. Petiolate and decussate leaves are present. Solitary, axillary, or paired, pendunculate, 1-4 mm long, narrowly winged, and pilose or glabrous are the distinctive features of its flowers. The seeds are black and fruits are green **(Van Bergen and Snoeijer, 1996)**.

Several studies evaluated the potency of *C. roseus* extracts as a bio-insecticides and recorded its antifeedant and larvicidal efficacy. Ethyl acetate fraction of *C. roseus* leaf extract showed highest larval mortality rate against larvae of *Helicoverpa armigera* **(Ramya, 2008).** Also, the pupicidal action against the pre-pupal stage of *S. litura* was assessed topically and the mortality percentage was found to be dose dependent **(Sandey and Summarwar, 2016).** Fumigant and contact toxicity of *C. roseus* aqueous extract to control rice weevil (*Sitophilus oryzae*) was studied and the $LD_{50}$ values were 0.027 mg $cm^{-2}$ and 0.083 mg $cm^{-2}$, respectively **(Majeed, 2011)**.

The current work aims to assay the toxicological effect of *Catharanthus roseus* extracts in addition to investigate the phytochemical profiling, biochemical and biological latent effects of the most active fractions against *Spodoptera littoralis* (Boisd.) larvae in their second instar.

## Materials and Methods

NMR spectra was recorded using Bruker 400 MHz at (Nuclear Magnetic Resonance Laboratory, Mansoura University). MS analysis was performed on a Waters Acquity UPLC system at Drug Discovery Research and Development Center, Faculty of Pharmacy, Ain Shams University. Column chromatography was achieved on basic alumina 70-290 mesh (50-200 mm); most are "approximately 150 mesh" of SIGMA-ALDRTCH. Preparative TLC silica gel 60 F254 (250 µm) of MERCK for purification. All solvents hexane, chloroform, ethyl acetate, butanol, ammonium hydroxide, acetone, benzene and methanol were HPLC grade from Loba Chemie Company.

### *Plant material*

*Catharanthus roseus* was collected from Hay El Ashgar Mansoura, Egypt in December 2021. Then identified according to **(Boulos et al. 2009)**, by Dr. Maha Mohamed Alshamy, associate professor - Botany Dept., Faculty of Science, MansouraUniversity

### *Extraction and Isolation*

Processing of *Catharanthus roseus* was carried out in two schemes. The first scheme according **(Al-Matani et al. 2015)** has been done by crushing and soaking (500 g) of the air-dried whole plant in methanol at room temperature for 3 days then, filtrated and evaporated till 1/3 primary volume. The methanol extract (46 g) was sequentially fractionated by solvent extraction using hexane, chloroform, ethyl acetate, and butanol. The second scheme was performed according to **(Yubin et al. 2014)**, by soaking (1kg) of the air dried crushed whole plant. Two-third of solvent volume was allowed to evaporate to its 1/3 volume by rotary evaporator. Defatting was subjected by extraction with hexane. The extract was acidified by diluted HCl to pH 2 and exhaustively extracted with chloroform to give chloroform extract (non-basic fraction). Then

the extract was basified by ammonium hydroxide to pH 12 and exhaustively extracted with chloroform (alkaloid fraction). The fractions of the two methods' were dried over anhydrous sodium sulfate and the solvents were evaporated to give alkaloid fraction (3.65 g), butanol fraction (2.52 g), ethyl acetate fraction (2.16 g), chloroform fraction (2.32 g), hexane fraction (14 g) and non-basic fraction (4.86 g). 3.65 g of the alkaloid fraction was performed by column chromatography over basic alumina as a stationary phase using hexane/ethyl acetate and chloroform/methanol of solvent system as an eluent with increasing polarity to yield five sub-fractions based on their TLC patterns. Sub-fraction II has been purified on plates of silica gel, eluted by a mixture of benzene/acetone/ ammonium hydroxide 9.4:0.5:0.1 to yield compound (**1**) ($R_f$ = 0.65, 340mg)and compound (**2**) ($R_f$ = 0.59, 260mg).Sub-fraction III applied on PTLC, eluted by a mixture of benzene/acetone/ ammonium hydroxide 9.1:0.7:0.2 to yield compound (**3**) ($R_f$ = 0.45, 115mg).

