Effects of Chlorpyrifos, Imidacloprid and neem seed powder on emergence of adults of the invasive fruit fly *Bactrocera invadens* (Diptera:Tephritidae)

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ABSTRACT

Bactrocera invadens (Drew, Tsuruta and White) (Diptera:Tephritidae) is an invasive fruit fly species of Asian origin which was detected in twenty African countries. Now, the species is well established in several parts of the Sudan. The effects of Chlorpyrifos, Imidacloprid and neem seed powder on emergence of *B. invadens* adults were determined under laboratory condition. Third instars larvae were exposed to treated and untreated (control) sand. Chlorpyrfos were tested at 2, 4,8,12,16 and 32 ppm, Imidacloprid at 2, 4, 8,16 and 32 ppm and neem seed powder at 312.5 (0.031%), 625 (0.063%),1250 (0.125%), 2500 (0.25%) and 5000 ppm (0.5%). Twenty mature third instars larvae were placed in petri dish (15 cm in diameter) to allow them to pupate in the treated and untreated sand. They were supplied with small pieces of mango fruit pulp to feed on. The experiment was replicated three times. Number of emerged adults was recorded after ten days and the mortality rate (%) was calculated. Observations on the effect of the tested chemicals on the pupae formation were taken. Data were analyzed using probit analysis. The three tested chemicals were found to be effective in inhibiting the emergence of adults but with varying degrees. The inhibition always increased with increasing the concentration of the chemicals. Chlorpyrifos was the most effective among the tested chemicals, with an LC_{50} of 4.13 ppm compared to Imidacloprid and neem seed powder which had an LC_{50} of 10.14 ppm and 1299.63 ppm, respectively. Emergence of adults was completely inhibited at concentration of 32 ppm of Chlorpyrifos. Pupation was much affected by Chlorpyrifos and Imidacloprid compared to neem seed powder.

INTRODUCTION

Fruit fly pests are members of the family Tephritidae which belongs to the order Diptera. They are of a highly economic importance as they infest both fruits and vegetables, worldwide. The most important genera are *Ceratitis*, *Bactrocera* and *Dacus*. Generally, *Dacus* spp. are restricted to vegetables while fruits were mostly attacked by *Bactrocera* and *Ceratitis* spp. The most important genera are *Ceratitis*, *Bactrocera* and *Dacus*. Generally, *Dacus* spp. was restricted to vegetables while fruits are mostly attacked by *Bactrocera* and *Ceratitis* spp. Most mango-producing countries are located in fruit-fly-infested areas, and producers suffer a significant direct and indirect economic losses resulting from fruit fly damage. Annual production of mangoes in Africa was estimated to be 1.9 million tonnes; about 40% of which was lost due to fruit flies (Gesmallah, 2009). Fruit fly infestation rates vary among countries and seasons, ranging from 5 to 100% (Gesmallah, 2009). In the Sudan, the production and export of fruits and vegetables is seriously affected by fruit flies. Infestation and damage in mango crop exceeded 80% (Gubara and Abulgasim, 2004).

The current trend in fruit fly control is to adopt a nationwide action control to overcome the often in effective and unsustainable control resulting from uncoordinated action by individual producers in small holdings. A national program for fruit fly control has started since 2008 and is now covering all parts of the Sudan through the Plant Protection Directorate offices in all States of the Sudan. Big achievements are being recovered of using an IPM program involving cultural practices, cleaning campaigns, pruning, and earthing up, use of pheromones such as Methyl euogenol, Trimidlure, Cuelure and Terpenyl acetate. Abbas (2008) concluded that cleaning of the orchards from infested and dropped fruits must be practiced to minimize the next infestation by the fruit flies, fallen over-ripe or damaged fruits can be destroyed by deep burying to a depth of
more than 50 cm. Bashir (2007) found that incorporation or treatment with neem seed kernel oil (NSKO), neem seed kernel powder (NSKP) and Spinosad 240 SC at the laboratory level proved effective in inhibiting emergence of *C. cosyra* adults. Spinosad was effective at very low doses and as low as 0.1% totally inhibited the emergence at *C. cosyra*.

