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Effects of Neem Leaf Dust and a Commercial Formulation of a Neem Compound on the Longevity, Fecundity and Ovarian Development of the Melon Fly, *Bactrocera cucurbitae* (Coquillett) and the Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae)

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Abstract: Neem leaf dust and a commercial formulation of neem were tested on adult *Bactrocera cucurbitae* (Coquillett) and *Bactrocera dorsalis* (Hendel) to determine their effects on the longevity, fecundity and ovarian development. Different combinations of neem leaf dust and a commercial formulation of a neem compound incorporated with sugar solution and adult rearing diets were tested. The Laboratory tests showed that ingestion of neem can significantly reduced the longevity and fertility of both the fly species. Significantly fewer pupae were collected from adults fed on laboratory rearing diet and nimbicidine as water source. Effect of neem treatment on the pupation and subsequent adult emergence of late-instar larvae was negligible. Microscopic observation indicated that the decreased fecundity was due to the block of ovarian development. Experimental results confirmed that neem can be effectively used as a safe alternative insecticide for the control of *Bactrocera s*pecies.

Key words: *Bactrocera cucurbitae*, *Bactrocera dorsalis*, neem, longevity, fecundity, ovarian development

INTRODUCTION

The Melon fly, *Bactrocera cucurbitae* (Coq.) and the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) under the family Tephritidae are considered as economically most important pests in most of the tropical and sub-tropical areas of the world. The fly species are multivoltine with explosive reproductive capacity and disperse widely. In Hawaii 125 and 173 host plant species have been reported for *B. cucurbitae* and *B. dorsalis*, respectively (Metcalf and Metcalf, 1992). While 42 host plants for *B. cucurbitae* and 117 for *B. dorsalis* in South-East Asia have been reported (Allwood *et al*., 1999) in addition to other alternate hosts (Uchida *et al*., 1990). The economic impact of fruit flies includes not only the direct loses of yield and increased cost of control, but they also seriously impede international trade because of quarantine regulations (Animal and Health Inspection Service, 1988).

The control measures adopted for the fly species mainly are contact poisons or bait traps for mature adults (McQuate *et al*., 2005; Vargas *et al*., 2003). Baits and sprays of conventional insecticides have toxic effects on non-target beneficial fauna including parasitoids of *Bactrocera* species. Some attention has been paid to the possibility of fly control by targeting late-instar larvae and pupae (Singh, 2003; Stark *et al*., 1990). Sterile Insect Techniques (SIT) have been shown to be an important component of integrated approaches to control flies under the family tephritidae (Hendrichs *et al*., 2002; Wong *et al*., 1992) and have also successfully able to eradicate the melon fly from isolated Island like, Okinawa, Japan (Koyama *et al*., 2004). However, because of polyandrous and long distance migratory abilities of the fly species with high population densities throughout the years SIT does not seem to be suitable for continental areas.

Neem (*Azadirachta indica* A. Juss.) (Meliaceae) has emerged as an excellent alternative to synthetic insecticides for the management of different insect pests. Azadirachtin is an example of natural chemical defense by plants, affecting feeding primarily through chemoreception (deterrence) and secondly through toxic effects (Mordue (Luntz.) and Blackwell, 1993). As many as 540 insect including all key insect pests of agriculture have already been found to be susceptible and exhibit various behavioural and physiological effects of neem (Schmutterer and Singh, 2002). However, there are only few reports on the effect of neem extracts on tephritids (Stark *et al*., 1990; Stefens and Schmutterer, 1983) and also few literature are available on the post-embryonic development against *B. cucurbitae* and *B. dorsalis* when fed as water source (Di Ilio *et al*., 1999; Singh, 2003).

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Therefore, to establish neem as an environmentally safe insecticide for integrated control of tephritid flies, the present investigation was undertaken to determine the effect of neem leaf dust and a commercial formulation of a neem compound on the longevity, fecundity and ovarian development of adult *B. cucurbitae* and *B. dorsalis* under laboratory condition.

MATERIALS AND METHODS

Adult *B. cucurbitae* and *B. dorsalis* used were from established colonies maintained in the laboratory of Insect Biotechnology Division, Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh, for about 100 generations at 28±2ºC temperature and 75±5% relative humidity.