*Identification and characterization of separated alkaloids*

Vindoline (**1**) was a yellowish-brown amorphous solid with a pseudo-molecular ion peak in its ESIMS spectra with the chemical formula $C_{25}H_{32}N_2O_6$ at m/z 457.8785 $(M+H)^+$.$^1$H-NMR ($CDCl_3$, 400 $MH_Z$): $\delta_H$ 3.74 (1H, s, H-2);3.50(1H, ddd,2.53, 4.74, 9.6 Hz, H-3α); 2.82 (1H, dt, 2.2, 13.68 $H_Z$ H-3β); 3.42 (1H, ddd, 4.56, 8.56, 13.84 Hz, H-5α); 2.52 (1H, dt, 8, 10.48 $H_Z$ H-5β); 2.31 (2H, m, H-6); 6.88 (1H, d,8.16 Hz, H-9); 6.30 (1H, dd, 2.24, 8.12 Hz, H-10); 6.07 (1H,d, 2.28 Hz, H-12); 5.43 (1H, ddd, 1.48, 5.04, 9.92 Hz, H-14); 5.2 (1H, brd, 10.56 Hz, H-15); 5.45 (1H, s, H-17); 0.48 (3H, t, 7.4 Hz, H-18); 1.64 (1H, q, 7.4 Hz H-19α); 1.11 (1H, q, 7.4 Hz, H-19β); 2.65 (1H, s, H-21); 2.07 (3H, s, COMe); 2,64 (3H, s, NMe); 3.77 (3H, s, 11-OMe); 3.76 (3H, s, 22-OMe). $^{13}$C-NMR (APT) (100 MHz, $CDCl_3$) $\delta_C$ 83.42 (C) (C-2); 51.10 ($CH_2$) (C-3); 51.97 ($CH_2$) (C-5); 44.07 ($CH_2$) (C-6); 52.80 (C) (C-7); 125.05 (C) (C-8); 122.70 (CH) (C-9); 104.62 (CH) (C-10); 161.17 (C) (C-11); 95.85 (CH) (C-12); 153.75 (C) (C-13); 124.12 (CH) (C-14); 130.50 (CH) (C-15); 79.58 (C) (C-16); 76.40 (CH) (C-17); 7.65 ($CH_3$) (C-18) ); 30.83 ($CH_2$) (C-19); 42.93 (C) (C-20); 67.05 (CH) (C-21); 171.95 (C) (C-22); 170.83 (C) (C-OCOMe); 21.08 ($CH_3$) (C-COMe); 38.28 ($CH_3$) (C-NMe); 55.39 ($CH_3$) (C-11-OMe); 52.83 ($CH_3$) (C-22-OMe).

Vindorosin (**2**) was a yellowish-brown amorphous solid with a *pseudo*-molecular ion peak in its ESIMS spectra with the chemical formula $C_{24}H_{30}N_2O_5$ at m/z 427.6880 $(M+H)^+$. $^1$H-NMR ($CDCl_3$, 400 $MH_Z$): $\delta_H$ 3.74 (1H, s, H-2); 3.52 (1H, ddd, 2.53, 4.74, 9.6 Hz, H-3α); 2.82 (1H, dt, 2.2, 13.68 $H_Z$ H-3β); 3.41 (1H, ddd, 4.56, 8.56, 13.84 Hz, H-5α); 2.52 (1H, dt, 8, 10.48 $H_Z$ H-5β); 2.37 (2H, m, H-6); ٧,٠٢ (1H, d, 7.4 Hz, H-9); 6.٧6 (1H, t, 7.4 Hz, H-10);7.14(1H, t, 7.4 Hz, H-11); 6.٥١ (1H,d, 7.4 Hz, H-12); 5.85 (1H, dd, 3.96, 9.96 Hz, H-14); 5.20 (1H, brd, 9.85 Hz, H-15); 5.47 (1H, s, H-17); 0.43 (3H, t, 7.4 Hz, H-18); 1.62 (1H, q, 8.85 Hz H-19α); 1.07 (1H, q, 8.85 Hz, H-19β); 2.65 (1H, s, H-21); 2.07 (3H, s, COMe); 2.66 (3H, s, NMe); 3.78 (3H, s, 22-OMe).$^{13}$C-NMR (APT) (100 MHz, $CDCl_3$) $\delta_C$82.91 (C) (C-2); 51.13 ($CH_2$) (C-3); 52.28 ($CH_2$) (C-5); 44.28 ($CH_2$) (C-6); 52.80 (C) (C-7); 133.03 (C) (C-8); 122.40 (CH) (C-9); 119.33 (CH) (C-10); 130.45 (CH) (C-11); 109.53 (CH) (C-12); 152.52 (C) (C-13); 124.12 (CH) (C-14); 130.50 (CH) (C-15); 79.64 (C) (C-16); 76.72 (CH) (C-17); 7.68 ($CH_3$) (C-18); 30.88 ($CH_2$) (C-19); 43.01 (C) (C-20); 67.35 (CH) (C-21); 171.95 (C) (C-22);170.83 (C) (C-OCOMe); 21.08 ($CH_3$) (C-COMe); 38.47 ($CH_3$) (C-NMe); 52.83 ($CH_3$) (C-22-OMe).

