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Study on variability among predatory stink bug, *Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae) population thriving in different agro-ecosystems of north-western Himalayas, India

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Abstract
Present investigation was carried out to study the relation of geographical distribution with biological attributes and genetic variability of *Eocanthecona furcellata* thriving in different crop ecosystems of North-Western Himalayas, India**.** The study was conducted on seven diverse locations of Uttarakhand and the result showed no significant differences in biological attributes and predatory efficiency between the populations of predatory bug of different locations. In the study of genetic variability, similarity coefficient of cluster analysis of individual predatory bug from high hills to plain areas was ranged from 0.07 to 0.77. The overall 131.55 bands were obtained from 5 primers and out of them 8 bands were monomorphic. RAPD primer EF6 showed highly efficient in determining the polymorphicity within species. Results indicated that *E. furcellata* was present in all seven diverse locations and it had adapted different climatic conditions to survive with at par predatory efficiency. Hence, utilization of native population of this potential predator could be effective in biological control programme for effective management of insect pests in Western Himalayas of Uttarakhand.

Keywords: *Eocanthecona furcellata*, genetic variability, north-western himalayas, predation efficiency

1. Introduction
Several predators of families Pentatomidae, Anthocoridae and Reduviidae have ability to suppressing the pest population of different crops ecosystems and they can be utilized as biological control agents against different crop pests. Predatory stink bugs (Sub-family Asopinaeae) have great potential for use as biocontrol agents by virtue of their wide host range and unique mode of predation. *Eocanthecona furcellata* (Wolff) (Hemiptera: Pentatomidae) is one of the predator that has received attention recently, in the field of biological control due to its potential to prey different orders of insect pests *viz*. lepidoptera, coleoptera and heteroptera [9]. In India, *E. furcellata* also has been considered as an important predator for several lepidopteran pests and also for the pest of cotton, chickpea and vegetable fields [44, 58]. The host specificity and eco-friendly nature of this predator enhancing its value in insect pest management programs because it has been found preying on larvae of leaf worm, spotted bollworm and American bollworm in Myanmar [43, 30]. *E. furcellata* may be used as a potential biological control agent to control pests in many agricultural crops [24] such as, *Prodenia litura* Fab. larvae of cotton [4, 27], slug caterpillar, *Latoia lepida* (Cramer) on mango [16, 53]. Leaf roller *Diaphania pulverulentalis* on mulberry [50]; Semiothisa pervolgeta Wlk, and *Terias hacabae* L. on Daincha (*Sesbania bispinosa*) [8] *Spilosoma obliqua* on soybean and sesame [54, 5] and pests of many other crops [49, 19, 57].

In Uttarakhand, the North-Western Himalayas region of India, the major prey of this bug are *Spodoptera litura*, *Maruca vitrata*, *Mythimna seprata*, *Clostera fulgurita*, *Cnaphalocrosis* sp. and many hairy caterpillars [59] found in pulse, poplar, soybean, wheat, rice and vegetable crops [1, 37]. But owing to diverse and extensive geographical stretch (High hill, mid hill, foot hill and tarai region) in Uttarakhand, knowledge of genetic diversity and population sub-divisions of predatory bug is important to understand its distribution patterns and their colonization. The diversity of insect population is dependent upon their phenotypic and ecological flexibility [39]. Even within a species, insect varies morphologically and in their

behavior which attributes to the complex interaction of insect population with the environment [10]. Hence the fitness and biological attributes of predators like predatory efficacy against various pest species, mortality, fecundity, host searching ability, host range and longevity may vary from place to place also within species. Therefore, it becomes mandatory to determine firstly the natural variability within the diverse location's predatory bug population to characterize a suitable strain of this bioagent for the particular geographical area to evolved Biological integrated pest management (BIPM).

The present investigation was carried out to study the variability among the population of predatory stink bug, *E. furcellata* in North-Western Himalayas, India. In order that, a suitable strain of a particular geographical area could be identified and same could be utilized in biocontrol program for that particular region and also providing first information about the effect of distribution pattern on biology and genetic variability.

2. Materials and Methods

The present investigation was carried out from 2016 to 2018 at Biological Control Laboratory, Department of Entomology, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand, India.

2.1 Sampling of *E. furcellata* from diverse locations of Kumaon region of Uttarakhand

To the study variability amongst the population of *E. furcellata*, the predatory bug was collected from seven locations of diverse geographical conditions from Kumaon region of North-western Himalayas. The choice of locations was made on the basis of differences in their geographical location and climate (Table 1) thus selected locations were: Ramnagar (river valley/ foothills), Pantnagar (Tarai), Majhera (river valley/ mid hills), Almora (river valley/ mid hills), Khati (high hills), Lohaghat (mid hills) and Pithoragarh (valley/ mid hills). Insect samples were collected from the different crop ecosystems of the specified locations. Adults, nymphs and eggs of *E. furcellata* were collected manually through hand picking method from different crop ecosystems of geographically diverse locations (Table 1). The predatory bug was collected from three different sites of a specified location.

Table 1: Selected locations of sampling for predatory bug, *E. furcellata* from different regions of Kumaon, Uttarakhand, India during 2016-2017.

S. No.	Location	Altitude	Latitude	Longitude	Code for Location
1	Pantnagar	235mt/771ft	$29^0 3' 0''$N	$79^0 31' 0''$E	P_n
2	Ramnagar	367mt/1204ft	$29^0 24' 20''$N	$76^0 358' 24''$ E	R_n
3	Majhera	922mt/3026ft	$29^0 16' 6''$ N	$80^0 5' 19''$ E	M_j
4	Almora (Matela)	1212mt/3976ft	$29^0 81' 50''$ N	$79^0 29' 02''$ E	A_l
5	Lohaghat	1649mt/5411ft	$29^0 25' 0''$ N	$80^0 6'$E	L_o
6	Pithoragarh	1569mt/5148ft	$29^0 35'$ N	$80^0 13'$E	P_i
7	Khati (Kapkot)	2872mt/9423ft	$29^0 57'$ N	$79^0 54'$ E	K_k

2.2 Study on variability of predatory bug, *E. furcellata*

The variability among the population of *E. furcellata* was investigated through the studies on their biology, predation efficiency and genetic diversity of the collected predatory bugs.