Effects of neem leaf dust integrated diet on the longevity, fecundity and ovarian development of *B. cucurbitae* and *B. dorsalis*: Fresh neem leaves were collected from AERE campus in the month of July, 2006. The leaves were then washed in tap water, freeze dried (Lab Conco., USA) and were ground in an electric blender (New Hartford, Conn., USA) to make fine powder. Aqueous extract of neem leaf was prepared by shaking weighed amounts of fresh neem leaf into beaker (1600 mL) containing distilled water for 24 h with an electric shaker and filtering through Whatman No. 1 filter paper.

Newly emerged sixty pairs of adult *B. cucurbitae* and *B. dorsalis* were placed in five nylon-netted cages (25×15×15 cm) separately and supplied with: (i) control (laboratory rearing diet, autolyzed brewers yeast: sugar, 1:4), (ii) negative control (no food), (iii) only sugar solution (10%), (iv) neem leaf dust and 10% sugar solution (w/v, 5 g: 5 mL) and (v) neem leaf dust: protein-hydrolysate:sugar (1:1:2). Adult survivals in the test cages were recorded until all flies died. Three replicates were conducted for each treatment for both the fly species. On day 14, ovaries of the treated and control flies were dissected in invertebrate physiological saline water (0.7% NaCl) and micro-photographed using Nikon microscope. The length and breadth of ovary of the treated as well as control flies were measured with a micrometer. Micro-photograph of the normal ovary as well as whole reproductive organs of female *B. cucurbitae* is also given (Fig. 1A-E).

Effects of a commercial formulation of neem compound on the longevity, fecundity and ovarian development of *B. cucurbitae* and *B. dorsalis*: Commercial formulation of a neem compound (Nimbicidine®, supplied by ACI Ltd., Dhaka, Bangladesh) was used in the present study. Required concentrations (1, 5, 10, 15 and 20 ppm) of azadirachtin (0.03% EC) were prepared by diluting into 10% sugar solution. Newly emerged fifty pairs of *B. cucurbitae* and *B. dorsalis* were kept in nylon-netted cages separately and were provided with nimbicidine at a concentration of 1, 5, 10, 15 and 20 ppm as water source, via cotton wicks in conical flask. Sugar solution (10%) was served as control. Treatments and controls were

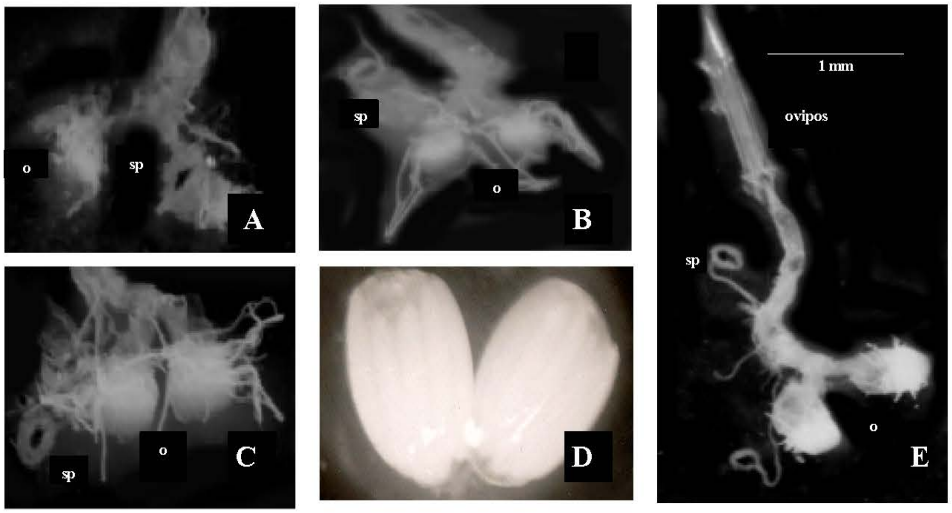

Fig. 1: Ovaries (ov), spermatheca (sp), ovipositor (ovipos) of 14 days old female flies fed on (A) neem leaf dust and sugar solution/only nimbicidine (10 ppm), (B) sugar solution only, (C) neem leaf dust:protein-hydrolysate:sugar (1:1:2), (D) control (autolized brewers yeast:sugar 1:4) and (E) whole reproductive organs of freshly emerged *Bactrocera cucurbitae*

changed every 24 h to prevent contamination through cotton wicks. After 24 h number of flies died was recorded per concentration per cage. Ovaries of treated flies which survived beyond 14 days were dissected in saline water, micro-photographed and the length and breadth were measured as mentioned earlier.

