Tea industry is greatly influenced by abiotic factors such as climatic conditions (rainfall, temperature, humidity) and also by biotic factors (pests and diseases) (Owuor et al., 2006). Both these factors in some way or the other influence tea cultivation and production.

5.1. *Helopeltis* infestation and agro-climatic condition

The survey study on the degree of *Helopeltis* infestation in the different clones suggests that TV1 and TV23 clones are more susceptible as compared to S3A3 and Tinali clones. This may be because of the genetic makeup of these clones. *Helopeltis theivora* infestation on 4 different tea clones viz; TV1, TV23, S3A3, Tinali was studied for four flush of the year namely: 1st flush (March-April), second flush (May-June), third flush (July-Aug) and 4th flush (Sept-Oct). The winter season was not considered for the present study as during this period the leaves are not abundant due to pruning of the tea bushes. A significant positive correlation has been found between *Helopeltis* infestation and climatic conditions like temperature, rainfall and humidity. This is because the high temperature, rainfall and humidity facilitate growth of the pest in general.

The findings are in agreement with the earlier study on brinjal plant that temperature, rainfall and relative humidity are responsible for the incidence of pest
like leaf hopper and white fly (Mathur et al., 2012). Study conducted on neem plant for *Helopeltis antonii* pest incidence showed positive relation with relative humidity and rainfall (Onkarappa, 1993). In yet another study in Indonesia, *Helopeltis antonii* attack was found to be more during the humid rainy months of the year (Zeiss et al., 2001).

Composition of soil plays a vital role in plant body in making it resistant or more susceptible to the pests (Phelan, 1995). Plant disease or their severity solely lies on the type of soil (Surendramohan, 1995). Agricultural practices which causes soil nutrient imbalance can lower plant’s resistance to the pest (Magdoff et al., 2000). While on the other hand, an increase in the soil nutrient makes plants more vulnerable to the pests as the plant serves as food source for the pest (Pimental et al., 1989). Soil organic carbon has direct relationship with soil organic matter. Therefore, decrease in soil organic carbon will indicate decrease in soil organic matter which acts as store for all the available nutrients for the plant (Nandwa, 2001).

For plants, carbon and sulphur are important nutrients for their life cycle and are cycled in the soil between organic matter and plant available nutrients. Soils containing high organic matter donot suffer from sulphur deficiency as sulphur mineralizes from the organic matter. Thus, soil with low organic carbon suffers from sulphur(S) deficiency. Also from previous reports, it is quite evident that sulphur deficiency is indicative of nitrogen (N) deficiency as S and N enter together into the plant system to form protein complexes (Killham, 1994).

Potassium is a macronutrient which is required by the plant system in large amount. It helps in activating many enzymes and thus is very vital for synthesis of
protein and carbohydrate. It also helps in hydrating the plant. Potassium must be adequate for maintaining optimum levels of minerals in the plant system. On the other hand, very high potash suppresses the uptake of calcium, magnesium, silicon, sugars and amino acids (FAO et al., 2000).

In the present investigation soils collected from the area of non-infected clones of all the tea estates showed normal range of organic carbon while the soil samples from area of infected clones of all the tea estates showed fluctuations from their normal range (> 0.80) which was found to be statistically non-significant. Similar findings were found for soil potash (normal range is >100 ppm), sulphur (>40 ppm (parts per million)) for all the clones of all the tea estates studied. Thus our study indicate that the soil nutrient parameters are in the normal range in all the tea estates studied and has no significant effect on the degree of infestation.

Soil pH indicates balance between cation elements (calcium, magnesium, sodium etc.) and soil acidity. Acidic pH range of soil is essential for proper working of fertilizers while very low pH can reduce the availability of nutrient. Therefore, adjusting soil pH is a must for proper growth of the crop plant. (Bationo et al., 1998)

In the present study, pH of both the soils collected from all the 7 tea estates showed normal range (4.5-5.5) required for growth of tea plants. As such Pearson correlation coefficient for soil parameters and Helopeltis infestation showed no significant relationship.

