

## **Effect of neem and antitranspirant products against *Aphis craccivora* Koch and its biology.**

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### **ABSTRACT**

Nimbecidine (Contains 0.03% azadirachtin) and Green Miracle (antitranspirant) were tested alone and in combination against *Aphis craccivora* Koch. Nimbecidine was found to be highly effective in compared with Green Miracle at all concentrations tested against this aphid species. Ninety and fifty percent lethal concentrations ( $LC_{90,50}$ ) were 0.4 and 0.13 ml/l for nimbecidine, 3.5 and 1.7 ml/l for Green Miracle, respectively.  $LC_{90}$  and  $LC_{50}$  were 2.2 and 1.3ml/l when mixing Nimbecidine and green miracle together. The Ninety and fifty percent lethal times ( $LT_{90,50}$ ) were 6.2 and 4.2 days at 0.6ml/l for Nimbecidine, 4.8 and 2.9 days at 5.0ml/l for Green Miracle. Mixing Nimbecidine and Green Miracle achieved  $LT_{90,50}$  values as 3.1 and 2.2 days(at 0.3 N + 3.0 G ml/l). The highest concentration for each formulation tested caused percentage reduction in the number of young born as 92.25% at 0.6 ml/l for Nimbecidine, 91.46 % for Green Miracle and 95.4% at 0.3 N+ 3.0G ml/l for combination. The natal period and longevity decreased as concentration increased. The different concentrations for Nimbecidine, Green Miracle and in combination caused retardation in the development of the fourth nymphal instar. Nimbecidine and Green Miracle could be used in combination in integrated pest control.

**Key words:** Nimbecidine, Green Miracle, neem, antitranspirant, *Aphis craccivora*.

### **INTRODUCTION**

The cowpea aphid, *Aphis craccivora* Koch is a serious pest of leguminous crop plants in Egypt (Ahmed *et al.*, 2007; Dimetry *et al.*, 2008) and in many Mediterranean nations (Tao and Chiu, 1971; Agarwala *et al.*, 1987; Waterhouse, 1998). It is known to inject a powerful toxin, which can stunt or kill a plant in case of heavy infestations and also cause growth of black sooty mould because of the copious amount of honeydew secreted (Summers *et al.*, 2004). Chemical methods involving organically certified insecticides such as neem products are part of the integrated pest management techniques for this pest (Summers *et al.*, 2004; Dimetry *et al.*, 2008).

The major function of the plant epidermis is to form the cuticle, a functional permeability barrier of the cell wall which prevents excessive water loss and the entry of harmful substances and pathogens into the host and ease feeding the insects, especially the piercing sucking insects. This type of cell wall modification is mainly composed of a polyester matrix, cutin and soluble waxes embedded in the matrix and deposited on the external surface.

Film forming polymers are widely used as spray adjuvants within the agricultural, forestry and horticultural industries (Backman, 1978). Their main functions are to reduce weathering and extend pesticide efficacy, act as

stickers/spreaders to improve distribution and adherence of agrochemicals, and decrease water loss and wilting of young transplants (Gale and Hagan, 1996).

Spraying of antitranspirant formulations on plants caused high germination and may decrease the infestation by sucking and piercing sucking insect.

In this study, we examined the effect of Nimbecidine (botanical insecticide) and Green Miracle (antitranspirant) formulations and its combination against *A. craccivora*.

## MATERIALS AND METHODS

### **1.1. Insect used:**

A stock culture of *A. craccivora* was maintained on broad bean, *Vicia faba* under laboratory conditions of  $20.0 \pm 5.0$  °C &  $70.0 \pm 5.0$  % R.H. and a photoperiod of 16 L: 8 D for several generations. In all experiments, the insects, whether adults or nymphs, were put on fresh 7 days old broad bean plants cultivated in small pots (8 cm in diameter, one plant / pot) and enclosed individually in glass cylinders, 10 cm diameter, 22 cm long, the tops of which were covered with muslin held in place with rubber bands.

