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Baseline sensitivity of brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenée) in South India to Cry1Ac insecticidal protein of *Bacillus thuringiensis*

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Studies were carried out to determine the baseline sensitivity of the brinjal shoot and fruit borer, *Leucinodes orbonalis* (Guenée) to Cry1Ac insecticidal protein of *Bacillus thuringiensis* (*Bt*) by a diet incorporation method for the populations collected from different locations in South India. A total of 14 districts from five South Indian states (Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu and Goa) were sampled during 2009 and 2010 cropping seasons to understand the spatial baseline sensitivity. Median lethal concentrations (LC_{50}) ranged between 0.020 and 0.042 ppm and moult inhibitory concentration (MIC_{50}) values for *L. orbonalis* ranged from 0.003 to 0.014 ppm for 14 populations across two seasons. The overall variability in the sensitivity was 1–4-fold between the study locations. These benchmark values will be referenced while monitoring resistance to Cry1Ac provided *Bt* brinjal hybrids expressing Cry1Ac are approved for commercial cultivation in India.

Keywords: *Bacillus thuringiensis*, baseline sensitivity, brinjal, Cry1Ac endotoxin, *Leucinodes orbonalis*.

BRINJAL, *Solanum melongena* (family Solanaceae), is a widely cultivated common man's vegetable in India. Indian people annually consume between 8 and 9 million metric tonnes of brinjal which is grown on > 500,000 ha. In spite of its popularity among small and resource-poor farmers, brinjal cultivation is often input-intensive, especially for insecticide applications. Like any other solanaceous vegetables, brinjal has a diverse pest complex, but the most serious is the shoot and fruit borer (SFB), *Leucinodes orbonalis* (Guenée) (family: Pyralidae). The pest poses a serious problem because of its high reproductive potential, rapid turnover of generations and intensive cultivation of brinjal both in wet and dry seasons of the year. The larva confines its feeding activities on the shoot in the early stages of crop causing wilting and dieback of the branch terminals, which reduces the fruit-bearing capacity of the plant, and later, on the fruits which become unfit for human consumption[1]. Fruit feeding is the major cause of damage. It feeds on brinjal shoots and

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fruits throughout the larval period, followed by pupation inside the shoot/fruit. Hence, preventing injury by this insect depends on the difficult task of using well-timed chemical insecticide applications before the eggs hatch into larvae that bore into the shoot or fruits of the plant[2]. The infestation rate and yield loss varies from season to season and location to location. SFB larvae bore into tender shoots and fruits, retarding plant growth and causing fruit damage as high as 92% (refs 3 and 4).

The genetic plasticity of *L. orbonalis* is apparent in its ability to detoxify many synthetic insecticides in addition to the secondary plant metabolites present in its narrow array of host plant species. In response to the stresses, the populations of *L. orbonalis* have adapted to the insecticide applications by changing their eco-behavioural pattern, feeding physiology and reproduction[5]. This in turn led to excessive and indiscriminate use of insecticides (spraying upwards of 40 times per season) by desperate farmers in many parts of the country[2]. Despite the concerted efforts, *L. orbonalis* remains a major recurring pest requiring considerable control, and there is little prospect of change in the immediate future unless novel approaches are adopted. Transgenic expression of *Bacillus thuringiensis* endotoxins (*Bt* Cry proteins) in several plant species has been proven to be effective against lepidopteran insect pests, including *L. orbonalis*[6]. Genetically engineered brinjal hybrids expressing *B. thuringiensis* Berliner insecticidal proteins offer an alternative strategy for the control of this tissue-boring pest and can also benefit the environment by reducing the application of conventional chemical insecticides[7].

Following the first commercial approval in 2002, the use of *Bt* cotton has quickly spread, with planting on 9.40 m ha in 2010 in India[8]. *Bt* technology has the potential to provide similar value in brinjal against brinjal borer pest in India. It has been demonstrated that Cry1Ac protein in *Bt* brinjal can control SFB in India under greenhouse conditions[9].