In general, fruit fly pest management depends largely upon the use of insecticides (Pen˜a, 1993). Pen˜a (1998) mentioned that without chemical control, the damage caused by *C. capitata* in mangoes would reach 60%. Soil treatment with insecticides is used under the fruit trees in many parts of the world to control fruit flies, because the prepupating larvae usually leave the fruit and drop into the ground and pupate in the soil (Saul *et al.*, 1983).

Various insecticides, such as Aldrin® dust 5% a.i., and Diazinone® have been evaluated for controlling fruit fly, particularly during the pre-pupating period. Neem have been traditionally used for protection of crops and products. The neem seed kernel is rich in azadirachtin (Rembold, 1991), and accordingly, it was selected in the present study, in addition to chlorpyrifos (Dursban®) and imidacloprid (Confidor®) to be used against the prepupating FF larvae.

This study was conducted to investigate the effects of Chlorpyrifos, Imidacloprid and neem seed powder on third instars larvae and emergence of adults of the invasive fruit fly (*B. invadens*) and to determine the effective doses which can be used to control this important pest.

MATERIALS AND METHODS

Laboratory experiments were conducted to study the effects of Chlorpyrifos (480 gm/l), Imidacloprid (200 gm/l) and neem seed powder on the third instars larvae, pupae and emergence of adults of the invasive fruit fly. Larvae were obtained from infested mango fruits collected from Sennar State. Mature third instar larvae (L3) were allowed to pupate in treated and untreated sand. Two hundred and fifty gram lots of dried sand were sieved through a 16 µm mesh screen and placed in 15cm glass Petri dishes. Treatments were as follows:

1. Chlorpyrifos: A series of concentrations, viz. 2, 4, 8, 12, 16 and 32 ppm were prepared. A volume of 25 ml from each concentration was mixed with sand to obtain a homogeneous mixture.
2. Imidacloprid: 2, 4, 8, 16 and 32 ppm solution were prepared. A volume of 25 ml from each concentration was mixed with sand to obtain a homogeneous mixture.
3. Neem seed powder (NSP) concentrations were prepared as follows: 312.5 (0.031%), 625 (0.063%), 1250 (0.125%), 2500 (0.25%) and 5000 ppm (0.5%). These concentrations were applied as powder to the sand to obtain a homogenous mixture. A volume of 25 ml of distilled water were added to this mixture.
4. A volume of 25 ml of distilled water was mixed with sand as a control.

In order to study the effects of the tested chemicals, 20 mature L3 were used for each concentration and replicated three times (i.e. 60 larvae/ concentration and 20 larvae for the control). The larvae were placed in Petri dishes to pupate in the treated and untreated sand. Small pieces of mango fruit pulp were provided for the larvae to feed on. The number of the emerged adults were recorded after 10 days and the mortality rate (%) was calculated. Observations and remarks regarding the effect of the tested chemicals on the pupal formation were taken. Data were subjected to Probit analysis.

RESULTS AND DISCUSSION

The three tested chemicals chlorpyrifos, imidacloprid and neem seed powder (NSP), were found to be effective in inhibiting the emergence of the adults, but with varying degrees. The inhibition of emergence always increased with increasing the concentration (Figs. 1, 2 and 3). The different concentrations of chlorpyrifos showed significant effect on the emergence of B. *invadens* adults and the highest mortality (93%) was obtained of using the highest concentrations (16 ppm) while the lowest mortality (35%) was corresponded to the lowest concentration (2 ppm) (Table 1). The inhibition was maximum (100%) mortality when using the concentration (32 ppm). This concentration was excluded from the analysis because it does not fit with the probit analysis. Chlorpyrifos had a great effect on development of larvae to pupae; all treated larvae died before reaching the pupal stage.

