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Physico-chemical analysis of textile dye effluent using microbial consortia mediated degradation process

M. N. Abubacker[1]* and T. Mehala[2]

[1]*Associate Professor and Head, PG & Research Department of Biotechnology, National College, Tiruchirappalli 620 001, Tamil Nadu, India*
[2]*PG & Research Department of Botany, National College, Tiruchirappalli 620 001, Tamil Nadu, India*

Email: : abubacker_nct@yahoo.com

ABSTRACT

Physico-chemical analysis of textile dye effluent have shown higher concentrations of total solids, total suspended solids, total dissolved solids, biological oxygen demand, chemical oxygen demand, dissolved oxygen, total hardness, carbonate, bicarbonate alkalinities, chloride, calcium, magnesium, sodium, sulphate, zinc, chromium, copper and lead was found to be above permissible levels of WHO standards which ensure the presence of pollutant overloaded in the textile effluent. Bacterial consortia was developed with *Bacillus subtilis* (NCBT 012), *Clostridium butyricum* (NCBT 017), *Enterobacter aerogenes* (NCBT 024) and *Pseudomonas fluorescens* (NCBT 046) and Fungal consortia was developed with *Aspergillus erythrocephalus* (NCBT 124), *Aspergillus fumigatus* (NCBT 126), *Cladosporium herbarum* (NCBT 142) and *Fusarium oxysporium* (NCBT 156). Between bacterial consortium and fungal consortium mediated process of textile effluent, the fungal consortium have shown more efficient and much reduction in all the physico-chemical parameters than the bacterial consortium mediated degradation. The textile effluent was the major source of pollution which will affect the natural environment. Thus, there is need for treatment of effluent before they are discharged into the environment.

Keywords: Bacterial consortium, Degradation, Effluent, Fungal consortium, Textile dye, Textile effluent

INTRODUCTION

Among different industrial sectors, textile is one of the largest and growing throughout the world. Textile industries consume an extensive and diverse array of chemicals in the form of dyes, dispersants, leveling agents, acids, alkalis, salts and sometimes heavy metals[1,2]. A huge amount of water is used and most of it latterly discharged as waste from the textile industry. Textile waste water accounts for about 22% of the total volume generated from all different types of industries. Generally, wastewater from textile industries has been characterized as; extremely coloured and alkaline with high load of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), dissolved and suspended solids[3,4].

Textile dyes are of various types depending upon their structures and mode of application[5]. They have been continuously exhausted (5-50%) in the water bodies because of their poor adsorbability to the fibers[6,7]. Mostly, dyes are noticeable in water at concentrations, as low as 1 mg/ml. Therefore, wastewaters, typically with dyes contents in the range of 10-200 mg/ml are extremely coloured[8]. Presence of dyes raise dissolve solids[9,10], cause rapid diminution of oxygen level in the water bodies[11]. Artificial dyes and pigments are made colorfast and stable by using certain additives like; ammonia, chloride, nitrate, phosphate, sulfate, and heavy metals. The recalcitrant nature of dyes in water bodies not only stops biological recycling of nutrients but also reduce the biotic productivity of aquatic ecosystems. In addition, certain dyes and their degradation products have been reported to have toxic to mutagenic affects in almost all forms of life including humans[12,13,14,15].

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Dyes, because of their diverse and stable chemical nature, are difficult to remove in wastewater by conventional treatments[16]. In this perspective, numerous physical, chemical and biological techniques have been researched in the last few decades. Relatively, biological treatments have been gaining much attention in terms of their low cost and eco-friendly nature. Amongst different bioremediation agents, the roles of bacteria and fungi have always been considered vital for chemical like dyes.

There are many conventional physico-chemical methods like precipitation, iron exchange, electro coagulation and reverse osmosis are available for the removal of pollutants from the textile dye effluent. But all these methods are expensive and need many techniques. Hence bioremediation, particularly microbial remediation is proved as an effective eco-friendly affordable technology for the degradations and removal of pollutants from textile dye effluents. In this investigation four indigenous bacterial strains and four fungal strains were selected as indicator microbes from textile dye effluent exposed soils, these microbes were used as consortia in degradation process.

MATERIALS AND METHODS

Collection of Samples:

The area under the study for this research work was identified based on the need, diversity and extend of pollutants produced by the small scale textile dying houses located on the banks of the Amaravathy River, Karur, state of Tamil Nadu, and peppered with a number of large and small scale textile dying houses and industries. Water samples from the field receiving discharge from industry were collected, at three sampling sites in the longitudinal section: site-1 (comprises a sample sources collected at the discharge site of the dying house), site-2 - the effluent discharge point, samples collected 100 meters, site 3 - 200 meters away from the discharge point. Generally, all samples were filled in properly cleaned, sterilized, wide mouth plastic bottles and stored in the dark at a temperature of 4°C.