However, if *Bt* brinjal is to be introduced into India, an appropriate plan must be developed to manage the risk of target pest evolving resistance. Regular monitoring of temporal and spatial baseline susceptibility changes and resistance after commercialization is an essential part of such a resistance management strategy for any *Bt* crop[10–13]. The first and most important step in resistance monitoring is to generate baseline susceptibility data before commercialization with the *Bt* protein expressed by the *Bt* crop.

Hence, a benchmark study was undertaken in 2009 and 2010 seasons to screen geographical populations of *L. orbonalis* from 14 locations of South India representing five states (Figure 1) for susceptibility to Cry1Ac protein in laboratory bioassays.

During kharif season of 2009 and 2010, *L. orbonalis* larvae were collected from Kolhapur (Maharashtra), Parbhani (Maharashtra), Dapoli (Maharashtra), Hyderabad (Andhra Pradesh), Vijayawada (Andhra Pradesh), West Godavari (Andhra Pradesh), Raichur (Karnataka), Dharwad (Karnataka), Bangalore (Karnataka), Bijapur (Karnataka), Coimbatore (Tamil Nadu), Madurai (Tamil Nadu), Aduthurai (Tamil Nadu) and Margao (Goa; Figure 1).

Field-collected larvae (F_0), along with brinjal fruit/shoot, were transported to the insect laboratory of the Main Agricultural Research Station, University of Agricultural Sciences (UAS), Raichur. The larvae were transferred onto freshly prepared semi-synthetic diet and allowed to pupate. Pupae were collected, surface-sterilized in 0.1% sodium hypochlorite, air-dried at room temperature and kept in separate jars for moth emergence.

Twenty-five pairs of freshly emerged adults were transferred to each oviposition cylindrical chamber (30 cm height and 15 cm diameter, lined with rough blue paper and single layer of 16-mesh nylon netting). Four-week-old potted eggplant seedling was placed inside the cylinder. Soil in the plastic pot was covered with aluminum foil and cotton swab dipped in 50% honey was placed next to the seedling in the oviposition cage. The top of the chamber was covered with purple paper and nylon net was secured with an elastic band. Oviposition chamber was placed in a room at 26–30°C and 65–70% relative humidity (RH).

After four days and daily thereafter, the oviposition chamber was examined for eggs on both the nylon netting and purple paper. Eggs were collected and kept for hatching at 26°C and 70% RH. The diet for *L. orbonalis* consisted of black gram flour 140 g, yeast 13 g, sucrose 20 g, agar–agar (powder) 20 g, methylparahydroxybenzoate 2.5 g, sorbic acid 1 g, ascorbic acid 4.3 g, formaldehyde

Figure 1. Sampling locations in South India for the collection of brinjal shoot and fruit borer populations.

Table 1. Median lethal concentration (LC, µg of Cry1Ac ml^{-1} diet) of brinjal shoot and fruit borer populations collected from brinjal-growing districts of South India during 2009–2010 to Cry1Ac protein bioassays