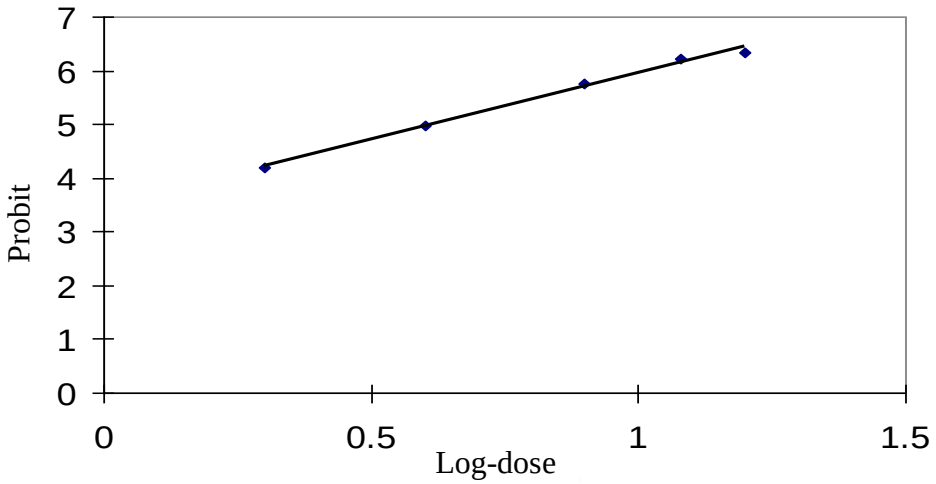

Fig. 1. Log dose–probit line of chlorpyrifos against the invasive fruit fly(*B. invadens*).

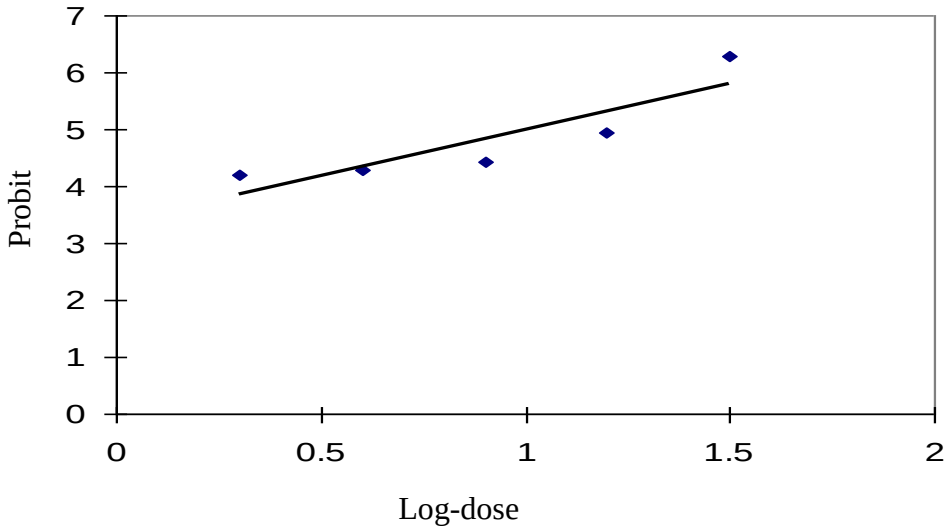

Fig. 2. Log dose–probit line of imidacloprid against the invasive fruit (*B. invadens*).

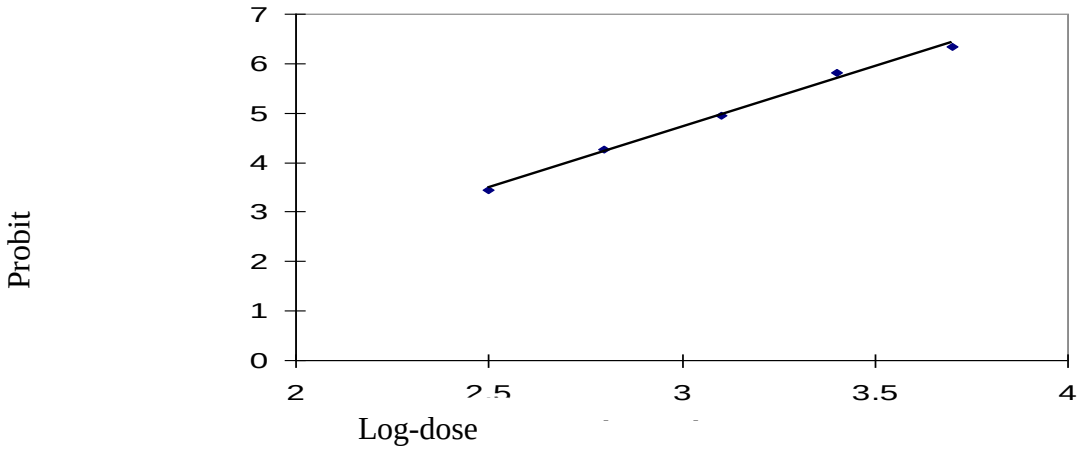

Fig. 3. Log dose–probit line oт neem seed powder against the invasive fruit fly(*B. invadens*).

