The effects of crude alkaloid extracted for *Nerium oleander* L. Leaves on biological performance of *Bemisia tabaci* Genn. (Homoptera: Aleyrodida)

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Summary

In laboratory bioassays to determine the toxicity of crude alkaloid extracts of *Nerium oleander* leaves to whitefly *Bemisia tabaci* . Crude alkaloids applied at concentrations of 0.1 , 0. 2 , 0.5 , 1 , 2 % . The egg was generally the least susceptible stage to all test treatments.The results indicated that the concentration of 2 % was the most effective . At this concentration eggs mortality reached 51.35 % in crude alkaloids , differences in nymphal mortality according to age was observed at all concentrations of alkaloid extract , the first larval instar and second namphal stadium had higher mortality when treated with alkaloid extract at concentrations of 1% and 2% than third nymphal stadium . Pupal and adults mortality reached 84.66 % and 71.84% when treated with crude alkaloids at concentration of 2% respectively .Cumulative mortality reached 100 % at concentration of 1 % and 2 % in second and third nymphal stadia when treated with crude alkaloids . Development time of immature stages of *B. tabaci* also , affected by the application of crude alkaloids extracts of *N. oleander* leaves , generally development period prolonged in all treatments of alkaloids as compared with control treatment .

Introduction

Plant may provide an alternative chemicals to currently used pesticides to control of plant pests , due to the extances of bioactive chemicals (8 , 15) . Recent studies have demonstrated that insecticidal properties of chemicals derived from plants are active against specific target species , biodegradable to non toxic products and potentially suitable for use in integrated management programs (18 , 27) . In recent years the whitefly *Bemisia tabaci* (Gennadius) has become an increasingly important pest of vegetables in Jordan (2) , in Iraq (1) , in Egypt (20) , and other countries in Middle East . Three types of economic damage to plants may be caused by *B. tabaci* (direct and indirect ways) :

1 – sucking sap from plants 2 – excretion of honeydew on fiber

3 - transmission of viruses

(22) and (14)carried out laboratory experiments with *Nerium oleander* leaves(hot and cold water extracts) and reported their insecticidal activity , the toxicity of various parts of the plant and their constituents has been studied by different groups of workers as well as *N.oleander* is widely distributed and easy grown . The potential of using leave extracts of *N. oleander* as alternatives to synthetic acaricides for controlling the *B. tabaci* . One of the largest groups of chemicals arsenals produced by plants are the alkaloids , more than 10000 different alkaloids have been discovered in species from over 300 plant families (16) . Alkaloids are derived from various amino acids and often contain one or more rings of carbon atoms , usually with a nitrogen atom in the ring . The position of the nitrogen atom in the carbon ring varies with different alkaloids and with different plant families (7 , 26) . It has been known for some time that veratrum and tobacco alkaloids are rather toxic for a variety of organisms and , in fact , since ancient times man has taken the advantage of such pesticidal properties to protect his cultured plants from insect attack , more ever , several modern pesticides aimed against sucking insect and mite species are based on alkaloid structure i.e. the comprise pyrazol , pyrrol , pyrimidine , benzimidazole , phtalmide or quinoxaline moieties (16) .

Materials & Methods

Whiteflie adults were collected from the field (Al-Rashidya city) and kept in cage containing young eggplant *Solanum melangena* L. (15 - 20 cm height) as a host plant . After an ovipositional period of 24 hours the adult whiteflies were removed . The egg – bearing leaves on plant were incubated at 25 ± 2 C° , relative humidity 60 ± 5 % and photoperiod of 14 : 10(light : dark) hours until adults emergence (1) . *N. oleander* fresh green leaves were collected during 2009 . The fresh leaves were washed to remove residual dust and air-dried at room temperature for two weeks . The dried leaves were pulverized to powdered form by electrical grinder and kept in plastic bags at 10C° (12) . Alkaloid extraction from *N. oleander* leaves was done according to (13) . 10 gm of dried and milled leaves were extracted in Soxhlet extractor for 24 hours at 40C° with 200ml of ethanol as a solvent, then it was evaporated at 50C° by a rotary evaporator . The dried extract was dissolved in 5 ml of 80% ethanol , 30ml of sulfuric acid was added , then it was dried in electrical oven to removed ethanol . The acidic extract was adjusted to pH = 9 using 10% amonium hydroxid solution . Then extracted with chloroform four times (10 ml each time) in the separation funnel . The extracts were collected and 10 gm of anhydrate sodium carbonate was added to removed humidity . Then it was filtered through filter paper (Whatman No. 1). The extract was dried by rotary evaporator and the concentrated extract was stored in a refrigerator until the next stage of the experiment .