1. Study on the biological attributes of *E. furcellata*

Study on biological attributes of the predator was started through field collected eggs of *E. furcellata*. The each egg masses collected from each location was counted and tagged to *Solanum nigrum* plants as 1^s instar nymphs of predator are non-predaceous and feed on plat sap as their initial food. The tagged plants were placed in plastic trays till they hatched and complete its 1st instar [20]. 2nd instar onwards all nymphs were maintained separately in plastic trough (20×20×15 cm) in the controlled laboratory conditions (Temperature: 27±2 °C, Relative humidity: 65±2% and Photoperiod: 12±1 hrs) by providing the ten larvae of rice meal moth, *Corcyra cephalonica* in each trough at a time and then trough were covered with muslin cloth for proper aeration. On transforming into adult, they were transferred to aluminum cages (3x3x2.5 ft) for mating and egg laying [59]. *C. cephalonica* is also reared in the laboratory [33]. The experiment was replicated thrice. The observations on number of individuals transforming into nymphal period, adult period, fecundity and incubation period were recorded separately for each location. The number of consumed larvae was recorded daily and fresh larvae were provided for further feeding at every 24 hrs. Predation by each nymphal and adult stage of predator collected from all seven locations was recorded separately. The data obtained through study of biological attributes were subjected to statistical analysis [18]. The significance of each location population were verified with one-way ANOVA at $P<0.05$ to study the predation efficiency.

2. Study on genetic variability of predatory bug, *E. furcellata*

The genetic variability among the population of *E. furcellata* was investigated. Through study the variation found in their nucleotides patterns by using Random Amplified Polymorphic DNA (RAPD) primers because RAPD-PCR will be an efficient tool to differentiate geographically isolated populations and measuring genetic differences between related species or within populations [14, 12, 15, 36, 56, 29, 28] which able to detect DNA polymorphism with in intra or inter population genetic diversity [60, 61, 22, 3, 25, 38]. During present study ten individual from each specified location were collected and stored in 80% ethanol under -80^{0}C conditions for further DNA isolation.

2.3 DNA extraction of predatory bug, *E. furcellata*

During DNA extraction firsty individual insect was wiped with 90% ethanol for surface cleaning. Being a predator in nature, the gut of *E. furcellata* might contain contamination in form of body fluid of pests. Hence, to avoid these contaminations in place to use whole insects, the abdominal portion of predatory bugs were removed and only thoracic muscles were utilised for DNA isolation [26, 51, 29]. Here 30 mg sample from individual insects was used for DNA isolation by using HiPurA^{TM} Insect DNA Purification Kit (HIMEDIA)

following the manufacture's protocol for isolation of genomic DNA [6, 52]. The concentration of DNA was determined spectrophotometrically using UV scanning spectrophotometer (Citizen: UV 1800, India).

2.4 RAPD analysis of predatory bug, *E. furcellata*

Genomic DNA of each *E. furcellata* individual was used for RAPD-PCR amplification with 8 random primers viz., EF1, EF2, EF3, EF4, EF5, EF6, EF7, EF9 (Chromos Biotech, India; Table 2) during the study. Out of them 3 primer EF1, EF5 and EF7 not showed any amplification. The PCR reaction was carried out in volume of 25 µl which consisting of 2.5 µl of Taq buffer (1X), 1.5 µl of dNTPs (10mM), 0.3 µl of Taq DNA polymerase (1U), 2µl of RAPD primer (10pmol) and 100ng template DNA. For optimization and reproducibility of RAPD, the reagent concentration was kept constant throughout the experiment. PCR amplification was carried out on the thermal cycler (Eppendorf, MJ Research PT 200) using 5 min initial denaturation at 94^0C, followed by 40 cycles of 45 second denaturation at 94^0C, 45 second annealing at 31^0C (according to primer it will vary) for 2 min extension at 72^0C, and finalized by 5 min at 72^0C [29, 41]. Duplicate amplification reactions were performed to test the reproducibility of the banding patterns. PCR products were separated electrophoretically with 100 bp ladder (Himedia) as molecular weight marker than gels were photographed on Gel Documentation System (Alpha InfoTech Alpha imager EC).

The data of the RAPD-primers were used to estimate the diversity within the species by calculating the genetic distances on the basis of the number of unshared amplified products or loci [7]. The data was firstly entered in a binary data matrix as discrete variables on excel sheets in which the Amplicon (amplified PCR products) were scored as binary presence (1) or absence (0) with each primer combination and similarity were determined [42]. Final scoring was done by analyzing the dendrogram designed by NTSys software (NTSYSpc 2.11) using unweighted pair group method with arithmetic mean (UPGMA) based on Jaccard's diversity coefficient. Present polymorphism, Polymorphism Information Content (PIC) and Resolving Power (RP) were also determined using the formula [55, 48]:

% Polymorphism of individual primer

$$= \frac{\text{No.of polymorphic bands amplified by an individual primer}}{\text{Total no.of bands amplified by an individual primer}}$$

Polymorphism Information Content (PIC) =1-ΣPij^2 (where, Pij = frequency of the j^{th} allele (marker) for the i^{th} RAPD locus).

Resolving Power (RP) = ΣIbi (where, Ibi=1- **(**2x|0.5-pi|); 'pi' is the proportion of accessions containing the i^{th} band and Ibi is the in formativeness of the i^{th} band).

