Table 1. The SSA at Kanpur during the week of the Diwali festival

<table>
<thead>
<tr>
<th>Days from Diwali</th>
<th>440 nm</th>
<th>675 nm</th>
<th>875 nm</th>
<th>1020 nm</th>
<th>440 nm</th>
<th>675 nm</th>
<th>875 nm</th>
<th>1020 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>0.89</td>
<td>0.883</td>
<td>0.866</td>
<td>0.861</td>
<td>0.909</td>
<td>0.861</td>
<td>0.855</td>
<td>0.85</td>
</tr>
<tr>
<td>0</td>
<td>0.917</td>
<td>0.913</td>
<td>0.912</td>
<td>0.91</td>
<td>0.869</td>
<td>0.867</td>
<td>0.85</td>
<td>0.874</td>
</tr>
<tr>
<td>1</td>
<td>0.902</td>
<td>0.88</td>
<td>0.872</td>
<td>0.865</td>
<td>0.85</td>
<td>0.843</td>
<td>0.805</td>
<td>0.855</td>
</tr>
<tr>
<td>2</td>
<td>0.875</td>
<td>0.874</td>
<td>0.852</td>
<td>0.846</td>
<td>0.852</td>
<td>0.804</td>
<td>0.849</td>
<td>0.859</td>
</tr>
<tr>
<td>3</td>
<td>0.872</td>
<td>0.87</td>
<td>0.865</td>
<td>0.85</td>
<td>0.855</td>
<td>0.771</td>
<td>0.828</td>
<td>0.842</td>
</tr>
<tr>
<td>4</td>
<td>0.90</td>
<td>0.899</td>
<td>0.894</td>
<td>0.891</td>
<td>0.863</td>
<td>0.81</td>
<td>0.853</td>
<td>0.861</td>
</tr>
</tbody>
</table>

**Absorbing characteristics** during 2001, the SSA is found to decrease by 0.05 after the Diwali festival and during 2002 the SSA is found to decrease by 0.07. The decrease of SSA is found from 15 November in 2001 (one day after the main Diwali day) and from 5 November in 2002 (one day after the main Diwali day), which is due to the higher concentrations of absorbing aerosols. The higher change in SSA after the Diwali festival during the year 2002 compared to those from the Diwali festival of the year 2001 may be attributed to the prevailing meteorological conditions and the background aerosol concentrations. The values of SSA at 440, 675, 875 and 1020 nm wavelengths are shown in Table 1. The decrease of SSA with higher wavelengths (Table 1) is due to the higher absorption by smaller particles. But during the year of 2002, SSA at 1020 nm is found to be higher compared to those at other wavelengths which is attributed to the large increase in the volume concentrations of the aerosols around 1 μm size range (Figure 2b) compared to the year 2001. The maximum decrease in SSA after the Diwali festival is found at 675 nm wavelength for both the years.

The increase of aerosols during the Diwali festival period, especially in urban areas is very common due to fireworks and crackers. Such increase in concentration of the aerosols has been found over Delhi. The present results show that the AOD increase during Diwali festival period is reflected in the size distribution curves. In view of the increasing population and the use of fireworks and crackers in major urban areas, the routine measurements of aerosols using sunphotometers will help in guiding people and local administrators for the use of fireworks and crackers during Diwali festival period and also in understanding the changing climatology of Indo-Gangetic basin.


