

Effect of crude phenolic extracts of *Nerium oleander* L. leaves on
the biological performance of *Bemisia tabaci*
(Genn.)(Homoptera: Aleyrodida)
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Abstract

Laboratory bioassays were done to determine the toxicity of crude phenolic extracts of *Nerium oleander* leaves to whitefly *Bemisia tabaci* . Crude phenolics 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 45.02%, in crude phenolic differences in nymphal mortality according to age was observed at all concentrations of phenolic extract , both first and third nymphal instars had higher mortality with phenolic extract at concentrations of 1% and 2% than second nymphal instar . Pupal and adults mortality reached 82.63% and 60.45% when treated with crude phenolics at concentration of 2% respectively . Cumulative mortality reached 100 % at concentration of 1% and 2 % in second nymphal instar and adults respectively when treated with crude phenolic. Development time of immature stages of *B.tabaci* also , affected by the application of crude phenolic extracts of *N. oleander* leaves , generally development period prolonged in all treatments of phenolics as compared with control treatment.

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Introduction

Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non target species, air, water, bottom sediments and food (Miller, 2004). Pesticide contaminate land and water when it escapes from production sites, storage tank, runs off from field, discarded, sprayed aerially and when it is sprayed in to water to kill algae, insecticide can kill bees and may be a cause of pollinator decline, the loss of bees that pollinate plants and colony collapse disorder (Colin *et al.*, 2004). Some pesticides contribute to global warming and the depletion of the ozone layer (Stapleton *et al.*, 2003).

Plant may provide an alternative to currently used pesticides for the control of plant pests, as they constitute a rich source of bioactive chemicals (Kim *et al.*, 2005; Daoubi *et al.*, 2005). 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 (Markouk *et al.*, 2000; Tare *et al.*, 2004).

In recent years the whitefly *Bemisia tabaci* (Genn.) has become an increasingly important pest of vegetables in Jordan (Al-musa *et al.*, 1987), in Iraq (Al-mansour, 1995), in Egypt (mohammad *et al.*, 2009), and other countries in Middle East. Three types of economic damage to plants may be caused by *B. tabaci*: 1- sucking sap from plants 2 - excretion of honeydew on fiber 3 - transmission of viruses

Rao (1957) and Hassan (1996) carried out laboratory experiments with *Nerium oleander* leaves 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

Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodida)

Materials & Methods

Whiteflies were collected from the field and kept in cage containing young eggplant *Solanum melangena* L. as a hostplant. After an ovipositional period of 24 hours the adult whiteflies were removed. The egg – bearing leaves on plant were incubated at 25 ± 2 C° , 60 ± 5 % relative humidity and photoperiod of 14 : 10 (light : dark) hours until adults emergence (Al- Mansour , 1995) . *N. oleander* fresh green leaves were collected during March , 2009 . The fresh leaves were washed to remove residual dust and air - dried at room temperature for two weeks , then were pulverized to powdered form by electrical grinder and kept in plastic bags at 10C° (Haikal and Omar , 1993) . Phenolic extraction from *N. oleander* leaves was done according to Ribereau – Gayone (1972). Two grams of dried phenolic extract were dissolved in 5ml. ethanol , the volume was made up to 100 ml. with distilled water. From this stock solution six different concentrations were prepared (2 , 1 , 0.5 , 0.25 , 0.125, 0.0) % , liquid paraffin 1% and 1 – 2 drops of tween were added to each concentration as adhesive agent and surfactant respectively (Al-Rubaei and Al- Zubeaidi , 2001) . The effects of crude phenolic with their different concentrations were evaluated against all developmental stages (egg , first larval instar , second and third nymphal instars, 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 phenolics were sprayed with a laboratory spray gun , the cages were kept at incubator conditions previously mentioned . The adults mortality was 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 instars 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 the developmental 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 instar was 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 instar as well as

Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodida)

pupae . As well as cumulative effects of crude phenolics were calculated , all the steps mentioned above were applied except for eggs which were left after treatment . Mortality rates of eggs, first larval instar , second , third nymphal instars , pupae and adults were recorder and corrected according to Abbots formula . The statistical analysis system – SAS (2004) 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

The effects of crude phenolic extracts on different stages of *B. tabaci* are shown in table 1 . The mortality rates were significantly varied with concentrations used in the treatments ($P < 0.05$) . A direct correlation between extract concentration and the different stages mortality was found .