Effective multiplex ratio (EMR) and Marker index (MI) were calculated [47, 40]

EMR= {np (np/n)}, where EMR = Effective multiplex ratio, np = No. of polymorphic bands, n = Total no. of bands.

MI=PIC*EMR where MI is the product of polymorphism information content value and effective multiplex ratio

3. Results and Discussion

3.1 Study on variability in biological and predatory efficiency of *E. furcellata*

The life cycle of *E. furcellata* passed through 5 nymphal instars with a maximum total nymphal period and adult period of 27.00±1.83 days and 16.67±1.52 days, respectively in population of Pithoragarh (Table 2). The longest duration of first instar was observed in population from Majhera i.e., 4.33±0.58 days, while the shortest duration of 2.67±0.57 days was seen in population from Lohaghat. The longest duration of second instar i.e., 5.67±1.53 days, was observed in population of predatory bug collected from Majhera, whereas, the shortest duration of 3.67±0.57 days was recorded in population from Pithoragarh. The longest duration of third instar was observed in population from Khati i.e., 5.67±1.15 days and shortest duration of 4.33±0.58 days was observed in the populations from Almora and Majhera. The longest duration of fourth instar was seen in population from Lohaghat i.e., 7.33±1.53 days and shortest duration of 4.67±1.52 days was recorded in population from Pithoragarh. The longest duration of fifth instar was seen in population from Lohaghat i.e., 6.67±1.15 days. While, the shortest duration of 4.67±0.58 days was observed in population from Pantnagar. The maximum total nymphal period of 27.00±1.83 was recorded in population collected from Lohaghat, whereas, the minimum total nymphal period of 24.33±1.32 days was recorded in population from Pantnagar. The adult period varied from 13.00±1.00 days to 16.67±1.52 in populations from Ramnagar and Pithoragarh, respectively. Total life period for populations of *E. furcellata* reared on *C. cepahlonica* was observed to be maximum 48.67±3.60 days in population from Pithoragarh and minimum 44.33±3.29 days in population from Ramnagar. Maximum number of eggs were laid by individuals from Pantnagar i.e., 52.00±2.65 and minimum number of eggs were laid by individuals collected from Lohaghat i.e., 39.67±3.79. Maximum incubation period of 7.00±1.00 days was observed in the population from Ramnagar and minimum 6.67±1.15 days incubation period was observed in population of Almora. It is evident from the study that the total nymphal period varied from 24.33±1.32 to 27.00±1.83 days in the populations of all seven locations. In accordance with past research it was evident that total nymphal period was ranged from 20.67±1.53 to 24.33±0.57 days, whereas, the total adult period and life period were ranged from 7.00±1.00 to 17.33±1.52 days and 31.33±0.57 to 39.33±2.30 days, respectively on different hosts [59]. Also adult longevity of male and female were 13.2 days and 22.6 days, respectively on *Maruca* sp [46]. Similar incubation period 6±1.05 days and total nymphal period of about 16±0.64days with male and female longevity of 12±1.05days and 14±1.09 days, respectively also reported [35]. Thus, during the present study no significant difference was found in the duration of life stages of various strains of *E. furcellata* collected from geographically and climatically varied locations.

The first nymphal instar in *E. furcellata* is not predatory and feeds on plant sap. Predation started from second nymphal instar onwards. The mean predation rate as observed was maximum in Pantnagar 79.28%, followed by Ramnagar 78.15%, Almora 76.67%, Majhera 75.4%, Pithoragarh 75.06%, Lohaghat 74.9 and minimum in Khati 79.89% (Table 3). In second instars, the maximum predation rate was observed in the strain from Pantnagar i.e., 74.27%. While the minimum predation rate of 69.86% was observed in strain from Khati. The third nymphal instars contained predation rate as high as 82.83% from the strain from Ramnagar and as low as 76.33% from the strain from Pithoragarh. The predation rate in fourth nymphal instar ranged from 78.07% to

84.73% in strains from Lohaghat and Pantnagar, respectively. Maximum predation rate in fifth nymphal instar was observed to be 88.6% in the strain from Pantnagar and minimum predation rate was recorded at 76.67% from the strain from Pithoragarh. The adults displayed a fall in predation rate in all the strains. The predation rate in adults ranged from minimum 69% to maximum 75.71% in strains from Majhera and Pantnagar, respectively. Past researchers [59] studied the predatory efficiency of *E. furcellata* and found that maximum per cent predation by third, fourth and fifty nymphal instar of *E. furcellata* was 90.85 %, 93.98 % and 93.98 %, respectively on *Maruca vitrata*, while the predation rate of adult was found significantly higher at 84.49%. Results of present study were align to a similar finding of past researchers who also reported maximum consumption of prey by third, fourth and fifth instar nymph of *E. furcellata* [34]. Thus, difference in environmental condition were not affected their predatory efficiency that express the positive impact of this predatory bug to use as biological controlling agent in different geographical condition on commercial level.

Table 2: Biology of predatory stink bug, *E. furcellata* on larvae of rice meal moth, *Corcyra cephalonica* reared on broken maize grains in laboratory (27±2^{0}C and 65% RH)

