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Laboratory Evaluation of The Susceptibility of Adult African Invader Fly *Bactrocera Invadens* (Diptera : Tephritidae) To Nulure-Based Bait of Some Selected Insecticides Commonly Used By Mango Farmers In Ghana

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Abstracts -True fruit flies are one of the world's worst pests of fruit and fruiting vegetables, causing millions of dollars worth of damage each year. While Africa has a number of native fruit flies of its own, some exotic species present in some continents has in recent years raised some serious biosecurity concerns among local experts. Bactrocera invadens is one of the exotic fruit flies that recently invaded Africa. Evidence of competitive displacement of indigent fruit flies in some mango agro ecosystem by this species has been recently documented. Effective control of this fruit flies can best be achieved through an IPM based control programme which will involve the use of bait in traps or spot treatments. However, no fruit flies bait is currently registered and is available on Ghanaian market for use in the management of this species by the farmers. The use of some readily available insecticidal products namely Cydim Super, Pyrinex 50 EC, Diazol 50 EW and Deltapaz as killing agents in the commercially available food bait Nulure was evaluated in the laboratory to ascertain their efficacy. The result show that Pyrinex and Diazol based bait were the most effective bait that can be used for the management of fruit flies in Ghana.

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Classification: *GJSFR-G Classification (FOR): 070308, 070399*

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Abstract-True fruit flies are one of the world's worst pests of fruit and fruiting vegetables, causing millions of dollars worth of damage each year. While Africa has a number of native fruit flies of its own, some exotic species present in some continents has in recent years raised some serious biosecurity concerns among local experts. Bactrocera invadens is one of the exotic fruit flies that recently invaded Africa. Evidence of competitive displacement of indigent fruit flies in some mango agro ecosystem by this species has been recently documented. Effective control of this fruit flies can best be achieved through an IPM based control programme which will involve the use of bait in traps or spot treatments. However, no fruit flies bait is currently registered and is available on Ghanaian market for use in the management of this species by the farmers. The use of some readily available insecticidal products namely Cydim Super, Pyrinex 50 EC, Diazol 50 EW and Deltapaz as killing agents in the commercially available food bait Nulure was evaluated in the laboratory to ascertain their efficacy. The result show that Pyrinex and Diazol based bait were the most effective bait that can be used for the management of fruit flies in Ghana.

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I. Introduction

Exporting fresh agricultural produce to high end markets in Europe and America is gaining importance in many developing countries (Braun, 2002). The Natural Resource Institute (NRI) estimates that up to 45 million people in the African, Caribbean and Pacific (ACP) countries depend on horticultural goods for export to Europe alone (Braun, 2000). In Africa, fruit and vegetable production is one of the fastest growing agricultural sectors providing both income and employment to growers and exporters (Lux *et al.*, 2003a; Ekesi and Billah, 2006). The range of fruits and vegetables grown is diverse. Mango, citrus, apple, papaya, passion, guava, avocado, cucumber, pumpkin and watermelon are among the most common fruits and vegetables grown for domestic urban markets and for export to major markets in Europe and the Middle East (Ekesi and Billah, 2006).

Mango Mangifera indica L. is the most widely cultivated fruit tree in the Sahel and one of the most important tree crops in the tropics (Deng and Janssen, 2004). It is a highly prized exotic fruit on the European market and one of the important fruit crops grown in tropical and sub-tropical regions (Nakasone and Paull, 1998; Nofal and Haggag, 2006). In recent years, mangoes have become well established as a fresh fruit and processed product in the global market (Lux et al., 2003). World demand for mango is now increasing, particularly in temperate countries where mangoes are gaining popularity. Sequel to this, increase in mango production in non-traditional mango producing areas has been notable and includes parts of Asia, West Africa, Australia, South America and Mexico (EMEX, 2000). The growth in mango production in these areas is as a result of change in the dietary pattern of the people leading to increased consumption of the fruits and processed products made from it (Lux et al., 2003a; Ekesi and Billah, 2006).