Two grams of dried alkaloid extract were dissolved in ethanol , the volume was made up to 100 ml with distilled water . From this stock solution six different concentration were prepared (2 , 1 , 0.5 , 0.25 , 0.125 , 0.0) mg / ml , liquid paraffin 1% and 1 – 2 drops of tween were added to each concentration as adhesive agent and surfactant respectively (3) .The effects of crude alkaloid with their different concentrations were evaluated against all developmental stages (egg , first larval instar , second and third nymphal stadia , pupae and adult) of *B.tabaci* by taking 30 adults and introduced to the experimental cage supplied with young host plant (15 - 20 cm height) . Five replicates for each concentration. The crud alkaloids were sprayed with a laboratory spray gun , the cages were kept at incubator conditions previously mentioned at 25 ± 2 C° , relative humidity 60 ± 5 % and photoperiod of 14 : 10(light : dark) . The adults mortality were recorder and corrected according to Abbots formula . All the steps previously mentioned were used in the treatment of eggs , first larval instar, second and third nymphal stadia as well as pupae ,surrounded with oil ring (five replicates were made for each treatment and kept at the same conditions of adults) . Mortality rates were recorder after 24 hrs. and continually the seven days post spraying and corrected according to Abbots formula .

To calculate development period , twenty five newly hatched first instar larvae (five each replicates) that lived from previous treatment with concentration that mentioned before .They were surrounded by oil ring and incubated at same condition of adults . The developmental period (days) from first larval instar to second nymphal stadium were counted and the mean of each was calculated . All the above steps were used to calculate the developmental period (days) of second and third nymphal stadia as well as pupae . As well as cumulative effects of crude alkaloids were calculated , all the steps mentioned above were applied except for eggs which were left after treatment . Mortality rates of eggs, first larva linstar , second , third nymphal stadia , pupae and adults were recorder and corrected according to Abbots formula . The statistical analysis system –SAS (24)was used to effect of concentration of extract in mortality percentage and other traits. The least significant difference –LSD test was used to compare between means.

Results & Discussion

Table 1 showed that crude alkaloids were affected different stages mortality , as well as the data showed a direct correlation between different stages mortality and extract concentration ,significant difference were found among the concentration at $P < 0.05$.

Egg mortality reached between 1.33 % at control treatment to 51.35% at concentration of 2% . (1)found that the alkaloid extracts of *I. lutea* was highly toxic to the eggs of *B. tabaci* , eggs mortality reached 64 % at

concentration of 2 % . Also , who found that the eggs mortality rate ranged between 0.6 – 21.3 % in cold water extract ,while it was between 2.6 – 12.3 % in hot water extract of *I. lutea* , (14) found that *Nicotiana gossei* extracts did not affect whitefly eggs. Eggs mortality of *B.tabaci* reached35%when treated with aqueous extract of *Melia azedrach* (9). This effect may be due to the mimic effect of juvenile hormone and other compounds which interfere with the embryonic development when eggs were treated in the early time after ovulation (23) . Also , eggs mortality may be due to embryo asphyxia inside the egg because the extract was formed as layer on the external shell (25).

The mortality rates of first larval instar , second and third nymphal stadia ranged between 17.81 – 100 , 24.99 – 93.98 and 7.33 – 86.88 % at concentration 0.1– 2% respectively ,compared with 1.33 , 2.66 and 0.0 % at control treatment .Present tudy revealed that nymphal mortality of *B. tabaci* increased as crude alkaloid concentrations increased (Table 1) .Similar results has been reported on *B.tabaci* treated with extract of *I. lutea* (1)and *A.indica* (9) . (1)mentioned that *I. lutea* extract was highly toxic to the first larval instar , second and third nymphal stadia (nymphal mortality reached 100 % at concentration of 2 %) . While (9) reported that neem seed extract was more efficient in reducing the number of first larval instar than the extracts of fresh fruit of *Melia azedarach* . Also ,(9) found that The highest reduction percentage of whitefly nymphs was recorder with 2.5 % concentration of garlic and black pepper extract (100 % reduction). (29) and (6) mentioned that the toxic extracts inhibit growth and development of many species of insects because it interfere with molting hormone and converted to physiologically inactive ecdysteroids . The extracts were more effective in first larval instar (crawlers) than second and third nymphal stadia , this may attributed to the crawlers usually move a few centimeter in search of a feeding site (exposed to toxic extracts more than second and third nympal stadia) while ,the second and third nymphal stadia are immobile .