Population	χ^2	Slope ± (95% CI)	LC_{50} (95% CI)	LC_{95} (95% CI)
Dharwad, KA	6.12	1.10 ± 0.05	0.031^{b} (0.020–0.058)	1.052^{ab} (0.524–1.842)
Bijapur, KA	8.48	1.04 ± 0.08	0.034^{bc} (0.025–0.046)	1.234^{ab} (0.724–1.045)
Bangalore, KA	7.93	1.12 ± 0.12	0.038^{bc} (0.030– 0.060)	1.643^{bc} (0.776–2.365)
Raichur, KA	5.69	1.10 ± 0.10	0.042^{c} (0.024–0.048)	1.829^{c} (0.627–2.127)
West Godavari, AP	6.62	1.20 ± 0.08	0.032^{b} (0.032–0.076)	1.156^{ab} (0.825–2.628)
Hyderabad, AP	9.79	1.14 ± 0.10	0.026^{b} (0.016–0.040)	0.721^{a} (0.442 –1.088)
Vijayawada, AP	6.76	1.16 ± 0.07	0.030^{b} (0.022–0.052)	0.823^{ab} (0.783–1.130)
Chennai, TN	7.40	1.18 ± 0.06	0.036^{bc} (0.024–0.074)	1.476^{b} (0.862–2.484)
Aduthurai, TN	8.24	1.10 ± 0.14	0.028^{b} (0.020–0.058)	0.883^{ab} (0.556–1.372)
Coimbatore, TN	6.65	1.12 ± 0.12	0.038^{bc} (0.032–0.082)	1.620^{bc} (0.962–2.178)
Margao, Goa	9.17	1.04 ± 0.10	0.020^{a} (0.018–0.042)	0.529^{a} (0.324–0.984)
Kolhapur, MH	8.15	1.08 ± 0.04	0.032^{b} (0.022– 0.068)	1.142^{ab} (0.864–1.587)
Dapoli, MH	7.32	1.12 ± 0.06	0.030^{bc} (0.022–0.048)	0.987^{ab} (0.265–1.478)
Parbhani, MH	7.41	1.00 ± 0.08	0.029^{b} (0.018–0.062)	0.868^{ab} (0.478–1.529)

LC_{50}: Concentration of Cry1Ac that killed 50% of test larval population in the observation period of 7 days.
LC_{95}: Concentration of Cry1Ac that killed 95% of test population. KA, Karnataka; AP, Andhra Pradesh; TN, Tamil Nadu; MH, Maharashtra. LC_{50} and LC_{95} values followed by different lower case letters for each population are significantly different from each other ($P < 0.05$); $n = 630$.

(10%) 2 ml, ethanol 5 ml, Wesson's salt 2.5 g, multivitamin mix 10 ml, brinjal fruit powder 100 g and distilled water 1100 ml. Bioassays were conducted with the F_1 generations.

Commercial formulation MVP II, which contained 19.7% (by weight) Cry1Ac protein was obtained from Monsanto Research Centre (MRC), Bangalore. Cry1Ac protein was assayed by the diet-incorporation method using seven graded concentrations. The insect diet was prepared using the ingredients listed in above, poured into sterile glass bottles and kept warm in a water bath maintained at 60°C. A volume of 111 µl of the primary stock was serially diluted for assaying against *L. orbonalis.* Protein concentrations in the assay ranged from 1.00 to 0.001 ppm. Diet with protein was mixed using a mixer and poured into 25-well insect bioassay trays at 750 µl per well. Newly hatched larvae were transferred onto the solidified diet in the bioassay trays at 1 larva $well^{-1}$ using a fine-hair brush. On completion of larval transfer, bioassay trays were covered with pull-n-peel tabs. Preparation of bioassay trays and inoculation of neonates were carried out on a laminar-flow clean bench. Infested trays were kept in a BOD incubator maintained at 27 ± 0.5°C. Thirty larvae were screened at each toxin concentration along with an untreated control. All assays were repeated three times with each population. Thus, for each population a total of 630 larvae were exposed.

Bioassays were rated after 7 days, and observations of mortality, stage of the surviving larvae and group weight of all the surviving larvae were recorded. Probit analysis of the data was carried out using Finney's method[14] to compute LC_{50}, LC_{95}, MIC_{50} and MIC_{95} for each population. The dose-response values were expressed as micrograms of Cry1Ac/ml of diet (= parts per million, ppm).

Cry1Ac protein was found to be toxic to all geographic populations tested (Tables 1 and 2). In the 2009 kharif season, LC_{50} for neonates ranged from 0.020 to 0.042 ppm, with the population from Margao having the lowest LC_{50} value and that from Raichur having the highest LC_{50} value (Table 1). LC_{95} values ranged from 0.529 to 1.829 ppm across the populations. MIC is a dose that prevents larvae from moulting to the second instar within the observation period of 7 days. The MIC_{50} values for *L. orbonalis* ranged from 0.003 to 0.014 ppm, and MIC_{95} values ranged from 0.028 to 0.145 ppm across populations (Table 2). The LC_{50} and MIC_{50} values (Tables 1 and 2) of the Raichur population were higher than those of the populations from other locations.