World production of mango in 2005 was estimated at 28.51 million tonnes (Mt) (Evans, 2008). Of this Africa produced only 2.5 million tonnes, accounting for about 10 per cent of fresh fruits and 11 per cent of processed mango. Ghana's current production is said to have increased from about 1,200 tonnes in 2007 to about 2,000 in 2008 (Qaurtey, 2008). In Ghana, it is targeted as the next non-traditional export crop that is expected to fetch the highest foreign exchange for the country and replacing cocoa (Qaurtey, 2008). Total land

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area under mango production in Ghana is estimated to be 4,166.7 ha with the largest farms measuring up to 416.7 ha. Export of 270 Mt in 2004 was valued at US $376,000 (Adongo, 2006).

However, different types of insect pests cause serious damage to mango in Africa and perhaps none have gained greater attention than the fruit flies (Ekesi and Billah, 2006). The problem of the fruit fly complex has increased in recent times by the incidence of the African invader fruit fly *Bactrocera invadens* Drew, Tsuruta and White, which has been reported to cause 15-50 % yield losses in some African countries (Vayssierres, *et al.,* 2005). *Bactrocera invadens* was first noticed in Ghana in 2005 (Drew *et al.,* 2005; Billah *et al.,* 2006) as an important quarantine pest. The presence of *B. invadens* in Ghana has already brought some setback in the mango trade between Ghana and South Africa (for example mango consignment from Ghana was rejected at the port and subsequent import was banned) (Wilson, 2006).

Bactrocera invadens belongs to the *Bactrocera dorsalis* complex of tropical fruit flies (French, 2005). This complex comprises more than 75 species largely endemic to South-East Asia (Drew and Hancock, 1994), with several undescribed species remaining in collections (Lawson *et al.,* 2003). The group is arguably one of the most important pest species complexes in world agriculture (Clarke *et al.,* 2005; Drew *et al.,* 2005).

Current control measures against fruit flies mostly incorporate the use of insecticides as a cover spray or bait and targeting the adult flies (Raga and Sato, 2006). Because female fruit flies lay eggs beneath the exocarp of the fruit and the larvae develop inside the fruit with little chance of insecticide affecting them (Moreno *et al.,* 1994), any integrated pest management strategy to control *B. invadens* in Ghana may involve the use of insecticides as spot sprays and/or bait on the adult flies. This is because if the fly population becomes too large, there is no control method that will be cost effective against the flies. One of the only effective strategies for interrupting the developmental cycle of the flies is by prevent adult flies from implanting eggs in the fruit by the use of baited traps and preventive insecticide treatment (CTA, 2007).

Several insecticides are registered in Ghana for the control of arthropod pests including fruit flies in the mango agro-ecosystem. Some of the most common ones include Chlorpyrifos, Cypermethrin, Deltamethrin, Lambda- cyhalothrin, bifenthrin, Azadirachtin, Dimethoate, White oils and Thiamethxam (EPA, 2008). However, there is limited or no registered bait for the control of fruit flies especially B. invadens. And current fruit fly control strategy under the IPM fashion lays much emphasis on the use of bait. Therefore, there is an urgent need to evaluate the efficacy of the baits of some of the most commonly used insecticides by farmers in Ghana to control this quarantine pest. The objective of this study was to evaluate the efficacy of the nulure-based baits of some commonly used insecticides by mango farmers in Ghana against adult B. invadens flies.

II. Materials and Methods

A. Collection of fruits for the establishment of B. invadens colony

Mango fruits were collected from the University of Ghana farm, at Legon, Agricultural Research Centre, at Kade and its environs, and from different locations within the University environment. Fruit samples collected included unripe, overripe and rotten ones as windfalls (Rwomushana et al., 2008). Fruits collected were packaged in bagco sacks (Sino PP woven bag, Danzaki Ltd., Kano, Nigeria) with the tops left open to provide adequate ventilation and taken to the laboratory for rearing out of the fruit flies.