Egg mortality ranged between 2.66 % in control treatment to 45.02 % at concentration of 2% . All nymphal instars were more susceptible by phenolic extracts than egg stages , mortality of the nymphal stages ranged between 18.36 - 91.49 , 10.96 - 79.97 and 9.65 - 86.89 in first nymphal stage, second and third nymphal instar at concentration of 0.1 - 2% respectively , compared with 2.00 , 2.66 and 3.33% at control treatment . Pupal stage was more susceptible by phenolic extracts as compared with egg stages , pupae mortality ranged between 4.00 % at control treatment to 82.63 % at concentration of 2% . Adults were also affected , the mortality rate reached 60.45 % at the concentration of 2% . In this respect Al –Mansour (1995) found that the adults mortality of *B. tabaci* reached 68.3% when treated with phenol extract of *Ibicella 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 (phenolics and alkaloids substances tend to be water soluble) . Bouchelta *et al.* (2005) found that flavonoids of *Capsicum frutescens* caused mortality 29 % of adults *B. tabaci* . Diethylether extract of *N. oleander* at concentration of 1000 part per million caused adults mortality 90 % , 89 % and 72 % of *Aphis crucivora* , *Acyrtosiphon solani* and *A. gossypii* respectively (Al– Dowri *et al.* 2005) . Mohammad *et al.* (2009) found that the best treatment against whitefly adults was observed with 2.5 % concentration extracted from capsicum and ginger. Also , Mazen *et al.* (2009) mentioned that the treatment with *Retama retama* extract killed adults of *B. tabaci* .

Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodida)

Adults mortality may be attributed either to an indirect effect of strong deterrence causing energy depletion or dehydration (Veierov ,1996) ,or to direct toxicity of the crude extract to very susceptible adults (Metcalf *et al.* , 1951) . Al- mansour (1995) found that phenolic extracts of *Ibicella lutea* was highly toxic to the eggs of *B. tabaci* , eggs mortality scored 67.5% at concentration of 2 % . Also, he found that the eggs mortality rate ranged between 0.6 – 21.3 % when treated with cold water extract ,while it was between 2.6 – 12.3 % in hot water extract of *I. lutea* . Liu and Stansly (1995) reported that *Nicotiana gossei* extracts did not affect whitefly egg hatch . Egg mortality of *B.tabaci* reached 35% when treated with aqueous extract of *M. azedrach* (De - Souza and Vendarmim, 2000) Mazen *et al.* (2009) found that aqueous extract of *Lepidium sativum* had a toxicity but not significantly different against eggs of *B. tabaci* while, Mohammed *et al.* (2009) reported that the most effective treatment against whitefly eggs was 2.5 % from the botanical water extract of capsicum + ginger + garlic + black pepper . Leaves phenolic extracts of *N. oleander* were significantly affected egg hatchability. 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 early after ovulation (Rockestein,1978). Also ,eggs mortality may be due to embryo asphyxia inside the egg because the extract was formed as layer on the external shell (Saxena *et al.* 1980) .

Present study revealed that nymphal mortality of *B. tabaci* increased as *N. oleander* extract concentrations increased (Table 1). Similar results has been reported on *B.tabaci* treated with extract of *I. lutea*. (Al- Mansour,1995) , *A.indica* (De - Souza , Vendramim, 2000) . Al- Mansour (1995) mentioned that *I. lutea* extract was highly toxic to all three nymphal instars (nymphal mortality recorded 100 % at concentration of 2 %) . While De - Souza and Vendramim (2000) reported that neem seed extract was more efficient in reducing the number of first nymphal stage than the extracts of fresh fruit of *Melia azedarach* .

Differences in nymphal mortality according to age was observed at all concentrations of phenolic extract treatments. Both first and third nymphal stages, showed higher mortality with phenolic extract at concentrations of 1% and 2% than second nymphal instar (Table 1) . Kumar and Poehlin (2006) reported that neem oil was highly toxic to first nyaphal stage after the hatching of viable eggs, corroborating that this stage is highly susceptible to the effect of extract . Patricia *et al.* (2009) found that the first nymphal stage mortality rate was significantly higher (53.4 %) when compared with that in third nymphal instar (31.8%) as well as , they

Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodida)

found that both first and second nymphal stages had higher mortality when treated with neem oil at 0.5 % than the third nymphal stage.