In the 2010 kharif season, the LC_{50} values of neonates ranged from 0.022 to 0.040 ppm and LC_{95} values ranged from 0.610 to 1.742 ppm across the 14 populations. The MIC_{50} values ranged from 0.003 to 0.012 ppm, and the MIC_{95} values ranged from 0.032 to 0.130 ppm (Tables 3 and 4), indicating a similar trend in the sensitivity of geographical population as in 2009. Dose response values of *L. orbonalis* population from Raichur were higher than those of the populations from other locations.

Geographic populations of *L. orbonalis* in South India showed susceptibility to Cry1Ac, with some natural variation among the populations. The overall variability in the sensitivity was 1–4-fold between the study locations without any location-specific pattern of dose–response values due to the lack of history of exposure to Cry1Ac protein.

The concentration–response values generated in this study can form benchmark values for post-commercialization monitoring of resistance to the *in planta* produced Cry1Ac. Earlier, workers from India had reported

Table 2. Median lethal concentration (LC, µg of Cry1Ac ml^{-1} diet) of brinjal shoot and fruit borer populations collected from brinjal-growing districts of South India during 2010–2011 to Cry1Ac protein bioassays

Population	χ^2	Slope ± (95% CI)	LC_{50} (95% CI)	LC_{95} (95% CI)
Dharwad, KA	7.13	1.04 ± 0.10	0.028^{ab} (0.018–0.042)	0.872^{ab} (0.428–2.140)
Bijapur, KA	6.42	1.03 ± 0.06	0.032^{ab} (0.022–0.068)	1.228^{ab} (0.720–2.240)
Bangalore, KA	8.23	1.14 ± 0.05	0.035^{b} (0.028–0.076)	1.360^{b} (0.630–1.818)
Raichur, KA	9.28	1.18 ± 0.10	0.040^{c} (0.028–0.074)	1.742^{c} (0.628–2.523)
West Godavari, AP	5.72	1.08 ± 0.12	0.026^{ab} (0.020–0.046)	0.762^{ab} (0.452–1.244)
Hyderabad, AP	7.63	1.07 ± 0.07	0.029^{ab} (0.024–0.064)	0.946^{ab} (0.710–1.950)
Vijayawada, AP	6.10	1.09 ± 0.08	0.031^{ab} (0.020–0.052)	1.146^{ab} (0.432–1.268)
Chennai, TN	8.21	1.18 ± 0.10	0.034^{b} (0.026–0.058)	1.386^{b} (0.728–1.382)
Aduthurai, TN	9.10	1.09 ± 0.12	0.030^{ab} (0.020–0.046)	1.046^{ab} (0.439–1.436)
Coimbatore, TN	8.73	1.16 ± 0.06	0.036^{b} (0.028–0.068)	1.543^{bc} (0.546–2.340)
Margao, Goa	6.00	1.04 ± 0.08	0.022^{a} (0.018–0.042)	0.610^{a} (0.373–0.852)
Kolhapur, MH	9.23	1.10 ± 0.14	0.030^{ab} (0.024–0.065)	0.973^{ab} (0.428–1.342)
Dapoli, MH	6.73	1.00 ± 0.07	0.027^{ab} (0.022–0.040)	0.764^{ab} (0.431–1.168)
Parbhani, MH	8.42	1.08 ± 0.08	0.031^{ab} (0.026–0.069)	1.272^{ab} (0.563–1.136)

Footnote: Same as Table 1.

