Indian Journal of Entomology, 81(4): 000-000 (2019) DoI No.:

RAPD- ANALYSIS OF POPULATIONS OF *SPODOPTERA LITURA* (F.) FROM UTTRAKHAND

Mritunjoy Barman*, Neeta Gaur, Rashmi Joshi, Rukesh Pramod and Pradeep Mishra

Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar 263145
*Email: mritubarman@gmail.com

ABSTRACT

RAPD analysis was carried out in *Spodoptera litura* (F.) collected from eight locations of Kumaon region, Uttarakhand. Using ten RAPD markers, a total of 126 loci were amplified, of which 113 loci were polymorphic showing 89.68% polymorphism. The results suggest the development of new populations with demographic variation, temperature and selection pressure of pesticides.

Key words: *Spodoptera litura*, RAPD markers, Uttarakhand, Kumaon, polymorphism

Spodoptera litura is one of the most devastating polyphagous leaf feeding insect, infesting more than one hundred plants around the Asia-Pacific region (Ahmad et al., 2013). This wide host range is considered as an important factor for distribution, genetic variation and survival (Lee et al., 2003). In India some commercially cultivated crops which have been majorly infested by this pest are cotton, groundnut, soybean, ladyfinger, chilli, cabbage, cauliflower and such others; inflicting annual crop damage worth 12000 million rupees (Baskar et al., 2012). Although the pest is having great economic importance, its genetic diversity and population structure information is scarce, this information is crucial for accurate prediction of local population dynamics (Peng et al., 2019).

It has been reported that *S. litura* being one of the major pests has always been exposed to indiscriminate pesticide dosages and thus evolved resistance against major pesticides. Different cases of pesticide résistance in *S. litura* reported includes BHC (Benzene Hexachloride) resistance in Rajasthan (Srivastava and Joshi,1965);endosulfan and carbaryl in Haryana (Verma et al.,1971); cypermethrin and fenvalerate in Andhra Pradesh (Rao et al.,1996); fenvalerate in Tamil Nadu (Kumar et al., 2011). Hence, the property of high insecticide resistance in addition to high adult dispersal and migration resulted in wide host range of this pest (Kranthi et al., 2002; Saito, 2001; Baskar et al., 2012). It also reported that geographical location plays an important role in changing phenotypic characteristics of a population which leads to the variation among the population (Lee and Roh, 2010).

Kumaon region of Uttarakhand is situated at the foot hills of North West Himalayaswith diverse climatic conditions and distinct demography which changes with change in microclimate of the area. This leads to varried weather conditions prevailing in the area ranging from hot and humid condition in summers to extreme cold in winters during October- February (Singh et al., 2014). So, to study the diversity in geographically isolated population of *S. litura*, it is necessary to check its genetic pattern (Gandhi and Patil, 2017). Molecular markers are important tools for analyzing the phylogeny, evaluation and population dynamics (Geetharajalakshmi et al., 2006). As a preliminary step, it is necessary to check the genetic patterns among geographically distinct populations. RAPD-PCR marker isa important methodologyused extensively to distinguish geographically isolated populations (Jain et al., 2010).In the present study, RAPD analysis was done to measure the genetic variability among populations of *S. litura* at various spatial scales in the Kumaon region of Uttarakhand. Eight populations were studied from different altitudes with transect spanning the high, moderate and low pesticide intensive crop ecosystems.