Ajmalicine (3) white powder, $^1$H-NMR ($CDCl_3$, 400 $MH_Z$): $\delta_H$ 8.20 (1H, brs, NH); 3.29 (1H, br dd, 1.96, 10,96 Hz, H-3α); 2.47 (1H, td, 11.32Hz, H-5α); 2.37 (1H, m, H-5β); 2.77 (1H, brd, 14.20Hz, H-6α); 3.08 (1H, m, H-6β);7.46 (1H, br d, 7.72Hz, H-9); 7.09 (1H, t, 7.28Hz, H-10); 7.15 (1H, t, 7.20Hz, H-11); 7.32 (1H, br d, 7.96Hz, H-12); 3.18 (1H, m,, H-14α); 1.37 (1H, q, 12.32Hz, H-14β); 2.34 (1H, br t, 2.88Hz, H-15α); 7.55 (1H, d, 1.76 Hz, H-17); 4.91 (1H, Br d, 13.05 Hz, H-18); 4.44 (1H, dq, 3.64Hz, H-19);2.23 (1H, t, 2.52 Hz, H-21α); 2.80 (1H, dd, 12.76 Hz, H-21β); 3.75 (3H, s, $OCH_3$).

*Preparation of the test concentration*

Assuming that the crude extract contained 100% of the active ingredient, a known weight of the crude was added to the smallest portion of solvent to create a stock solution that was already manufactured. Each extract was prepared as a stock solution before usage, and the first dilution was homogenized with the addition of 0.1% tween 80. Subsequent

dilutions were then carried out using water to obtain a series of concentrations that would be examined.

*Insect's culture*

A lab-grown strain of *S. littoralis* was obtained from Plant Protection Research Institute, ARC, Sakha, Kafr El-Sheikh, Egypt. This strain was nurtured at Mansoura Agricultural Research Station for 20 generations, further than not previously been exposed to any to become a susceptible and homogenous strain as defined by **(El-Defrawi et al. 1964)**. At 25 °C and 75% RH, larvae were raised on fresh castor bean leaves. The pupae and adults were moved to appropriate enclosures for mating and egg-laying. Moths that had just emerged were fed a 10% sugar solution.

*Leaf dipping technique*

To investigate the toxicity of the various plant extracts tested against the *S. littoralis* second-instar larvae, laboratory tests were conducted utilizing the leaf dipping technique as described by **(Sadek et al. 2003)**. Larval mortality was determined 7 days after exposure, corrected in accordance with **(Abbott et al. 1925)**, and then submitted to probit analysis. Toxicology lines were drawn on log concentration-probit paper, and statistical analysis was performed in accordance with **(Finney et al. 1982)** to obtain the $LC_{25}$, $LC_{50}$ and $LC_{90}$ values of different extracts to determine the more effective fractions. Mortality percentages were calculated using the following formula:

% Mortality = No. of dead larvae / Total No. of larvae x 100

Using (Sun's 1950) equation, the toxicity index for several extracts was calculated by contrasting these materials with the most effective extract.

Toxicity index = $LC_{50}$ of compound A / $LC_{50}$ of compound B x 100

Where: A is the most efficient component B is the other tested compounds

*Biochemical Responses*

The insects were prepared as designated by **(Amin et al. 1998)**. In distilled water (50 mg/ml), they were homogenized. In a chilled centrifuge, homogenates were spun for 20 minutes at 8000 rpm. The supernatants, when held at 50°C, were kept for at least a week without experiencing any discernible activity loss while the deposits were rejected.

According to **(Ishaaya et al. 1976)**, the activity of the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured. Acetylcholine Esterase (AchE) activity was assessed using the stated methodology by **(Simpson et al. 1964)**. According to the procedure outlined by **(Bade et al. 1981)**, chitinase was tested using 3,5-dinitrosalicylic acid reagent to detect the free aldehydic groups of hexosamine liberated on chitin digestion.

*Biological responses*

The bioassays were carried out using newly 2$^{nd}$ larval instar of *S. littoralis* having homogeneous masses, by using sublethal concentrations ($LC_{25}$) of both alkaloid 52.23 ppm and butanol 198.16 ppm extracts to evaluate their effects on the biological parametrs of *S. littoralis* according to **(Dahi et al. 2015)**. Ten of 2$^{nd}$ larval instar were familiarized to each Petri dish as a replicate. Three replicates of castor bean oil leaves treated with concentration $LC_{25}$ were carried out for 72h. At that point, untreated leaves were given larvae.

The biological parameters, such as larval and pupal duration, pupation percentage, pupal weight, percentages of normal pupae, deformed pupae and adult emergence percentage were assessed. Each female moth's total number of eggs laid (fecundity) was calculated. The egg masses were then allowed to hatch, and the proportion of hatchable eggs and the adult longevity for males and females were estimated in accordance with **(Crystal et al. 1963)** as follows:

% Fecundity =No. eggs (treated female)/No. eggs (untreated female) x 1000

*Statistical analysis*

Using (Abbott's 1925) formula, the percentage mortality of the treated larvae was multiplied by the percentage mortality of the untreated larvae and then imperiled to LdP Line to evaluate the $LC_{25}$, $LC_{50}$ and $LC_{90}$ for each treatment. The oxidative stress enzyme and life history parameters data, were statistically estimated according to costat software using one-way

analysis of variance (ANOVA) and the significance between means using the Duncan's multiple range test at 95% confidence interval. Data were expressed as mean values ± SE. Means were calculated by using the Duncan multiple range test at the 5% level.