Mazen *et al.* (2009) found that the extract of *L. sativum* had toxicity that was not significantly different against early nymphal stage of *B. tabaci*. Also, Mohammad *et al.* (2009) found that the highest reduction percentage of whitefly nymphs was recorded with 2.5 % concentration of garlic and black pepper extract (100 % reduction). The extracts were more effective in first nymphal instar (crawlers) than second and third nymphal instars, this may be due to the crawlers usually move a few centimeter in search of a feeding site (exposed to toxic extracts more than second and third nymphal instar) while, the second and third nymphal stage are immobile. Also, the increasing in mortality of *B. tabaci* nymphs may be due to the *N. oleander* extracts were affected in 20-hydroxyecdysone hormone (molting hormone), the 20-hydroxyecdysone hormone stimulate molting in nymphs of *B. tabaci* (Dale *et al.*, 2005). Zhang and Kubo (1993) and Blackford and Dinan (1997) 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. Pupal mortality reached 82.63 % in crude phenolics at concentration of 2% (Table 1). Al-mansour (1995) mentioned that the alkaloid extract of *I. lutea* affected less than the phenolic extract at different concentrations on pupae of *B. tabaci*. Mazen *et al.* (2009) revealed that the aqueous extract of *L. sativum* had toxicity that was not significantly different against pupae of *B. tabaci*. First, second and third nymphal instars were more susceptible to neem oil than pupae Kumar *et al.* (2005), who recorded different rates of pupal mortality of *B. tabaci* after treatment with some formulations of neem oil (0.5, 22.5, 36.9, 41.2, 68.0, 86.0 % at concentrations of 0.0, 0.1, 0.25, 0.5, 1.0 and 2.0 %). The existence of different degrees of susceptibility among nymphal stages and pupae was also observed by (Patricia *et al.*, 2009). The toxicity of extract on pupal mortality may be due to abundant presented of extract, which blocks the synthesis and release of molting hormone (Isman, 2006). Kumar *et al.* (2005) observed that pupal stage was 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 Elling *et al.* (2002) and Kumar *et al.* (2005) based on the

Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodida)

evidence that the pupal stage is divided into three sub stages , and the pupae feed in the first substage only (Gill , 1990) . These authors affirmed that since pupae feed only in their first substage , they are more capable of avoiding the effects of neem oil by ingestion . Extract compounds can penetrate into the leaves as observed by De – Souza & Vendramim (2000) . According to Isman (2006) many plant species seems to cause toxic effect by ingestion , with deleterious physiological consequences to insects as observed by Bleicher *et al.* (2007) and Singha *et al.* (2007) .

Figure 1 shows that crude phenolics was affected the cumulative mortality .Significant differences were observed among the concentration mention earlier ($P < 0.05$). Mortality rate reached 100% at concentration of 2% and 1% in second nymphal instar and adults respectively while it was 68.75 , 47.91 , 48.46 and 4.00% in adults at concentrations of 0.5 , 0.2 , 0.1% and control respectively. These results agrees with those of Al- mansoure (1995),who found that phenolic extracts 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,, he Also found that the cumulative mortality of immature stages were highest in ethyl alcohol and ethyl acetate extracts than in hexane extract . Al- mansoure (1995) found that the cumulative mortality rates of whitefly immature stages reached 81.6 % in nymphal stages and 86.6 % in pupal stage when treated with hot water extract of *I. lutea* .

El-Shafie and Basedow (2003) found that the cumulative mortality reached 78 % and 61 % in nymphal instars 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 .

Table 2 shows that crude phenolics were significantly affected the development period, the data also showed a direct correlation between developmental period and extract concentrations, significant differences were observed among the concentrations at $P < 0.05$. The developmental time increased from about 3.2 , 3.3 , 3.2 and 4.4 days in control treatment to about 6.7 , 6.9 , 6.8 and 8.1 days when treated at concentrations 1, 2, 2, 2 % of crude phenolics respectively. Al –Mansour *et al.* (1995) found that both aqueous extract and solvent extract of *I. lutea* affected the development period of *B.tabaci* . Al- Mansour (1995) mentioned that the development period of *B.tabaci* generally prolonged in all treatment of phenolic extracts as compared with control treatment . He also found that the development period of first, second and third nymphal stages and pupae increased from about 3.4 , 3.2 , 4.0 and 4.8 days in control treatment to about

Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.)(Homoptera: Aleyrodida)

6.8 , 6.5 , 9.1 and 9.2 days at concentration of 0.5 % when treated with crude phenolics . Coudriet *et al.* (1985) found that treatment with neem seed extract prolong larval development period of *B .tabaci* also , Al – Zubaidi *et al.* (1996) indicated that leaves extract 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 referred 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 (Al –Sharook and Girjees , 1993) .