MATERIALS AND METHODS

Larval population were collected from different agroecosystems with diverse geographical locations viz., plain (Tarai), foot hills, mid hills and river valley of Kumaon region of Uttarakhand during *kharif* 2018. The details of these in the order S. No.; Location; Coordinates; Area described; Elevation; Host crop; and Code for Locationare as follows:

1. Pantnagar, 29^{0}3$'$0$''$N 79^{0}31$'$0$''$E, Tarai region, 235 masl, soybean (G); 2. Ramnagar, 29^{0}24$'$20$''$N76^{0}358$'$24$''$ E, River (Kosi) valley/foot hills(Himalaya), 367masl, soybean (R); 3. Majhera, 29^{0}16$'$6$''$ N 80^{0}5$'$19$''$ E, River

valley/ mid hills, 922 masl, Bhat (wild soybean) (M); 4. Almora, 29^{0}81$^{'}$ 50$^{"}$ N 79^0 29$^{'}$ 02$^{"}$ E, River valley (Kosi, Suyal)/ mid hills, 1212 masl, Colocasia (A); 5. Lohaghat, 29^{0}25$^{'}$0$^{"}$ N 80^0 6$^{'}$E, Mid hills, 1754 masl, castor (L); 6. Naogaon, 29^0 26$^{'}$ N 76^0 354$^{'}$E, foot hills, 345 masl, soybean (N); 7. Pithoragarh, 29^0 35$^{'}$ N 80^0 13$^{'}$E, Valley/ mid hills, 1569 masl, Bh Bhat (wild soybean) (P); and 8. Aathgaon, 29^0 35$^{'}$ N 80^0 13$^{'}$E, Mid hills, 1501 masl, castor (T).

From each geographical location, agriculture fields were surveyed and samples of *S. litura* were collected by following zig-zag pattern (Ellango et al., 2015).Healthy 5th instar larvae, six individual from each location were selectedand were starved for 24 hrs before DNA isolation.These were killed with the help of chloroform and later digestive system and fat bodies were dissected out. Only head and skin portion from the larvae were used during DNA isolation and amplification. All the samples were preserved in 90% ethanol and stored at -20^{0}C before DNA extraction. During DNA extraction process the instructions given in Hipur A^{TM} Insect DNA Purification Kit (Himedia) were duly followed.

DNA amplification was performed with sets of 10 arbitrary deca-nucleotide RAPD primers in PCR thermocycler. The RAPD primers were synthesized by Genei Laboratories Pvt. Ltd., Bengaluru. Each RAPD-PCR was performed in a total volume of 20 µl containing DNA template (2 µl), 10 Mm primer (2 µl), 10X PCR Buffer with MgCl2 (2 µl), 10Mm dNTP mix (2 µl), 5U/µl *Taq* DNA polymerase (0.6 µl), molecular biology grade water (11.4 µl). Thermal cycler programmed a denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 30°C for 45 sec and extension at 72°C for 2 min with a final extension at 72°C for 5 min.

After obtaining the amplified DNA, PCR product was employed to electrophoresis on 2.5% agarose gel at constant voltage of 80V for 2 hr and 30 min with TAE (50X) gelelectrophoresis buffer. Gel image and bands were captured and visualized on UV gel documentation system. Scoring of gel image credited (1) for presence and (0) for absence of bands of different molecular weight in the outline of binary medium. All the gelimages were scored thrice manually, independently and unbiasedly. All single bands were credited and incorporated in data analysis.Genetic distance based on Jaccard coefficient (Jaccard,1908) was calculated using NTSYSpc 2.11a software (Dice, 1945). Distance coefficients obtained were used to construct the dendrogram using UPGMA employing the SHAN clustering algorithm.

RESULTS AND DISCUSSION

Amplification of genomic DNA of all populations produced large numbers of distinct fragmentsfor each RAPD marker; the RAPD loci sizes ranged from 200bp to 2000bp (Table 1; Fig. 1). A total of 126 RAPD loci were obtained from all the 10 primers, of which 113 were polymorphic (89.68%) across eight populations. The number of loci generated by individual primers