B. Rearing of fruit flies

Fruits collected from various locations were processed in the laboratory for rearing of the fruit flies as described by Copeland (2006) and Rwomushana *et al.*, (2008). Fruits were placed on large plastic racks with trays of pre-sterilized moistened sand under the container to collect exiting larvae. Fruits were also incubated in plastic bowls lined with sand. The sand was heat-sterilized at 100 ^{0}C for 12 h. Fruits were dissected after 2 weeks of incubation to remove any remaining larvae as larvae may sometimes prefer to remain inside the rotting fruit if the outside environment is not conducive (Billah, personal communication). After 3 days, the sand was sieved to recover puparia, which were placed in petri dishes lined with moist filter paper. Daily checks for emerged insect was done and insects transferred into holding cages. Adult insects were allowed to stay for 3-4 days to achieve full adult characteristics to facilitate proper identification. They were provided with water-soaked cotton wool and fed on a diet containing pure baker's yeast and sugar (1 part yeast:3 part sugar, vol./vol. combination). Insects were separated according to species and kept in different cages. Parasitoids and non-tephritid flies were also separated.

C. Identification of flies.

Flies were identified using literature and keys of White and Elson-Harris (1992), De Meyer (1996, 1998, 2000) and Billah and Mansell (2006). Where there was doubt, flies were sent to Dr. M. K. Billah (Zoology Department, University of Ghana) for confirmation. Flies were sorted according to species. Non-tephritids flies were killed.

D. *Culturing of flies*

The emerged adults were cultured in cages on artificial diet prepared with three parts sugar and one part pure bakers' yeast (vol./ vol.) diet (Ekesi *et al.*, 2006). Few drops of honey were added /spotted on the underside of the roof of the cage and cotton wool soaked with tap water was placed in small container on the floor of the cage. The cages were occasionally cleaned to maintain good environment.

E. *Bait bioassay*

Baits of four insecticides namely; Cydim Super, Pyrinex 48 EC, Diazol 50 EW and Deltapaz were evaluated during the study. Due low effects of the Delpaz bait at the freshly treated panels trials, its 3 and 6-day old treated panels were not tried. The insecticides used for the studies were purchased from two Agro-chemical dealers; Agri-mat and Dizengoff Ghana Ltd. Nulure was obtained from Dr. M. K. Billah, Zoology Department University of Ghana.

The bait solution for the respective insecticides was obtained by mixing a given dose of the test insecticides with pre-mixed 2% Nulure solution. Thus, cydim super bait was prepared by mixing 1.6, 2.3 and 3.0 ml of cydim super per litre of 2% Nulure. Pyrinex bait was prepared by mixing 0.9, 1.0 and 1.1 ml of Pyrinex 48 EC per litre of 2 % Nulure solution, Diazol bait was obtained by using 1.8, 2.5 and 3.2 ml of Diazol 50 EW per litre of 2% Nulure solution and Deltapaz bait was similarly obtained by adding 2.6, 3.4 and 4.0 ml of the insecticide per litre of 2% Nulure solution. This represents the application of 10 ml below the manufacturers recommended dose (Lowest doses), the manufacturers recommended doses (median doses), and 10 ml above the manufacturers recommended doses (Highest doses) for each insecticide product. Each experiment was repeated three times, including control (panels immersed in 2% Nulure® solution) set ups.

Bait efficacy was determined by calculating the lethal time (LT_{50} and LT_{90}) values for the field strain of adult *B. invadens* for each formulation. All bioassays were conducted in the research Laboratory at the African Regional Postgraduate Programme in Insect Science (ARPPIS) building, University of Ghana, Legon, under 28 ± 2°C, RH 68-81% and photoperiod of 12:12 D:L hours.

Bioassays were conducted in one litre transparent bottles fitted with yellow caps, from which treated hardboard panels (4 x4 cm) were hanged. A hundred and fifty (150) holes of 1.5 mm diameter were made on the sides of the bottles for ventilation during the bioassay (Cheng *et al.,* 2009). A hole of 1.5 mm in diameter was made at the centre of the bottles' yellow cap to hang the treated panels with the aid of a string attached to the panels.