The pupal mortality percentage reached between 0.0 at the control treatment to 84.66 % at concentration of 2% . (1) mentioned that the alkaloid extract of *I. lutea* affected less than the phenolic extract at different concentrations on pupae of *B.tabaci* . (19) Revealed that the aqueous extract of *L. sativum* had toxicity that was not significantly different against pupae of *B. tabaci* .

(15) observed that pupae were the least susceptible to another commercial neem oil products , the least susceptibility of the pupae can be explained by considering the two modes of action of neem oil : contact or ingestion , one possible explanation is that pupae have a cuticular layer preventing their contact with the neem oil applied on leaves , another explanation was given by (10) based on the evidence that the pupal stage is divided into three sub stages , and the pupae feed in the first sub stage only. These authors affirmed

that since pupae feed only in their first sub stage , they are more capable of avoiding the effects of neem oil by ingestion . Extract compounds can penetrate into the leaves as observed by (9)

The adults mortality rate were also affected and reached 71.84 % at the concentration of 2% . I n this respect (1) found that the adults mortality of *B. tabaci* reached 85.6 % when treated with alkaloid extract of *I. lutea* at concentration of 2%. Also he found that the adults mortality reached 90% in hot water extract and 61% in cold water extract at 100 % extract concentration . (20) found that the best treatment against whitefly adults was observed with 5 % concentration extracted from capsicum and ginger. Also, (19) mentioned that the treatment with *Retama retama* extract killed adults of *B. tabaci* . Adults mortality may be attributed either to an indirect effect of strong deterrence causing energy depletion or dehydration (28) ,or to direct toxicity of the crude extract to very susceptible adults (21) .

Study results indicated that crude alkaloids of *N .oleande*r leaves significantly affect accumulative mortality (P < 0.05). Figure 1 shows the percentages of accumulative mortality of *B,tabaci* which treated with concentration mention earlier. Mortality rate reached 100 % at concentration of 2% and 1% in second and third nymphal stadia stages respectively , while 78.61 , 42.7 5 , 24.13 and 3.33% in adults at concentration of 0.5 , 0.2 , 0.1% and control respectively. These results agrees with those of (1) , who found that the alkaloid extract of *I. lutea* was poisonous to *B.tabaci* due to the accumulative of active compound in the digestive system which lead to the poisonous effect , Also he found that the cumulative mortality of immature stages were highest in ethyl alcohol and ethyl acetate extracts than in hexane extract . (11)found that the cumulative mortality reached 78 % and 61 % in nymphal stages of *Aphis gossypi* and *B. tabaci* respectively when treated with Neem Azal – T/S® while it was 57.1% and 52.4 % when treated with neem oil .

The development period of first larval instar , second and third nymphal stadia and pupae was significantly increased (P < 0.05) as the concentration of crude alkaloid increased (Table 2) .The developmental time increased from about 3.4 , 3.3 , 3.2 and 4.7 days in control treatment to about 6.7 , 6.4 ,7.8 and 9.2 days when treated at concentrations 1 ,1 ,2 ,2%of crude alkaloids respectively. (1) mentioned that the development period of *B. tabaci* generally prolonged in all treatment of alkaloid extracts as compared with control treatment . He found that the development period of first larval instar , second , third nymphal stadia and pupae increased from about 3.8 , 3.9 , 4.8 and 5.4 days in control treatment to about 9.5 , 8.5 , 9.1 and 9.6 days at concentration of 1% when reated with crude alkaloids .(5) indicated that leaves extracts of *Lagenaria siceraria* prolong the development period of immature stages of *B .tabaci* . The increasing

developmental period of immature stages that treated with extracts may be attributed to decreased larval efficiency of food conversion which affected negatively on growth and increased developmental period , or due to the interference with the action of endocrine system (4) .