Table 1. Effect of Chlorpyrifos concentrations on the mortality rate of *B. invadens*.

R^2 = 0.993 Regression equation : Y = 3.48 + 2.47X
Slope = 2.47 SE_X = 0.12
SE_Y = 0.105 LC_{50} = 4.13 ppm.
LC_{95} = 19.03 ppm

Concentration		Mortality		Probit	
Ppm	In log	% tested	% corrected	Tabulated	Expected
2	0.3	35	21	4.19	4.22
4	0.6	58	49	4.97	5.1
8	0.9	82	78	5.77	5.71
12	1.08	91.0	89	6.23	6.15
16	1.2	93	91	6.34	6.45

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LC_{50} and LC_{95} values for chlorpyrifos were found to be 4.13 ppm and 19.03 ppm, respectively, while the log –dose probit regression line was:

$$Y = 3.48 + 2.47X$$

The heterogeneity factor (chi square = 0.993), concentration, log concentration, tested mortality, corrected mortality, tabulated Probit and expected Probit were shown in Table (1). The calculated slope of the Ld-p line was 2.47. LC_{50} and LC_{95} values for imidacloprid were found to be 10.14 ppm and 105.89 ppm, respectively. The Ld-p line equation: Y = 3.38 + 1.61X, and heterogeneity factor (chi square = 0.792). The rest of the parameters are presented in Table (2). The slope was 1.61, i.e. the population is less homogeneous to this chemical than the former. Similar studies were carried out by Hu and Prokopy (2009) who tested the imidacloprid technical ingredient in the laboratory to determine

ingestion/contact or contact alone toxicity to apple maggot flies *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) and they found that imidacloprid has high lethal and sublethal effects on the maggot of this pest.

Table 2. Effect of Imidacloprid concentrations on the mortality rate of *B. invadens*.

Concentration		Mortality		Probit	
Ppm	In log	% tested	% corrected	Tabulated	Expected
2	0.3	35	21	4.19	3.86
4	0.6	38	24	4.29	4.34
8	0.9	42	29	4.43	4.83
16	1.2	57	48	4.95	5.31
32	1.51	92	90	6.28	5.81

R^2 = 0.792 Regression equation : Y = 3.38 + 1.61X
Slope = 1.61 SE_X = 0.476
SE_Y = 0.475 LC_{50} = 10.14 ppm.
LC_{95} = 105.89 ppm

Regarding the neem seed powder (NSP) all the tested concentrations showed reduction on adult emergence. The highest mortality (93%) was corresponded to the concentration (5000 ppm) while the lowest mortality (23%) was corresponded to the lowest concentration (312 ppm) (Table 3). Naheed *et al.* (2004) found that solvent extract of neem seed when applied to guava fruit in different concentrations showed significant effect of settling response, formation of pupae and emergence of adults of the fruit fly species *B. zonata* (Saunders). The LC_{50} and LC_{95} values were calculated to be 1,299.63 ppm and 6,033 ppm, respectively. The regression line was: Y= -2.66 + 2.46X, and heterogeneity factor (Chi square = 0.995). The slope of the Ld-p line is 2.46, which is almost similar to that of chlorpyrifos.

Table 3. Effect of neem seed powder concentrations on the mortality rate of *B. invadens*.