The hardboard panels were treated by dipping into the respective concentrations of the baits, viz: 10 ml below the manufacturers recommended dose (Lowest doses), the manufacturers recommended doses (median doses), and 10 ml above the manufactures recommended doses (Highest doses) (Moore and Miller, 2006). Ten adult flies of mixed sexes (5 males and 5 females) aged 4- 12 days were collected from the rearing cages using a 1.5 cm diameter vial. These were introduced into the bioassay bottles containing the treated panels. The bottles were placed upright.

Observations for mortalities were made at least once every 5 minutes. Adult flies that were knocked down and could not move any appendages for 30 seconds after responding to the baits were considered, and scored dead. The times taken for 50 and 90% of the introduced flies to die were recorded to represent LT_{50} and LT_{90}, respectively for a treatment and dosage level. Three set of experiments were conducted based on the age (in days) of the treated panels;

(1) Freshly treated panels (10 minutes after dipping).

(2) 3-day old treated panels (72 hours after dipping).

(3) 6-day old treated panels (144 hours after dipping) (Urbaneja *et al.*, 2009).

This gave the residual toxicity (persistence) of each bait under study over six days period. A similar panel treated with only nulure before exposure to insects, was used as a control for the different doses of the four insecticides baits.

F. *Data analysis*

Data from laboratory bioassay were analysed using ANOVA with the GENSTAT statistical packages (GenStat Release 9.2 (PC/Windows) 2007, Lawes Agricultural Trust (Rothamsted Experimental Station) 9th Edition). The results were presented as tables and figures. Means were separated using Least Significant difference (LSD, P< 0.05). Mortality exceeding 10% in the control was corrected using Abbotts' formula (Abbott, 1925).

III. Results

A. *Freshly treated panels with bait solution*

The results for panels freshly treated with bait solutions are presented on table 1. On panels treated with the lowest doses of bait solutions, the longest mortality times were recorded with Deltapaz bait , mean LT_{50} and LT_{90} = 1066.00 ± 208.47 and 1410.00 ± 281.71 min, respectively; and these times were significantly different (P < 0.05) from the other treatments.

For both the median and the highest doses of bait solutions, there was a dose-dependent reduction in the mean mortality times (LT_{50s} and LT_{90s}) for all insecticides bait treatments. The mean mortality times for the control (2% Nulure® solution with no insecticide applied) being significantly longer (P < 0.05) than all the treatments. The mean mortality time for Deltapaz bait was significantly longer (P < 0.05) than the other insecticides bait.

Table 1 Mean lethal time (LT) for freshly treated panels with insecticides bait

			Mean± S E. lethal	time (min)
Dose levels	Treatment	Dose (ml/L)	LT_{50}	LT_{90}
LD	Control	0.0	2880.00 ± 0.00	2880.00 ± 0.0
	Cydim Super bait	1.6	47.00 ± 10.80	78.33 ± 7.86
	Pyrinex bait	0.9	39.00 ± 1.53	63.33 ± 7.00
	Diazol bait	1.8	20.33 ± 4.50	36.67 ± 3.71
	Deltapaz	2.6	1066.00 ± 208.47	1410.00 ± 281.7
	LSD (P < 0.05)		294.20	397.30
MD	Control	0.0	2880.00 ± 0.00	2880.00 ± 0.0
	Cydim Super bait	2.3	42.67 ± 14.50	70.67 ± 4.18
	Pyrinex bait	1.0	35.33 ± 1.45	54.33 ± 4.67
	Diazol bait	2.5	18.33 ± 1.67	29.67 ± 3.18
	Deltapaz bait	3.4	599.67 ± 249.73	1336.00 ± 140.70
	LSD (P < 0.05)		352.50	198.50
HD	Control	0.0	2880.00 ± 0.00	2880.00 ± 0.0
	Cydim Super bait	3.0	42.67 ± 0.67	60.00 ± 1.15
	Pyrinex bait	1.1	29.33 ± 1.00	43.00 ± 5.51
	Diazol bait	3.2	14.00 ± 0.58	19.00 ± 0.58
	Deltapaz bait	4.0	217.33 ± 26.30	658.00 ± 325.71
	LSD (P < 0.05)		37.10	459.10