### **1.2. Compounds and concentrations used:**

Commercial liquid formulation, Nimbecidine contains 0.03 % azadirachtin as active ingredient and Green Miracle antitranspirant and stomatal regulation based on long chain fatty alcohol group (C8 to C18) were used. These products were obtained from T. Stanes Company Limited, India. Serial dilutions were prepared as 0.6, 0.3 and 0.15 ml/l distilled water for Nimbecidine, 5.0, 3.0 and 1.0 ml/l distilled water for Green Miracle and prepared mixture from nimbecidine and green miracle as 0.075 + 0.75, 0.15 + 1.5 and 0.3 + 3.0 ml/l distilled water for each experiments.

### **1.3. Bioassay procedure.**

#### **1.3.1. Toxic effect of the compounds against the adult stage.**

The toxicity of the compounds was determined by using seedling broad bean plants (7 days old). Five newly formed adults (1 day old) were placed on each plant, settled for 5 hours before treatment. After spraying plant by hand sprayer (capacity 200ml) with compounds and / or its mixture by the concentrations mentioned, the plant was covered with glass cylinder. The mortality of aphids was recorded daily. Another plants were inoculated with five newly formed adults and sprayed with distilled water served as control.

$LT_{50, 90}$  and  $LC_{50, 90}$  were determined using the Probit analysis (Finney 1971). Ten replicates were used for each experiments as well as for the control.

#### **1.3.2. Effect of the different tested materials on aphid reproduction.**

Ten newly formed adults (1 old day) were put singly on the previously treated plants with different concentrations for each compound and / or its mixture. Another plants were sprayed with distilled water and infested with one newly formed adults as control. The cumulative number of young born deposited per each adult / concentration and the natal period were recorded daily. The percentage reduction in the number of young born was calculated by Abbot's Equation (1925). Ten replicates were carried out. (one adult / plant / replicate). The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) and the means were compared using the Least Significant Difference test (LSD,  $P = 0.05$ ) (Computer program Micro-stat version 2.5, 1991).

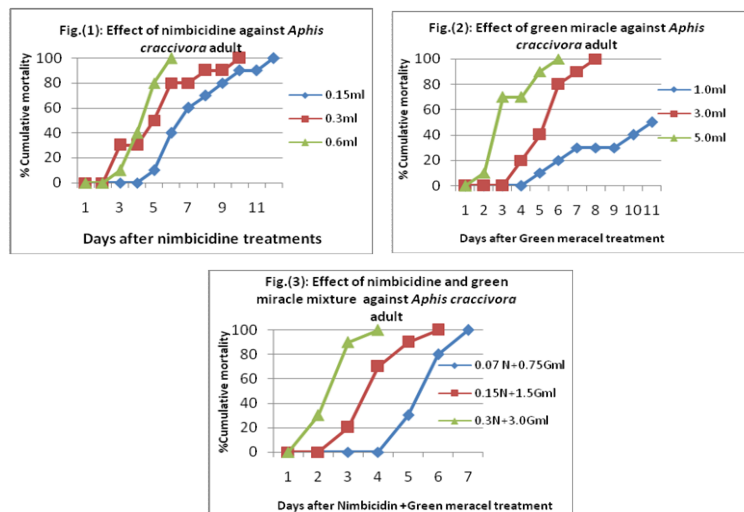
### 1.3.3. Effect of the different tested materials on developmental period of nymphal stage.

Ten newly moulted 2<sup>nd</sup> nymphal instar of *A. craccivora* were put singly on the previously treated bean seedlings using the previous concentrations used of Nimbecidine, Green Miracle and its mixture. The developmental time for each instar to reach adult stage was recorded. Another group from plants were sprayed with distilled water and served as control. Ten replicates were carried out. (Individual / plant / replicate). All experiments were carried out under laboratory conditions of 20.0 ± 5.0 °C & 70.0 ± 5.0% R.H and a photoperiod of 16 L: 8 D.