In a separate experiment, eighty pairs of freshly emerged adult *B. cucurbitae* and *B. dorsalis* provided with laboratory rearing diet (autolyzed brewers yeast and sugar, 1:4) and 20 ppm nimbicidine as drinking source. After 14 days 10 pairs of flies were transferred into another cage separately containing oviposition medium (sweet gourd and banana paste for *B. cucurbitae* and *B. dorsalis*, respectively) for egg collection. Total five cages were maintained for both the species. After 24 h of egg collection, sweet gourd and banana paste per cage per fly species were transferred into artificial rearing medium and kept for pupation. The same procedure was repeated on day 20 to collect egg from *B. cucrbitae* and *B. dorsalis*. Mean pupation and subsequent adult emergence was recorded.

Effect of aqueous extract of neem leaf and neem compound on the pupation of *B. cucurbitae* and *B. dorsalis* larvae: Previously sterilized 100 g saw dust were mixed with 100 mL nimbicidine (0.03%) and air dried properly. Ten late-instar (popping) larvae were allowed to pupate on 10 g neem treated saw dust in Petri dishes (9 cm). Sterilized 100 g saw dust were also mixed with 100 mL neem leaf extract for the same purposes. Ten replicates per treatment group for both the fly species were maintained to determine the percentage of pupation and subsequent adult emergence.

Statistical analysis: Data obtained from the present study were analysed with statistical software Minitab, version 13.2. DMRT, one way Analysis of Variance (ANOVA) and Tukey's pair-wise comparison test were performed.

RESULTS

Effects of neem leaf dust integrated diet on the longevity, fecundity and ovarian development of *B. cucurbitae* and *B. dorsalis*: Mean longevity of adult *B. cucurbitae* and *B. dorsalis* fed on neem integrated diet was significantly lower from that of control. Flies fed only 10% sugar solution had the higher longevity than the flies fed on neem leaf dust mixed sugar solution (Table 1). No flies in both the species survived up to 3/5 days with negative control (no food) under laboratory condition. Significant reduction in the longevity of both the fly species also observed when fed on neem leaf dust integrated diet (protein hydrolysate:sugar: neem leaf dust, 1:1:2) than control (autolyzed brewers yeast and sugar 1:4). The mean longevity was 41±5 and 39±2 days (Table 1) for neem leaf dust integrated diet fed *B. cucurbitae* and *B. dorsalis*, respectively. *Bactrocera* species fed on neem leaf dust integrated diet laid eggs after 38 and 35 days which was 21-24 days away from the egg laying (14 days after adult emergence) of control flies. No flies were observed to lay egg fed on either only sugar solution or neem leaf dust mixed sugar solution.

Table 1: Mean (±SE) longevity of adult *B. cucurbitae* and *B. dorsalis* fed on different adult diets

	Mean longevity (days)	
Adult diets	*B. cucurbitae*	*B. dorsalis*
Control (laboratory rearing diet)	80±5.0[a]	75±5.0[a]
Negative control (no food)	4±1.0[e]	3±0.5[e]
Only sugar solution (10%)	55±2.0[b]	50±4.0[b]
Neem leaf dust and 10% sugar solution (1:1)	18±3.0[d]	17±0.5[d]
Neem leaf dust: protein-hydrolysate: sugar (1:1:2)	41±5.0[c]	39±2.0[c]

Values are mean of three replications per treatment group for both the fly species, Mean in each column having different letter(s) differ significantly at 0.05% (DMRT)

A series of microscopic observation on the ovaries of flies fed on neem leaf dust mixed sugar solution, sugar solution only, neem leaf dust integrated diet and same day old control flies are shown in Fig. 1A-D. Ovaries of all the treated flies appeared reduced in size compared to control. The accessory glands on the contrary appeared well developed in both the treated and control flies. The length and breadth were 0.37±0.01 and 0.29±0.02 mm, 0.39±0.02 and 0.32±0.01 mm, 0.87±0.02 and 0.79±0.01 mm and 2.56±0.01 and 1.95±0.02 mm, respectively, for the neem leaf dust mixed sugar solution, sugar solution only, neem leaf dust integrated diet and same day old control flies, respectively.

