POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) AS A DETECTION METHOD FOR IRRADIATED STERILE MELON FLY, BACTROCERA CUCURBITAE (COQ.) (DIPTERA: TEPHRITIDAE)

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Abstract: Mature pupae (seven days old) of the melon fly, Bactrocera cucurbitae (Coq.) were exposed to Co-60 gamma irradiation to establish the dose-response-banding pattern of esterase isozyme for the detection of radiation sensitive protein marker in adult stage using Polyacrylamide Gel Electrophoresis (PAGE). The banding sequence of esterase isozyme in 5% PAGE was not precisely isolated in 10, 20 and 50 Gy, whereas the bands were distinctly separated at 30 to 40 Gy doses which are the sterility dose of B. cucurbitae. These bands could be used as a biochemical marker for the identification of sterile flies.

Key words: Melon fly, Bactrocera cucurbitae, radiation, esterase, PAGE

INTRODUCTION

Fruits and vegetables produced in the tropical and sub-tropical countries are subjected to infested by different species of fruit fly of the family Tephritidae. They are subject to strict quarantine in many countries, especially USA (Vockeroth 2002). Females of the fly lay eggs under the skin of fresh fruits, and subsequent larval feeding tracks provide entry points for bacteria and fungi that cause the fruits to rot. Bangladesh produced 15 to 16 lac tons of vegetables from 2.5 lac hectare of land out of which 0.32% are exported to 60 countries in the year 2003-04 (Bangladesh Bureau of Statistics, 2004). So, vegetables have an important share in the export market of our country.

In the field application of Sterile Insect Technique (SIT), a detection method is necessary to distinguish sterile flies from the wild ones during post-release assessment. Several detection methods have been suggested (Walder and Calkins 1992, Dyby and Sailer 1999 and Anwar et al. 1971). Saha et al. (2005) reported that the reduced size of testes and ovary could be used as an indicator of irradiation in trapped adults flies during the field release for SIT. Nazarea and Manoto (1992) also reported that separation of macromolecules as well as bands using PAGE show some potential for adaptation as an analytical technique for detecting radiation induced changes in adult flies. Hasanuzzaman (2003) studied the esterase isozyme banding patterns in B. cucurbitae on 5% PAGE
during the different life stages and also in sweet gourd, which was used as larval food media.

This study evaluated the effectiveness of an autocidal control method to suppress the melon fly population through radiation. The objectives of this research was to determine (1) Dose-response banding pattern of esterase isozyme in the adult fly on 5% PAGE and (2) To develop the methodology needed to identify the sterile flies from wild flies in the field application of SIT programme.

MATERIAL AND METHODS

Rearing of B. cucurbitae: The eggs of B. cucurbitae were obtained from the colony (50♂ x 50♀) of 14 days old flies that had been maintained in the laboratory of the AERE at 25 ± 2°C temperature and 60-65% relative humidity. The eggs were then placed on an artificial larval diet mixed with local ingredients. This situation continued until pupation. Pupae were collected after seven days of egg collection. These were then irradiated one day before adult emergence with the doses of 10, 20, 30, 40 and 50 Gy. Each group of pupae treated with these different doses were maintained separately and waited for adult emergence. The emerged adults were fed on artificial diet prepared by proteose peptone: sugar (1: 4) and water in a conical flask with a cotton wade soaked with the food.

Electrophoresis: In order to observe the banding pattern of esterase isozyme, 5% PAGE electrophoresis was performed following the standard procedures described by Hasanuzzaman (2003 and 2004) and Raymond et al. (1996) with slight modifications in staining procedure, which are as follow:

Substrate mixture: 120 ml substrate mixture was prepared using 1.07 g of NaH₂PO₄, 2.67 g of Na₂HPO₄, 0.09 g of α-Naphthyl acetate and 0.09 g of β-Naphthyl acetate and 2 ml of acetone in distilled water.

Stain solution: The stain solution consisted of 0.09 g of Fast blue RR salt and 150 ml distilled water. The gel was kept in substrate mixture for 15 minutes. Afterwards stain solution was added and incubated at 37°C for 10-15 minutes.

Photography and Scanning: Gels with clear banding patterns were photographed using Fujii 100 colour film. These photographs were scanned at 1200 dpi with Adobe Photoshop 5.5 (Scanner, Hewlett Packard 1125). Finally, the photographs were printed on a HP 1175 printer.
RESULTS AND DISCUSSION

The banding pattern of esterase isozyme of irradiated (10-50 Gy) and non-irradiated flies were examined electrophoretically through 5% PAGE as shown in Fig. 1. Two clearly separated bands were found in both 30 Gy and 40 Gy treated fly (Fig. 1 A: slot no. 7-10, B: slot no. 17-20, C: 30-33). A diagrammatic sketch was provided to identify the bands (Fig. 2). Nahar (2004) reported that the sterility dose of the melon fly lies between 30 to 40 Gy. The non-irradiated male and female fly did not show any clear separated banding sequence (Fig. 1, A: 1,2; B: 25, 26; C: 38, 39). The bands were also not precisely isolated in 10, 20 and 50 Gy male and female fly (Fig. 1, A: slot no. 3-6, 11-12; B: slot no. 21-24, 15, 16; C: slot no. 34-37, 28, 29). There were two separated bands in 20 Gy treated female fly (Fig. 1 A: slot no. 6), but those bands were not observed in the others cases of the same slots (Fig. 1, B: slot no. 21 and C: slot no. 24). So, these bands at 20 Gy were not considered. Two bands, Est-1 and Est-2, showing seven allelomorphs were observed in B. cucurbitae (Hasanuzzaman 2003). In the present study, the esterase isozyme banding patterns of adult melon fly (male and female) irradiated at 10-50 Gy were observed. Clearly separated bands at 30-40 Gy through the analysis of 39 slots in the gel were considered.

Fig. 1. Electrophoretic banding patterns of esterase isozyme in fertile adults of B. cucurbitae. Slot No. 1, 13, 26, 39 = control male; Slot No. 2, 25, 38 = control female; Slot No. 3, 24, 37 = 10 Gy treated E; Slot No. 4, 23, 36 = 10 Gy treated F; Slot No. 5, 22, 35 = 20 Gy treated F; Slot No. 6, 21, 34 = 20 Gy treated E; Slot No. 7, 20, 33 = 30 Gy treated F; Slot No. 8, 19, 32 = 30 Gy treated E; Slot No. 9, 18 = 40 Gy treated F; Slot No. 10, 17 = 40 Gy treated E; Slot No. 11, 16, 29 = 50 Gy treated F; Slot No. 12, 14, 15, 27, 28 = 50 Gy treated E.
Anwar *et al.* (1971) reported that the fluorescent powder marking of the released flies, which were later caught in traps, was not sufficiently reliable. Irradiated sterile flies could be detected by the reduced size of testis and ovary. Distinct banding patterns were observed in the electrophoretic profile at each stage of development. Thus each profile could be used as an electrophoretic signature for a particular stage of insect (Nazarea *et al.* 1991). Esterases in insects have been implicated in reproductive behaviour.

![Fig. 2 Diagrammatic sketch of the electrophoretic banding patterns of esterase isoenzyme in fertile adults of *B. cucurbitae*.](image)

Pheromone and hormone metabolism, digestion, neurotransmission, and the action of, and resistance to, insecticides, particularly organophosphates (Richmond *et al.* 1990 and Parker *et al.* 1991). In this paper, we found prominent bands at 30 and 40 Gy doses, which may be used as a biochemical marker in quarantine purpose and also in SIT programme.

**LITERATURE CITED**


A detection method for irradiated sterile melon fly


