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Susceptibility And Some Biological Aspects Of *Ceratitis capitata* (Wiedemann) As Influenced By Different Crude Extracts Of *Nerium oleander* L.

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ABSTRACT:

A trial was conducted to evaluate the toxic impact of Nerium oleander L. leaf extracts against Ceratitis capitata (Wiedemann), adults. Acetone, ethanol, petroleum ether, and water were used to extract the N. oleander leaves contents.

Leaves crude extract with ethanol was the most toxic than the other three extracts based on lethal concentrations (LC_{50} and LC_{90}), recording the values of (2.62 & 4.75), (1.68 & 3.29), and (1.10&d 2.23) µg/ml post 24, 48 and 72 h of application. Also, the ethanol extract at the LC_{50} showed the ability to decline some biological aspects such as the fecundity (number of eggs laid), hatchability percentages, and triggered retardation in larval duration in comparison with the control.

Conclusively, *the research recommends that Nerium oleander, extracted with different solvents, has a toxic impact against Ceratitis capitata under laboratory conditions.*

Keywords: *Nerium oleander, Ceratitis capitata,* leaf crude extract, toxicity, biological parameters.

INTRODUCTION:

Ceratitis capitata is a graver and destructing pest of fruits and vegetables in tropical and subtropical areas, triggering numerous disadvantages to the fruits causing a considerable wastage of fruit outputting annually (Khan *et al.,* 2005).

Recurrently and anarchically, chemical pesticides dominate harmful implications like environmental pollution, residual toxicity on food, and the

development of more resistant strains of pests to chemicals. Motivate to use novel techniques to obviate these hazards, such as natural products, and Plant extracts that take marvelous react for their availability, impact, degradability, and amicable to the environment (Aqil *et al.*, 2010). More than 2000 plant species are known to have the potential to be vital to insects (Souza *et al.*, 2017).

Nerium oleander L. is known as a butter plant that is commonplace and found in numerous Mediterranean regions, it also has been broadly planted as an ornamental plant in tropical and subtropical areas. This plant belongs to the Apocynaceae family (Akal and Matrood, 2020). The leaves of *N. oleander* include oleandrin, which is responsible for toxicity (Fakoorziba *et al*., 2015), and else useful as an insecticide as well as an anti-feedant (Senthilkumar *et al*., 2020), also, lately used in controlling agricultural pests and rodents (Sivakumar *et al*., 2022). Many research mentioned that *N. oleander* only has larvicidal activity against various insect pests (El-Akhal *et al.*, 2015), subacute toxicity (Abdou *et al*., 2019), and toxic influence (Nasir *et al*., 2021).

Therefore, it was necessary to study the toxicity and other biological impacts of *N. oleander* plant extract on *C. capitat* adults on one of the most dangerous pests.

MATERIALS AND METHODS:

Insect rearing:

The culture of *Ceratitis capitata* was initiated from the pupal stage that obtained from the Plant Protection Research Institute, Agricultural Research Center at Giza, Egypt, and the mass rearing was completed in Plant Protection Research Laboratory's Zagazig branch–Sharqia, Egypt. The relative humidity (RH) was kept at 65.5%±3C^0 while, the temperature of fruit fly colonies was maintained at 25.2±3^{0}C.

The adult flies were reared on an artificial diet according to Tanaka *et al*. (1969) that was composed of 1 part protein hydrolysate: 3 parts sugar, and water. Cages measured (30×30×30 cm), made from plastic with several tiny pores placed within the cage to valeting as oviposition pots contained water inside it to keep moisture for eggs. Eggs were collected 2-3 times per week and reposed on an artificial diet in plastic trays covered with cloth lids and left for hatching and larval development. The artificial diet for larvae included wheat bran, brewer's yeast, sodium benzoate, HCL, and water. For jumping larvae to pupate, the diet trays were put in a sizable wooden box filled with sand at the bottom (Shehata *et al*., 2006). To muster pupae, the sand was sifted and relayed into the adult rearing cage, which was made of a wooden frame coated with wire screen from different sides

except one side, which had a sleeve opening, and the cage floor was made of wooden sheet until the emergence of flies.