Biological Parameter	Period (days ± SD)						
	Pantnagar	Ramnagar	Khati	Lohaghat	Pithoragarh	Almora	Majhera
No. of eggs released	40.67±5.25*	38.67±3.51	39.67±2.31	42.67±4.51	39.67±2.51	40.67±5.13	41.33±3.21
No. of eggs hatched	39.33±3.78	37.33±3.5	36.67±1.52	41.00±3.60	33.67±2.08	34.33±2.89	38.00±2.65
Incubation period	6.33±0.57	7.00±1.00	6.33±2.08	6.33±1.52	7.33±1.52	6.67±1.15	6.67±1.53
First instar	3.00±1.00	2.67±1.15	3.33±0.58	2.67±0.57	3.67±1.15	3.33±0.58	4.33±0.58
Second instar	5.33±1.53	4.67±0.58	4.00±1.00	4.67±2.08	3.67±0.57	5.33±1.53	5.67±1.53
Third instar	4.67±0.58	4.33±1.53	5.67±1.15	5.67±0.58	5.33±2.08	4.33±0.58	4.33±0.58
Fourth instar	6.67±0.58	6.67±1.53	7.00±1.00	7.33±1.53	4.67±1.52	6.76±1.15	5.67±1.15
Fifth instar	4.67±0.58	6.00±1.00	6.33±1.15	6.67±1.15	5.33±0.57	6.33±1.53	6.00±1.15
Total Nymphal Period	24.33±1.32	24.33±1.55	26.33±1.55	27.00±1.83	24.67±3.21	25.67±3.79	26.00±3.00
Adult	14.00±2.00	13.00±1.00	13.67±3.21	14.67±3.06	16.67±1.52	15.67±2.08	13.67±1.15
Total life period	44.67±3.57	44.33±3.29	46.33±3.38	48.00±3.77	49.67±3.60	48.68±4.04	46.34±2.08
No. of egg laid	52.00±2.65	42.67±2.08	46.33±3.06	37.67±3.79	41.67±2.30	42.67±1.53	43.67±1.53

*Values represent mean±SD of three replications.

Table 3: Predatory efficiency of the predatory stink bug, *E. furcellata* on *Corcyra cephalonica*

Locations	Life stage of *E. furcellata*						
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Adult	Mean predation (%)
Pantnagar	-*	74.27 (59.55)**	82.75 (65.64)	84.73 (67.01)	88.60 (70.31)	75.7 (60.50)	79.28
Ramnagar	-	71.40(57.70)	82.83 (65.62)	83.67 (66.39)	87.73 (69.56)	74.73 (59.85)	78.15
Khati	-	69.86 (56.73)	78.87 (62.85)	79.20 (62.96)	84.13 (66.93)	70.13 (56.98)	74.89
Almora	-	69.86 (58.49)	80.67 (63.99)	80.00 (63.49)	79.33 (63.08)	70.67 (57.22)	76.67
Pithoragarh	-	72.33 (58.30)	76.33 (60.93)	79.00 (62.90)	76.67 (61.15)	71.00 (57.46)	75.06
Majhera	-	73.33 (58.92)	79.67 (63.30)	75.67 (60.49)	79.33 (62.99)	69 (56.16)	75.40
Lohaghat	-	70.60 (57.19)	80.93 (64.29)	78.07 (62.26)	82.47 (65.46)	74.53 (56.71)	74.90
SEm±	-	(0.93)	(1.75)	(1.57)	(1.69)	(1.15)	-
CD (0.05)	-	(2.79)	(5.24)	(4.71)	(5.09)	(3.46)	-

*No feeding

** Values in parentheses are angular transformed values

3.2 Study on genetic variability of *E. furcellata*

In the present study, the RAPD-PCR DNAs were used to find out the genetic variability within the populations of *E. furcellata* from Western Himalayan region of Uttarakhand. The major advantage of RAPD-PCR has that there is no need of prior knowledge of the genome that made it a simple and rapid method for determining genetic diversity and similarity in various organisms [13, 31].

During molecular study, ten adults of predatory bug from each location were analysed. Hence, total seventy individuals of *E. furcellata* were collected from all seven diverse locations and subjected to the genetic variability study. The band patterns were scored from gel photograph, against 5 primers-EF2 (GTCGCCGTCA); EF3(TTGGCACGGG); EF4 (CAGCGACAAG); EF6(GAGCCCTCCA); EF9 (ACCCCGCCAA) which produced number of discrete band of different intensity (Figures 1 to 5) which showed that the amplified bands pattern were not uniform even with same primer in individuals of same location. The monomorphic bands varied with primer to primer. DNA from predatory bug was separated successfully with primer EF2 (Fig.1.D) which resulted maximum 41 bands with individuals of Pantnagar location and minimum 13 bands with individuals from Khati at different 18 loci (Photoplate1.A). EF2 primer showed 92.41% polymorphism (Table 4) with two monomorphic bands which were common in all individuals at ~1400 bp and ~900 bp. EF3 primer has 94.62% polymorphism (Table 4) with one common band in all individuals at 900 bp except in Khati samples (Fig.2.A). EF3 primer showed maximum 25 bands with individuals of Pantnagar (Fig.2.D) and minimum 12 bands with individuals from Pithoragarh at different 18 loci (Fig.2.C). Primer EF 4 had 100% polymorphism because none of monomorphic band was obtained and this primer separated DNA in maximum 45 bands from the individuals of Majhera (Photoplate3.B) and minimum in 8 bands in individuals from Pithoragarh at different 22 loci (Fig.3.C). Primer EF6 showed 92.84% polymorphism (Table 4) and had two species specific bands at 450 bp and 800 bp, except in individuals from Khati (Fig.4.A). Maximum 38 and minimum 20 bands were produced with individuals from Khati and Almora at 20 different loci respectively (Fig.4.B). Primer EF9 showed minimum polymorphism in comparison to other

primers *i.e.*, 89.11% as it yielded 3 monomorphic bands with DNA samples of seventy individuals at 250 bp, 600bp and 900bp (Fig.5. A; B; C) with maximum 41 bands in Khati and minimum 7 bands in Pithoragarh at 27 different loci. Overall 131.55 bands were obtained from 5 primers out of them 8 bands were monomorphic that mean 123.55 amplified products exhibited polymorphism (Table 4). The Polymorphism information content (PIC) values of different primers were ranged from 0.17 to 0.22 with 16.20 to 22.00 Effective Multiplex ratio (EMR) values. The Marker Index were ranged (MI) from 2.49 to 4.91 and resolving power (RP) as observed in case of 5 primers ranged between 0.98 to 2.12 (Table 4).