Effects of a commercial formulation of neem compound on the longevity, fecundity and ovarian development of *B. cucurbitae* and *B. dorsalis*: Lethal mortality of *B. cucurbitae* and *B. dorsalis* were determined at 15 and 20 ppm concentration within 24 h of exposure. *B. cucurbitae* and *B. dorsalis* were observed to survive up to 22 and 19 days, respectively, when supplied with 10 ppm nibmicidine. The percentage survival was 20 and 10% for *B. cucurbitae* and *B. dorsalis* fed on 5 ppm concentration of nimbicidine during this period (Fig. 2). No differences were observed between 1 ppm nimbicidine fed than control for both the fly species. The micro-photograph, the length and breadth of 10 ppm nimbicidine fed flies ovary were same to that of the neem leaf dust mixed sugar solution fed flies as mentioned earlier.

Table 2: Mean (±SE) pupation and adult emergence of 10 pairs of *B. cucurbitae* and *B. dorsalis* fed on protein diet and 20 ppm nimbicidine as water source at 14 and 20 days after adult emergence

		B. cucurbitae		*B. dorsalis*	
Age of flies	Diet	Pupation	Adult emergence	Pupation	Adult emergence
14 days	Nimbicidine fed	127±5.0^c	120±5.5^c	58±5.6^c	45±5.5^c
	Control	200±5.6^a	195±6.5^a	120±5.5^a	105±5.0^a
20 days	Nimbicidine fed	155±4.5^b	150±5.5^b	95±4.65^b	87±2.5^b
	Control	210±5.2^a	200±5.0^a	125±4.5^a	115±6.8^a

Values are mean of five replications, 10 pairs per replicate, In a column mean values followed by same letter(s) do not differ significantly at 0.05% (DMRT)

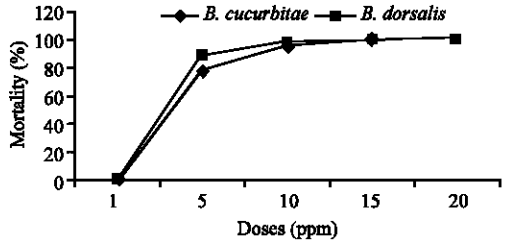

Fig. 2: Percentage mortality of adult *B. cucurbitae* and *B. dorsalis* after 14 days, fed on different concentrations of nimbicidine

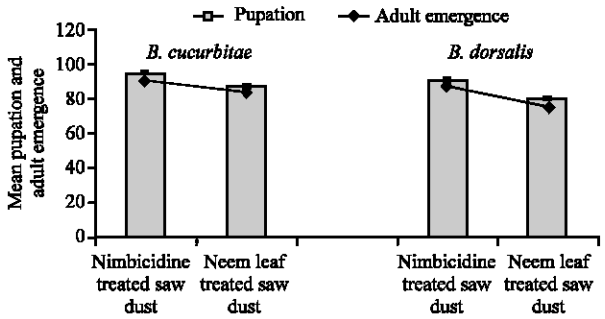

Fig. 3: Mean (±SE) pupation and adult emergence of *B. cucurbitae* and *B. dorsalis* from late-instar larvae on nimbicidine as well as neem leaf extract treated saw dust

The adult *B. cucurbitae* and *B. dorsalis* supplied with protein sources (autolized brewers yeast and sugar) and 20 ppm nimbicidine as drinking source were observed to lay egg as control flies after 14 days of adult emergence. During this period, nearly 14-33% *B. cucurbitae* and *B. dorsalis*, respectively, were observed died. However, the mean pupation and adult emergence were significantly lower than that of control (Table 2).

Effect of aqueous extract of neem leaf and neem compound on the pupation of *B. cucurbitae* and *B. dorsalis* larvae: Aqueous extract of neem leaves and nimbicidine treated saw dust did not exert any effect on the pupation of late-instar larvae of both the *Bactrocera* species. Larvae were observed to escape the effect of nimbicidine and neem leaf extract mixed saw dust and formed the puparia. However, pupal duration delayed for 2 to 3 days from that of control. Mean Pupation and subsequent adult emergence are shown in Fig. 3.