Table 3. Moult inhibitory concentration (MIC, µg of Cry1Ac ml^{-1} diet) of brinjal shoot and fruit borer populations collected from brinjal-growing districts of South India during 2009–2010

Population	χ^2	Slope ± (95% CI)	MIC_{50} (95% CI)	MIC_{95} (95% CI)
Dharwad, KA	9.14	1.27 ± 0.08	0.011^{bc} (0.003–0.016)	0.102^{bc} (0.0823–0.1561)
Bijapur, KA	7.89	1.17 ± 0.10	0.007^{b} (0.004–0.009)	0.080^{b} (0.0621–0.1268)
Bangalore, KA	8.32	1.21 ± 0.09	0.010^{bc} (0.007–0.012)	0.095^{bc} (0.0812–0.4121)
Raichur, KA	9.36	1.36 ± 0.12	0.014^{c} (0.005–0.020)	0.145^{c} (0.0908–0.1612)
West Godavari, AP	6.57	1.22 ± 0.06	0.005^{ab} (0.003–0.008)	0.061^{ab} (0.0428–0.0891)
Hyderabad, AP	7.59	1.14 ± 0.09	0.007^{b} (0.005–0.010)	0.091^{bc} (0.0734–0.1382)
Vijayawada, AP	8.67	1.33 ± 0.07	0.009^{bc} (0.005–0.011)	0.098^{bc} (0.0759–0.1469)
Chennai, TN	4.27	1.15 ± 0.08	0.005^{ab} (0.004–0.007)	0.045^{ab} (0.0310–0.0789)
Aduthurai, TN	6.24	1.14 ± 0.06	0.004^{ab} (0.002–0.006)	0.037^{ab} (0.0218–0.0590)
Coimbatore, TN	7.28	1.13 ± 0.07	0.006^{ab} (0.004–0.007)	0.062^{ab} (0.0456–0.0872)
Margao, Goa	5.33	1.06 ± 0.06	0.003^{a} (0.001–0.005)	0.028^{a} (0.0170–0.0428)
Kolhapur, MH	8.84	1.31 ± 0.10	0.012^{bc} (0.006–0.025)	0.113^{bc} (0.0810–0.1561)
Dapoli, MH	7.52	1.12 ± 0.08	0.008^{bc} (0.006–0.010)	0.084^{b} (0.0632–0.1249)
Parbhani, MH	6.79	1.08 ± 0.06	0.004^{ab} (0.003–0.006)	0.033^{a} (0.0242–0.0572)

MIC_{50}: Concentration of Cry1Ac that inhibited moulting of 50% of test larval population into the second instar in the observation period of 7 days. MIC_{95}: Concentration of Cry1Ac that inhibited moulting of 95% of test population. KA, Karnataka; AP, Andhra Pradesh; TN, Tamil Nadu; MH, Maharashtra. MIC_{50} and MIC_{95} values followed by different lower-case letters for each population are significantly different from each other ($P < 0.05$); $n = 630$.

significant activity of Cry1Ac against first instars of *L. orbonalis.* Baseline susceptibility data revealed 12-fold variability in LC_{50} value of 29 populations tested for Cry1Ac susceptibility[9]. The field populations demonstrated 70-fold inter-population variation in MIC_{50} values. The variability was 14-fold when MIC_{95} was considered and values ranged from 0.020 to 0.138 ppm of diet. Average MIC_{95} is found to be 0.059 ppm (ref. 15). The three crystal proteins CryIB, CryIA and CryIIA with the highest toxicity to eggplant fruit and shoot borer larvae were considered to be prime candidates for transgenic eggplant studies[16]. Efficacy of seven lepidopteran-specific delta-endotoxins of *Bt* was studied against the second instar larvae of the brinjal shoot and fruit borer[17]. Insect bioassay was done by coating the *Bt* toxins onto the modified semisynthetic diet for *L. orbonalis*. Larval mortality recorded every 24 h with the final mortality on the fourth day revealed that Cry2Aa protein was the most potent toxin tested followed by Cry1C, Cry1Ac, Cry1Ab and Cry1B in descending order[17].