## Results and Discussion

The development of new products that could be good alternatives for pest control has become a universal demand. Processing of the *Catharanthus roseus* air-dried whole plant was performed using two schemes, the first one yielded four main fractions hexane, chloroform, ethyl acetate, and butanol while the alkaloidal extraction scheme yielded non-basic chloroform fraction and the basic chloroform fraction (Alkaloid fraction).

Data concerning the toxic effect of *C. roseus* fractions against *Spodoptera littoralis* (Boisd.) larvae in their second instar after seven days of exposure using leaf dipping technique are shown in table 1. The obtained results proved that the alkaloid fraction was most efficient at $LC_{50}$ and $LC_{90}$ levels, followed by butanol, ethyl acetate, chloroform, hexane and non-basic fractions, respectively. The insecticidal action ($LC_{50}$ value and toxicity index) of the above arranged fractions were (234.58 ppm and 100), (481.09 ppm and 48.76), (1270.28 ppm and 18.47), (5223.02 ppm and 4.49), (31699.22 ppm and 1.16) and (1137000 ppm and 0.02), respectively. The toxicity lines slopes were calculated to be fluctuated between 0.48 using non-basic fraction to 1.75 using butanol fraction.

The above-mentioned results proved that the alkaloidal fraction was superior over the rest tested fractions. Alkaloids from different plants exhibit insecticidal properties against various insect species by causing a range of toxic effects such as malfunction of the midgut, redox imbalance, disruptions of biological membranes, inhibition of cholinesterase, disturbing development and metabolism, reproductive and acute toxicity **(Chowanski, 2016)**. The lipophilic crude extracts of *Stemona curtisii* and *Stemona cochinchinens* roots showed very strong insecticidal activity against *S. littoralis* as a result of accumulation of stemofoline, dehydroprotostemonine and oxystemokerrin alkaloids. $LC_{50}$ values of these alkaloids were 2.0, 6.1 and 5.9 ppm, respectively after 5 days of exposure **(Kalteneggera et al., 2003).** *C. roseus* is rich by monoterpenoid indole alkaloids, which presented excellent insecticidal properties against cabbage moth (*Plutella xylostella* L.) larvae **(Trindade, 2008)**.

Table 1. Toxicity of *C. roseus* fractions against *S. littoralis* (Boisd.) larvae in their second instar after exposure for seven days

| Plant extract | $LC_{50}$ (ppm) | $LC_{90}$ (ppm) | Slope | Toxicity index ( $LC_{50}$) |
|---|---|---|---|---|
| Hexane fraction | 31699.22<br>9946.32 2211600 | 1413600<br>115130 27662 E+6 | 0.78 ± 0.24 | 1.16 |
| Chloroform fraction | 5223.02<br>3337.36 11088.65 | 63615.67<br>23454.9 518120 | 1.18 ± 0.22 | 4.49 |
| Ethyl acetate fraction | 1270.28<br>687.93 2219.29 | 43052.46<br>1374.68 744270 | 0.84 ± 0.19 | 18.47 |
| Butanol fraction | 481.09<br>317.23 653.94 | 2595.23<br>1788.99 21654.44 | 1.75 ± 0.27 | 48.76 |
| Alkaloid fraction | 234.58<br>69.47 426.54 | 4071.7<br>2297.98 12691.9 | 1.03 ± 0.22 | 100 |
| Non basic fraction | 1137000<br>823987 1487645 | 5.42E+06<br>6.32E+06 3.87E+011 | 0.48 ± 0.27 | 0.02 |

Bioassay guided phytochemical investigation of the most promising alkaloidal fraction through a series of extensive chromatographic techniques (CC, TLC) and spectral elucidations (1D and 2D NMR) *(c.f* expermintal part) and identified three major monoterpenoid indole alkaloids **(1-3)**. The identified compounds were vindoline **(1) (Jackson, 1989)**, vindorosin **(2) (Tiong, 2014)** and ajmalicine **(3) (Höfle, 1980)**.