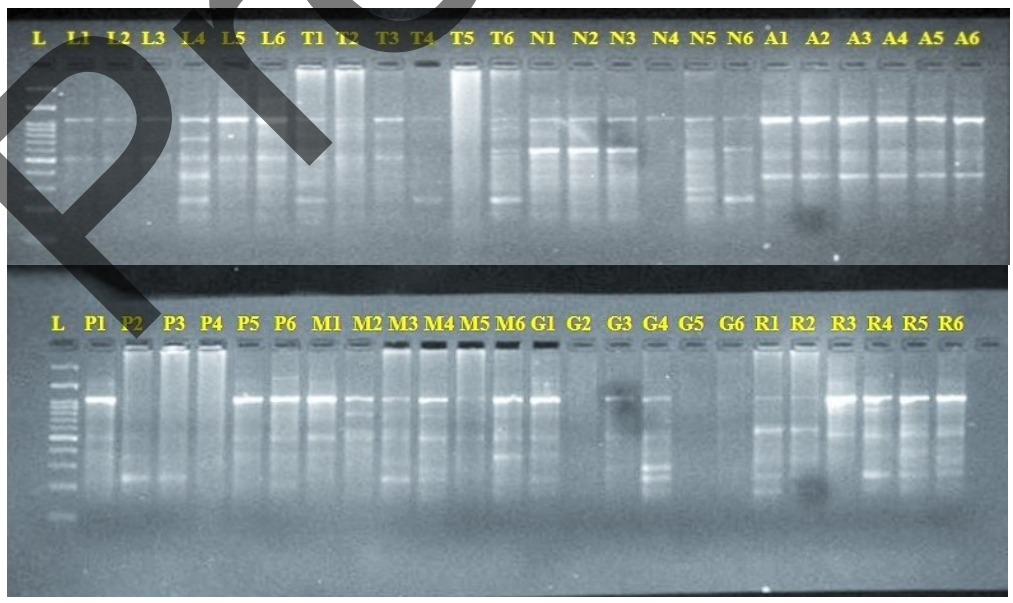

Fig. 1. Amplification profile of S. litura- from Lohaghat (L), Aatgaon (T), Naogaon (N), Almora (A), Pithoragarh (P), Majhera (M), Pantnagar (G), Ramnagar (R)- RAPD marker EF 9

Table 1. RAPD analog polymorphisms among the *S.litura* samples with ten arbitrary primers

S.No	Primer	No of amplicon in six individuals from individual population								Total no of amplicon	Range of allele	No of allele	NPA	NMA	%PA	%MA	PIC	EMR	MI	RP
		L	T	N	A	P	M	G	R											
1	EF1 GGACCCAACC	37	33	39	55	44	40	30	52	330	240-1300	13	11	2	84.61	15.39	0.42	9.30	3.93	6.38
2	EF3 TTGGCACGGG	50	44	36	44	63	37	24	28	326	250-1900	12	9	3	75.00	25.00	0.36	6.75	2.44	6.09
3	EF4 CACCGACAAG	52	31	30	54	61	34	25	36	323	250-1500	13	12	1	92.30	07.70	0.35	11.07	3.96	5.88
4	EF5 TCTCCGCCCT	18	31	32	13	29	39	13	15	190	300-1500	10	10	0	100.00	00.00	0.39	10.00	3.95	3.96
5	EF6 GAGCCCTCCA	51	36	12	31	54	33	09	41	267	250-1500	13	13	0	100.00	00.00	0.40	13.00	5.23	4.99
6	EF8 GGCTAACCGA	39	45	42	35	41	31	23	32	288	250-1500	9	6	3	66.66	33.34	0.38	4.00	1.53	5.21
7	EF9 ACCCCGCCAA	38	45	67	37	29	57	29	61	363	250-2000	17	15	2	88.23	11.77	0.40	13.23	5.32	5.65
8	EF10 AGGCCCGATG	64	44	47	50	39	16	18	19	297	200-1400	17	16	1	94.11	05.89	0.39	15.05	5.93	5.66
9	EF11 CTGGGCACGA	26	26	11	36	29	34	28	54	244	300-1100	10	10	0	100.00	00.00	0.47	10.00	4.73	5.12
10	OPM10 TCTGGCGCAC	55	56	29	26	36	20	46	41	309	270-1200	12	11	1	91.66	08.34	0.40	10.08	4.12	6.65

