Effect on Soil Microflora

Studies were carried out to determine the effect of Bt-cotton on soil microbial diversity.

Soil samples were collected from of the rhizosphere of Bt-cotton and counterpart non-Bt cotton plants grown in the trial plots at our Isarwadi farm. Such samples were collected at a depth of about 20 cm and 10-15 from the main stem of the plant, at 60, 120 and 225 days after sowing. Three such soil samples collected from the same test cotton material, Bt as well as non-Bt, were thoroughly mixed so as to draw a single common representative sample. Soil sample suspensions were prepared in distilled water by suspending about 5 gm of soil sample in 50 ml of distilled water. Such suspensions were thoroughly mixed for about 5-10 minutes on a rotary
shaker and then further diluted (1:10) with distilled water and plated onto selective medium (such as LB + Cyclohexamide for establishing bacterial cultures and Streptomycin supplemented Nutritive agar for fungi). The plates were incubated at 28 ± 1°C for 3-4 days.

The data for the total number of culturable bacteria and fungi for the Bt rhizosphere and non-Bt rhizosphere soil samples of Isarwadi Bt cotton test plot are presented in Tables 3 & 4. The results did not reveal any differences, either between Bt and non-Bt cotton hybrids or between any of the test hybrids.

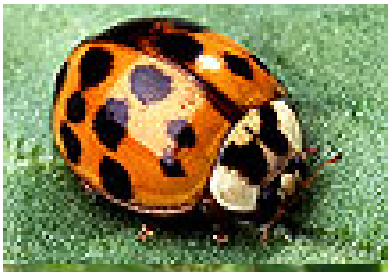
Table 5: Effect of Bt-cotton on Bacterial Population of Soil Rhizosphere at 60, 120 and 225 days after sowing.

Test Hybrid (Bt & Non-Bt)	Bacterial Population of Soil Rhizosphere (Mean± SE) × 10^6 CFU/g soil Days after sowing		
	60	120	225
NCEH-2R Bt	19.8± 0.48	21.8±0.75	24.3±1.10
NCEH-2R NBt	19.3±0.67	20.5±1.26	23.8±1.18
NCEH-3R Bt	22.6±1.34	21.2±0.88	21.5±0.85
NCEH-3R NBt	21.1±1.12	22.6±1.03	18.7±1.3
NCEH-6 Bt	20.6±1.09	21.7±0.88	21.4±0.87

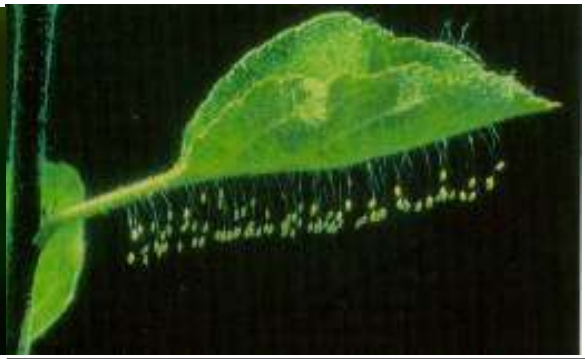
Table 6: Effect of Bt-cotton on Fungal Population of Soil Rhizosphere at 60, 120 and 225 days after sowing.

Test Hybrid (Bt & Non-Bt)	Fungal Population of Soil Rhizosphere (Mean± SE) × 10^4 CFU/g soil Days after sowing		
	60	120	225
NCEH-2R Bt	1.67± 0.08	1.52±0.06	1.71±0.04
NCEH-2R NBt	1.74±0.02	1.75±0.09	1.70 ±0.06
NCEH-3R Bt	2.22±0.16	1.68±0.05	1.65±0.12
NCEH-3R NBt	2.14±0.09	1.63±0.14	1.66±0.17
NCEH-6 Bt	1.99±0.15	1.94±0.08	1.50±0.11

Study on Beneficial Insects Predator such as Coccinelids, Syrphid, Chrysopa and Spiders were recorded from five randomly selected plants and their abundance, relative to the controls in general and counter part Non-Bt Hybrids in particular was evaluated at 30, 60, 90 and 120 DAS of crop age. The data revealed that the population of predators / natural enemies was not different among Bt and Non-Bt hybrids.