5.2. Changes in biochemical parameters due to infestation

Significant decrease in the amount of total protein and carbohydrate due to Helopeltis infestation is observed in the present study. For the decrease in protein and
carbohydrate might be due to decrease in the rate of their synthesis and/or increase in
the rate of their degradation due to infestation stress. The result was in accordance
with the study of feeding of plants by aphidoidae (Miles, 1989) who reported that
insects draw nutrients from the host plant for their food.

The findings indicate that infestation of tea leaves by *Helopeltis theivora*
causes a significant increase in the oxidative enzymes viz; peroxidase (POX) and
polyphenol oxidase (PPO). The increase in the activities of these enzymes in the
leaves of tea plant might be necessary to counter the stress produced by *Helopeltis*
infestation. Both these oxidative enzymes play a role in defense mechanism by
oxidation of phenolic compounds to quinones (Shimzy *et al*., 2006). Quinones are
toxic to the pathogen causing cell death in affected area which prevent further spread
of infection to nearby sites (Bi *et al*., 1995). Quinones being highly reactive
intermediate compound react with amino acid and cross-link proteins thus reducing
the protein content (Zhang *et al*., 2008). This is also observed in the present study.

The findings are in accordance with the earlier study of chocolate spot disease
of broad bean (*Vicia faba*) where increase in peroxidase is considered as indicator for
resistance (Nawar *et al*., 2003). It has also been reported that increased PPO in
infected *S.lycopersicum* leaves lead to disease resistance (Li *et al*., 2002). Reaction of
host to insect results in oxidative state of the plant which produces reactive oxygen
species that are removed by oxidative enzymes (Zhao *et al*., 2009). It has been
reported that increase in POX and PPO activities increases the reactive oxygen
species which act as scavenger to prevent the spread of infection (Stout *et al*., 1999).
The secondary metabolite produced by the plants are the phenolics which defend themselves from the pathogen (Heil et al., 2002). In the present study, total phenol was found to be lesser in infected plant than the healthy one. With the increase of POX and PPO activities, more phenol is used as it acts as substrate for antioxidant enzymes. This lead to the decrease of phenols in the infected plant. Similar result has been recorded in the study of cabbage against aphid (Khattab, 2007) stating that phenol oxidation by antioxidant enzyme is a potential defense mechanism in plants against insect attack. Phenols activate defensive enzymes by reduction of reactive oxygen species and play a role in host pathogen reaction against herbivore and insects (Maffei et al., 2007; Usha et al., 2010; Sharma et al., 2009). Several studies also reported PPO to play a vital role in plant defence against insect attack (He et al., 2011; Bhonwong et al., 2009).

5.3. Genetic variability of the clones by RAPD analysis

From the RAPD study, the banding pattern generated and the polymorphism reflected in these patterns was used to identify the 27 accessions consisting of 4 varieties (TV1, TV23, S3A3, Tinali) collected from 7 tea estates of undivided Dibrugarh district, Assam, India. All the 10 amplifying arbitrary RAPD primers produced polymorphic bands. The ability of a primer to distinguish between unrelated strains can be determined by the number of types (pattern types) defined by the primer and the relative frequencies of their types. A single numerical index of discrimination ‘D’ (Hunter et al., 1988) was calculated based on the probability that two unrelated genotypes amplified from the test population will be placed into different typing
groups. The D value greater than 0.90 is desirable to distinguish between two unrelated strains (Looveran et al., 1999). The value of D in this study ranged from 0.92 to 0.97 (primer A, B, C, D, E, F, G, H, I) for single primer based RAPD patterns except for 0.81 (primer J).

Polymorphic information content (PIC) of a primer determines the value of a marker by analyzing its linkage with other loci. PIC values exceeding 0.50 indicates locus to be highly informative, values between 0.50 to 0.25 indicates a medium informative locus while values below 0.25 indicates locus to be non-informative (Botstein et al. 1980). In the present study, PIC (Polymorphic information content) of 4 primers (B, D, E, H) ranges from 0.25 (primer E) to 0.35 (primer B & D) indicating a medium informative locus.