DISCUSSION

The present experimental results confirmed that neem leaf dust and neem compound have significant detrimental effects on the physiology and development of *B. dorsalis* and *B. cucurbitae*. Flies fed on neem leaf dust mixed sugar solution, negative control and nimbicidine alone at 1, 5 and 10 ppm concentration were not able to produce eggs and led us hypothesize that neem may act on *B. cucurbitae* and *B. dorsalis* by disrupting the function of ovarian development as reported by Di Ilio *et al.* (1999) in case of medfly, *Ceratitis capitata* (Wied). Role of azadirachtin as a compound responsible for these effects were also reported by Burkhard (1989). The author noted that azadirachtin caused a reduction in egg deposition, weight of the ovaries and free ecdysteroid in the hemolymph and the ovaries respectively, on blow fly, *Phormia terraenovae* (RD). Singh (2003) confirmed that neem seed kernel extract as well as pure azadirachtin at 2.0 ppm completely inhibit egg laying in *B. cucurbitae* and *B. dorsalis* when fed as water source. Complete and irreversible sterility was also reported in female *C. capitata* by feeding azadirachtin mixed adult rearing diet (Di Ilio *et al.*, 1999).

In insect, yolk synthesis is dependent on Juvenile Hormone (JH) and 20-OH ecdysone (Handler and Postlethwait, 1978). Azadirachtin has been reported to affect both the processes by inhibiting oogenesis and ovarian ecdysteroid synthesis (Rembold, 1988; Schulz and Schluter, 1984). Changes in hemolymph protein expression of *Ostrinia furnacalis* (Guenée) was also reported to be induced by azadirachtin treatment (Huang *et al.*, 2007). In the present experiment neem leaf dust mixed sugar solution and nimbicidine feeding completely inhibit the fecundity of both the fly species. Mean pupal collection

from adults fed on protein and nimbicidine fed was significantly less from that of control flies. The present observation have some similarities with the findings of Steets (1976) who noted that Colorado Potato beetle, *Leptinotarsa decimlineata* (Say) females fed with azadirachtin rich neem seed kernel extract for 5 days resulted reduced fecundity which was greater than 98%. However, unlike to the findings of Singh (2003), *B. dorsalis* seem to be more sensitive than *B. cucurbitae* to the tested neem compound.

The present microscopic observation also confirmed the developmental redundancy of the ovaries of the treated flies. The observed maturity of the accessory glands in the treated females indicated that neem compound should possess specific activity on the ovaries only, without affecting the whole female reproductive organs as reported by Di Ilio *et al.* (1999) in case of *C. capitata*. Further physiological assays are necessary to analyze which metabolic pathways are involved in the chemosterilant activity of neem extracts on *Bactrocera* species.

Exposure to unfavorable environmental conditions during pupation is known to have a negative effect on the survival of tephritid fruit flies (Jackson *et al.*, 1998). Gaabour and Hayes (1984) reported a correlation between increasing azadirachtin concentration and reduction in pupation/adult emergence in case of the face fly, *Musca autumnalis* De Geer. The effect was explained due to the possible disruption in the neuroendocrine center of moulting insects (Rembold, 1988; Rembold and Sieber, 1981). The present finding is partially similar with the observation of Stark *et al.* (1990) who recorded no significant effect on fecundity and fertility of the medfly, *C. capitata*, oriental fruit fly *B. dorsalis* and melon fly, *B. cucurbitae*, that survived the larval-pupal stage when exposed as late third instar larvae in azadirachtin treated sand.

Neem contains an array of chemicals having different complex mode of action on insects. In the present endeavour only popping larvae were exposed to azadirachtin treated saw dust (contact toxicity) and were limited only till adult emergence. The effect of oral toxicity (feeding azadirachtin at third instar larvae) and the fecundity and post embryonic development of the emerged adult flies should be investigated to speculate the effect of azadirachtin on F1 generation. Commercial nimbicidine used in the present investigation contained approximately 0.03% azadirachtin, in addition to other substances. Hence, per unit weight of azadirachtin in nimbicidine must have been less than pure technical azadirachtin. However, it is presumed that the presence of 0.03% azadirachtin is responsible for the recorded effects on *B. dorsalis* and *B. cucurbitae* under this study.

The present experimental results clearly indicate that the commercial formulation nimbicidine as well as neem leaf dust can efficiently inhibit the fecundity and fertility of both the *Bactrocera* species under laboratory condition. Field use of neem may be problematic because azadiracthin degrades rapidly after exposure to UV radiation (Barnby *et al.*, 1989). However, the use of azadirachtin based compounds in insecticidal baits appears promising when mixed with attractant substances such as heptanol, protein hydrolysate (Prokopy and Vargas, 1996; Roessler, 1989). Further investigations are needed to develop the strategies for integrated pest management techniques for *B. cucurbitae* and *B. dorsalis* using neem as a cheap, effective and renewable source of eco-friendly botanical insecticide.

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