Extraction of Nerium oleander leaves:

According to the method of Macneel *et al*. (1975) leaves of *N. oleander* was collected from healthy trees and minced into small pieces. The pieces were blended in a blender and then weighed and extracted in each solvent separately in the ratio of 1:5 w:v. Each extraction suspension was mixed with a vortex mixer (VM-300 Axiom, Germany) and placed on a rotary shaker for 1 h. at (170 rpm) then filtered using filter paper (Whitman filter paper grade No.1).

The extracts were concentrated to 1 ml on a rotary evaporator by removing the excess solvent under vacuum, adding 20 ml of a solvent to the solvent-plant mixture (1ml), then centrifuging at 6000 rpm for 10 min. Supernatants from the extractions from each solvent were combined and concentrated to dryness under vacuum before being mixed with sterile distilled water, weight extracts, adding 45 ml with distilled water. These extracts were either used immediately or kept in the refrigerator at 4±1°C until further use. Each plant extract stock solution was serially diluted with distilled water to obtain the different extract concentrations.

Toxicity experiments:

The plant extract was assayed towards the adults of *C. capitata* under laboratory requisites. Different concentrations (0.5, 1, 1.5, and 2%) of each tested solvent extract were prepared. Small glass jars were used for the toxicity experiments, each jar contained ten adult males and ten adult females, the jars were kept separately, and they starved before treatment. Cotton pieces were submerged in a series of five concentrations, with five replicates for each concentration. Five untreated replicates were also put as a control. Tested flies were examined daily, the dead flies were excluded and the mortality percentages were recorded after 24, 48, and 72 hours. The average of mortality percentages was corrected using Abbott's (1925) formula also, LC_{50} and LC_{90} values were calculated using, Biostat 2007, (professional Build 3200).

Some biological aspects of C. capitata as influenced by ethanol extract:

Some biological parameters were observed to evaluate the impact of *N. oleander* at LC_{50} under laboratory conditions. For experiments Three replicates were prepared the newly emerged adults (25 pairs) each were starved for 24 h., and confined in cages separate, then the diet was mixed with LC_{50} of *N. oleander* ethanol extract. Adults were equipped with water in small plastic (perform the plastic fruits) with small pores as vessels for ovo-position. Three replicates were prepared. The recorded biological aspects were: No. of eggs laid/♀, incubation

period (the period from the beginning of the first laid egg to the first egg hatching), fecundity, hatchability %, larval and pupal duration, emergence, and the adult longevity according to Murtaza *et al*.(2021).

Statistical analysis:

The lethal concentrations (LC_{50}), (LC_{90}), toxicity index (T.I) and the relative potency (R.P) were determined using the formula of Sun (1950) and Zidan and Abdel-Maged (1988).

RESULTS AND DISCUSSION:

Toxicity of N. oleander extracts to C. capitata:

Data recorded in (Table 1) and (Figures1d, e & f) demonstrated that the LC_{50} and LC_{90} of the four different extracts against *Ceratitis capitata*, adults were (2.71&5.06), (2.62 & 4.75), (23.11&5.69), and (3.26 &5.76) µg/ml after 24h. post-treatment, while, after 48h. the values recorded were (2.39&5.07), (1.68&3.29),(1.73&3.38), and (2.95&5.55)µg/ml; these figures after 72h were (1.59&3.54), (1.10&2.23), (1.25&2.75), and (2.29&4.67) µg/ml post-treatment for *N. oleander* leaf extracted with acetone, ethanol, petroleum ether, and water, respectively.