Of the dose-response parameters estimated in this study, MIC is the best indicator of 'functional mortality' because neonates that are unable to progress to the second instar within 7 days in bioassays are likely to die under field conditions without damaging the plants any further[18]. Therefore, comparing a parameter such as the MIC_{95} to *in planta* concentrations of Cry1Ac is a fair approach for predicting the potential effectiveness of *Bt* brinjal hybrids.

In India, shoot and fruit borer-resistant *Bt* brinjal was developed in 2007 by the Maharashtra Hybrid Seeds Company (Mahyco) using a transformation process

Table 4. Moult inhibitory concentration (MIC, µg of Cry1Ac ml^{-1} diet) of brinjal shoot and fruit borer populations collected from brinjal-growing districts of South India during 2010–2011

Population	χ^2	Slope ± (95% CI)	MIC_{50} (95% CI)	MIC_{95} (95% CI)
Dharwad, KA	9.18	1.19 ± 0.09	0.010^{bc} (0.006–0.012)	0.098^{bc} (0.0765–0.1439)
Bijapur, KA	8.24	1.15 ± 0.07	0.009^{bc} (0.006–0.010)	0.084^{b} (0.0645–0.1292)
Bangalore, KA	9.27	1.28 ± 0.10	0.011^{bc} (0.007–0.016)	0.110^{bc} (0.0767–0.1857)
Raichur, KA	9.33	1.33 ± 0.09	0.012^{c} (0.008–0.018)	0.130^{c} (0.0942–0.1672)
West Godavari, AP	6.34	1.22 ± 0.06	0.004^{ab} (0.002–0.007)	0.064^{ab} (0.0368–0.0721)
Hyderabad, AP	7.82	1.18 ± 0.08	0.006^{ab} (0.004–0.009)	0.070^{ab} (0.0487–0.1074)
Vijayawada, AP	8.29	1.28 ± 0.09	0.008^{b} (0.006–0.010)	0.090^{bc} (0.0721–0.1352)
Chennai, TN	5.67	1.12 ± 0.06	0.005^{ab} (0.004–0.008)	0.062^{ab} (0.0392–0.912)
Aduthurai, TN	6.54	1.16 ± 0.06	0.003^{a} (0.002–0.005)	0.032^{a} (0.0194–0.0628)
Coimbatore, TN	7.28	1.19 ± 0.08	0.007^{ab} (0.004–0.008)	0.084^{b} (0.0608–0.1168)
Margao, Goa	6.23	1.06 ± 0.06	0.004^{ab} (0.002–0.006)	0.055^{ab} (0.0327–0.0789)
Kolhapur, MH	8.79	1.20 ± 0.10	0.009^{bc} (0.006–0.014)	0.089^{bc} (0.0613–0.1240)
Dapoli, MH	6.67	1.12 ± 0.06	0.007^{ab} (0.005–0.012)	0.079^{b} (0.0542–0.0971)
Parbhani, MH	6.12	1.10 ± 0.07	0.005^{ab} (0.003–0.008)	0.045^{ab} (0.0267–0.0632)

Footnote: Same as Table 3.

similar to the one used in the development of *Bt* cotton using *cry1Ac* gene from the soil bacterium *Bt*, and a single copy elite event named EE-1 was selected for introgression into brinjal hybrids. A study of the reported tissue-concentrations of Cry1Ac in shoots and fruits of brinjal (EE-1 *Bt* event) and the highest upper fiducial limit of MIC_{95} estimated among South Indian populations of *L. orbonalis* in this study (0.161 and 0.167 ppm respectively, for 2009 and 2010 for Raichur population) indicates that EE-1 hybrids could provide effective management of *L. orbonalis*, the neonates of which primarily feed on the shoots and fruits. Thus, this technology could provide considerable value to Indian brinjal farmers[9].