Concentration		Mortality		Probit	
Ppm	In log	% tested	% corrected	Tabulated	Expected
312.5	2.5	23	6	3.43	3.48
625	2.8	37	23	4.26	4.22
1250	3.1	57	48	4.95	4.96
2500	3.4	83	79	5.81	5.7
5000	3.7	93	91	6.34	6.43

R^2 = 0.995 Regression equation : Y = -2.66 + 2.46X
Slope = 2.46 SE_X = 0.098
SE_Y = 0.308 LC_{50} = 1299.63 ppm.
LC_{95} = 6032.37 ppm

Chlorpyrifos appears to be by far the most effective among the tested chemicals, LC_{50}, is 4.13 ppm, compared to imidacloprid and NSP (LC_{50} were 10.14 ppm and 1299.63 ppm, respectively). The same trend was found for the LC_{95}. The emergence of adults was completely inhibited at the concentration of 32 ppm of chlorpyrifos. Chlorpyrifos and imidacloprid were found to be more effective in inhibiting

the development of larvae to pupae compared to NSP. They caused the death of larvae before reaching the pupal stage.

A series of laboratory experiments were carried out to investigate the fruit fly pupation habitat, it mostly concludes that the larvae of this pest showed strong preference toward pupating in shaded rather than brightly area, in moist rather than dry soil and in soil with larger particle sizes, (Abbas, 1998; Alyokhin *et al.*, 2001). The above findings play an important role when planning to apply the tested chemicals in the field.

In conclusion Chlorpyrifos, Imidacloprid and neem seed powder could be used at a rate of 12, 48 gm active ingredient and 1.4 kg (NSP)/feddan (60 mango trees) under the mature mango trees in coincidence with peak of the pre-pupating period to reduce the reservoir of the resident population of *B. invadens*.

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تأثير مبيدات الكلوروبيروفوس، إميداكلوبريد وبدرة بذور النيم على خروج الحشرات الكاملة لنوع ذبابة الفاكهة *Bactrocera invadens* (Diptera:Tephritidae)

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الخلاصة

هي إحدى أنواع ذبابة الفاكهة *Bactrocera invadens* (Drew, Tsuruta and White) (Diptera:Tephritidae) دولة أفريقية وهي الآن موجودة في أجزاء مختلفة من السودان. 20الغازية. يعتبر موطنها الأصلي هو آسيا وقد تم رصده في عدد حيث تم *B. invadens*تم إختبار أثر مبيدات الكلوروبيروفوس ، إميداكلوبريد وبدرة بذور النيم على خروج الحشرات الكاملة للنوع الإختبار على الطور اليرقي الأخير الذي سمح له بالتعذر داخل تربة معاملة بالمركبات المذكورة أعلاه. التركيزات التي تم إختبارها ، (0.063%))، 625 0.031%هي 2، 4 ، 8 ،12، 16 و32 جزء من المليون، 2، 4، 8 ، 16 و32 جزء من المليون و 312.5 (جزء من المليون للكلوروبيروفوس ، إميداكلوبريد وبدرة بذور النيم (0.5%) و5000 (0.25%) ، 2500 (0.125%)1250 على التوالي. تم وضع 20 يرقة من الطور اليرقي الأخير في طبق بتري وسمح له بالتعذر في تربة معاملة بالمركبات أعلاه وأخرى غير معاملة (الشاهد). وضعت قطع صغيرة من المانجو على سطح التربة لكي تتغذى عليها اليرقات قبل التعذر. كررت التجربة ثلاثة مرات إضافة إلى الشاهد. بعد عشرة أيام تم تسجيل الحشرات الكاملة التي خرجت ومنها تم تحديد نسبة الموت (%). أيضاً تم ملاحظة أثر المركبات المختبرة على تكوين العذارى. تم تحليل النتائج بإستخدام تحليل البروبت. أوضحت نتائج الدراسة أن المركبات المختبرة الثلاثة ذات كفاءة في تثبيط خروج الحشرات الكاملة بدرجات متباينة، وجد أن التثبيط بشكل عام يزيد بزيادة التركيز. كما وجد أن 10,14 و LC_{50} 4,13 جزء من المليون مقارنة بالإميداكلوبريد وبدرة بذور النيم LC_{50}الكلوروبيرفوس هو الأكثر كفاءةً بـ 1299,63 جزء من المليون على التوالي. خروج الحشرات الكاملة ثبط تماماً عند تركيز الكلوروبيرفوس بـ 32 جزء من المليون.