Table1): Toxicity of the different *Nerium oleander* extracts to *Ceratitis capitata* adults

Nerium oleander extracts	LC_{50} Mg/ml	LC_{90} Mg/ml	Toxicity index % at		Relative toxicity at		Slope ±SE
			LC_{50}	LC_{90}	LC_{50}	LC_{90}	
Post 24 hours of treatment							
Acetone	2.71	5.06	96.67	93.67	1.20	1.13	0.60±0.13
Ethanol	2.62	4.75	100	100	1.24	1.21	0.54±0.08
Petroleum ether	3.11	5.69	84.24	83.47	1.04	1.01	1.45±0.15
Water	3.26	5.76	80.36	82.46	1.00	1.00	1.51±0.37
Post 48 hours of treatment							
Acetone	2.39	5.07	70.29	64.89	1.23	1.09	0. 79±0.14
Ethanol	1.68	3.29	100	100	1.75	1.68	0.47±0.11
Petroleum ether	1.73	3.38	97.10	97.33	1.70	1.64	1.13±0.26
Water	2.95	5.55	56.94	59.27	1.00	1.00	2.90±0.83
Post 72 hours of treatment							
Acetone	1.59	3.54	69.18	63.00	1.44	1.32	0.85±0.10
Ethanol	1.10	2.23	100	100	2.08	2.10	0.69±0.43
Petroleum ether	1.25	2.75	88.00	81.09	1.83	1.70	1.77±0.62
Water	2.29	4.69	54.58	47.54	1.00	1.00	2.96±0.89