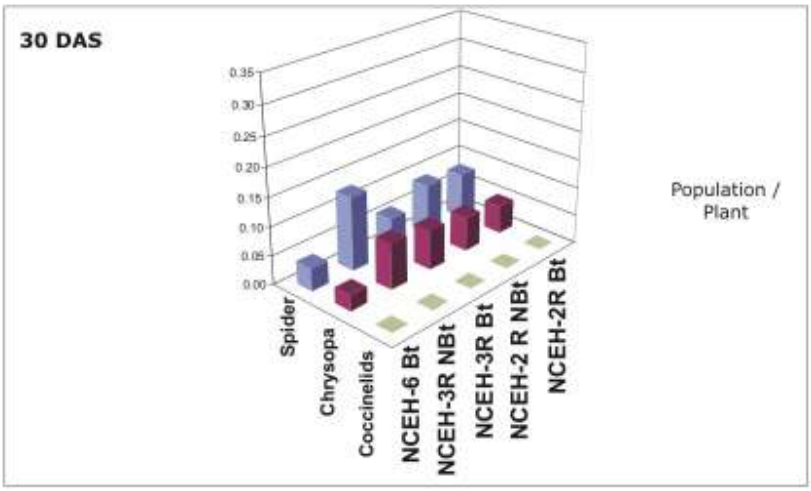
Adults and grubs of Lady Bird Beetle feed on Aphids. They are found on upper as well as lower surface of the cotton leaves. Observation were recorded from five randomly selected and tagged plants from 15 DAS to 60 DAS, of each of test Bt-cotton cultivar as well as the counter-part non-Bt cotton hybrids and checks.

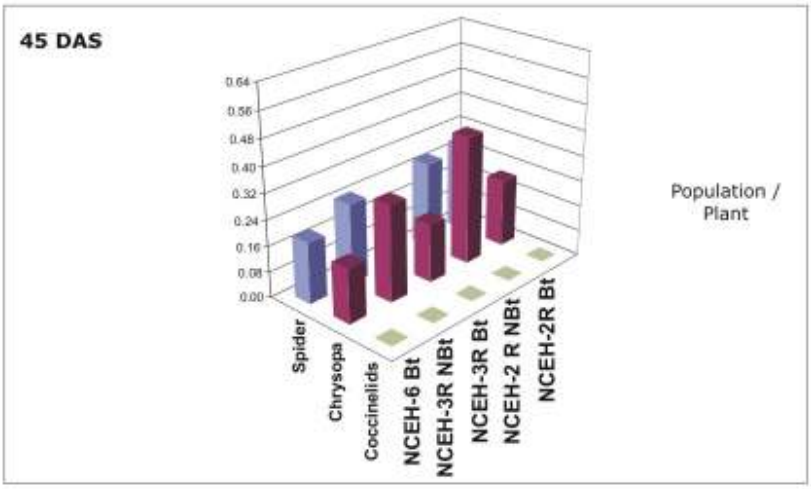


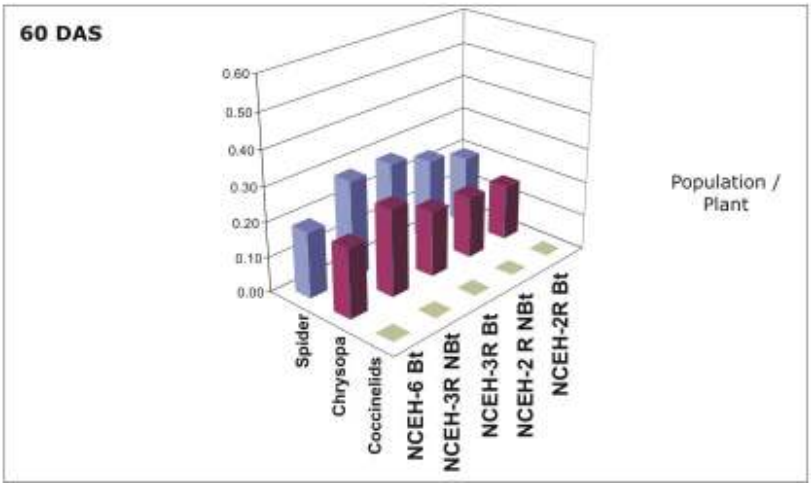
Larvae of Chrysopa feed on sucking pests as well as eggs and young larvae of Lepidopteran pests. Observation are recorded from five randomly selected and tagged plants for larvae only (Adults fly away) from 15 DAS to 45 DAS.