In present study EF6 primer was able to work as efficient marker for discrimination the different individual of *E. furcellata* within the species from different sites because it produced two monomorphic bands with 92.84% polymorphism and had highest 2.12 RP value incomparion to other primers. While the primer EF4 showed 100% polymorphism with no any monomorphic bands and not showed any genetic differentiation within the populations [11]. On the other hand primer EF3 had 94.62 % polymorphism but having lowest 0.98 RP value. Hence, EF6 may serve as genetic markers and act as basis of genetic differentiation for the considered population. The high RP values of primers indicated that the primers were more informative for diversity among the population because the characterize the ability of primers in combination to detect the difference between samples [48, 17]. DNA from the individuals of the same location produced varying banding patterns when amplified with same primers. This led us to the conclusion that RAPD markers are highly efficient in determining the polymorph city in the population.

By scoring bands from all samples of *E. furcellata* a unique RAPD profile was generated in the form of dendogharm (Figure 6) which revealed cluster formation and resulted a main Cluster C with 0.07 or 7% Jaccard's similarity cofficient with in the individuals which further subdivided into specific subcluster C1 and broad C2 cluster. Results revealed that C1 cluster represented all the individuals of Khati (K_{k1}-K_{k10}) by splitting into C1A and C1B with 14.92% genetic similarity. K_{k2} and K_{k9} individuals were very closly related to each other with 77% genetic similarity because they grow from same egg mass, while other were collected separately from field condition. On the other hand the broad cluster C2 subdivided into C2.A and C2.B with 10.24% genetic similarity with in them which covers rest all *E. furcellata* individuals from different altitude. C2.B cluster showed mixed population in form of C2.Ba and C2.Bb cluster of individual from Pithoragarh and Majhera with 19.24% genetic similarity, while C2.A resembled as major cluster because it covered 54 individual of different sites by spliting into C2.Aa and C2.Ab with 13.84 % genetic similarity. C2.Aa cluster again subdivided in C2.Aa.I and C2. Aa. II with 17.08% similarity between individuals of mixed population from hill and plain area. C2.Aa.I showed close relation between individuals of Lohaghat, Almora and with one individual of Ramnagar (R_{n9}). Cluster C2.Aa.II represented the relationship between Pantnagar and Pithoragarh individuals. On the other hand the cluster C2.A.b was further divided into C2.Ab.I and C2.Ab.II with 14.2% similarity with in individuals of Almora, Ramnagar, Pithoragarh and Majhera.

The RAPD technique has also been applied to assess polymorphism in *Coccinella septempunctata*, *Rhynchiphorus ferrugineus*, *Nezara viridula*, *Microctonus aethiopoides*, *Aedes aegypti* [22, 15, 29, 45, 2]. For determination of population structure and strain differentiation without prior knowledge of DNA sequences of natural populations [21, 23] and geographically isolated populations [62].

Cluster analysis of the UPGMA dendrogram reveal that strains collected from areas of very high altitude are genetically distant from other strains. Whereas, the biology and predatory efficiency of the predator does not vary significantly in strains from geographically diverse areas. During the study it was found that individuals from Khati (2872 mt/9423ft) were found to be genetically distant from rest of the population and were placed in a separate cluster (Jaccard's similarity coefficient: 0.14). The individuals from Lohaghat (1649mt/ 5411ft), Almora (1212mt/3976ft), Pithoragarh (1569mt/ 5148ft), and Pantnagar (235mt/771ft) were grouped together and most of the individuals from Majhera (922mt/3026ft) and Ramnagar (367mt/1204ft) were placed in the same cluster. A few individuals from Pithoragarh and Majhera were clustered separately, though not very distantly. It implies that the predatory bug has somehow adapted itself according to different climatic conditions while maintaining its biology and predatory efficiency. Each strain has made adjustments to preserve its potential as a predator. The population was considered as nearly co-related while positioned quite distant to other. Here the basis of discrimination was the differences in their geographical location which may affect their DNA banding pattern. Therefore, high altitude samples were genetically distant to middle hill, foot hill and Plain (Tarai) samples. Similar pattern of differentiation were obtained from RAPD analysis of Southern green stink bug during genetic diversity study [29]. RAPD-PCR also used in the study of genetic variability and phylogenetic relationship of geometrid moth (Geometridae) in India [32].

Table 4: RAPD analog polymorphisms among the *E. furcellata* individuals with five arbitrary primers.

Primer with sequence	No. of Bands/primer							Total No. of Bands/Primer	No. of Loci	Monomorphic bands	Percent polymorphism	PIC value	EMR	MI	RP value
	K_k	L_o	A_l	P_i	M_j	R_n	P_n								
EF2GTCGCCGTCA	13	38	22	22	16	32	41	184	18	2	92.41%	0.21	14.22	3.10	1.36
EF3TTGGCACGGG	24	13	19	12	19	18	25	130	18	1	94.62%	0.15	16.05	2.49	0.98
EF4CAGCGACAAG	23	56	36	8	45	30	21	222	22	0	100%	0.22	22.00	4.91	1.33
EF6GAGCCCTCCA	38	23	20	23	30	26	35	195	20	2	92.84%	0.18	16.2	2.90	2.12
EF9ACCCCGCCAA	41	26	29	7	40	26	24	193	27	3	89.11%	0.17	21.33	3.66	1.01
								131.55							

Kk: Khati; Lo: Lohaghat; Al: Almora; Pi: Pithoragar; Mj: Majhera; Rn: Ramnagar; Pn: Pantnagar.

