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Physiological impacts of Euphorbia pulcherrrima Willed extracts on Cotton Leaf Worm, Spodoptera littoralis larvae

Abdel-Raouf Mohamed Sallam[1], *Heba Youssif El-Sayed Ibrahim[2], and Fatma Jamal Salh Hamdi[3]

[1]*Professor of Entomology, Zoology Department, Faculty of Science, Mansoura University, e-mail: abdelraouf.sallam@gmail.com*

[2]*Plant Protection Research Institute, Agriculture Research Center (ARC), Dokki, Giza, 12618, Egypt, e-mail: hyei_youssif@yahoo.com*

[3] *Faculty of Science, Zoology Department, Tripoli University, e-mail: fatma.j.hamdi@gmail.com*

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Abstract: Different fractions of Euphorbia pulcherrima Willed. aerial parts extract were evaluated for their physiological effect on the fourth instar larvae of Spodoptera littoralis under laboratory conditions. The effects of these extract fractions on the transaminases enzyme; Glutamic Pyruvic transaminase (GPT), Glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase enzymes (AlP) in S. littoralis were studied. All E. pulcherrima extract fractions showed marked disruption of the tested enzymes. This proved worthy of E. pulcherrima extracts to be used as green alternatives of the traditional synthetic insecticides.

keywords: *Euphorbia pulcherrrima*, transaminases, alkaline phosphatase, *Spodoptera littoralis*.

1.Introduction

The cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a serious polyphagous agricultural pest. It attacks many plants of various families that spread over tropical and subtropical regions. So, the use of pesticides has become imperative to control this pest. But, by the time, resistance towards many registered insecticides has developed [1]. Residual toxicity and negative impacts of the traditional synthetic insecticides on the environment and beneficial insects were also worsened. So, it was necessary to find other environmentally friendly insecticides.

Plant extracts are promising safe alternatives used for insect pest management. *E. pulcherrima* contains numerous chemical groups such as terpenoids, alkaloids, flavonoids, steroids, saponins, glycosides, reducing sugars [2,3] . Based on the principle that the insecticidal properties of any plant extract depend upon its chemical constituents, *E. pulcherrima* extracts are promising candidate for insect pest management. Here, this study aimed to assess the physiological impacts (enzymatic disruption) of *E. pulcherrrima* extracts on the fourth instar larvae of *S. littoralis* .

2. Materials and methods

2.1. Plant Material:

Aerial parts of *E. pullcherima* were collected from the Mansoura University garden in November 2019. Identification of the plant was confirmed by Botany Department, Faculty of Science, Mansoura University., Egypt.

2.2. Extraction of *E. pulcherima* chemical constituents:

Aerial parts of the plant were cleaned and dried at room temperature then, grounded to a powder and soaked in methanol three times, each time for three days. The methanolic extract of *E. pulcherrima* was filtered and evaporated to 1/3 of its volume, then successively extracted by different organic solvents of ascending polarity (petroleum ether, methylene chloride and ethyl acetate) by using a separating funnel. All fractions were dried

over anhydrous sodium sulphate then evaporated to dryness.

2.3. The tested insect pest

Susceptible culture of *S. littoralis* was obtained from Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. It was maintained on fresh castor leaves under laboratory controlled condition (27± 2°C, 65±5% R.H).

2.4. Biochemical study:

The fourth larval instar were treated with LC_{25} of each tested fraction. They were collected at 1st, 3rd, 5th, 7th and 10th days post-treatment. For each trail 3 larvae were kept in clean jars in the refrigerator at 5° C until biochemical determination. Larvae were homogenized in 3 ml bio-saline solution (0.9% NaCl) then centrifuged at 2000 rpm for 2 min. The supernatants were immediately tested for enzymes activities by the kinetic method according to the Expert Panel of the (IFCC) without pyridoxalphosphate activation for the quantitative determination of GPT and GOT according to [4] and ALP according to [5] .

3. Results and Discussion

2.1. Effect of different fractions of *E. pulcherrima* extracts on transaminases activity of *S. littoralis* 4th larval instar :

Data presented in Table 1 and illustrated in Fig. 1 showed that the disruption of GPT activity in *S. littoralis* as a result of treatment with LC_{25} of different fractions of *E. pulcherrima* extract. It was noticed that all extract fractions except ethyl acetate fraction revealed a great inhibition of GPT activity comparing with the control at 1st and 3rd day post treatment. On the 5th day post- treatment, almost all fractions revealed a marked increase in GPT activity. Methylene chloride fraction showed the highest value reaching 542.91% more than the control.