## RESULTS AND DISSECTION

### 2.1.1. Toxicity of the different tested materials against the adult stage.

Percentage mortality of *A. craccivora* adults are shown in Figs. (1, 2, 3) as affected by Nimbecidine, Green Miracle and their mixture against. Mortality of adults increased as the concentration increased (i.e. mortality is concentration dependent). Mixing Nimbecidine with Green Miracle caused 100% mortality to the adult aphids at 0.3 + 3.0 ml after 3 days while treatment of the adults with Nimbecidine or Green Miracle alone gave 100% mortality at 0.6 ml after 5 days or after 6 days at 5.0 ml, respectively. The present findings are in agreement with Sanjeev and Singh, (2008) found that the plots treated with NSKE 5% for control mustard aphid, *Lipaphis erysimi* (Kalt.) recorded 13.8 individual/plant compared with 40.0 individual/plant in control and yield recorded 11.5 ton / ha. Rathikannu and Sivasubramanian, (2008) found that the high azadirachtin formulation neem seed kernel extract at 5.0% and neem azal T/S at 1.0% were enough to reduce the incidence of sucking pests of *Phyllanthus amarus*. The highest percent reduction of whitefly population (58.04%) was observed in plots treated with 5.0% NSKE. The percent reduction of thrips, aphids and whitefly were high after the first spray (54.12, 54.99 and 58.04%, respectively). Carvalho *et al.*, (2008) found that neem oil at all the tested concentrations (0.25, 0.5, 0.75, 1.0 and 2.0%) was efficient to control *Brevicoryne brassicae* aphid while it is toxic only at 1.0 and 2.0 % concentrations to *Myzus persicae*. Singh *et al.*, (2007) evaluated number of neem products against the mustard aphids and found that plots treated with Achook (at 1000 ml/ha) and Nimbecidine (at 1500 ml/ha) achieved high mummified aphid population and the population of *Coccinella septempunctata* was higher in these plots.



Goncalves and Bleicher, (2006) found that azadirachtin at 192-1536 ppm showed efficacy in range 39.16-83.81 % mortality on nymphs *A. craccivora* when treated the soil with neem. Singh, (2006) stated that neem oil decreased the incidence of mustard aphid and increased the yield of rai when applied as mixture or alternate combination with the insecticide methyl – O- demeton(at 1.0 neem + 0.012% demeton and 2.0 neem + 0.025 demeton %). Also, Neerja *et al.*, (2005) when mixed Phorate at 1 kg /ha with NSKE at 5.0 % resulted in the minimum mustard aphid infestation observed 15 days after spraying. The authors stated that neem oil at 1.0% was the least effective in controlling the aphid population and therefore, recorded the lowest mustard yield. Bright and Regupathy,( 2001) are in consistent with the present finding that Nimbecidine and TNAU Neem increased with increasing dose from 200 to 1000 ml/ha and decreased as the days advanced.

Data in Table (1) show that treatment with Nimbecidine was superior to the other treatments. Whereas, the LC<sub>90</sub> was 0.4 ml for Nimbecidine, 2.2 ml for the mixture and 3.5 ml for Green Miracle. Also, the lethal time of 90 % was 3.1 days at 0.3 + 3.0 ml for the mixture, 4.8 days at 5.0 ml for Green Miracle and 6.2 days at 0.6 ml for nimbecidine.

The present findings are in agreement with Kumar *et al.*, (2007) who found that the combined formulation consisting of methanolic extract of neem and karanj oil was very effective (LC<sub>50</sub> = 0.11%) and showed 70 and 11.36 fold increase in activity over neem (7.0%) and karanj ( 1.25%) alone in the laboratory against *Tetranychus* species. Also, the same authors stated that combined formulation showed synergism between neem and karanj against aphid *Macrosiphoniella sanborni* causing 100% protection compared to 68.4 and 52.9% of neem and karanj alone, respectively, after 48 hour at 0.5 % concentration.