References

1- **Al – Mansour ,N.; A. 1995 . The effect of different extracts of *Ibicella letea* in the biological performance of whitefly *Bemisia tabaci* Ph.D. Thesis. College of Science , University of Basrha , Iraq . 126**

2- **Al – Musa , A. ; Nazer , I. K. & Sharaf , N. 1987 . Effect of certain combined agricultural treatment on whitefly population . Dirasat J. , 14 (11) : 127 – 134 .**

3- **Al – Rubaei , H. & Al –Zubaidi , F. 2001 . The effects of aqueous extract *Datura innoxa* Mill on the biological performance of the housefly , of *Musca demostica* L. (Diptera : Muscidae) . J. Babylon Univ. , 6 (3) : 642 – 648 .**

4- **Al – Sharook ,Z. & Girjees , E. 1993 . Evaluation of five plant extracts for biological activity against the development stages of *Culex molestus* . Mosquito. J. Edu. & Sci., 18 : 62 – 70 .**

5- **Al – Zubaidi , F. ; Al – Mansour , N. ; & Akber , M. 1996 . Resistance of Bottle gourd,*Lagenaria siccarig* (molina) to whitefly, *Bemisia tabaci* (Genn.) J. Babylon Univ. 1 (3) : 237- 241 .**

6- **Blackford ,M. & Dinan ,L. 1997.The effects of ingested 20 – hydroxy ecdysone on the larvae of *Aglais urticae* , *Inachis io* , *Cynthia cardui* (Lepidoptera : Nymphalidae) and *Tyria jacobaeae* (Lepidoptera : Artiidae) . J. Ins. Physiol. ,43 : 315 – 327 .**

7- **Ghosh , B. 2000 . Poly amines and plant alkaloids . Ind. J. Exper. Biol. Arch. Oral. Biol., 43: 717 – 728.**

8- **Daoubi , M. ; Macias , A. ; Hermamdez , R. ; Hitchcoch , P. ; Hanson, J. & Collado , I. 2005 . Antifungal activity and biotransmation of disophorone by Botrytiscinerea . J. Agricul. Food Chem. , 53 : 6035 – 6039 .**

9- **De – Souze , A. & Vendramim , J. 2000 . Ovicidal activity of aqueous extract of Meliaceae on the silver leaf whitefly for tomato .Sci. Agricol. , 57 (3) : 403 – 406 .**

10- **Elling ,K. ;Borgemeister ,C. ; Setamou ,M. & Poehling ,H. 2002 . The**

effect of Neem Azal – T/S , a commercial neem products , on different developmental stages of the common greenhouse whitefly *Trialeurodes vaporiorum* west wood (Homoptera : Aleyrodidae) . J. Appli. Entomol. , 126 : 40 – 45 .

11- El – Shafie , H. & Basedow , T. 2003 . The efficacy of different neem preparations for the control of insects damaging potatoes and eggplants in the sudan . Crop Protec. , 22 : 1015 – 1021 .

12- Hiakal , M. A. & Omer , A. A. 1993 . Medicinal and aromatic plants. Escandrya , Egypt . 514p.

13- Harborn , J.B. 1984 . Phytochemical methods : A guide to modern techniques of plant analysis . Academic press , Chapman and Hall , 2nd . ed. New York . 288 p.

14- Hassan ,A. J.1996 .The effect of different extracts of *Nerium oleander* (Apocynaceae) on the biological performance of the housefly *Musca domestica* (Diptera : Muscidae) . M. Sc., College of Science , University of Babylon , Iraq . 79 p.

15- Kim ,H.G.;Jeon , J.H. ,Kim ,M.K. & Lee ,H.S. 2005 . Pharmacological ectsofasaron aldehyde isolated from acorusgrum in eusrhizome. Food Sci. Biotechnol. , 14 (5) : 685 – 688 .

16- Levinson , H. Z. 1976 . The defensive role of alkaloids in insects and Plants . Cell. Molecul. Sci. , 32 (4) : 408 - 411 .

17- Liu , T. , X. & Stansly , A. 1995 . Toxicity of biorational insecticides to *Bemisia tabaci*(Homoptera : Aleyrodidae) on tomato leaves . J. Econ. Entomol. , 88 (3) : 564 –568 .

18- Markouk ,M.; Bekkouche ,K.; Larhsini ,M.; Bousaid , M.; Lazrek , H. & Jana , M. 2000 . Evalution of some Moroccan medicinal extracts for larvicidal activity . J. Ethanopharmacol. ,73:293 – 297.