Genetic similarity estimates based on RAPD banding patterns were calculated using method of Jaccard’s coefficient analysis (Jaccard, 1908). The similarity coefficient matrix generated was subjected to algorithm “Unweighted Pair Group Method for Arithmetic Average (UPGMA)” to generate clusters using NTSYS 2.02 pc program (Rohlf, 1998). The Jaccard’s pairwise similarity coefficient values ranged from 0.26% (Ethel.TV1 and Deo. Tinali) to 0.71% (Bor.S3A3 and Mor. S3A3) with an average of 0.49%, for single primer based RAPD patterns. The clusters constructed through NTSYS (2.02 pc) presented in the form of dendrogram has generated three major cluster (cluster A, B and C). Cluster A consists of 7 tea plant samples, two from S3A3 Groups, (Lep. S3A3, Ana.S3A3) and five from Tinali, (Ethel.Tinali, Mor. Tinali, Bor. Tinali, Lep. Tinali, and Ana. Tinali). Cluster B consist of 10 plant samples, two from TV1 (Lep. TV1 and Ana.TV1) three from TV23
Cluster C consists of 5 plant samples, three from TV1 (Deo., Mor., Bor.) and two from TV23 (Tip. and Deo.). Five samples were found with exclusive lookouts in the dendrogram that was Mor.TV23 which was recorded most distinguishing plant sample, while rest four sample found in two set with two samples in each. Two plant samples of the Tinali group separated at 48% similarity coefficient consisting of Deo.Tinali and Tip.Tinali. Genetic similarity showed higher similarity coefficient for within groups compared to between groups. Within groups, least similarity was observed for Tinali groups 26 % ((I) Ethelwood Tinali and Deohal Tinali and (II) Ethelwood Tinali and Tippuk Tinali), whereas S3A3 group showed highest similarity (71%) between Moran S3A3 and Borborooah S3A3. High similarity value suggests that these tea clones have narrow gene pool within the genus Camellia (Mishra et al., 2009). Between groups minimum genetic similarity 37 percent were observed among the three groups i.e. TV1 and TV23 groups (between sample Mor.TV1 and Deo.TV23), TV1 and S3A3 (between sample Ethel.TV1 and Bor.S3A3) and TV1 and Tinali groups (Ethel.TV1 and Bor. Tinali) and maximum genetic similarity was observed between S3A3 and Tinali groups accession, Ethel. S3A3 and Bor. Tinali (67%) followed by TV23 and S3A3 accession, Ethel.TV23 and Ethel. S3A3.

On the basis of Jaccard’s similarity coefficient, on an average the relationships were TV1 group were 44 per cent diverse, TV23 group 48 per cent diverse, S3A3 group 51 per cent diverse, and Tinali groups were 54.5 per cent diverse. In the present study, TV1 and TV23 group shows minimum diversity which
might be due to intensive selection program on this variety (Sui et al., 2008) so are more prone to Helopeltis infestation as plant population which are genetically less diverse are more susceptible to pathogens (Bandyopadhyay, 2011). While S3A3 and Tinali group shows maximum diversity so are less prone to infestation as increase in host genetic diversity reduces the risk of infestation (Lively, 2010). Also from the cluster visualization in the dendogram; it is quite evident that different varieties (Tinali, S3A3, TV1) are different from each other, as they are occupying different clusters and hence must have some diversity in genes. In the present study we have found TV1 and TV23 sharing cluster C which indicates TV1 to have some similarity with TV23. This might explain the higher susceptibility of TV1 and TV23 clones to Helopeltis infestation. Again S3A3 and Tinali was found sharing cluster A which suggests S3A3 to have some similar genes with Tinali. Thus, Tinali and some S3A3 samples are less prone to Helopeltis infestation and thus might harbour some resistant genes.

Thus from the present study, RAPD markers has proved efficient tool in differentiating all 27 accessions consisting of 4 tea clones (TV1, TV23, S3A3, Tinali) showing high polymorphism among them which determine their genetic diversity. Also the dendogram has placed the moderately susceptible clones in separate cluster indicating that they might harbour some resistant genes.