Protocol for Large Scale Field Trials of Bt Brinjal Hybrids MHB-4 Bt, MHB-9 Bt, MHB-10 Bt, MHB-80 Bt and MHBJ-99 Bt Containing the cry1Ac Gene for Testing of Efficacy and Yield During Kharif 2006

Objectives
This study will be conducted with the two promising Bt brinjal hybrids that have passed through one year of RCGM approved replicated field trials and one year of ICAR trials. The following objectives will be achieved:

1. To generate information on yield and demonstrate agronomic performance of Bt brinjal hybrids as compared to their non-Bt counterparts and commercial check hybrids.
2. To generate information on incidence of beneficial and non-target insects among Bt brinjal and their non-Bt counterparts.
3. To generate information on insect infestation on Bt brinjal, their non-Bt counterparts and non-Bt commercial check hybrids.

Experimental Design

Treatments
T1 = Non-Bt version of T2
T2 = Bt brinjal hybrid
T3 = Non-Bt commercial check.

Number of trials : 20

Description of Field Plots

Treatments : 3
Row – row spacing : 90 cm
Spacing between plants within row : 60 cm
Passage between two treatments : 2 m
Plot size of each treatment : 432 sq. m each
Plants per treatment : 800
Net experimental plot area : 1296 sq m

Isolation shall be maintained as per the permission order.
Field Layout of Limited Field Trial

Note: An isolation distance of 200 meters on all sides of the trial plot shall be maintained from any other brinjal crop.

Segment types for Bt brinjal hybrids

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Segment Type</th>
<th>Bt Brinjal Hybrid</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Oval, Purple with white stripes, Green Non-spiny calyx</td>
<td>MHB-11 Bt</td>
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<tr>
<td>2</td>
<td>Oval, reddish purple, Green Non-spiny calyx</td>
<td>MHB-39 Bt</td>
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Observations to be recorded

A. Yield parameters
1. Number of healthy (marketable) fruits and those damaged by borers.
2. Weight of healthy (marketable) fruits and those damaged by borers.
3. Economic benefits from cultivation of Bt brinjal is to be calculated based on marketable yield and cost involved in taking up the ETL based sprays and comparing with Non Bt counterpart.
B. Insect Observations:

1. **Shoot Damage**: Count the number of healthy shoots and number of damaged shoots due to fruit and shoot borer attack from 20 selected plants randomly in block of each treatment at 15 days interval starting from 15 DAT (Days After Transplanting).

2. **Fruit borer larvae**: Count the number of fruit and shoot borer larvae (*Leucinodes orbonalis*) and fruit borer larvae (*Helicoverpa armigera*) on 20 selected plants randomly in block of each treatment at 15 days interval starting from 30 DAT.

3. **Fruit Damage**: Count percent fruits damaged by borer/s on 20 selected plants randomly in block of each treatment. Count Number of fruits damaged by borers and record the weight of the fruits damaged.

   Dissect 20 fruits of approximately similar size from the pre-determined plants and count the total no of larvae from Bt and non-Bt fruits (*Leucinodes orbonalis* and *Helicoverpa armigera*).

4. **Stem borer damage (%)**: Count the number of stem borer infested stems from 20 randomly uprooted plants in block of each treatment at the end of the cropping season. Length of the stem (cm) and length of the stem tunnelled (cm) will be recorded to calculate percent stem tunnelling.

5. **Non target and beneficial insect pests**: Count the number of larvae/adults of other insect pests and accordingly monitor their incidence at regular intervals.

All above observations shall be taken on same 20 selected plants throughout the crop duration.

**Agronomic and Pest Management**

Trials will be managed as per prevalent agronomic practices in the region. Insecticide sprays will be taken based on the ETL of the target pest and will be given for each treatment separately. Disease management will be done by taking up recommended practices prevalent in the region. Need based sprays will be given to control sucking pests and spray records will be maintained.
ETL criteria – ETL criteria for brinjal fruit and shoot borer will be observed as under:

1. Shoot damage 5%.
2. Fruit damage 10%

Data analysis
Data will be analyzed by using the statistical procedure using - General linear model of AGROBASE software.

Experimental Risk Management

- “Recombinant DNA Safety Guidelines-1990” and “Revised Guidelines for research in transgenic plants & Toxicity and Allergenicity evaluation of transgenic seeds, plants, plant parts 1998” put in place by Government of India, would be followed strictly.
- Complete account of the transgenic materials and seeds thereof would be kept as per the directives given.
- Only company authorized personnel and Government nominees would be permitted to visit the experimental plot and records thereof shall be maintained.