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**Baseline resistance to Cry1Ac toxin in cotton bollworm, Helicoverpa armigera** (Hubner) in south Indian cotton ecosystem

Insecticidal protein genes coding for crystal (Cry) toxins of Cry1A group from the bacterium Bacillus thuringiensis (Bt) have been transferred to and expressed in a number of crops in order to confer resistance against lepidopteron insect pests. Several transgenic crop species incorporating cry1A genes have been commercialized and cultivated in a number of countries over the past few years. Bt transgenic cotton was cleared by the Department of Biotechnology, Government of India for commercial cultivation from the year 2002, after a long debate and discussion. The primary target pest of this technology in India and several other countries is the cotton bollworm, Helicoverpa armigera (Hubner). It is a polyphagous pest with a wide host range of 181 plant species including cotton, pigeonpea, tomato, chickpea, maize, sun-
flower and several vegetable crops which is currently estimated to cause economic losses up to about Rs 250 billion in India.4 Lately, the problems of pest management in cotton and other crops have been compounded by the development of resistance to insecticides in H. armigera.5 This led to excessive and indiscriminate use of insecticides by desperate farmers in many parts of the country. Outbreak of H. armigera in south Indian cotton and pigeonpea ecosystem usually leads to severe socioeconomical disturbances, including several cases of suicide by farmers. Introduction of insect-resistant transgenic crops, especially Bt transgenics, is expected to be useful in management and effective control of lepidopteran pests with a significant reduction in the overall use of insecticides. However, long-term exposure to Bt transgenic crops is likely to render lepidopteran pests resistant to the Cry toxins due to continuous selection pressure.5 Moreover, the introduction of transgenic plants expressing a Cry toxin under the influence of constitutive promoters, is likely to hasten this process. The development of resistance to Bt toxins can be quite distinct, depending upon the species, selection regime or geographical origin of the founder colony. Hence, initial survey to assess the susceptibility of test insect to the Cry toxins will establish a baseline that can be used in monitoring resistance development in future. We report the resistance of H. armigera to Cry1Ac toxin in 11 distinct geographic populations representing the entire south Indian cotton ecosystem.

The Cry1Ac protein was produced according to the method in Albert et al.6 from E. coli strain containing hyperexpressivity recombinant plasmid vector pKK223, kindly provided by Daniel R. Zeigler, Ohio State University, USA. The toxin was purified from over-expressing cells by sonication and extensive washing with sodium bromide. Proteins were quantified according to Lowry et al.7 and the toxin was quantified by SDS-PAGE densitometry before preparing dilutions (ranging from 10 to 20000 fold) in distilled water.8 Forty per cent of the protein extracted from the recombinant E. coli cultures was found to comprise Cry1Ac toxin. LC50 values were determined for the toxin.

Laboratory strains of H. armigera were established from those collected in cotton fields during cropping season of 2001–02 from major cotton-growing regions of south Indian cotton ecosystem: Nagpur and Nanded (Maharashtra); Guntur, Madhira and Nalgonda (Andhra Pradesh); Dharwad, Raichur and Mysore (Karnataka); Coimbatore, Madurai and Kollipatti (Tamil Nadu). These 11 sampling locations represent the cotton growing ecosystems of south India (Figure 1). An insecticide-susceptible H. armigera obtained from ICRISAT, Patancheru, Hyderabad was used as baseline susceptible strain for comparison. Larvae were reared individually on chickpea-based semi-synthetic diet, in 32-well multivacity trays till pupation. Moths were kept in glass jar at 27°C ± 1°C and 70% RH and fed with 10% honey solution. A layer of muslin cloth was placed on the inner surface of jar for oviposition.