Soil samples were collected at the above mentioned sites. After thorough mixing, 200 g representative samples from each point was collected and stored in polythene bags, transported to the microbiology research lab in sealed, labeled containers and maintained a temperature of 4°C, or less to ensure minimal biological activity. Soil samples are processed in 24 h for isolation, enumeration, identification and characterization of microbial flora. A portion of the effluent and soil samples were acidified to pH ≤ 2 with Conc. HNO_3, refrigerated to prevent the volume change due to evaporation and the concentration of various heavy metal present in all the samples. A portion of moist soil samples was dried and sieved to remove the particles greater than 2 mm, homogenized and stored in a plastic bag for further analysis.

Physico-chemical Analysis of Textile Dying House Wastewater:

Standard methods[17] were used for analyses of various physicochemical parameters of the effluent. The physico-chemical parameters like color, odour, pH (pH meter Model EC1O), temperature (thermometer) were recorded at the spot.

Enumeration, isolation and identification of bacteria:

The soil samples were analyzed to identify microbial groups *viz*. bacteria and fungi by following standard procedures with their specific Nutrient agar and Sabourand Dextrose Agar media (Hi-Media Laboratories Pvt. Ltd., Mumbai, India) was used for isolating bacteria and fungi.

Scanning Electron Micrography:

The surface structure of bacteria and fungi were analyzed by scanning electron microscopy (SEM-TESCON). The samples were mounted on aluminum stab sequenced by sputter coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs[18].

RESULTS

The results of physico-chemical analysis of untreated textile dye effluent and treated effluent by bacterial and fungal consortia were presented in Table-1.

The textile dye effluent samples were highly colored and fishy odour. Colour is the first contaminant to be recognized due to the presence of unused dye stuff in water, which affects the aesthetic as well as the biological activity of the ecosystem[19]. Photosynthesis activity was affected due to dark coloration and disagreeable odor in water may be because of the presence of decaying vegetation, inorganic and organic materials in the effluent[20].

The *pH* of textile dye effluent was slightly alkaline 7.8 ± 0.21 whereas it was reduced to 7.2 ± 0.21 and 7.2 ± 0.15 for bacterial and fungal consortia degraded samples. In the present study, TS 2700 □ 0.84 for the untreated effluent and 1800 □ 0.21 for bacterial consortium degraded sample whereas 1700 □ 0.64 mg/l for fungal consortium degraded sample. The TSS 1780 □ 0.75 for the untreated effluent, reduced to 1320 □ 0.63 and 950 □ 0.84 mg/l for bacterial and fungal consortia mediated degradation samples. The TDS 3420 □ 0.65 for the untreated effluent, reduced to 2150 □ 0.84 and 1850 □ 0.41 mg/l for bacterial and fungal consortia mediated degradation process. The results are in line with Arun Prasad and Bhaskara Rao (2011)[21] as mentioned earlier and also with Furaha *et al*. (2015)[22]. TSS (1750 mg l^{-1}) 5875 mg l^{-1} TDS. Different industries have different amount of solid particulate matter either as suspended solids or total dissolved solids in the effluent. The TS, TSS and TDS affect the light intensity of water, influencing turbidity and transparency. Avasan Maruthi and Ramakrishna (2001)[23] observed the TSS

of sugar mill effluent was 220 to 790 mg l^{-1}. Senthil Kumar *et al.* (2001)[24] observed the discharged effluent from mill with total solids of 4485 - 1520 mg l^{-1}. The probable reason for the fluctuation in total solids and subsequent dissolved solids may be due to collision of the colliding particles, influenced by pH of the effluents. The EC remortality reduced from 4200 □ 0.80 in the untreated effluent to 2720 □ 1.21 for bacterial consortium degraded effluent and 2150 □ 0.63 μmhos/cm for fungal consortium degraded effluent. Jamaluddin Ahmed and Nizamuddin (2012)[25] reported EC range from 487 to 6225 μmhos/cm falls in line with the present investigation.