The obtained data in accordance with the previous protocol [6] who recorded a noticeable decrease of GPT activity% in cowpea aphid, *Aphis craccivora* treated with *Cladosporium cladosporioides* and its secondary metabolites extracts at 1st day post-treatment, then subsequent increase of the enzyme activity was recorded till reach the normal levels and decreased again at 7th day post-treatmen

Table 1: Glutamic Pyruvic transaminase (GPT) activity (U/L) in *S. littoralis* 4th larval instar treated with LC_{25} of *E. pulcherrima* extracts

Treatmnt	GPT activity at different times post treatment									
	1st day		3rd day		5th day		7th day		10th day	
	Mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %	Mean ± SD (U/L)	Change %	Mean± SD (U/L)	Chang%
Pet.ether fraction	91.33 ±5.033	-41.58	22.67 ± 2.45	69.08	9.67 ± 0.75	-13.89	6.7 ± 0.46	16.98	6.7 ± 0.4	1.98
Methylene chloride	119.67 ±3.51	-23.45	24.63 ± 1.16	66.41	72.2 ± 0.4	542.92	13.07± 0.35	61.96	6.3 ± 0.4	-4.11
Ethyl acetate	319.33 ±7.02	104.27	73.33 ± 4.04	0	38.03±1.00	238.65	8.67 ± 0.32	7.43	4.83±0.35	-26.48
Control	156.33 ±2.52	-----	73.33 ± 4.04	------	11.23±0.47	-------	8.07 ± 0.31	------	6.57 ±0.35	------

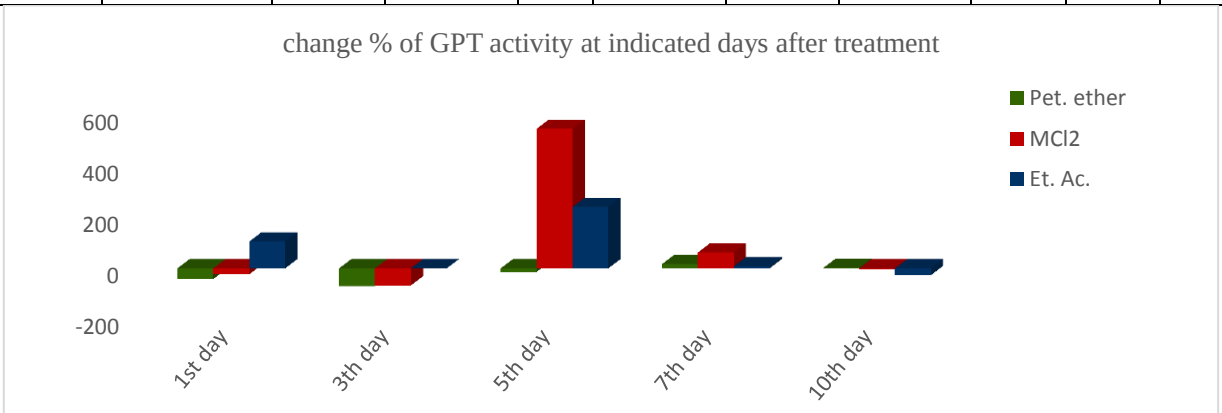

Fig. 1: GPT activity (U/L) in *S. littoralis* 4th larval instar treated with LC_{25} of *E. pulcherrima* extracts

Regarding GOT activity, data in Table 3 and Fig. 2 revealed that all extract fractions at a sub-lethal concentration (LC_{25}) caused marked inhibition of GOT activity almost along the experiment period except in the 1st day post-treatment, whereas ethyl acetate fraction exhibited a dramatic increase of GOT activity reached to 443.37% more than the untreated control, followed by methylene chloride fraction recording 86.51% more than control. The resulted obtained are in agreement with [7,8,9] , when treated *S. littoralis* with certain IGRs or insecticides, e.g., flufenoxuron, hexaflumuron, pyriproxyfen. Also, the increasing of GOT activity in *S. littoralis* larvae has been recorded before by [10] .

Aminotransferases are the responsible for maintenance of "amino acid pool" balance in the insect body [11] . Insecticides have a great effect on enzymes catalyzing the metabolism of amino acids in insects [12]. So, the recorded disruptions of these enzymes in the present study proved worthy of *E. pulcherrima* extracts to be used as green insecticides.