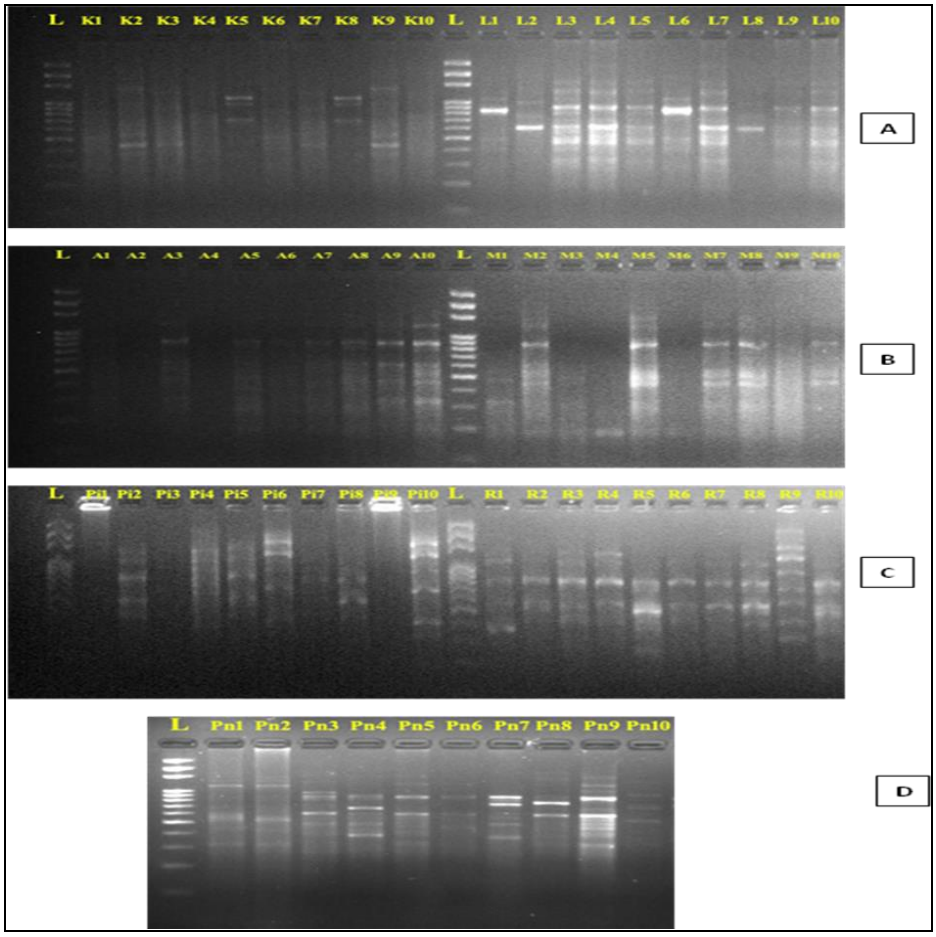

Fig 1: Amplification profile of *E. furcellata* Wolff. Against RAPD primer EF2. (A) Khati & Lohaghat (B) Almora & Majhera (C) Pithoragarh & Ramnagar and (D) Pantnagar.

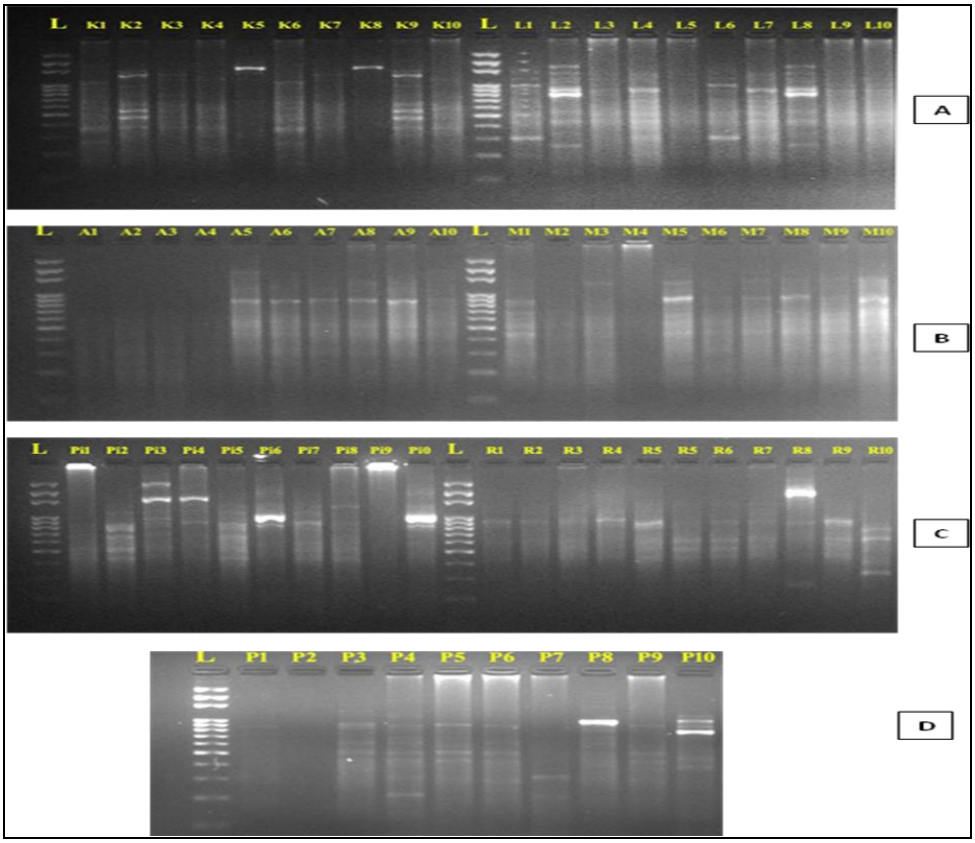

Fig 2: Amplification profile of *E. furcellata* Wolff. against RAPD primer EF3. (A) Khati & Lohaghat (B) Almora &Majhera (C) Pithoragarh & Ramnagar and (D) Pantnagar.