Laboratory cultures were established for each population of 500–650 moths and reared to get homogenous F1 populations before conducting bioassays. Bioassays were carried out in 32-well multivacity culture trays. Six-day-old juvenile larvae (ca 30–40 mg) were tested, one per well, on cotton leaves dipped in different concentrations of the toxin. In all, 30 larvae in three replicates were tested for each treatment. Mortality was recorded daily for six days. All assays were repeated three times and pooled data were subjected to statistical analysis. Assays were performed in the laboratory at 27°C ± 1°C and 70% RH. Median lethal concentrations (LC50) presented in Table 1 were derived from log dose probit calculations12 using MLP 0.38 statistical package.13 Cry1Ac protein was found to be toxic to all geographic populations tested (Table 1). The insecticide-susceptible ICRISAT laboratory strain was the most susceptible. Compared with the others, geographic populations from Nagpur, Nanded, Guntur, Nalgonda, Madhira and Raichur were found tolerant to the toxin. Mortality of different populations is presented in Table 1. LC30 values for Cry1Ac ranged from 0.147 to 1.095 μg/ml. The fiducial limits (at P = 0.95) of the probit assay data indicated that there was a good deal of variability in response of different populations to Cry1Ac. Kollipatti (Tamil Nadu; southern most part of south Indian cotton ecosystem) population was found to be as susceptible as the laboratory strain for Cry1Ac. Coimbatore and Dharwad populations were similar to each other at a resistance factor (RF) of 1.5. Geographic populations of Guntur and Nanded recorded highest RF 8.03 and 8.42, respectively. The LC30 values of the test populations can be considered as the baseline susceptibility LC30 values for these individual populations and used for monitoring resistance in future.

For resistance management programmes to be effective, monitoring, surveillance and early detection of resistant pheno-

![Figure 1. Geographic distribution of cotton bollworm populations in four states of south Indian cotton ecosystem used in the present investigation.](image-url)
Table 1. Log dose probit response of south Indian cotton ecosystem field populations of *H. armigera* to Cry1Ac toxin

<table>
<thead>
<tr>
<th>Location</th>
<th>LC50 (µg/ml)</th>
<th>Lower</th>
<th>Upper</th>
<th>Slope</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICRISAT^1</td>
<td>0.130^1</td>
<td>0.080</td>
<td>0.180</td>
<td>1.15</td>
<td>1.00</td>
</tr>
<tr>
<td>Nagpur</td>
<td>0.927^1</td>
<td>0.688</td>
<td>1.087</td>
<td>4.43</td>
<td>7.131</td>
</tr>
<tr>
<td>Nanded</td>
<td>1.095^1</td>
<td>1.032</td>
<td>1.479</td>
<td>4.13</td>
<td>8.423</td>
</tr>
<tr>
<td>Guntur</td>
<td>1.044^1</td>
<td>0.980</td>
<td>1.154</td>
<td>8.67</td>
<td>8.031</td>
</tr>
<tr>
<td>Nalgonda</td>
<td>1.001^1</td>
<td>0.909</td>
<td>1.204</td>
<td>4.99</td>
<td>7.700</td>
</tr>
<tr>
<td>Madhira</td>
<td>0.927^1</td>
<td>0.688</td>
<td>1.087</td>
<td>4.43</td>
<td>7.131</td>
</tr>
<tr>
<td>Raichur</td>
<td>0.884^1</td>
<td>0.647</td>
<td>0.975</td>
<td>4.57</td>
<td>6.800</td>
</tr>
<tr>
<td>Dharwad</td>
<td>0.191^1</td>
<td>0.094</td>
<td>0.277</td>
<td>1.07</td>
<td>1.469</td>
</tr>
<tr>
<td>Mysore</td>
<td>0.260^1</td>
<td>0.121</td>
<td>0.408</td>
<td>0.87</td>
<td>2.000</td>
</tr>
<tr>
<td>Coimbatore</td>
<td>0.191^1</td>
<td>0.094</td>
<td>0.277</td>
<td>1.07</td>
<td>1.469</td>
</tr>
<tr>
<td>Madurai</td>
<td>0.177^1</td>
<td>0.061</td>
<td>0.302</td>
<td>0.77</td>
<td>1.362</td>
</tr>
<tr>
<td>Kovilpatti</td>
<td>0.147^1</td>
<td>0.057</td>
<td>0.306</td>
<td>0.54</td>
<td>1.131</td>
</tr>
</tbody>
</table>

^1Test for heterogeneity; ^2significant at 5% level of significance. LC50 values designated by different letters are significantly different from each other through non-overlap of 95% fiducial limits. LC50, Median lethal concentrations expressed in µg/ml diet; FL, Fiducial Limits; RF, Resistant Factors calculated using ICRISAT strain as susceptible reference.