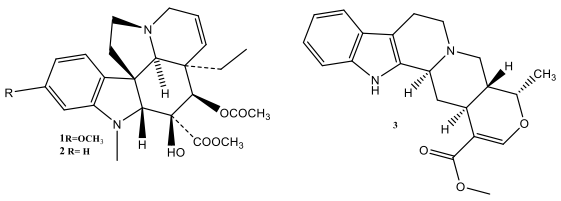


Figure 1: Effect on transaminases enzymes

Results presented table 2, indicated the

exposure of *S. littoralis* larvae in their second instar at $LC_{50}$ of alkaloid and butanol fractions of *C. roseus* that produced a significant activation on aspartate aminotransferase (AST). The activation values were 2.17±0.06 (-456.41%) and 1.62±0.06 (-315.38%) (mU/mg protein) for the $LC_{50}$ of alkaloid and butanol fractions, respectively, while recorded 0.39±0.006 (mU/mg protein) with control. Also, slightly increase occurs in alanine aminotransferase (ALT) activity of haemolymph larvae in their second instar of *S. littoralis* after treatment with $LC_{50}$ of alkaloid and butanol fractions 0.32±0.06 (-14.28%) and 0.34±0.06 (-21.43%) (mU/mg protein), respectively, as compared with control 0.28±0.01 (mU/mg protein).

It is obvious that applying the $LC_{50}$ of both fractions resulted in a noticeable increase in AST and ALT activity. Our findings were in accordance with **Giboney, (2005)**, the entrance of any toxic substance, tissue injury or microbial infections in insect's body cause a physiological challenge followed by a shift in the level of ALT and AST enzymes.

*Effect on acetylcholinesterase and chitinase*

AchE is a serine proteolytic enzyme in insect's central nervous system. Many plant secondary metabolites are well-known to exert an influence on AChE characters **(Liu et al. 2022)**. AchE of haemolymph larvae in their second instar of *S. Littoralis* was tabulated in table 2 which significantly inhibited with $LC_{50}$ of alkaloid and butanol fractions 75.34±5.76 (82.14%) and 105.1±5.23 (75.09%), respectively, as compared with control 421.94±29.07 (ug AchBr/min/g.b.wt). So, two fractions might be used as AchE inhibitors. Our findings were in agreements with the significant inhibitions obtained by the total alkaloids of *Chelidonium majus* against the AchE activity of *Lymantria dispar* **(Zou et al. 2017)**. Also, the methanolic extract of *Areca nut* beside the alkaloids arecaidine, guvacine and arecoline recorded AchE activity inhibition in *Plutella xylostella* larvae **(Liu et al. 2022)**.

Application of $LC_{50}$ of alkaloid and butanol fractions on the activity of Chitinase of larvae in their second instar was recorded in table 2. Significant increase in the enzyme activity were obtained for both alkaloid and butanol fractions, 9±0.43 (µg NAGA/min/g.b.wt) (-95.65%) and 4.91±0.01 (µg NAGA/min/g.b.wt) (-6.74%), respectively, comparing with control 4.60±0.71 (µg NAGA/min/g.b.wt). This increasing in the chitinase activity suggest the ability of both fractions in biodegradation of chitin, leading to *S. littoralis* death.

Insect growth regulators of plant origin impede with chitin synthesis or the endocrine system. Several studies indicated the effect of plant extracts and their metabolites in the disruption of the endocrine system and hormone balance of insects. Our results agreed with those of **Sun et al. (2012)** who stated that *Cynanchum komarovii* alkaloids may disrupt hormone balances of insects as obtained previously by azadirachtin, causing larval malformation and prolonging the larval development period of *Spodoptera litura* (Fab).

*The development parameters of S. littoralis affected by sublethal effect of the butanol and alkaloid fractions.*

The most promising toxicological properties of both alkaloid and butanol fractions was chosen for more biological studies on *S. littoralis* immature and adult stages.

The results in table 3 showed that treatment of larvae of *S. littoralis* in their second instar at $LC_{25}$ concentrations of alkaloid and butanol fractions significantly prolonged larval and pupal duration time. The larval duration recorded 28.67 days for alkaloid fraction and 26.67 days for butanol fractions, while for control gave 20.67 days. The mean pupal duration of alkaloid, butanol fractions and control were 6.33, 10.33 and 14,33 days, respectively, in addition to a marked decrease in pupal weigh was recorded for the treated larvae over the untreated one, the alkaloid fraction and butanol fraction reported 426.17 and 497.22mg, respectively compared with the untreated larvae 580.74 mg. The percentage of adult emergency for larvae in their second instar treated with alkaloid and butanol fractions were 44.44 and 58.33%and the control recorded 95.24%.The deformed pupae percentage in alkaloid and butanol fractions treatment reached to 66.67 and 42.68 %, while it did not exceed than 4.76 % in untreated larvae.

Regarding the latent effect of alkaloid and butanol fractions on the females fecundity developed for treated larvae, a significant decrease were recorded to be 52.10, 59.53% for both fractions compared with the untreated one 100 %.The average of total number of eggs

deposited by females whose received butanol and alkaloid treatments throughout its life span were 950.0 and 1146.67eggs/female, respectively and reached 1950.0 eggs/female for the untreated females. In comparison to the control (16.67and 13.33 days), the adult longevity of both females and males was severely reduced, measuring as follows: alkaloid (8.33 and 5 days), butanol (11.33 and 8.33 days), for males and females, respectively. The above mentioned results in table 4 agreement with those reported by **(Dimetry et al. 1998; Ismail et al. 2002; Dahi et al. 2015)**.