C. capitated laboratory colony was used.

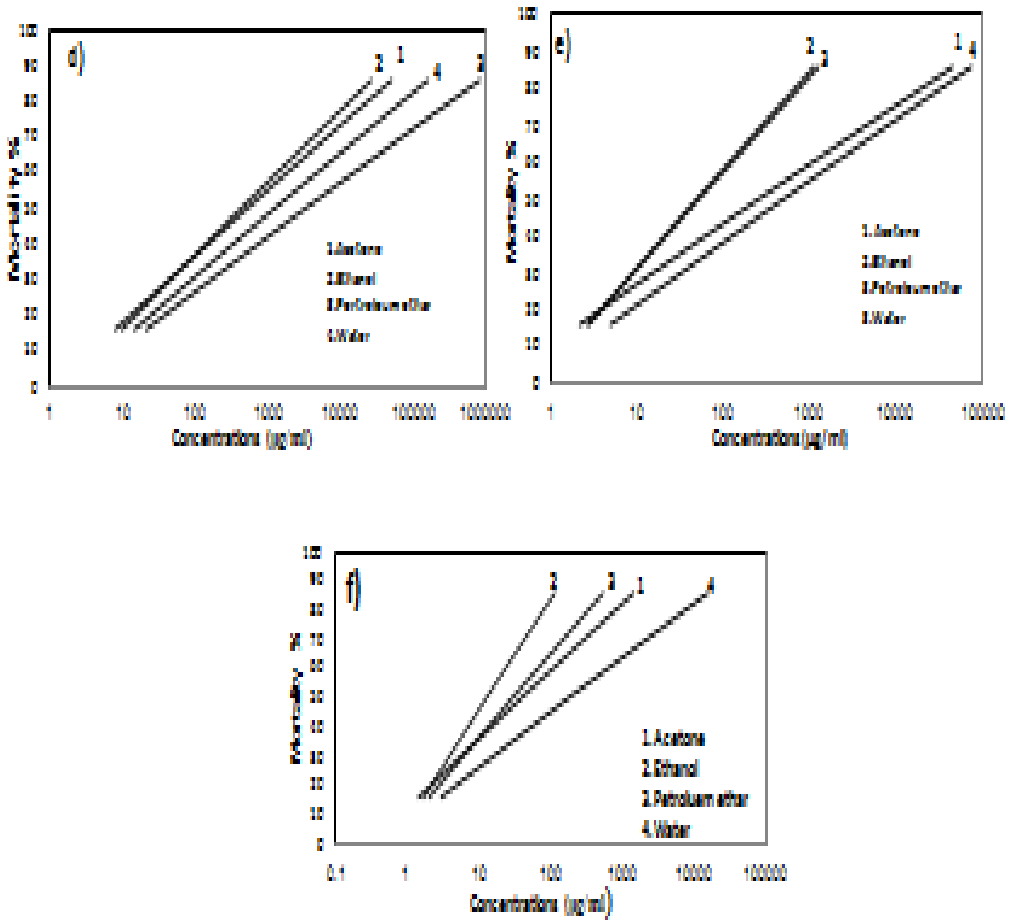

Fig.(1): Toxicity regression line of *Nerium oleander* leaf extracts from different solvents against *C.capitata* after, d), 24h.; e), 48h and f), 72h.post applied.

Toana *et al*.,(2023) stated that the concentrations of *N. oleander* extract were effective and caused mortality of *C. binotalis* larvae, from 1.25% to 2.5%, and the LC_{50} was 1.915% with a minimum concentration of 1.425% and a maximum concentration of 2.375%. Regarding toxicity indexes, the values were (96.67&93.7), (84.24& 83.47), and (80.36& 82.46)% after one day of treatment in comparison with the second and the third days the values observed with (70.29 & 64.89), (97.10&97.33), (56.94 & 59.27), (69.18&63.00), (88.00& 81.09), (54.58

&47.54)% which recorded with acetone, petroleum ether, and water in comparison with the most toxic solvent (ethanol) which had 100% toxicity index and the values were (1.20&1.13), (1.24&1.21), and (1.04&1.01) fold were the relative toxicity compared with the lowest solvent (water), respectively.
After 24h., for 48 and 72h. the values showed were (1.23&1.09), (1.75&1.68), (1.70&1.64), (1.44&1.09), (1.75&1.68), and (1.70&1.64) fold for acetone, petroleum ether and water, respectively. Nasir *et al*. (2021) observed that the *N. oleander* leaf extract given the level of stomach poison that enters the insect's body would increase, resulting in the death of the insect. Also, results noticed that values of slope after 24, 48and 72h. were as follows; (0.60±0.13), (0.54±0.08), (1.45±0.15), (1.51±0.37)& (0.79± 0.14), (0.47±0.11), (1.13±0.26), (2.90±0.83)& (0.85±0.10), (0.69± 0.43), (1.77±0.62), and (2.96±0,.89) for acetone,

Biological aspects:

Based on the LC_{50} of *N. oleander*, extract ethanol, which caused the highest toxic effect compared with other solvents, some biological aspects were studied for *C. capitata* adults that survived the ethanol extract and the resulted were recorded in the Table 2.

Data in Table (2) show that *N. oleander* ethanol extract shortened the oviposition-period to 29.33±0.66 days compared with the control which recorded with 54.33±0.19 days. Also, the ethanol plant extract reduced the fecundity to 154 ±1.85 eggs/♀, while, the control recorded 331±2.03 eggs/♀ during their longevity. For the incubation period, the *N. oleander* caused a slight increase in the incubation period being (4.00± 0.35) days in comparison with the control (2.45±0.33) day. On the other hand, *N. oleander* induced a highly decrease in the hatchability percentage recording 69.20±1.33%, while, this value of the control was 94.51±0.9%. Plant extract led to a high retardation in larval duration of the larvae descending from the treated adults recording 12.23±0.88 day compared with the control (7.47±0.43 day). Elimem *et al.* (2022) compared the efficiency of basalt as foliar spray with two doses (1.5% and 3%), in the ground as a fertilizer (1.5%), and as a combined application (1.5%) compared to two botanical aqueous extracts of bitter orange (*Citrus aurantium)* and Nerium (*Nerium oleander*) (50g/l) against *C. capitata* and *Phyllocnistis citrella* in citrus orchard. A live larvae population decreased considerably after one day of treatment with basalt and in the ground compared to plant extracts, in contrast with Moustafa *et al*.,(2018) observed that the Stem extract of *Nerium oleander* was the most toxic against 1st instar larvae of *Pectinophora gossypiella*, when evaluation 70% hydro-ethanolic extracts of *N. oleander* (leaves, stems and flowers) under laboratory conditions.