L: Lohaghat, A:Almora, P: Pithoragar, M: Majhera, R: Ramnagar, G:Pantnagar, N: Naogaon and T: Aatgaon
NPA-No of polymorphic allele, NPA- No of monomorphic allele, PIC-Polymorphic information content, (EMR)-Effective multiplex ratio, MI- Marker Index (R_p)- Resolution power

varied from 9 to 17 within the size range of 200- 2000bp. The highest number (17) of loci was produced by EF9 and EF10 primers while the least number (9) was recorded with EF8 primer, and polymorphism produced by each primer is given in **Table 2.** An average of 12.60 loci per primer was recorded. Polymorphic information content (PIC) of individual primer varied with highest 0.47(EF11) to lowest 0.34(EF4).

The benefits of the RAPD markers for study of insect genetic diversity is known.Jainet al. (2010) stated that RAPD-PCR markers can be a tool to distinguish geographically isolated insect populations. Janarthanan et al. (2002) found genetic difference between *S. litura* populations collected from six cotton growing fields of Tamil Nadu, using 40 RAPD markers. Gandhi and Patil (2016) demonstrated the value of RAPD markers for assessing the genetic variability between *S. litura* populations collected from eight soybean growing areas of India. A total of 3 random primers (OPA-01, OPA-10, OPM-10) were screened to reveal the existence of polymorphism. In the present study, RAPD polymorphism is analyzed with a phenetic distance measure (Jacquard's coefficient) and dendrogram constructedto indicate diversity.

The computation of similarity values was based on the presence or absence of discrete characters (PCR fragments) indicating number of PCR fragments shared (or not shared) between two individuals. The genetic similarity ranged from 0.32 to 0.88 (Fig. 2). The highest genetic similarity (0.88) was found between individuals