Multi-Location Trials (2004) North Zone

Population of Natural Enemies on Bt and Non-Bt Hybrids

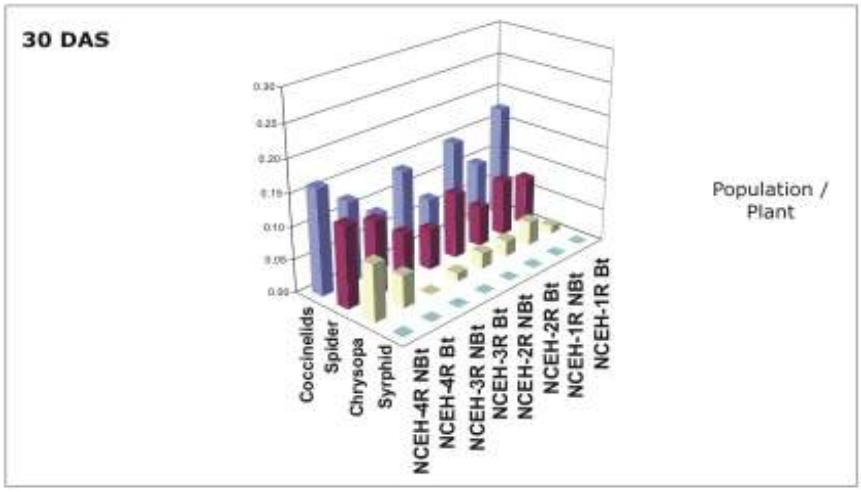


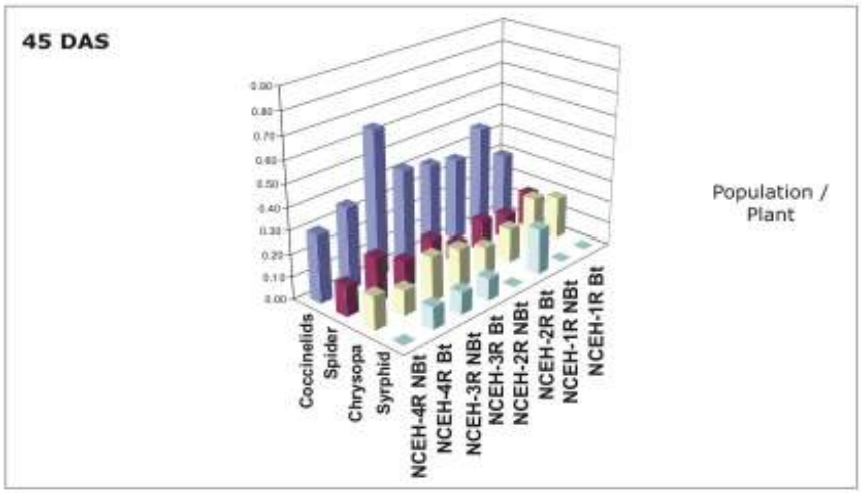


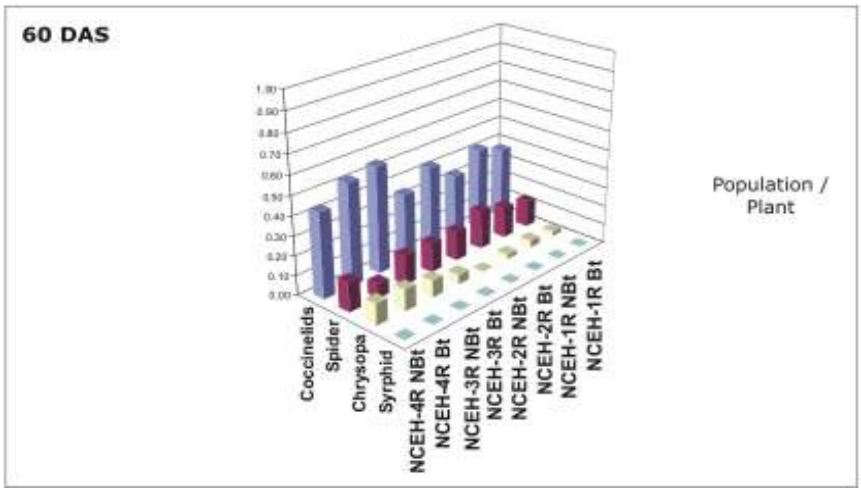