much of its success will depend on the sustained susceptibility of the target pests to the *Bt* toxins used in transgenic crops. *Bt* transgenic crops, which express Cry1Ac, were found to cause 100% and 75–90% mortality in susceptible *H. virescens* and *H. zea* respectively, in the US^20^ The same level of expression caused less than 90% mortality of *H. armigera* and *H. punctigera* in Australia^21^, indicating that *Helicoverpa* species appears to have certain level of tolerance to the *Bt* toxin, Cry1Ac, whether or not previously exposed to *Bt* toxin, when compared with the *Heliothis* species. It is important to note in this study that a few individuals of *H. armigera* in almost all the populations tested were found to survive even the highest concentrations of Cry1Ac. Under field conditions, tolerant individuals are likely to persist despite high expression of the Cry1A toxins and may subsequently contribute to the resistant gene pool. Daly^22^ reported that transgenic cotton plants in Australia killed susceptible larvae early in the season but the effect significantly declined later (95–100 days after sowing), when an increasing proportion of first instar larvae placed on transgenic leaves survived to late instars. The studies on *Bt* cotton in the USA and Australia have shown that Cry1Ac protein production decreased over the growing season and that the bio-efficacy of the protein was reduced by interaction with increasing levels of secondary plant metabolites^23,24^.

Differential expression in plant tissues may contribute towards a reduced efficacy of the *Bt* transgenic crops. If proper resistance management strategies are not implemented, the efficacy of pest management through *Bt* transgenic crops will be severely diminished due to widespread development of resistance. Such strategies have not yet been developed for the small farmer and predominantly non-irrigated cotton growing systems found in India and elsewhere.

4. Manjunatha, T. M., Bhatnagar, V. S., Pawan, C. S. and Sibhanatham, S., Pro-
Restoration of *Eremostachys superba* Royle ex Benth. – a critically endangered species

The Botanical Survey of India was the first to report the threatened status of *Eremostachys superba*, a member of Lamiaceae in the *Indian Plant Red Data Book*. This report may have gone unnoticed if Rao and Garg had not raised an alarm regarding the further deteriorating status of the species at Mohand (Dehradun), its type locality in 1994 and highlighted the need for conservation measures. The highly endangered nature of *Eremostachys superba* was also highlighted by the press.

As a follow-up of Rao and Garg’s report, we initiated extensive explorations of Jammu Shivaliks in 1996, as the species was also reported from this area. Up to 2001 we located five populations of the species containing about 1,300 (ref 6) individuals. In April 2003, one large population of approximately 2,000 individuals was discovered in Sunderbani (District Rajouri) area about 80 km in the north-west of Jammu. The latest discovery has swollen the number to a total of 3,300 plants in nature. This report reviews the present status of the species to indicate its restoration.

One of the reasons for reduction in plant number is the use of root tubers for curing mastitis and restoration of milching process in cattle, including cows and buffaloes (Figure 1a, b). The other cause is low fruit and seed set largely due to pollinator limitation.

In view of the magnitude of threat which the species is facing and its status, a number of measures were tried for its conservation. We have been able to raise plants *ex situ* from seeds and tubers collected from their natural habitat in the University Conservatory. At present more than 1,000 plants have been raised (Figure 1c).

**ACKNOWLEDGEMENT.** We thank Dr Daniel R. Zeigler, Ohio State University, US for providing the Cry1Ac over-expressing clone. This research work was supported by the DBT, GOI project grants to BF. Thanks are also due to Department of Agricultural Entomology, UAS Dharwad for providing infra-structure facilities and all those scientists who helped us during the visit to different locations across south India for *H. armigera* collection. We thank Dr G. T. Gujar, IARI, New Delhi, for his useful suggestions during the study and encouragement.

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