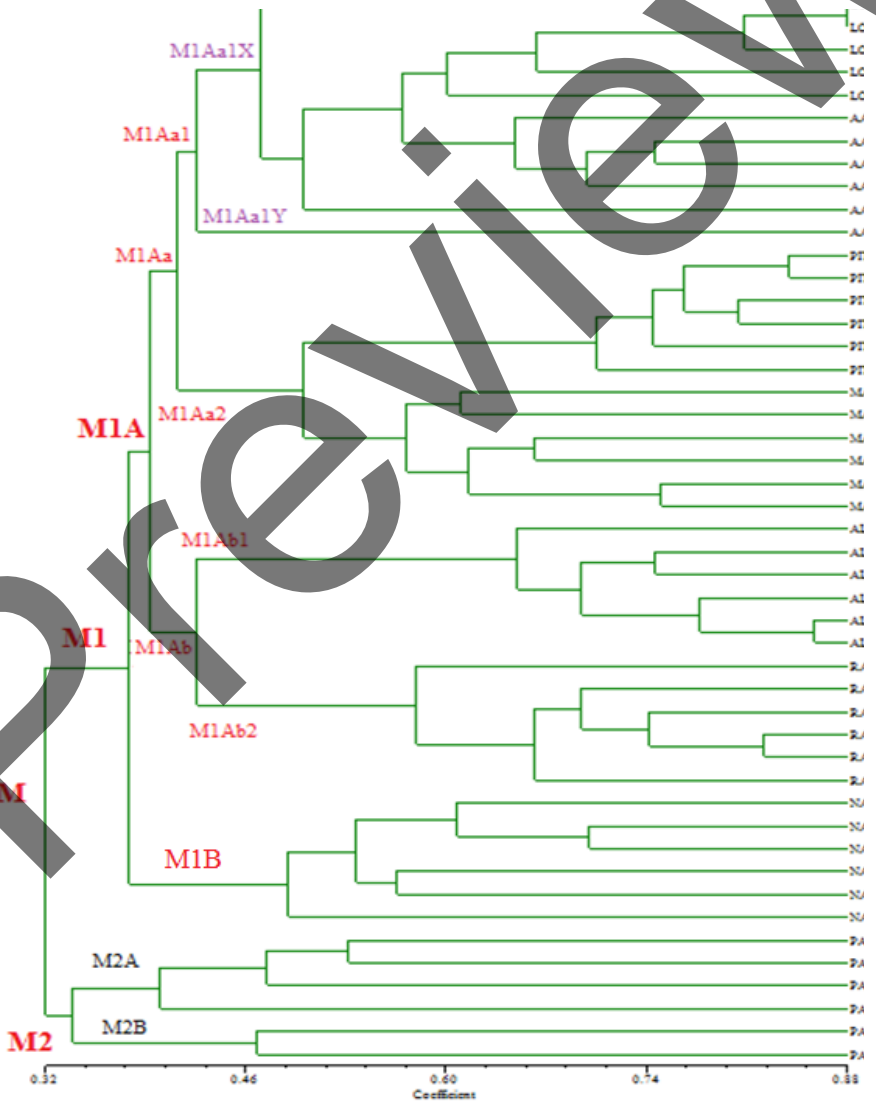

Fig. 2. UPGMA dendrogram constructed using NTSYSpc 2.11a.
Lohaghat (LOHA), Aatgaon (AAT), Naogaon (NAO), Almora(ALMOR), Pithoragarh (PITH), Majhera (MAJHE), Pantnagar (ANT), Ramnagar (RAM)

of population Lohaghat, and the least (0.32) with those of Pantnagar.The similarity values were used for phylogenetic analysis of *S. litura* population using UPGMA. Populations of *S. litura* collected from various geographic locations fall into two distinct cluster. The first group (M1) comprised seven populations while the distinct second group (M2) consisted of only one population (Pantnagar). The M2 group is subdivided into M2A and M2B at a similarity coefficient of 0.34, where M2B comprised only two individuals and M2A comprised left four individuals from Pantnagar.

The M1 group comprising seven different geographical populations included two distinct groups (at a similarity coefficient of 0.37) M1A and M1B; M1B group cluster consisting individuals from the population of Naogaon which basically tarai region of Uttarakhand, while M1A group is further subdivided into M1Aa and M1Ab at a similarity coefficient of 0.39; M1Ab further subdivided into two cluster (M1Ab1 and M1Ab2) at similarity coefficient of 0.42, one cluster comprised individual population from Ramnagar which basically located on foot hills of Himalaya and Other cluster (M1Ab1) comprised the population of Almora which is mid hills. M1Aa cluster further subdivided into two major cluster (M1Aa1 and M1Aa2) at a similarity coefficient of 0.41; M1Aa2 consisting individuals of two population Majhera and pithoragarh both consisting of mid hills,separated at 0.50 similarity coefficient. M1Aa1 group consisting individuals from Aatgaon and Lohaghat with 0.42 similarity coefficient both consisting of mid hills.The dendrogram shows that there is a close relationship between populations of Kumaon hills and a wide genetic distance (0.32) between plane and other hills' populations.

The Kumaun region of Uttarakhand is enriched with wide geographical and demographic variations with different farming practices making the hill agriculture organic by default while the plain areas are involved in chemical farming (Galvin, 2014). Aspects like biological characteristics (e.g. dispersal capability or breeding system), biogeographical history (e.g. colonization processes), as well as contemporary processes (linked to landscape features, demography or natural selection) must be taken into consideration while doing genetic diversity analysis (Corrnetti et al.,2016). Different parameters which could be responsible for outcome of the present study are discussed here.