Multi-Location Trials (2004) Central Zone

Population of Natural Enemies on Bt, Non-Bt and Check Hybrids

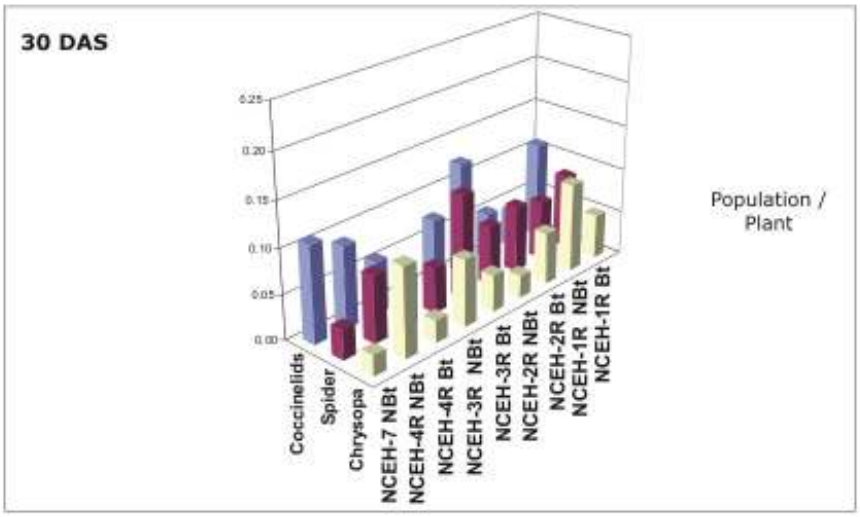


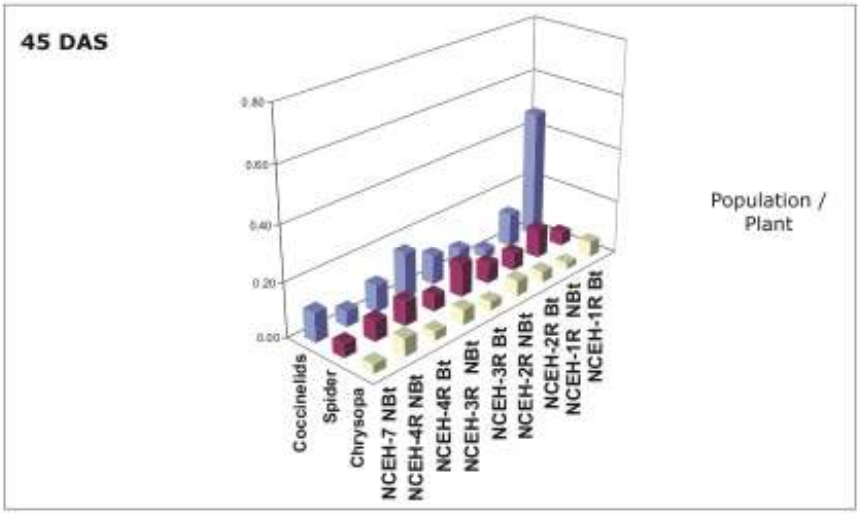


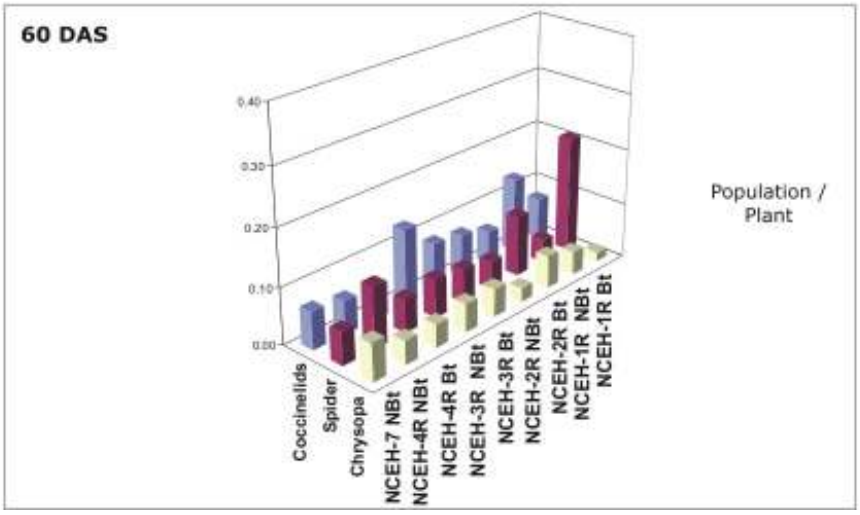