Table (1):The LC<sub>50,90</sub> and LT<sub>50,90</sub> of Nimbecidine, Green Miracle and their mixtures against *A. craccivora* adults.

Formulations	LC <sub>50</sub> ml/L	LC <sub>90</sub> ml/L	Conc. ml/L	LT <sub>50</sub>	LT <sub>90</sub>
Nimbecidine	0.13	0.4	0.15	6.7	10.9
			0.3	4.5	8.4
			0.6	4.2	6.2
Green miracle	1.7	3.5	1.0	12.1	33.5
			3.0	4.9	7.1
			5.0	2.9	4.8
Nimbecidine + Green miracle	1.3	2.2	0.075 + 0.75	5.3	6.9
			0.15 + 1.5	3.6	4.9
			0.3 + 3.0	2.2	3.1

Time calculated as days

### 2.1.2. Effect of the tested materials and their mixture on aphid reproduction.

The results in Table (2) show that when adults were exposed to treated plants with Nimbecidine, Green Miracle and its mixture, the nymphal production was reduced significantly at all tested concentrations. At concentrations of 0.6, 0.3 and 0.15 ml/l for Nimbecidine achieved 92.25, 91.86 and 87.1 % reduction in the number of the nymphs produced by adults, respectively. The natal period as well as the number of young produced daily were significantly reduced when using any one of products tested in compared with that of the control aphids.

For treated plants by *Green Miracle* the concentrations of 5.0, 3.0 and 1.0 ml/l caused 91.46, 82.67 and 68.37 % reduction in the number of the nymphs produced by adults, respectively. While the mixture revealed that the highest effect, whereas, achieving 95.4, 91.2 and 86.87 % reduction in the number of the nymphs produced by adults at the concentrations of 0.3 + 3.0 , 0.15 + 1.5 and 0.075 + 0.75 ml/l, respectively. The results revealed that the natal period decreased when the concentration increased. Longevity of the adult stage which exposed to treated plants with the mixture (0.3 + 3.0 ml) , green miracle (5.0 ml) and nimbecidine (0.6 ml) were reduced to 1.8 , 2.6 and 3.7 days ,respectively compared to 17.9 days in control.

Table (2): Effect of Nimbecidine, Green Miracle and their mixtures against some biological aspects of *Aphis craccivora* adult stage.

Formulations	Conc. ml / L	Mean No. young borne $\pm$ SE (progeny)	% Reduction	Mean Natal period in days $\pm$ SE	Mean Longevity of adult in days $\pm$ SE (days)
Nimbecidine	0.15	9.8 $\pm$ 1.4cd	87.13	4.7 $\pm$ 0.21bc	6.6 $\pm$ 0.68b
	0.3	6.2 $\pm$ 1.3cd	91.86	3.4 $\pm$ 0.61d	4.5 $\pm$ 0.71cd
	0.6	5.9 $\pm$ 0.9cd	92.25	3.0 $\pm$ 0.33de	3.7 $\pm$ 0.3cde
Green miracle	1.0	24.1 $\pm$ 1.5b	68.37	5.4 $\pm$ 0.45b	6.8 $\pm$ 0.77b
	3.0	13.2 $\pm$ 2.1c	82.67	2.4 $\pm$ 0.22de	4.7 $\pm$ 0.39cd
	5.0	6.5 $\pm$ 1.0cd	91.46	1.8 $\pm$ 0.25e	2.6 $\pm$ 0.4ef
Nimbecidine + Green miracle	0.075+ 0.75	10.0 $\pm$ 0.94cd	86.87	3.6 $\pm$ 0.34d	4.9 $\pm$ 0.23c
	0.15+ 1.5	6.7 $\pm$ 0.61cd	91.2	2.4 $\pm$ 0.22de	3.2 $\pm$ 0.29def
	0.3+ 3.0	3.5 $\pm$ 0.58d	95.4	1.4 $\pm$ 0.16e	1.8 $\pm$ 0.2f
Control	0.0	76.2 $\pm$ 8.71a	-----	15.0 $\pm$ 1.15a	17.9 $\pm$ 1.3a
F		53.25*		54.93*	60.58*
LSD <sub>05</sub>		8.41		1.7	1.4

Mean values within columns followed by a different letter differ significantly ( $P < 0.05$ ) by LSD test.