Table 2. Effects of *C. roseus* alkaloid and butanol fractions at respective $LC_{50}$ on the average activity of detoxifying enzymes of second-instar larvae of a lab-grown strain of *S. littoralis* (Boisd.) larvae in their second instar

| Plant extract | $LC_{50}$ mg/L | AST (mU/mg protein) Mean±SE | Change % | ALT (mU/mg protein) Mean±SE | Change % | AchE (ug AchBr/min/gm body weight) Mean±SE | Change % | Chitinase (µg NAGA/ min /g body weight) Mean ± SE | Change % |
|---|---|---|---|---|---|---|---|---|---|
| Control | | $0.39^{c}$±0.006 | | $0.28^{a}$±0.01 | | $421.94^{a}$±29.07 | | $4.60^{b}$±0.71 | |
| Alkaloid fraction | 234.58 | $2.17^{a}$±0.06 | -456.41 | $0.32^{a}$±0.06 | -14.28 | $75.34^{b}$±5.76 | 82.14 | $9^{a}$±0.43 | -95.65 |
| Butanol fraction | 481.09 | $1.62^{b}$±0.06 | -315.38 | $0.34^{a}$ ±0.06 | -21.43 | $105.1^{b}$±5.23 | 75.09 | $4.91^{b}$±0.01 | -6.74 |
| $LSD_{0.05}$ | | 0.16 | | 0.16 | | 60.11 | | 1.66 | |
| p | | 0.000 | | 0.64 | | 0.000 | | 0.001 | |
| f | | 373.69 | | 0.48 | | 122.28 | | 26.13 | |

LSD=less significant differences, F=F-test, P=P-value; According to Duncan's, letters represent the substantial variations between treatments; the data represent the average and standard errors of three replicates, each containing a 30-second larval instar.

Table 3. Effects of butanol and alkaloid fractions from *C. roseus* at respective $LC_{25}$ values on various biological properties of a lab-grown strain of *S. littoralis* (Boisd.) larvae in their second instar

| Fractions | LC25 mg/L | Larval duration Days ±SE | Pupal Duration Days ±SE | Pupal Weight (mg) ±SE | Normal Pupae% | Deformed Pupae % | Adult emergence % |
|---|---|---|---|---|---|---|---|
| Control | | $20.67^{a}$±0.88 | $14.33^{a}$±0.33 | $580.74^{a}$±34.16 | 95.17 | 4.76 | 95.24 |
| Alkaloid fraction | 52.23 | $28.67^{a}$±1.45 | $6.33^{c}$±0.33 | $426.17^{b}$±2.9 | 35.35 | 66.67 | 44.44 |
| Butanol fraction | 198.16 | $26.67^{b}$±0.67 | $10.33^{b}$±0.33 | $497.22^{ab}$±30.13 | 57.14 | 42.68 | 58.33 |
| $LSD_{0.05}$ | | 3.46 | 1.15 | 91.19 | | | |
| p | | 0.0032 | 0.0001 | ٠.٠١٧٢ | | | |
| f | | 17.33 | 68.16 | 8.62 | | | |

There is no statistically significant difference between values in the same column that have the same letter as them (P > 0.05: Duncan's test).

Table 4. Effects of *C. roseus* alkaloid and butanol fractions at respective $LC_{25}$ values on adult longevity, fecundity, and fertility for a lab-grown strain of *S. littoralis* (Boisd.) larvae in their second instar.

| Fractions | No. of eggs /female ±SE | Fecundity % | Egg hatchability % | Adult longevity Days ±SE | |
|---|---|---|---|---|---|
| | | | | Male | Female |
| Control | $1950^{a}$±45.83 | 100.00 | 95.18 | $13.33^{a}$±0.88 | $16.67^{a}$±0.33 |
| Alkaloid fraction | $950^{b}$±63.51 | 48.72 | 39.55 | $5^{c}$±0.57 | $8.33^{c}$±0.88 |
| Butanol fraction | $1146.67^{b}$±161.28 | 58.80 | 55.57 | $8.33^{b}$±0.33 | $11.33^{b}$±0.33 |
| $LSD_{0.05}$ | 358.2 | | | 2.21 | 2.00 |
| p | 0.0011 | | | 0.0003 | 0.0002 |
| f | 8.50 | | | 43.18 | 53.44 |

According to Duncan's test, letters represent the significant changes between treatments.

## Conclusion

The bio-insecticidal properties of *Catharanthus roseus* extracts were assessed and the alkaloid and butanol extracts were the most potent fractions against the larvae of *S. littoralis*. Phytochemical investigations of the promising fraction identified the major three monoterpenoid indole alkaloids, vindoline, vindorosin and ajmalicine. The results of biochemical and the biological aspects of *S. littoralis* suggest that *C. roseus* alkaloid and butanol extracts holds a potential to be used as bio-insecticide.