With regards, the pupal duration was slightly raised (9.30±0.31) day, whilst the control was (8.60±1.33) day. Toana *et al.* (2023) observed that the LC_{50} of *N. oleander* leaf extract, against *C. binotalis* larvae, was 1.915% (0.01915ml/ml), with a minimum concentration of 1.425% (0.01425ml/ml) and a maximum concentration of 2.375% (0.02375ml/ml). The higher the concentration of *N. oleander* leaf extract, the higher the feeding inhibition, causing a decrease in the feeding of *C. binotalis* larvae.

Also, the data obtained monitored a reduction in emergence percentage with (77.03±5.36) %, in contrast with the control. Data showed that the plant extract strongly diminished the male and female longevity to (44.84±2.07/♂& 40.01±1.01/♀) days, comparing with the control which noticed (75.42± 2.5& 79.21±1.83) day, consecutively. Zohara *et al*. (2024) evaluated the effect of *Nerium oleanderand* and *Artemisia campestris* as powders and methanolic extract against adult and larvae of the wheat beetles, *Tribolium castaneum* and *T. confusum*. Data recorded that *N. oleanderand* caused 100% mortality in adults up to 80 % on larvae at 0.5% post 7 days of treatment, compared with adults of *T. confusum* which showed some resistance to the powders of both plants, with a mortality percentage of 50% for adults.

Moreover, data noticed that the powder of *A. campestris*, against adults of *T. castaneum* was more toxic and caused 100% mortality, while methanolic extracts of *N. oleander* and *A. campestris* showed the effectiveness of this treatment against adults of *T. castaneumas* well as the larvae of both insects compared with *T. confusum* adults which have some resistance to methanolic extracts of plants.

Conclusively, the research recommends that *Nerium oleander,* extracted with different solvents, has a toxic impact against *Ceratitis capitata* under laboratory conditions.

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تأثير المستخلصات الخام لأوراق نبات الدفلة على ذبابة البحر المتوسط وبعض صفاتها البيولوجية

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تم إجراء هذه الدراسة تحت الظروف المعملية، لتقيم فاعلية تأثير المستخلص الخام لنبات الدفلة على الأطوار البالغة لحشرة ذبابة الفاكهة وبعض صفاتها البيولوجية. ثم باستخدام بعض المذيبات المختلفة كمذيب الأسيتون والإيثانول و البترولیوم إيثر والماء. وأظهرت النتائج أن نبات الدفلة المستخلص أوراقهُ بمذيب الإيثانول كان الأكثر سمية مقارنةً بالمذيبات الأخرى.

وكانت القيم المتحصل عليها للتركيزات المميتة لـ ٥٠% و ٩٠ % للحشرات الكاملة هي ، (٢.٦٢ و٤.٧٥) و(١.٦٨ و٣.٢٩) و(١.١٠ و٢.٢٣) ميكروغرام/مل بعد ٢٤ و٤٨ و٧٢ ساعة من المعاملة. كذلك أدى المستخلص الايثانولى لنبات الدفلة الى خفض بعض النواحى البيولوجية لذرية الحشرات المعاملة مثل الخصوبة (عدد النسل)، و نسبة الفقس وكذلك تسبب في امتداد الطور اليرقي وطور العذراء وانخفاض % لخروج الحشرات الكاملة مقارنةً مع المجموعة الضابطة.

التوصية: يوصى البحث باستخدام مستخلص نبات الدفلة والنعمل بمذيباتمختلفة لما له من تاثير سام تجاه حشرة ذبابة الفاكهة تحت الروف المعملية