LD = Lowest Doses, MD = Median Doses, HD= Highest Doses

IV. Three-day old treated panels with bait solutions

On the 3- day old panels treated with baited insecticides (Table 2), the lowest doses of Cydim Super bait gave the highest mean mortality times (LT50 = 78.67 ± 7.70 and LT90 = 125.00 ± 12.51 min) among the insecticides bait treatments; and was equally significantly higher (P< 0.05) than other treatments, but lower than the control (>2880.00 ± 0.00 min).

On panels treated with median doses of the bait solutions, the mean mortality times for all treatments were significantly different (P< 0.05) from each other and the control, which gave a significantly longer mean mortality time than all the treatments (Table 2).

The results on panels treated with the highest doses of the baits showed that, among the treatments, significant difference (P< 0.05) was only found between the mean LT50s for Cydim Super (LT50 = 64.67 ± 6.17 min) and Diazol (LT50 =28.00 ± 0.58 min) bait solutions treated panels (Table 2). Comparisons of the LT90 for all treatment indicated that, significantly longer (P < 0.05) mean LT90 was only found on panels treated with Cydim Super bait solution. The difference between the mean mortality time for the control (panels treated with only 2% Nulure solution) and all treatments were also significant ($P < 0.05$).

The results for mean mortality times for 90% (LT_{90}) of the adult flies indicated that, difference between mean values for panel treated with Pyrinex and Diazol bait solutions (LT_{90} = 43.33 ± 2.40 and 28.33 ± 1.0 min, respectively) were not significant ($P > 0.05$) (Table 2). However, LT_{90} for Cydim Super bait treated panels (64.67 ± 9.98 min) were significantly higher than those for both Diazol and Pyrinex. The difference between Nulure treated panels (control) and all other insecticides bait treated panels were significant ($P < 0.05$) (Table 2).

Table 2. Mean lethal time (LT) for 3- day old treated panels with insecticides bait

			Mean± S E. lethal	time (min)
Dose level	Treatment	Dose (ml/L)	LT_{50}	LT_{90}
LD	Control	0.0	>2880.00 ± 0.00	>2880.00 ± 0.0
	Cydim Super bait	1.6	78.67 ± 7.70	125.00 ± 12.51
	Pyrinex bait	0.9	41.00 ± 3.21	59.67 ± 3.84
	Diazol bait	1.8	31.33 ± 3.18	53.33 ± 3.53
	LSD (P < 0.05)		14.54	23.64
MD	Control	0.0	>2880.00 ± 0.00	>2880.00 ± 0.0
	Cydim Super bait	2.3	64.67 ± 6.17	87.00 ± 4.73
	Pyrinex bait	1.0	45.67 ± 6.36	59.67 ± 5.50
	Diazol bait	2.5	28.00 ± 0.58	36.67 ± 2.03
	LSD (< 0.05)		14.48	12.26
HD	Control	0.0	>2880.00 ± 0.00	>2880.00 ± 0.0
	Cydim Super bait	3.0	41.33 ± 8.51	64.67 ± 9.98
	Pyrinex bait	1.1	30.67 ± 0.67	43.33 ± 2.40
	Diazol bait	3.2	21.00 ± 3.21	28.33 ± 1.0
	LSD (< 0.05)		14.88	16.58

LD = Lowest Doses, MD = Median Doses, HD= Highest Dose

V. Six-day-old treated panels with bait solution

The results for mean lethal time for 6-day-old panels treated with bait solution are shown on Table 3. On panels treated with the lowest doses, LT_{50}s and LT_{90}s for Pyrinex and Diazol baits were not significantly different ($P > 0.05$). The LT values for Cydim Super bait were significantly longer ($P < 0.05$) than those for the other treatments, but significantly shorter ($P < 0.05$) than control (panels treated with 2% Nulure® solution only). The mortality times for the control were, however, significantly higher ($P < 0.05$) than all the bait treated panels.