The Aquatic ecosystem totally depends on dissolved oxygen, various biochemical changes and its effects on metabolic activities of microorganism. All the literature collectively indicated the importance of the dissolved oxygen concentration. The recommended DO in normal drinking water standard was 6 mg/l. DO is an index of physical and biological process in water and the most important parameter to assess the water quality. It measures the molecular oxygen utilized during specified incubation period for degradation of organic material and the oxygen used to oxidize inorganic material. In general, BOD reflects the high concentration of substances that can be biologically degradable and consumption of oxygen by microbes, potentially results in dissolved oxygen. The results of the present study 160 □ 1.23 for the untreated textile effluent, 42 □ 1.04 and 18 □ 0.54 mg/l for the bacterial and fungal consortia remediated samples shows much less values when compare with the work of Okunade and Adekalu (2013)[26] reported BOD of Cassava industry varied between 70 to 290 mg l^{-1}. Ameer Basha and Rajaganesh (2014)[27] reported BOD ranging between 243 to 1842 mg/l in textile dye effluent.

In textile effluent, pollution gets aggravated by the presence of free chlorine and toxic heavy metals leads to an oxygen sag in receiving water. In other words, a high degree of pollution is associated with high COD[28]. In present study the COD ranges from 570 □ 1.41 in textile untreated effluent to 260 □ 1.63 mg/l for bacterial consortium bioremediared effluent and 180 □ 0.48 mg/l for fungal consortium remediated samples in a decreasing order due to bioremediation. However, high and low COD levels, 584 mg l^{-1} and 160mg l^{-1} have also been reported to textile effluents. Mohabansi *et al*. (2011)[19] reported textile effluent released from textile mill are within the WHO standard limits, though BOD and other parameters were found to be high. Rajeswari *et al*. (2013)[29] reported high COD (2865-997 mg l^{-1} from textile dyeing effluent. If the Ratio of BOD:COD is above 0.5, the waste water is considered to be highly biodegradable. Azbar *et al*. (2004)[30] reported textile wastewaters exhibit low BOD: COD ratios (< 0.1), indicating non-biodegradable nature of dyes which is opposing to the present study.

The Chloride content in the effluent also contributes to the increased TDS and found to be positively correlated with increased EC, TDS, TSS,% NaCl, alkalinity and sulfate content. The present study also agrees the report and found to vary between 980□□□□□.08 for untreated sample, 620 □ 1.06 and 580 □ 0.21 mg/l for bacterial and fungal consortia degraded samples. Pratibha Mahawar and Azra Akhtar (2015)[31] reported that physico-chemical properties like EC (1470-2200 μS/cm), BOD (488-1090 mg l^{-1}) and COD (3120-6864 mg l^{-1}) TDS (980-1440 mg l^{-1}), Total hardness (230-2250 mg l^{-1}) of the textile dye effluent were found to be higher than the WHO standards.

The Total Hardness (TH) of waste water which is a combined effect of Calcium (Ca^{2+}) and Magnesium (Mg^{2+}) metal cations present effluent, the present study revealed 1870 □ 0.64 in the case of untreated effluent, whereas 1070 □ 0.81 for bacterial consortium, 780 □ 0.51 mg/l for fungal consortium degraded of effluent. Okunade and Adekalu (2013)[26] reported a range of 195 to 376 mg/l in different location of samples tested. According to Deepali Gangwar (2011)[32], the TH value was 676.95 mg/l in Textile industry effluent. These results show much lesser value than the present investigation.

The major problem associated with the textile processing effluents is the presence of heavy metal ions which arise from material used in the dyeing process or in considerably higher amount, from metal containing dyes. Metal based complex dyes release heavy metals are not only the source of pollution to nearby water bodies and the surrounding soil. The concentration of heavy metals present in the effluent samples were above the recommended CPCB limits and guideline for irrigation. Metals like calcium, magnesium, nickel, sodium, zinc, chromium, manganese, copper, lead and iron have been detected in the untreated textile effluent. The concentration of these metals are much reduced during the degradation by both bacterial and fungal consortia. Calcium level 330 □ 0.21 was reduced to 220 □ 0.42 and 210 □ 0.32 mg/l, magnesium 180 □ 0.48 to 140 □ 0.21, 130 □ 0.46 mg/l, Nickel 1.06 □ 0.73 to 1.02 □ 0.43, 1.00 □ 0.61 mg/l, sodium 980 □ 0.84 to 520 □ 0.61, 500 □ 0.89 mg/l, zinc 1.12 □ 0.21 to 1.00 □ 0.21, 1.00 □ 0.21 mg/l, chromium 4.38 □ 0.05 to 2.16 □ 0.31, 1.80 □ 0.85 mg/l, manganese 1.24 □ 0.05 to 1.12 □ 0.21, 1.10 □ 0.42 mg/l, copper 1.26 □ 0.02 to 0.10 □ 0.15, 0.10 □ 0.01 mg/l respectively for untreated effluent and treated effluent by bacterial and fungal consortia. Lead level 0.80 □ 0.02 was reduced to 0.15 □ 0.22 and 0.10 □ 0.85 mg/l, iron 2.14 □ 0.15 to 1.82 □ 0.30 and 1.80 □ 0.45 mg/l.