Diverse altitude of the state starting from 210 m to 7817 m above MSL and the extreme temperature ranging from minimum -4^{0}C to maximum 37.9^{0}C (Singh, 2014) may create tremendous selection pressure for survival which may be responsible for the diversity of insect population. Hence, there may be chances of variablity in the gene pool due to adoption and survival of the insect population in diverse environment. Result shows that huge variablity in lower and upper hill population bcz they remain in two different cluster. One of the most important factor for the difference in the population could be the collection of samples from widely different location. Due to which, higher genetic difference among the population could be examined (Vijaykumar et al., 2008). But it is not totally true for *S. litura* as it having high dispersal rate,resulting into no boundary or geographical limitations effect (Saito, 2000).

Tremendous selection pressure of Insectisides may also create the gene level alteration of the population *S. litura* (Karuppaiah et al., 2017). In upper hills of Uttarakhand farmers always tend to use the indigenous technology for controlling the pest population they don't use any input from outsidebut in plane region due to incredible use of pesticides population may suppose to create tremendous selection which ultimately make the alteration in the gene level (Chandola et al., 2011).

ACKNOWLEDGEMENTS

The authors thank the Department of Entomology and DES (Directorate of Experimental Station) Pantnagar for providing financial assistance.

REFERENCES

Ahmad M, Ghaffar A, Rafiq M. 2013. Host plants of leaf worm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. Asian Journal of Agriculture and Biology 1: 23-28.

Baskar K, Kingsley S, Vendan S E, Paulraj M G, Duraipandiyan V, Ignacimuthu S. 2009. Antifeedant, larvicidal and pupicidal activities of *Atalantiamonphylla* (L.) Correa against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). Chemosphere 75: 355-359.

Behere G T, Tay W T, Russell D A, Kranthi K R. 2013. Population genetic structure of the cotton bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in India as inferred from EPIC-PCR DNA markers. PLoS One 8(5): 34-48.

Chandola M, Rathore S, Kumar B. 2011. Indigenous pest management practices prevalent among hill farmers of Uttarakhand. Indian Journal of Traditional Knowledge 10(2): 311-315.

Cornetti L, Lemoine M, Hilfiker D, Morger J, Reeh K, Tschirren B. 2016. Higher genetic diversity on mountain tops: the role of historical and contemporary processes in shaping genetic variation in the bank vole. Biological Journal of the Linnean Society 118(2): 233-244.

Coulson S J, Hodkinson I D, Strathdee A T, Block W, Webb N R, Bale J S, Worland M. R. 1995. Thermal environments of Arctic soil organisms during winter. Arctic and Alpine Research 27(4): 364-370.

Dice L R, 1945. Measures of the amount of ecological association between species. Ecology 26: 629-634.

Ellango R, Singh S T, Rana V S, Priya N, Raina H, Chaubey R, Rajagopal R. 2015. Distribution of *Bemisiatabaci* genetic groups in India. Environmental Entomology 44(4): 1258-1264.

Galvin S S. 2014. Organic designs and agrarian practice in Uttarakhand, India. Culture, Agriculture, Food and Environment 36(2): 118-128.

Gandhi B K, Patil R H. 2017. Genetic diversity in *Spodoptera litura* (Fab.) from major soybean growing states of India. Legume Research: An International Journal 40(6): 1119-1125.

Geetharajalakshmi S, Subramanian S, Shanmugasundaram P S, Mohankumar S. 2006. Molecular analysis of *Leucinodesorbonlalis*Guen. Populations within Tamil Nadu using lepidopteran specific random primers. Pest Management in Horticultural Ecosystems 12(1): 29-36.

Greenberg S M, Sappington T W, Legaspi B C, Liu T X, Setamou M. 2001. Feeding and life history of *Spodoptera exigua* (Lepidoptera: Noctuidae) on different host plants. Annals of the Entomological Society of America 94(4): 566-575.

Jaccard P. 1908. Nouvelles recherches sur la distribution florale. Bulletin de la Société vaudoise des Sciences Naturelles 44: 223-270.