Panels treated with median and highest doses of the baits gave a similar pattern of responses. Mean lethal times for all panels treated with the baits were significantly different ($P < 0.05$) from each other. The mortality times for the controls were, however, significantly longer ($P < 0.05$) than all the insecticides bait treated panels. The least mean mortality times were generally obtained from panels treated with Diazol bait.

Table 3. Mean lethal time (LT) 6 days old treated panels with insecticides bait

			Mean± S E. lethal	**time (min)**
Dose level	Treatment	Dose (ml/L)	LT_{50}	LT_{90}
LD	Control	0.0	>2880.00 ± 0.00	>2880.00 ± 0.0
	Cydim Super bait	1.6	132.33 ± 21.00	190.67 ± 11.35
	Pyrinex bait	0.9	80.00 ± 2.52	103.00 ± 2.52
	Diazol bait	1.8	56.67 ± 2.40	83.67 ± 7.31
	LSD (P< 0.05)		34.57	22.39
MD	Control	0.0	>2880.00 ± 0.00	>2880.00 ± 0.0
	Cydim Super bait	2.3	108.33 ± 3.38	167.00 ± 14.30
	Pyrinex bait	1.0	75.33 ± 2.03	99.00 ± 4.00
	Diazol bait	2.5	38.67 ± 6.44	63.00 ± 6.03
	LSD (P< 0.05)		12.21	26.12
HD	Control	0.0	>2880.00 ± 0.00	>2880.00 ± 0.0
	Cydim Super bait	3.0	75.67 ± 7.67	112.67 ± 13.17
	Pyrinex bait	1.1	53.33 ± 2.33	81.67 ± 7.86
	Diazol bait	3.2	30.00 ± 3.05	57.00 ± 5.13
	LSD (P< 0.05)		14.00	26.37

LD = Lowest Doses, MD = Median Doses, HD= Highest Doses

VI. Discussion

The results for the experiment with baits of the candidate insecticides studied indicated that, Diazol with both contact and stomach poison properties and gave the fastest response. However, the application rate of Pyrinex was the lowest in ml/L compared to those of the other insecticides. Pyrinex was used at the 0.09% (0.9 ml/L), 0.1% (1.0 ml/L) and 0.11% (1.1 ml/L) compared to the other insecticides.

Diazinon (Diazol) and Chlopyrifos (Pyrinex) can therefore, be insecticides of choice for use against *B. invadens* especially in bait sprays because of their contact and stomach poison properties (EXTOXNET, 1996). where acute toxicities results from increased ingestion of the active ingredient because of the feeding stimulant/attractant (Barry and Polavarapu 2004, 2005) and comparatively of better residual toxicities. Bait sprays containing feeding stimulants (e.g., sucrose), have several advantages to conventional sprays. Lower concentrations of insecticides are needed in bait sprays than conventional sprays because mortality is primarily from oral toxicity, which has lower LC_{50} thresholds than dermal toxicity. More insecticide active ingredients are consumed because of the presence of the feeding stimulants (Reissig, 2003; Barry and Polavarapu, 2004). After mixing the bait with a pesticide, it is normally applied to 1-square metre spot on the canopy of each tree in the orchard. This is applied on weekly basis starting from when the fruits are about 1.3 cm in size and continues until the very end of the harvest (Ekesi and Billah, 2006). Therefore, bait sprays can be applied at a lower rate of active ingredient per hectare than conventional sprays (Prokopy *et al.,* 2003), and, since food-based protein baits are attractive to both male and female fruit flies, and, by reducing adult populations, have historically been a primary means of pest management (Mangan, 2009).

The results of the bait trials also show that, with little training, the resource poor farmers can mix a bait solution that may give effective control of the pestiferous fruit flies including *B. invadens* on their farms. The use of baits for *B. invadens* control will reduce the risk of over-dosing the trees with insecticide solutions that will leave unwanted residues on the fruit. It also has the advantage of reducing the amount of insecticide applied on the farm over time within the production cycle.

VII. Acknowledgement

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