Kraiss and Cullen,(2008) found that azadirachtin and neem seed oil significantly increased aphid nymphal mortality (80.0 and 77.0 % , respectively) while significantly increasing development time of those surviving to adult of *A. glycines*. Both azadirachtin and neem seed oil affected the development time and fecundity of *A. glycines* adults. Also, Bayhan *et al.*,(2006) found that neem clearly reduced adult longevity, survival rate (zero survival), fecundity and life table parameter of the cotton aphid. Liu *et al.*,(2001) stated that jasmonic acid induced the defense reaction of wheat whereas significant change was found in the feeding behaviour of *Sitobion avenae* and *Rhopalosiphum padi* aphids. After induction by jasmonic acid, the feeding suitability of wheat for the two aphids species was reduced. The author elucidated this reduction in feeding behaviour due to the volatile repellent of the wheat seedling and the change of nutritional components in the vascular bundle of jasmonic acid which induced wheat.

### 2.1.3. Effect of tested materials and their mixture on some biological aspects of *A. craccivora* nymphal stage.

Second instar nymphs of *A. craccivora* were transferred to bean plants treated with different concentrations of *Nimbecidine* ( 0.6, 0.3 and 0.15 ml/l ), *Green Miracle* (5.0, 3.0 and 1.0 ml / l ) and the mixture ( 0.3 + 3.0, 0.15 + 1.5 and 0.075 + 0.75 ml/ l). Results obtained in Table (3) show that the percentage of nymphal survival on treated plants with *Nimbecidine*, *Green Miracle* or its mixture decreased as the concentration of tested compounds increased. The developmental periods of 2<sup>nd</sup> nymphal instar prolonged significantly with increasing concentration of the *Nimbecidine* formulations or the mixture. Except, the developmental period of 2<sup>nd</sup>

nymphal instar which exposed to treated green miracle at concentration of 5.0 ml/l was lower than the developmental period of 2<sup>nd</sup> nymphal instar which exposed to untreated plants (i.e. 1.4 days in treated and 2.0 days in control), respectively. As to the form produced the results obtained show that there was differences in the percentage of winged form in the tested formulations ranging between 10 to 50%, while in the check treatment no winged individuals formed and 100% apterous were produced. The results also indicated that the developmental period of 3<sup>rd</sup> and 4<sup>th</sup> nymphal instars were prolonged compared with the untreated nymphs.

Table (3): Impact of Nimbecidine, Green Miracle and their mixtures on nymphal duration of *Aphis craccivora*.

Formulations	Conc. ml/L	% Survival	Mean of Developmental period (days) ±SE			Total Mean of Developmental period (days)	Formed produced %	
			2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>		apterous	winged
Nimbecidine	0.15	40.0	1.75±0.25	2.0±0.0	2.5±0.28	6.25±0.53	40.0	0.0
	0.3	40.0	2.2±0.2	2.0±0.0	2.75±0.25	6.95±0.45	40.0	0.0
	0.6	30.0	2.0±0.4	2.0±0.57	2.33±0.33	6.33±1.30	30.0	0.0
Green miracle	1.0	40.0	2.0±0.26	2.4±0.25	2.6±0.25	7.0±0.76	40.0	0.0
	3.0	60.0	1.66±0.21	2.2±0.2	2.33±0.33	6.19±0.74	40.0	20.0
	5.0	50.0	1.4±0.25	2.0±0.0	1.8±0.2	5.2±0.45	10.0	40.0
Nimbecidine + Green miracle	0.075 + 0.75	50.0	2.6±0.25	3.2±0.37	2.8±0.2	8.6±0.82	50.0	0.0
	0.15 + 1.5	30.0	3.0±0.5	2.6±0.45	2.0±0.0	7.6±0.5	30.0	0.0
	0.3 + 3.0	30.0	2.0±0.0	3.7±0.33	2.33±0.33	8.03±0.66	10.0	20.0
Control	0.0	100	1.6±0.27	2.0±0.21	2.2±0.33	5.8±8.1	100	0.0