Table 2: Glutamic oxaloacetic transaminase (GOT) activity (U/L) in *S. littoralis* 4th larval instar treated with LC_{25} of *E. pulcherrima* extracts:

Treatment	GOT activity at different times post treatment									
	1st day		3rd day		5th day		7th day		10th day	
	mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %	Mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %
Pet.ether fraction	291.33 ±7.37	-291.33	132.33 ±4.51	-132.33	92.37 ± 0.85	-92.37	36.13 ±0.75	-36.13	10.03 ±0.55	-10.03
Methylene chloride	576.33 ±7.64	86.51	278.17 ±5.35	1.47	66.5 ±0.61	-69.12	45.7 ±0.75	-43.65	10.4 ±0.75	-8.77
Ethyl acetate	1679 ±7.55	443.37	228 ±6	-19.24	195.33 ±7.37	-9.31	72.5 ±3.77	-10.6	13.77 ±0.59	20.79
Control	309 ±2.65	------	282.33 ±3.52	------	215.37 ±1.98	------	81.1 ±1.68	------	11.4 ±0.4	-------

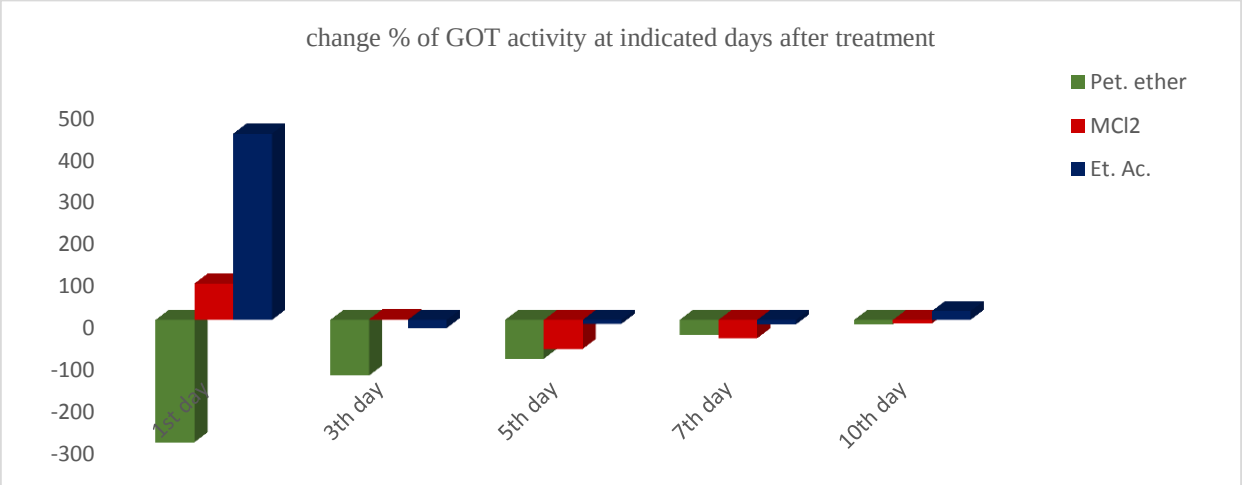

Fig. 2: GOT activity (U/L) in *S. littoralis* 4th larval instar treated with LC_{25} of *E. pulcherrima* extracts

2.2. Effect of different fractions of *E. pulcherrima* extracts on alkaline phosphatase activity of *S. littoralis* 4th larval instar:

Data arrranged in Table 3, and demonstrated in Fig. 3 cleared that petroleum ether fraction at sub-lethal concentration (LC_{25}) revealed significant inhibition in AlP activity comparing with the control along the period of experiment recording maximum inhibition at the 10th day post-treatment reaching -77.45% less than the untreated control.

On the other hand, ethyl acetate fraction revealed a significant increase in alkaline phosphatase activity at the first three days of the experiment reaching maximum increase (74.07%) more than control at the 3rd day post-treatment. Also, methylene chloride fraction exhibited a moderate increase in the

enzyme activity at the first three days then, it revealed a slight decrease at the 5th and7th day post- treatment.

The present data agreed with [13] who recorded a significant increase of the alkaline phosphatase activity of *S. littoralis* at 0 to 72 hrs post-treatment with *Bacillus thuringiensis* compared with control.