Multi-Location Trials (2004) South Zone

Population of Natural Enemies on Bt, Non-Bt and Check Hybrids





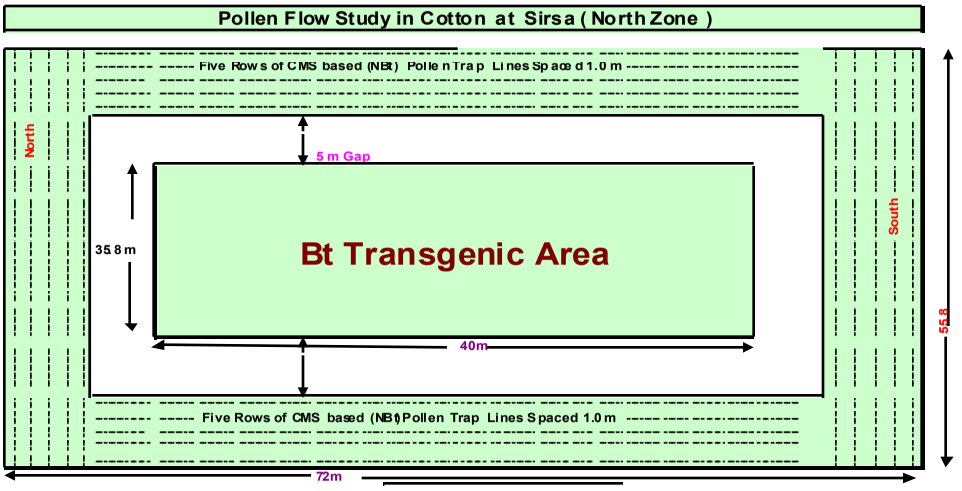


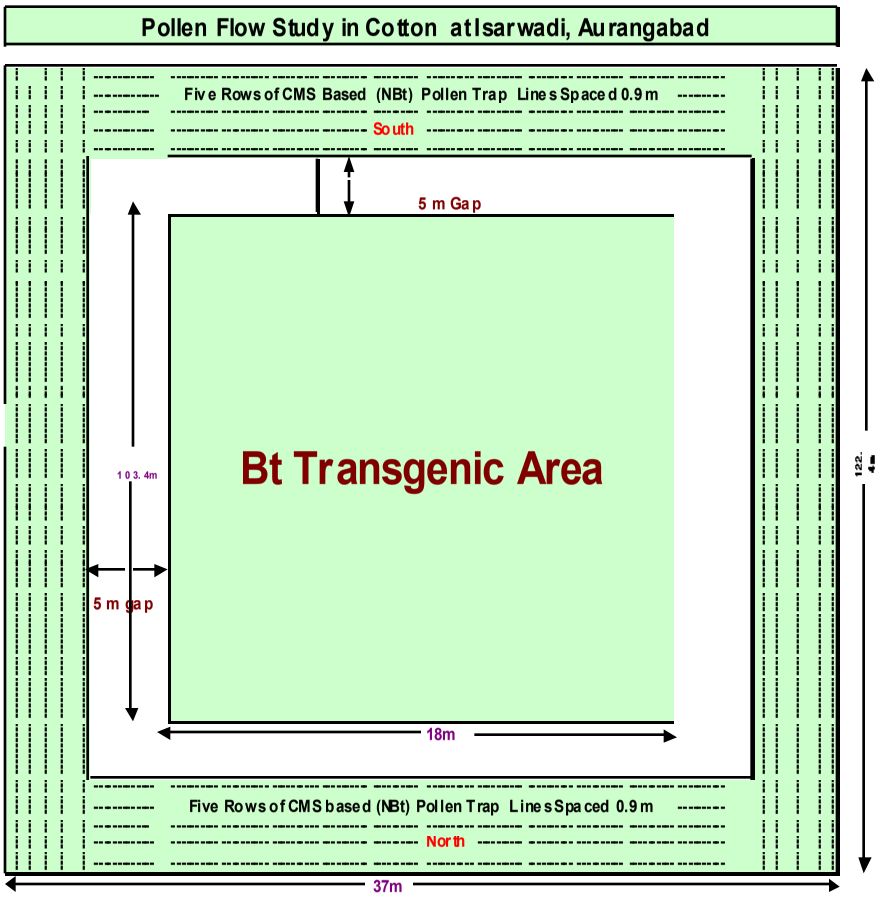
1. *Studies on Pollen Escape/Out Crossing*