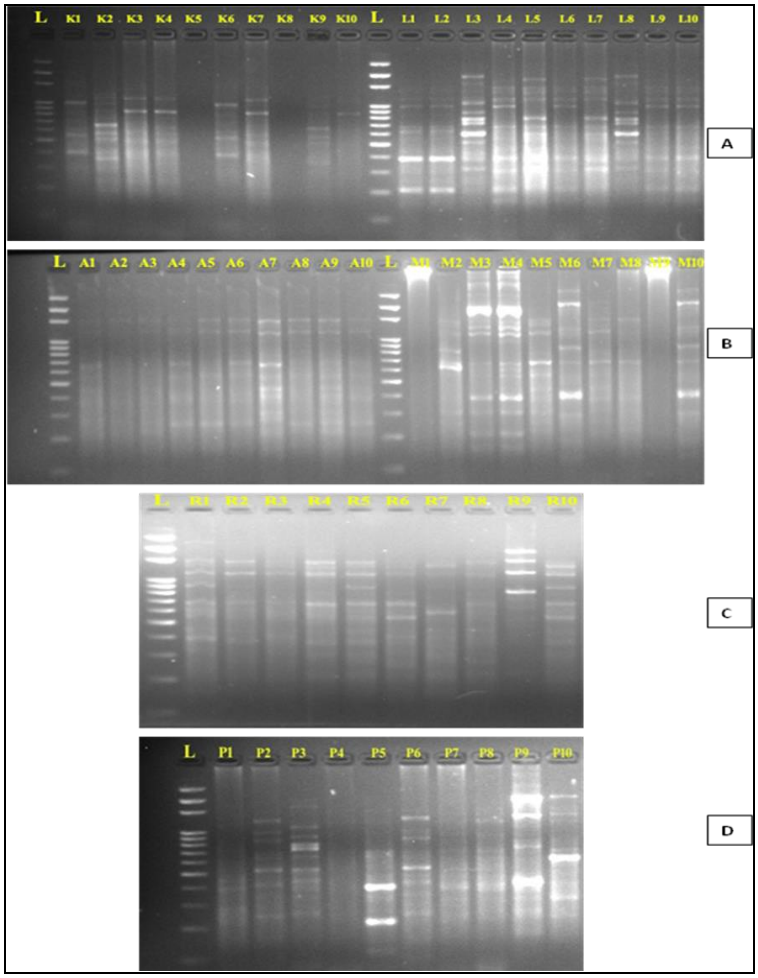

Fig 3: Amplification profile of *Eocanthecona furcellata* Wolff. Samples collected from (A) Khati and Lohaghat (B) Almora and Majhera (C) Pithoragarh and Ramnagar and (D) Pantnagar, generated by RAPD marker EF 4

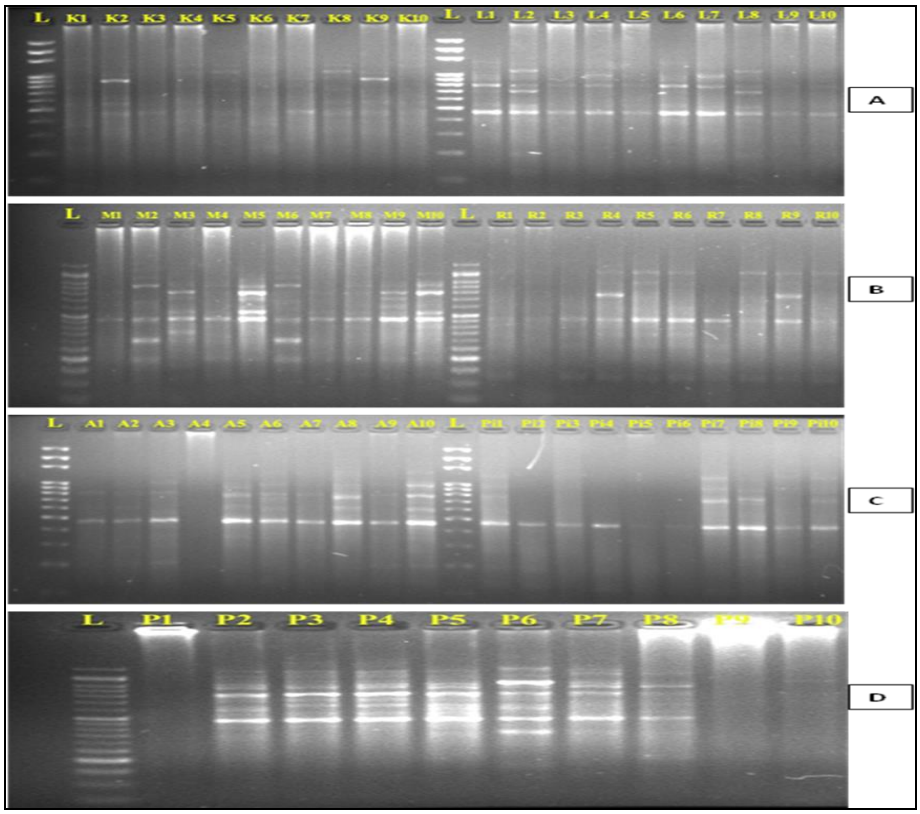

Fig 4: Amplification profile of *Eocanthecona furcellata* Wolff. samples collected from (A) Khati and Lohaghat (B) Almora and Majhera (C) Pithoragarh and Ramnagar and (D) Pantnagar, generated by RAPD marker EF