Jain S K, Neekhra B, Pandey D, Jain K. 2010. RAPD marker system in insect study: A review. Indian Journal of Biotechnology 9: 7-12.

Janarthanan S, Seshadri S, Kathiravan K, Ignacimuthu S. 2002. Use of RAPD in assessing the genetic variability in *Spodoptera litura* Fab. Indian Journal of Experimental Biology 40: 839-841.

Karuppaiah V, Srivastava C, Subramanian S. 2017. Toxicity and effectiveness of newer insecticides, conventional insecticides mixtures to field populations of *Spodoptera litura* (Noctuidae: Lepidoptera). Journal of Entomology and Zoology Studies 5(6): 1893-1897.

Kranthi K R, Jadhav D R, Kranthi S, Wanjari R R, Ali S S, Russell D A. 2002. Insecticide resistance in five major insect pests of cotton in India. CropProtection21(6): 449-460.

Kumar N, Regupathy A. 2001. Status of insecticide resistance in tobacco caterpillar *Spodoptera litura* (Fabricius) in Tamil Nadu. Pesticide Research Journal 13(1): 86-89.

Lee K P, Raubenheimer D, Behmer S T, Simpson S J. 2003. A correlation between macronutrient balancing and insect host-plant range: evidence from the specialist caterpillar *Spodoptera exempta* (Walker). Journal of Insect Physiology 49: 1161-1171.

Lee K P, Roh C. 2010. Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. Entomologia Experimenta et Applicata 136: 151-163.

Lopes H M, Bastos C S, Boiteux L S, Foresti J, Suinaga F A. 2017. A RAPD-PCR-based genetic diversity analysis of *Helicoverpa armigera* and *H. zea* populations in Brazil. Genetics and Molecular Research 16(3): gmr16038757.

McMichael M, Prowell D. P. 1999. Differences in amplified fragment-length polymorphisms in fall armyworm (Lepidoptera: Noctuidae) host strains. Annals of the Entomological Society of America 92: 175-181.

Peng W A N, Wu H H, Huang M S, Lei C L. 2019. Microsatellites reveal strong genetic structure in the common cutworm, *Spodoptera litura*. Journal of Integrative Agriculture 18(3): 636-643.

Prevost A, Wilkinson M J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and applied Genetics **98(1): 107-112.**

Rao G R, Dhingra S. 1996. Shift in the susceptibility level of *Spodoptera litura* (Delhi and Guntur populations) to cypermethrin and fenvalerate. Journal of Entomological Research 20: 225-228.

Saito O. 2000. Flight activity of three *Spodoptera* spp., *Spodoptera litura*, *S. exigua* and *S. depravata*, measured by flight actograph. Physiological Entomology 25(2): 112-119.

Singh S. 2014. Farm mechanization in hills of Uttarakhand, India-A review. Agriculture for Sustainable Development 2(1): 65-70.

Srivastava B K, Joshi H C. 1965. Occurrence of resistance to BHC in *Prodenia litura* Fab. (Lepidoptera: Noctuidae). Indian Journal of Entomology 27: 102-104.

Subramanian S, Mohankumar S. 2006. Genetic variability of the bollworm, *Helicoverpa armigera*, occurring on different host plants. Journal of Insect Science 6(1): 26.

Verma A N, Verma N D, Singh R. 1971. Chemical control of *Prodenia litura* Fab. (Lepidoptera: Noctuidae) on cauliflower. Indian Journal of Horticulture 28: 240-243.

Vijaykumar, Fakrudin B, Krishnareddy K B, Kuruvinashetti M S, Patil B V. 2008. Genetic differentiation among cotton bollworm, *Helicoverpa armigera* (Hübner) populations of south Indian cotton ecosystems using mitochondrial DNA markers. Italian Journal of Zoology 75(4): 437-443.

(Manuscript Received: May, 2019; Revised: November, 2019;
Accepted: November, 2019; Online Published: November, 2019)