## References


Abbott WS. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18(2):265-267.

Abdou M., & Zyaan O. (2023). The proficiency of silver nanoparticles in controlling cotton leafworm, Spodoptera littoralis (boisd.), under the laboratory conditions. Egyptian Journal of Zoology. 1-17.

Al-Matani SK, Al-Wahaibi RNS, & Hossain MA. (2015). Total flavonoids content and antimicrobial activity of crude extract from leaves of Ficus sycomorus native to Sultanate of Oman. Karbala International Journal of Modern Science. 1(3): 166-171.

Amin TR. (1998). Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, Spodoptera littoralis (Boisd.). Ph.D. Thesis, Faculty of Science, Cairo Univ. Egypt.

Bade ML, & Stinson A. (1981). Biochemistry of insect differentiation. A system for studying the mechanism of chitinase activity in vitro. Archs Biochem. Biophys. 206: 213-221.

Boulos L. (2009). Flora of Egypt checklist, revised annotated edition. Al-Hadara Publishing, Cairo, 198-201.

Chowański S, Adamski Z, Marciniak P, Rosiński G, Büyükgüzel E, Büyükgüzel K, & Bufo SA. (2016). A review of bioinsecticidal activity of Solanaceae alkaloids. Toxins. 8(3): 60-85.

Dahi HF, Walaa E, & Mona SA. (2015). Activity of castor oil seeds Ricinus communis ethanol extract against cotton leafworm Spodoptera littoralis (boisd.), J. Plant Prot. and Path., Mansoura Univ., 6 (12): 1711– 1725.

Deep A, Kumar D, Bansal N, Narasimhan B, Marwaha RK, & Sharma PC. (2023). Understanding mechanistic aspects and therapeutic potential of natural substances as anticancer agents. Phytomedicine Plus. 3(2):100418.

Dimetry NZ, El-Gengaihi SS, & Mohamed M. (1998). The effect of lipoid lmatter of neem fruits and leaves on Spodoptera littoralis development and reproduction. Insect Science and its Application. 18 (4): 325-331.

Divekar P. (2023). Botanical Pesticides: An Eco-Friendly Approach for Management of Insect Pests. Acta Scientific agriculture. 7(2): 75-81.

El defrawi, M. E., Toppozada, A., Mansour, N., & Zeid, M. 1964. Toxicological studies on the Egyptian cotton leafworm, Prodenia litura. I. Susceptibility of different larval instars of Prodenia to insecticides. Journal of Economic Entomology. 57(4):591-3.

Finney DJ. (1982). Probit analysis: a statistical treatment of the sigmoid response curve. Cambridge: Cambridge University Press. 1947. 256.

Giboney PT. (2005). Mildly elevated liver transaminase levels in the asymptomatic patient. American family physician. 71(6): 1105-1110.

Höfle G, Heinstein P, Stöckigt J, & Zenk MH. (1980). 1H-NMR Analysis of Ajmalicine-Type Alkaloids of the 3α Series. Planta medica. 40(10), 120-126.

Ishaaya I, & Swirski E. (1976). Trehalase, invertase and amylase activities in the black scale Saissetia oleae, and their relation to host adaptability. J. Insect Physiol. 22: 1025-1029.

Ismail AI, KAA ES, MM EM, & MM SEN. (2002). Biological and insecticidal effects of total glucosinolates and different extracts of Carrichtera annua (L.) DC. and Farsetia aegyptia Turra. On Spodoptera littoralis (Boisd.). Bulletin of the National Research Centre Cairo. 27(1): 51-62.

Jackson GE. (1989). 2D NMR Study of Vindoline. Spectroscopy letters, 22(1): 31-39.

Kaltenegger E, Brem B, Mereiter K, Kalchhauser H, Kahlig H, Hofer O, Vajrodaya S, & Greger H. (2003). Insecticidal pyrido[1,2-a]azepine alkaloids and related derivatives from Stemona species. Phytochemistry. 63, 803-816.

Kandil MA, El-Medany WA, Shalaby MAM, & El-Habal NA. (2023). Comparative Efficacy of Uphold and Closer Insecticide on the Developmental, Reproductive, Biochimical and Histological of the Spiny Bollworm, Earias insulana (Boisd.). Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control. 15(1): 11-24.

Kaur J, & Singh PK. (2023). Aptamer-based approaches for sensing harmful synthetic and natural toxins. In Sensing of Deadly Toxic Chemical Warfare Agents, Nerve Agent Simulants, and their Toxicological Aspects (pp. 247-268). Elsevier.

Liu R, Zheng M, Yuan L, Liu Z, Bao J, Yang W, & Feng J. (2022). Determination of the main alkaloids and their insecticidal activity of extract of Areca catechu nuts against Plutella xylostella. International Journal of Tropical Insect Science, 42(5): 3563-3570.