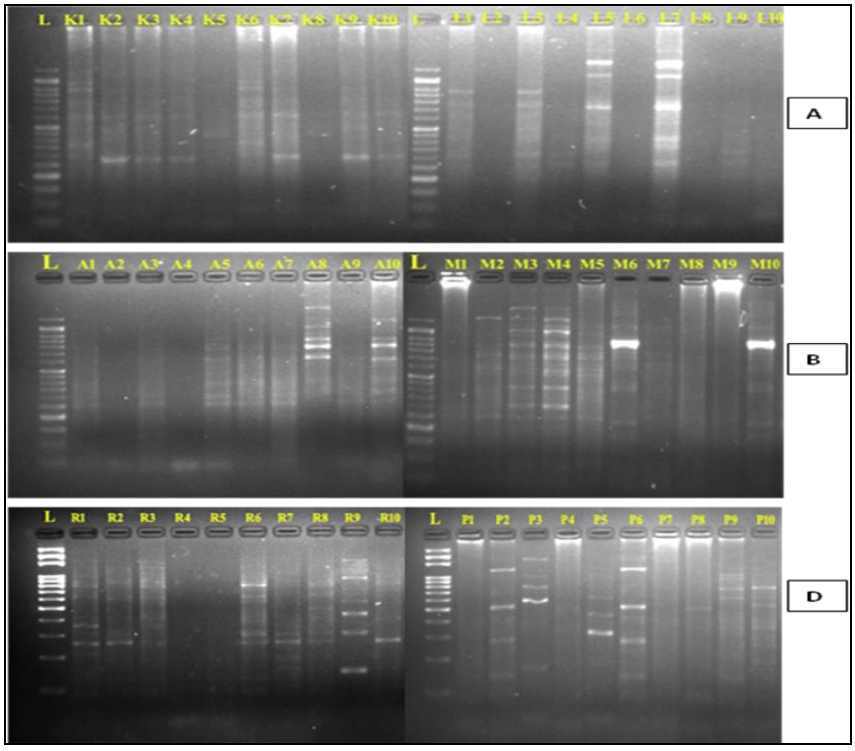

Fig 5: Amplification profile of *Eocanthecona furcellata* Wolff. samples collected from (A) Khati and Lohaghat (B) Almora and Majhera (C) Ramnagar and Pantnagar, generated by RAPD marker EF

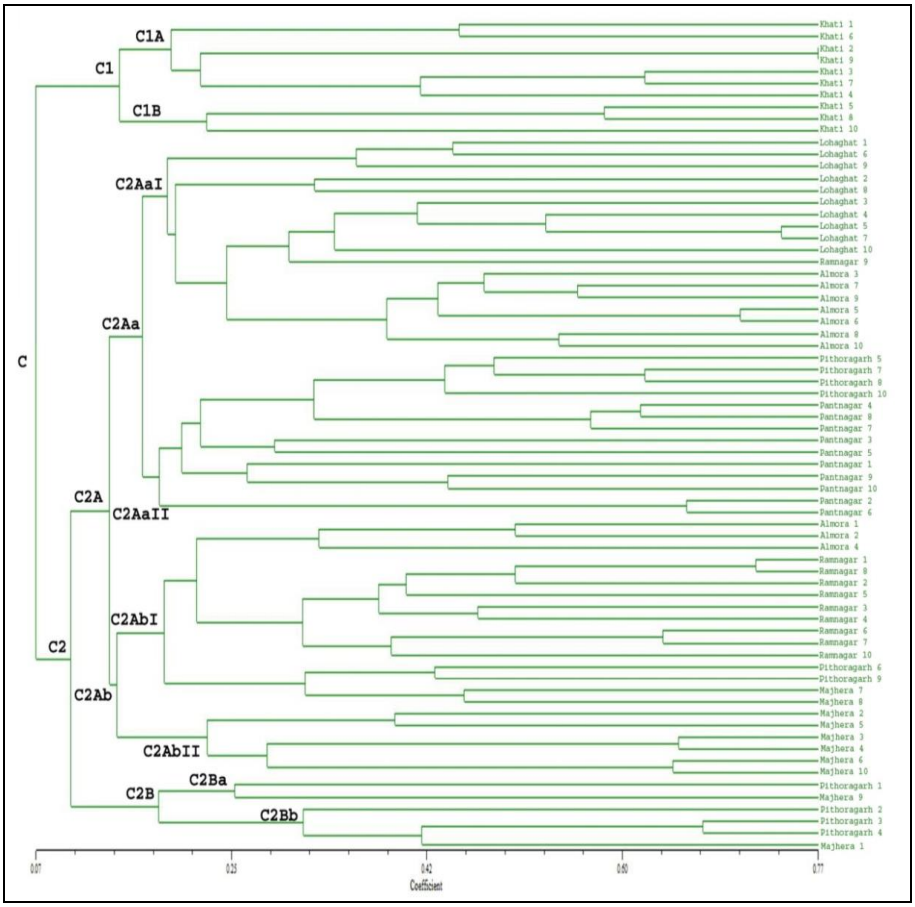

Fig 6: UPGMA dendrogram for 70 individuals of *E. furcellata* constructed using NTSYSpc 2.11.

4. Conclusion

The study provides firsthand information on the genetic variability and effect of environment on life cycle and predatory efficiency of *E. furcellata.* The results on genetic variability indicated that populations, even within a species, were affected by environmental factors and their geographical locations but it did not significantly affect their biological attributes *viz*., predation efficiency. This finding highlights the potential of *E. furcellata* to be used as a biocontrol agent on commercial level as it displays the ability to adapt itself

according to the environmental conditions while maintaining its predatory potential. Each strain has made adjustments to preserve its potential as a predator. The study showed that populations of *E. furcellata* prevailing in different locations/altitude of Western Himalayas of Uttarakhand vary genetically from location to location. Hence, it is concluded that locally available strains of *E. furcellata* will be effective to be utilized in biocontrol programme.

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