19- Mazen , A. ; Mohammad , A. & Talal , R. 2009 . Aqueous extracts of some medicinal plants are as imidacloprid to the sweet potato whitefly *Bemisia tabaci* ., J. Ins. Sci. , 9 (15) : 1 – 6 .

20- Mohammad , G. & Syed , F. 2009 . The role of mixing different plant extracts to boiled linseed oil for the control of whitefly *Bemisia tabaci* . J. Agri. Environ. Sci., 5(6) : 813 - 824.

21- Metacalf , C. , L. ; Flint , W. , P. & Metacalf ,R. , I. 1951 . Destructive and useful insects . 3rd. ed . McGraw – Hill , New York . 1208 p.

22- Rao , D.F. 1957 . Insecticidal properties of petals of several common Plants in india . Econ. Bot. , 11 : 247 – 76 .

23- Rockestein , M. 1978 . Biochemistry of insects . Acad. Press, London . 649 p.

24- S
AS Institute , 2004 . SAS / STAT Users Guide for Personal Computers. Release 6.12. SAS Institute Inc., Cary, North Carolina . U.S.A. 956 p.
25- Sexena , R. ; Waldbauwr ,G. ; Liquido , N. & Puma , B. 1980 . Effect of neem seed oil on the rice leaffolder *Cnaphalocrocis medinalis* In : Schmutterer , H. ; Ascher, K. & Rembold , H. (Eds.) Proc. 1st . ed . Int. Neem Conf. Rottach. Egern. FRG. 189 – 204 .
26- Steven, J.;Hart, H. ;Van-Ham,R. ; Elema, E.;Van- Den, E.; Wildebeer, M. & Zwaving, J. 1995 . Distribution of alkaloids and tannins in the crassulaceae. Biochem. Syst. Ecol. , 23 (2) : 157 – 165.
27- Tare , V. ; Deshpande , S. & Sharma , R. 2004 . Susceptibility of two different strains of *Aedes aegypti* (Diptera : Culicidae) to plant oils . J. Econ. Entomol. , 97 : 1734 – 1736 .
28- Veierov , D. 1996 . Physically and behaviorally active formulation for control of *Bemisia* . In Geling D. & Mayer , R. (Eds.) . *Bemisia* taxonomy , biology , damage , control & manegemant . Intercept , Andover , UK. : 557 – 576 .
29- Zhang , M. & Kubo , I. 1993 . Metabolic fate of ecdysteroids in larval *Bombyx mori* & *Heliothis virescens* . Ins. Biochem. Molecul. Biol. , 2 : 831 – 843 .

Table 1: The effects of crude alkaloids of *N. oleander* leaves on the mortality of different developmental stages of *B.tabaci*

Extract conc. mg/ml.	Eggs mortality (%)	Nympal mortality (%)			Pupal mortality (%)	Adults mortality (%)
		1st	2nd	3rd		
Control	1.33	2.66	1.33	0.00	0.00	13.33
o.1	10.81	17.81	24.99	7.33	13.33	20.76
o.2	22.97	43.83	31.98	16.66	31.33	29.99
o.5	32.43	59.59	56.75	33.00	40.00	35.38
1	38.51	80.88	78.37	52.66	66.66	52.30
2	51.35	100	93.98	86.88	84.66	71.84
LSD	5.844	7.362	6.458	6.458	6.359	5.847