Majeed SA. (2011). Study on fumigant and contact toxicity of Catharanthus roseus against Sitophilus oryzae. International journal of pharmaceutical and Biological Archives, 2(2), 751-755.

Mostafa ME, Alshamy MM, Abdelmonem A, & Abdel-Mogib M. (2017). Acaricidal activity of Chrozophora oblongifolia on the two spotted spider mite, Tetranychus urticae Koch. Journal

of Entomology and Nematology. 9(3): 23-28.

Mostafa MEH. (2021). Acaricidal potential and phytochemical analysis of the indigenous Centaurea aegyptiaca L. against Tetranychus urticae Koch (Acari: Tetranychidae). International Journal of Entomology Research. 6(1): 40-45.

Nataraj R, Jagadish MS, Varadaraju KR, & Bindu J. (2023). Isolation, purification and characterization of vindoline from Catharanthus roseus. International Journal of Science and Research Archive. 8(1): 310-319.

Ramya S. (2008). Biopesticidal effect of leaf extracts of Catharanthus roseus L (G) Don. on the larvae of gram pod borer-Helicoverpa armígera (Hübner). Ethnobotanical Leaflets. 12: 1096-1101.

Sadek MM. (2003). Antifeedant and toxic activity of Adhatoda vasica leaf extract against Spodoptera littoralis (Lep., Noctuidae). Journal of applied entomology. 127(7):396-404.

Sandey J, & Summarwar S. (2016). Pupicidal activity of plant extracts of Catharanthus roseus against 6th instar of Spodoptera litura. International Journal of Fauna and Biological Studies. 3(4): 9-10.

Simpson DR, Bull DL, &Lindquist DA. (1964). A semi microtechnique for the estimation of cholinesterase activity in boll weevils. Annals of the Entomological Society of America. 57(3):367-371.

Soliman MMM. (2001). Phytochemicals and toxicological studies of some plant extracts against Aphis craccivora Koch. Cairo University. PhD Thesis, Faculty of Agriculture.

Sun H, Blanford S, Guo Y, Fu X, & Shi W. (2012). Toxicity and influences of the alkaloids from Cynanchum komarovii AL. Iljinski (Asclepiadaceae) on growth and cuticle components of Spodoptera litura Fabricius (Noctuidae) larvae. Natural product research. 26(10): 903-912.

Sun YP. (1950). Toxicity Index-an Improved Method of comparing the relative Toxicity of Insecticides. Journal of Economic Entomology. 43(1):45-53.

Tiong SH. (2014). Alkaloids of Catharanthus roseus and their hypoglycemic activity/Tiong Soon Huat (Doctoral dissertation). PhD Thesis. University of Malaya.

Trindade RCP, Silva PPD, Araújo-Júnior JXD, Lima ISD, Paula JED, & Sant'Ana AEG. (2008). Mortality of Plutella xylostella larvae treated with Aspidosperma pyrifolium ethanol extracts. Pesquisa Agropecuária Brasileira. 43: 1813-1816.

Van Bergen MA, & Snoeijer W. (1996). Revision of Catharanthus G. Don: Series of Revisions of Apocynaceae XLI. Wageningen Agricultural University.

Yubin JI, Miao Y, Bing W, & Yao Z. (2014). The extraction, separation and purification of alkaloids in the natural medicine. Journal of Chemical and Pharmaceutical Research. 6(1): 338-345.

Zou C, Lv C, Wang Y, Cao C, & Zhang G. (2017). Larvicidal activity and insecticidal mechanism of Chelidonium majus on Lymantria dispar. Pestic Biochem Physiol. 142:123–132.

## الملخص العربي

## عنوان البحث: التقييم الأبادى والكيميائى لمستخلصات نبات كاثارانثوس روزوس ضد دودة ورق القطن

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أسفر الفصل الكروماتوغرافي لـ "كاثارانثوس روزوس" عن ستة خلاصات. كانت خلاصه القلويات والبيوتانول هي أكثر الخلاصات فعالية ضد يرقات دودة ورق القطن في عمرها الثاني. بعد سبعة أيام من التعرض لهاتين الخلاصتين باستخدام تقنية غمس الأوراق بتركيز LC50 والذى بلغ ٢٣٤,٥٨ و ٤٨١,٠٩ جزء في المليون على التوالي. وقد تم فصل وتعريف الخلاصات الأكثر سمية واوضحت النتائج وجود ثلاثة قلويدات إندول أحادية التربينات هي، vindoline (١) ، vindorosin (٢) وajmalicine (٣) تم فصلها في صورة نقية بواسطة طرق الفصل الكروماتوجرافي المتعددة، وأيضا عن طريق استخدام العديد من القياسات الطيفية. تمت دراسة تأثير خلاصه القلويات والبيوتانول على الجوانب البيوكيميائية والفسيولوجية ليرقات دودة ورق القطن.