Currently, frequent applications of insecticide is under practice to counter the menace of SFB, but normally the larvae of the borer escape insecticide sprays as they are concealed within shoots and fruits of brinjal. Hence growers tend to spray insecticides indiscriminately based on the visual presence of the borer pest, incurring additional financial cost for sprays and posing a serious risk to the health and safety of consumers due to unacceptable higher residues of pesticides. There are no confirmed sources of resistance in the crop germplasm against this monophagous pest to develop resistant cultivars through traditional plant breeding. Accordingly, a genetically modified brinjal (*Bt* brinjal) has been developed which has offered promise of sustainable management of SFB[19,20].

The amount of insecticides used against SFB was reduced by 80% with an overall insecticide reduction of 42% for the crop as indicated through the data on field trials submitted to GEAC by Mahyco, which would be a major achievement from the perspective of health and welfare of the Indian people[7].

Plants that are resistant to key pests should be the foundation for integrated pest management (IPM) and the introduction of *Bt* transgenic brinjal can be an important addition to the existing components of IPM[21].

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Deccan Traps-associated obsidian glass: a nuclear waste containment

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Alteration of obsidian collected from Osham Hill, Gujarat after treatment under hydrothermal-like conditions is compared with the naturally altered obsidian for its assessment as a nuclear waste glass. Experimental data have been obtained for ionic release, glass alteration and its retention in the residue. Geochemical evolution of obsidian shows partial to complete leaching of all the ions, but profusely of Si and Na ions. The ionic release is found in the order of Na > Si > K > Ca > Al = Mg > Fe > Mn > Ti. SEM-BSE images show distinct microstructures of smectite, montmorillonite and illite inside as well as outside the secondary layers, resulting from paragenesis of alteration products at various temperatures (100–300°C) and pressures (50, 250 and 1260 psi). It has been found that the octahedral cation occupancies of smectite are consistent with the dioctahedral smectite. The secondary layer composition shows retention for Si, Al and Mg ions, indicating their fixation in the alteration products, but remarkably high retention of Ti, Mn and Fe ions suggests release of a very small fraction of these elements into the solution. Devitrification of glass along the cracks, formation of spherulite-like structures, yellowish-brown palagonite, chlorite, calcite, zeolite and finally white-coloured clays that yielded after experiments, largely correspond to the minerals which are found in the residual soil profile (developed over fresh obsidian outcrops), formed as a result of weathering in the natural environment.

Keywords: Clay mineralogy, microtexture, neo-formed minerals, obsidian.

THE process of vitrification involves assimilation of nuclear waste into high-silica glass to develop a corrosion-resistant and highly durable matrix for its safe disposal in the geological repository. The heat generated due to decay of stored radionuclide raises the temperature; thus, hydrothermal-like conditions form within the glass matrix which causes release of radionuclide into the surrounding medium[1]. Prediction of glass dissolution rate on long-term basis requires an understanding of glass and environmental reactions[2]. In order to establish alteration mechanism and mineral paragenesis by inducing alteration in glass under hydrothermal-like conditions, Shrivastava *et al.*[3] discussed chemico-mineralogical attributes of surface layers and alteration products. Basaltic glass is considered as a natural analogue for the evaluation of the long-term stability of the nuclear waste[4]. However, good quality of obsidian that occurs in Osham Hill, Gujarat, India[5] is also considered as a potential natural analogue for long term stability of nuclear waste. In contrast to several studies focused on dissolution rate of basaltic and borosilicate glasses, corresponding studies on acid volcanic glasses (such as obsidian, rhyolite and impact glasses) are rare. Liritzis and Laskaris[6] used hydration rim and diffusion profiles to determine diffusion rates and mechanism as formed by hydration/diffusion during alterations in obsidian. However, complexity of phases due to variability in the properties is noted in case of obsidian collected from different locations[6]. To address these issues and to understand the chemico-mineralogical changes that occur at or near the surface, the present experimental study on obsidian was performed under hydrothermal-like conditions. Alteration mechanism is studied to assess its performance in the geological repository under hydrothermal-like conditions as sufficient water and radioactive heat is available in the system. The induced microtextural and mineralogical changes have

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