Generally, all nymphal stage that exposed to treated plants resulted in prolongation of the developmental period, i.e. (6.19 to 8.03 days) compared to 5.8 days in control. In case of *Nimbecidine*, the 4<sup>th</sup> nymphal period of aphids was 2.5, 2.75 and 2.33 days at concentrations of 0.15, 0.3 and 0.6 ml/l, respectively. This is similar to data obtained Dimetry and El-Hawary (1995) who found that neem Azal-F had an aphicidal effect against *A. craccivora*, hindered development of the nymphs, prolonged the duration of the nymphal stage and resulted in high percentage of winged form. Santos *et al.*, (2004) found that the net reproductive rate of *Aphis gossypii* was 35.0 nymphs/female when female was exposed to cotton leaf treated with 1410.0 mg neem/100 ml distilled water. The neem seeds extracted were efficient against *A. gossypii* causing nymph mortality and reducing the survival and fecundity.

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### ARABIC SUMMARY

#### تأثير مستحضرات النيم ومضادات النتح ضد حشره *Aphis craccivora*

محمد على الهوارى -

- شارع التحرير - القاهرة -

وقاية -

تأثير مركب النيمبيسيدين وجرين ميراكل منفردين ومخلوط من كلا المركبين معا ضد حشره مركب النيمبيسيدين كان الأكثر فعالية مقارنة *Aphis craccivora* مقارنة بمركب جرين ميراكل مع كل التركيزات هذه . مركب النيمبيسيدين سجلت التركيزات المميتة 50 & 90 % / 0.13 0.4 على التوالي بينما مركب جرين ميراكل التركيزات المميتة 50 & 90 % / 1.7 & 3.5 .

حاله استخدام مخلوط من كلا المركبين معا كانت التركيزات المميتة ل50 & 90% سجلت 1.3&2.2 / أيضا الوقت المميت ل 50 & 90% مع مركبات النيمبيسيدين (6.2 & 4.2 يوم مع التركيز 0.6 / ) مركب جرين ميراكل ( 4.8 & 2.9 يوم مع التركيز 5.0 مل/لتر)، مخلوط كلا المركبين ( 3.1 & 2.2 يوم مع التركيز 0.3 نيمبيسيدين + 3.0 جرين ميراكل مل / ) .

أيضا التركيز الأعلى من مركب النيمبيسيدين ( 0.6 مل/لتر) حقق أعلى نسبة خفض 92.25 % عدد الموالي من الحوريات الناتجة من الحشرات الكاملة المعرضة لنباتات معاملة بالمركب يليه 91.46% لمركب جرين ميراكل بينما النباتات المعاملة بمخلوط المركبين عند التركيز 0.3 نيمبيسيدين + 3.0 جرين ميراكل مل / نسبة خفض 95.4% فى عدد الموالي .

انخفضت مع زيادة التركيز المستخدم من المركبات. أيضا التركيزات غيره للهورية ، كما وان النتائج المتحصل عليها كبين كان فعالية ضد حشره المن مقارنة بكل مركب على حده.

بإمكانية أضافه مخلوط مركبي النيمبيسيدين وجرين ميراكل برامج المكافحة اقل بالاضافه الى فعل مركب جرين ميراكل كمحسن لحيويه النبات. حيث الفعالية