We have conducted a novel method of assessing the flow of pollen from the transgenic Bt-Cotton test plots used for the Multi-Location Trials.

The specific studies of pollen flow were carried out at Isaarwadi (Aurangabad) and at Sirsa (Haryana). The layout plans of the test plots are given in Figure 1A (for Isarwadi) and 1B (for Sirsa). As indicated in these diagrammatic sketches, pollen trap lines were located at a distance of five meters on all four sides of test plots. Total of five trap lines were planted at a distance of 0.9 meter (Row to Row) at Isarwadi and one meter distance (Row to Row) at Sirsa. We used a CMS cotton line for raising pollen trap lines and the plants raised from such seeds were completely sterile. Since no other cotton material was growing in the vicinity (about 50 meters), therefore, it would be reasonable to conclude that most, if not all, the boll formation on these CMS plants would be the result of pollination due to pollen flown by wind or carried to CMS flowers by honey bees / other insects, from the transgenic test plot. The results obtained have shown distinct difference between the two locations. Whereas, some seed set (one boll / plant) was observed even in the last cotton trap line at Isarwadi, however, the flow of pollen, whether by bees or wind, was very low at Sirsa (Figure 1A & 1B). The results of this study very clearly indicate that pollen flow would vary from one location to another, depending upon the climatic conditions, crops in the vicinity (attracting bees) etc.

The conclusion from this study is that cotton pollen does flow to rather long distances, affecting pollinations, though at a low frequency. Obviously, therefore, Bt transgenes will be carried to any neighboring population of cotton. However, since safety issues have been addressed comprehensively, such transgene escape should not be a cause of concern in terms of bio-safety. As regards possibility of transfer to other *Gossypium* species, the two 'Desi' cottons (*G. arboreum* and *G. herbaceum*) are both diploids (whereas *G. hirsutum* and *G. barbadense* are tetraploids) and, therefore, gene flow to these two diploid species is not possible due to sexual and genomic incompatibility barriers.


Pollen Flow Study in Cotton at Sirsa (North Zone)
Five Rows of CMS based (NBt) Pollen Trap Lines Spaced 1.0 m
North
5 m Gap
35.8 m
Bt Transgenic Area
South
40m
55.8
Five Rows of CMS based (NBt) Pollen Trap Lines Spaced 1.0 m
72m


Pollen Flow Study in Cotton at Isarwadi, Aurangabad
Five Rows of CMS Based (NBt) Pollen Trap Lines Spaced 0.9 m
South
5 m Gap
103.4m
Bt Transgenic Area
5 m gap
18m
122.4
Five Rows of CMS based (NBt) Pollen Trap Lines Spaced 0.9 m
North
37m

Pictures of the Contained Open Field Trial of various Bt-cotton test hybrids at the GTL research farm, Isarwadi, near Aurangabad.