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Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.)(Homoptera: Aleyrodida)

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Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.)(Homoptera: Aleyrodida)

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Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.)(Homoptera: Aleyrodida)

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Effect of crude phenolic extracts of *Nerium oleander* L. leaves on the biological performance of *Bemisia tabaci* (Genn.)(Homoptera: Aleyrodida)

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

Extract conc.(%)	Eggs mort. (%)	Nymphal mort. (%)			Pupal mort. (%)	Adults mort.(%)
		1 st	2 nd	3 rd		
Control	2.66	2.00	2.66	3.33	4.00	10.66
0.1	9.30	18.36	10.96	9.65	7.63	16.42
0.2	17.12	32.18	24.65	24.82	24.30	21.64
0.5	22.60	48.97	45.89	43.44	34.71	31.34
1.0	35.61	72.78	61.63	59.99	57.63	45.52
2.0	45.02	91.49	79.97	86.89	82.63	60.45
LSD	5.022	6.847	6.035	7.118	6.938	5.882

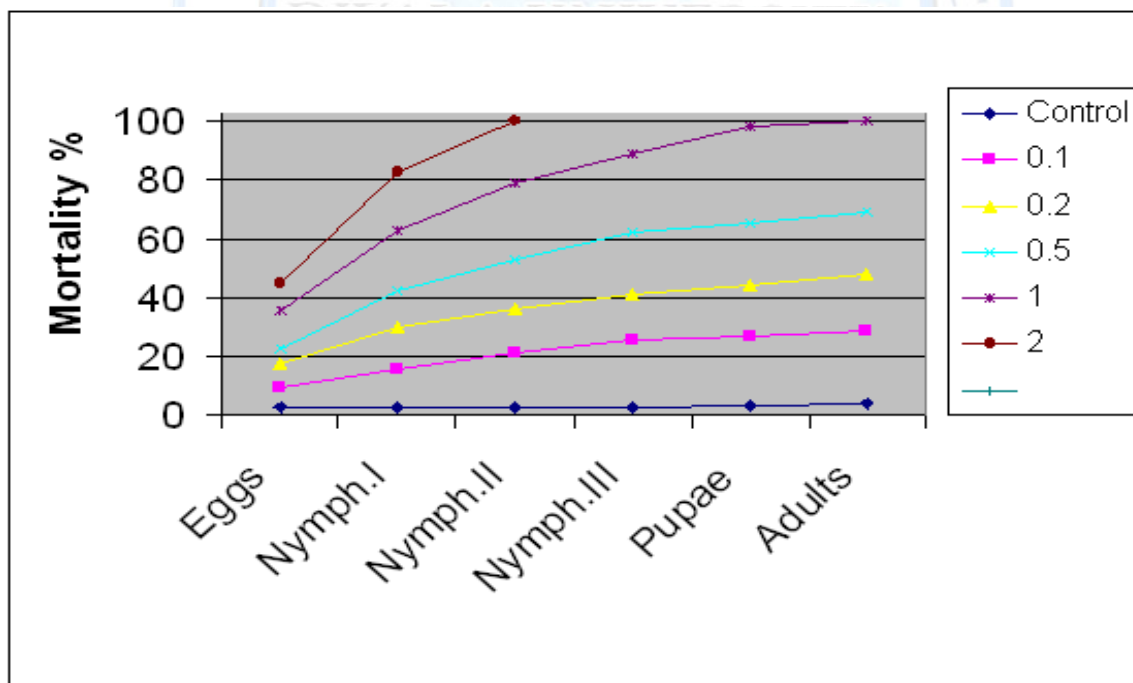


Fig. 1 : The effects of leaves crude phenolics of *N.oleander* on the cumulative mortality of *B.tabaci*

**Effect of crude phenolic extracts of *Nerium oleander* L. leaves on
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**Table 2: The effects of leaves crude phenolics of *N. oleander* on the developmental period of
immature stages of *B. tabaci***

Extract conc. (%)	Nymphal development period (days)			Dev. Period pupae (days)
	1 st	2 nd	3 rd	
Control	3.2	3.3	3.2	4.4
o.1	3.5	3.5	3.6	4.7
o.2	4.1	4.3	4.5	6.0
o.5	5.2	4.8	4.9	6.1
1	6.7	6.1	6.3	6.3
2	-	6.9	6.8	8.1
LSD	1.049	0.945	1.277	1.682