Alkaline phosphatase enzymes (ALP) is a vital indispensable enzyme for transphosphorylation reaction catalysis[14] . Any impairment in its activity will disrupt the physiology of the insect. Also, AlP enzyme is intimately linked to insect development and moults. So any inhibition of this enzymes could affect the normal development of the treated insect [15] .

Table 4: Alkaline phosphatase enzymes (AlP) activity (U/L) in *S. littoralis* 4th larval instar treated with LC_{25} of *E. pulcherrima* extracts

Treatment	**AlP activity at different times post treatment**									
	1st day		**3rd day**		**5th day**		**7th day**		**10th day**	
	mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %	mean ± SD (U/L)	Change %
Pet.ether fraction	33.37± 1.50	-13.44	28.4 ±1.2	2.42	18.53 ±0.81	-32.62	11.57 ±0.35	-22.51	2.96 ±0.25	-77.45
Methylene chloride	44.13 ±1.11	14.47	31.73 ±0.7	14.42	21.63 ±0.67	-21.35	13.9 ±0.3	-6.9	11.17 ±0.15	15.19
Ethyl acetate	56.27 ±1.07	45.97	48.27 ±0.87	74.07	24.17 ±0.4	-12.11	15.03 ±0.25	0.67	14.37 ±0.42	9.11
Control	38.55 ±0.5	------	38.55 ± 0.55	------	27.5 ± 0.6	------	14.93 ± 0.70	------	13.17 ±0.38	-------

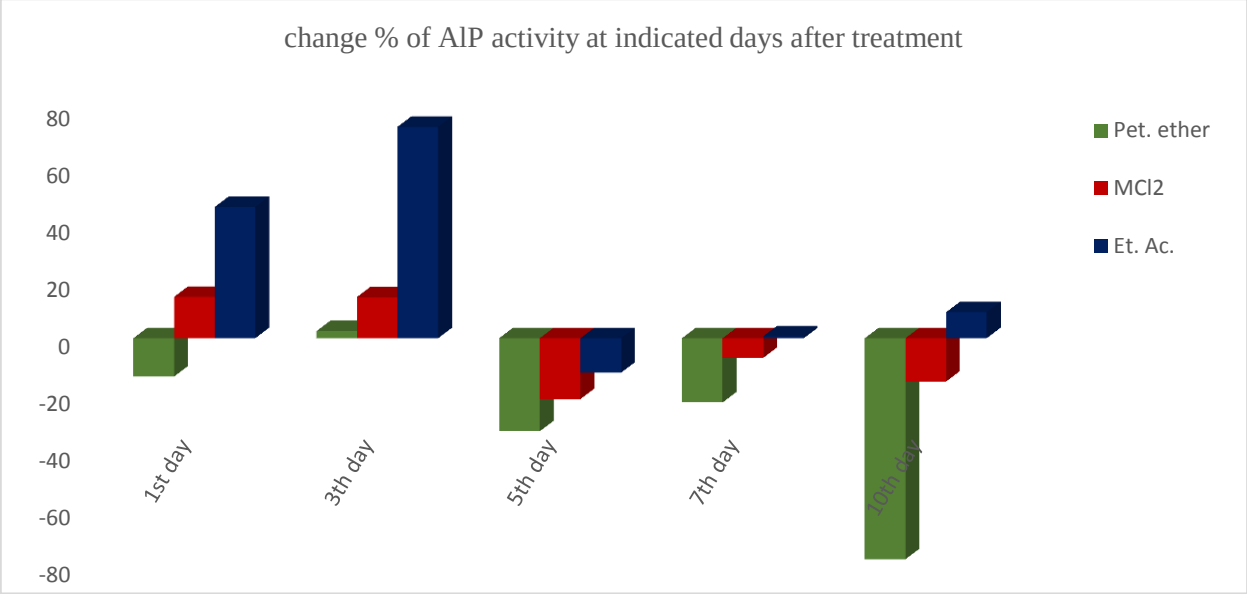

Fig.3: AlP activity (U/L) in *S. littoralis* 4th larval instar treated with LC_{25} of *E. pulcherrima* extracts**.**

Conclusion

The present study investigated the physiological impacts of *E. pulcherrima*

extract on *S. littoralis* 4th larval instar. These physiological impacts are represented by marked disruption of the transaminases and alkaline phosphatases activity. The current data indicated that *E. pulcherrima* secondary metabolites interfere with *S. littoralis* enzymes, causing significant disruption in the tested insect's growth and development.

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