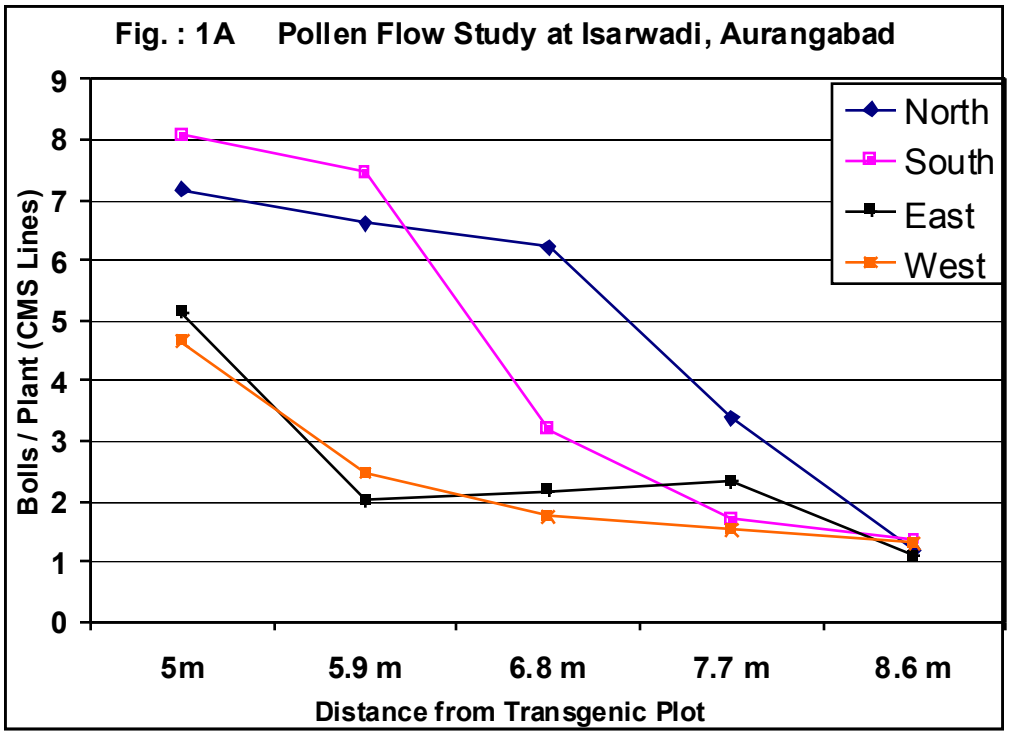


Fig. : 1A Pollen Flow Study at Isarwadi, Aurangabad
Bolls / Plant (CMS Lines)
0
1
2
3
4
5
6
7
8
9
5m
5.9 m
6.8 m
7.7 m
8.6 m
Distance from Transgenic Plot
North
South
East
West

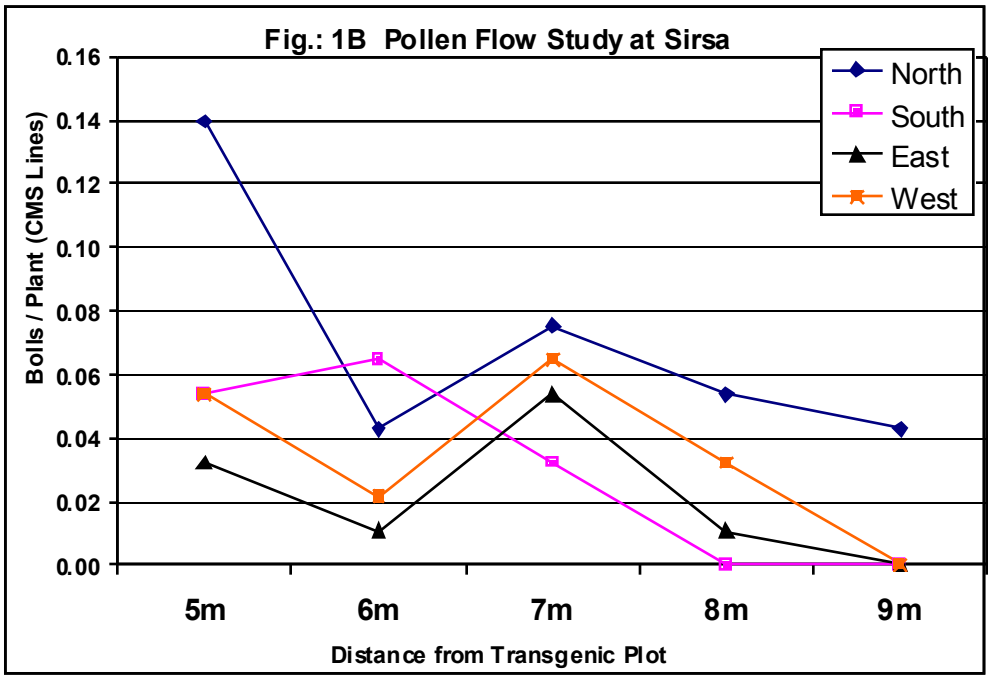


Fig.: 1B Pollen Flow Study at Sirsa
Bolls / Plant (CMS Lines)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
5m
6m
7m
8m
9m
Distance from Transgenic Plot
North
South
East
West