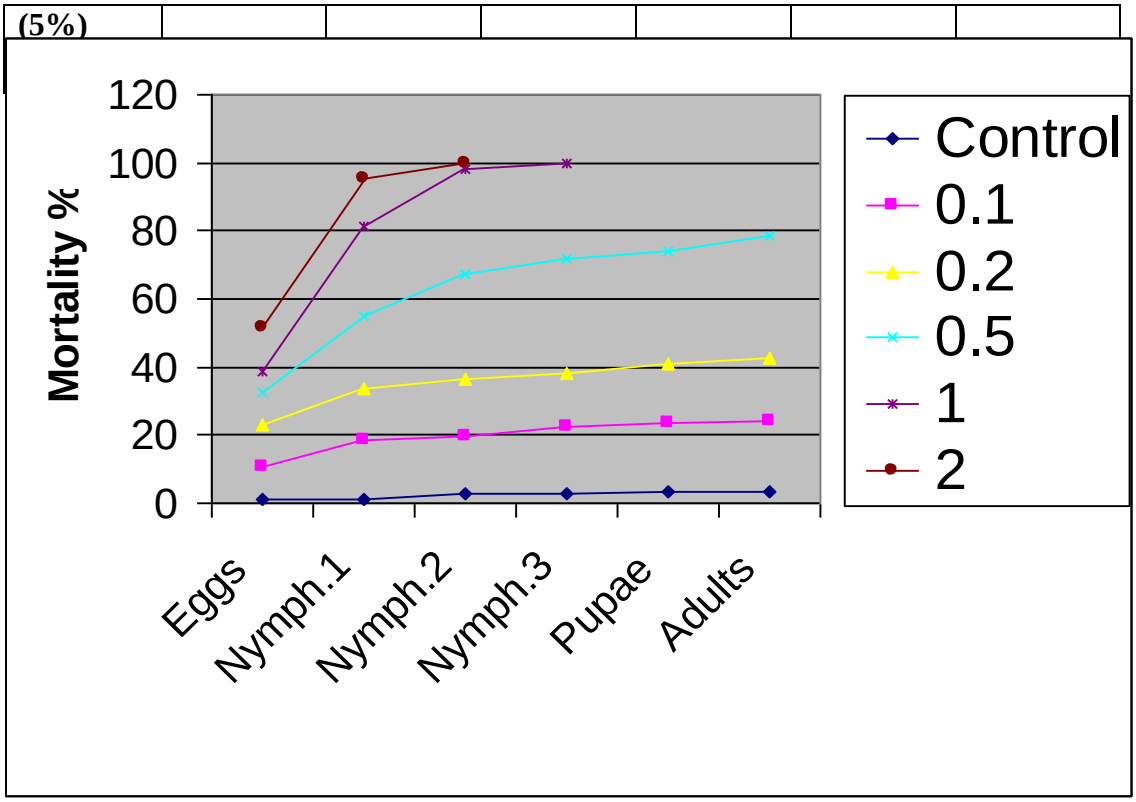

Fig.1: the effects of crude alkaloids of N .oleander leaves on the cumulative mortality of *B.tabaci*

Table 2 : The effects of crude alkaloids of *N. oleander* leaves on the developmental period of immature stages of *B. tabaci*

Extract conc. mg/ml.	Nymphal development period (days) 1st	2nd	3rd	Dev. Period pupae (days)
Control	3.4	3.3	3.2	4.4
o.1	3.8	3.6	4.0	5.3
o.2	4.3	4.1	4.2	5.6
o.5	5.1	5.5	5.9	6.2
1	6.7	6.4	6.8	7.4
2	- *	- *	7.8	9.2
LSD(5%)	1.037	0.894	1.366	1.893

*The numbers of individuals don't enough

الخلاصة

أجريت دراسة مختبريه لتقدير الفعالية السمية للمستخلصات القلوانية لاوراق نبات الدفلة ***Nerium oleander*** في بعض جوانب الاداء الحياتي للذبابة البيضاء ***Bemisia tabaci***. أختبرت أالمستخلصات القلوانية الخام بالتراكيز 0.1 ، 0.2 ، 0.5 ، 1 و 2 % البيض عموما كان أقل حساسية في جميع المعاملات ، أشارت النتائج الى ان التركيز 2% كان الأكثر تأثيراً . في هذا التركيز كانت نسبة هلاك البيض 51,35 % للمستخلص القلواني الخام ، هلاكات متباينة لوحظت في الحوريات لجميع تراكيز المستخلص القلواني . الطور الأول والثاني أمتلك أعلى نسبة هلاك عند المعاملة مع المستخلص القلواني بتركيز 1% و 2% بالمقارنة مع الطور الحوري الثالث . العذارى والكاملات وصلت نسبة الهلاكات الى 84.66 و 71.84 % عند معاملتها مع المستخلص القلواني بتركيز 2% على التوالي . الهلاكات التراكمية وصلت الى 100% بتركيز 1% و2% في الطور الثاني والثالث ، عموماً فترة النمو للأطوار غير البالغة أزدادت عند المعاملة بالتراكيز المختلفة للمستخلصات القلوانية بالمقارنة مع مجموعة السيطرة .