Results of Sabour *et al*. (2002)[33], Malik *et al*. (2002)[34] and Jaishree and Khan (2014)[35] are similar to the present study, they reported high concentration of Cd, Cr, Cu, Ni and Pd and Zn from the fabric industry effluent. Similarly Joshi and Kumar (2011)[36] reported Cr (0-0.1126 mg l^{-1}), Zinc (1.05 to 8.77 mg l^{-1}), Pb (0.0029 to 0.1394), Co (0 to 0.0290 mg l^{-1}), Ni (0 to 0.3721 mg l^{-1}), Cu (0 to 0.3517 mg l^{-1}), Ameer Basha and Rajaganesh (2014)[27] reported lead 11.12 to 32.10 mg/l and zinc 13.03 to 36.13 mg/l in the textile effluents are complied within the permissible limit and paradoxical to

the present study. Studies conducted by Pratibha Mahawar and Azra Akhtar (2015)[31] reported the metal concentrations Zn (0.3-6.8 mg g^{-1}), Cu (0.8-48.65 mg g^{-1}), Mn (4.3- 11.5 mg g^{-1}) and Fe (0.2-9.0 mg g^{-1}) were found to be higher than the guidelines for irrigation.

Degradation of Textile Effluent and Dyes by Bacterial and Fungal Consortia:

The isolates *B. subtilis* (NCBT 012), *C. butyricum* (NCBT 017), *E. aerogens* (NCBT 024) and *P. fluorescens* (NCBT 046) were used as consortium for the degradation of textile effluent. The fungal isolates *Aspergillus erythrocephalus* (NCBT (NCBT 124), *Aspergillus fumigatus* (NCBT 126), *Cladosporium herbarum* (NCBT 142) and *Fusarium oxysporium* (NCBT 156) were used as consortium for the degradation of textile effluent. There are many reports reveals the efficiency of degradation by bacterial and fungal consortia rather than the individual microb. The reports of Tony *et al*. (2009)[37], Jadhav *et al*. (2008, 2010)[38,39], Khadijah *et al.* (2009)[40] and Ajao *et al.* (2011)[41] are supporting evidence of the present report regarding the efficiency of degradation of textile dyes effluent by bacterial and fungal consortia.

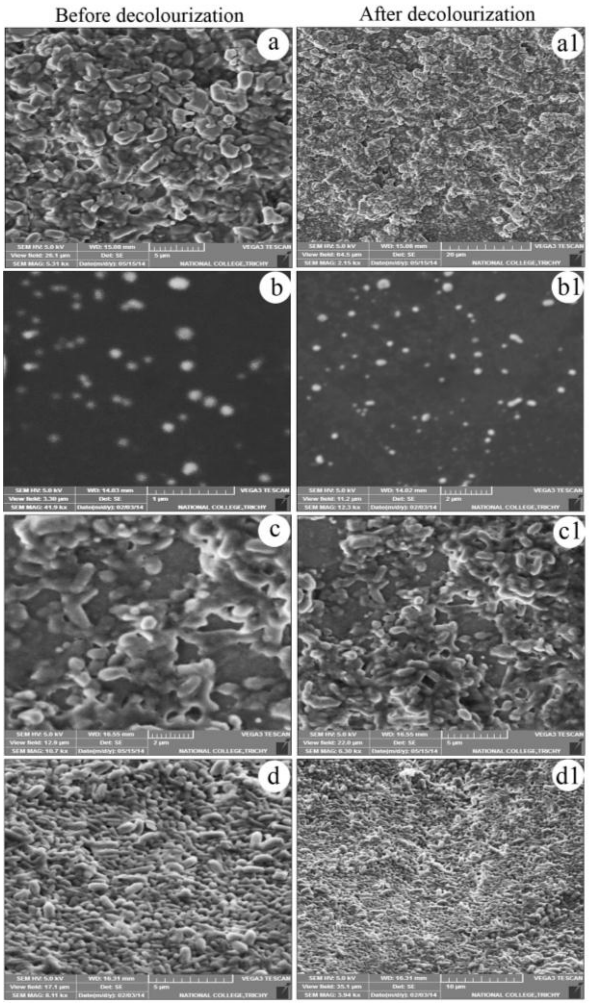
SEM Image Study of Bacterial and Fungal Strains:

The scanning electron microscopy image of bacteria

Table-1. Physicochemical analysis of textile dye house effluent using microbial consortium mediated degradation process

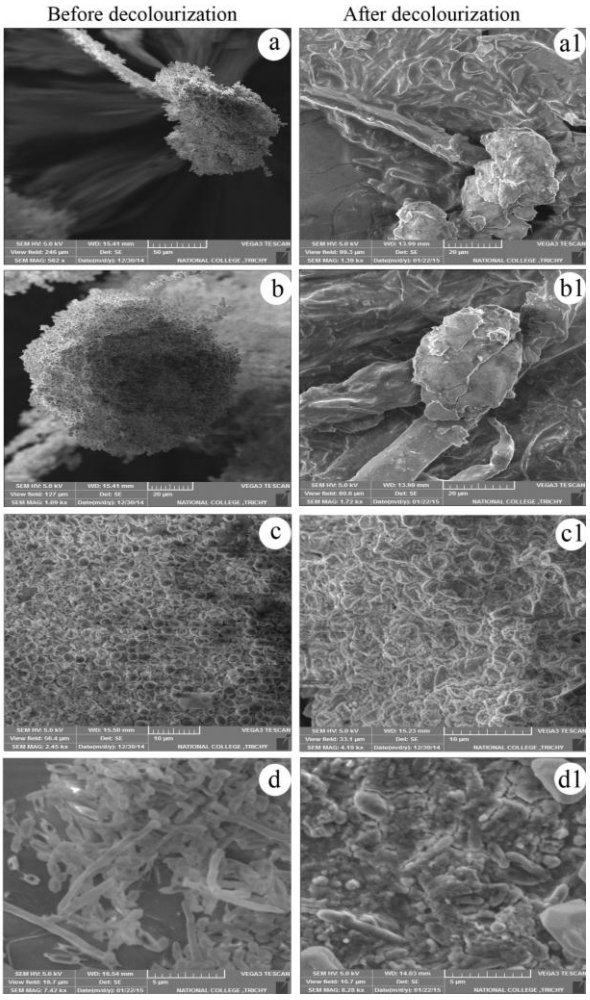
S.N.	Parameters	Unit	Untreated sample	Bacterial consortium	Fungal consortium	WHO standards
1	Colour	-	Tann	Light Tann	Light Tann	-
2	Odour	-	Unpleasant	No odour	No odour	-
3	pH	-	7.8 □□□□□□	7.2 □ 0.21	7.2 □□□□□□	7.0-8.5
4	Total Solids	mg/l	2700 □□□□□□	1800 □□□□□□	1700 □□□□□□	500-1500
5	Total Suspended Solids	mg/l	1780 □□□□□□	1320 □□□□□□	950 □□□□□□	100-600
6	Total Dissolve Solids	mg/l	3420 □□□□□□	2150 □□□□□□	1850 □□□□□□	850-1500
7	Electrical Conductivity	µmhos/cm	4200 □□□□□□	2720 □□□□□□	2150 □□□□□□	500-1500
8	Biological Oxygen Demand	mg/l	160 □□□□□□	42 □□□□□□	18 □□□□□□	≥ 5.0
9	Chemical Oxygen Demand	mg/l	570 □ 1.41	260 □□□□□□	180 □□□□□□	250
10.	Dissolved Oxygen	mg/l	20 □□□□□□	12 □□□□□□	8.0 □□□□□□	≤ 6.0
11.	Total Hardness as $CaCO_3$	mg/l	1870 □□□□□□	1070 □□□□□□	780 □□□□□□	500
12.	Carbonate alkalinity	mg/l	36 □□□□□□	28 □□□□□□	28 □□□□□□	200
13.	Bicarbonate alkalinity	mg/l	370 □□□□□□	190 □□□□□□	170 □□□□□□	50
14.	Chloride	mg/l	980 □□□□□□	620 □□□□□□	580 □□□□□□	200-600
15.	Calcium	mg/l	330 □□□□□□	220 □□□□□□	210 □□□□□□	75-200
16.	Magnesium	mg/l	180 □□□□□□	140 □□□□□□	130 □□□□□□	50-150
17.	Nickel	mg/l	1.06 □□□□□□	1.02 □□□□□□	1.00 □□□□□□	-
18.	Sodium	mg/l	980 □□□□□□	520 □□□□□□	500 □□□□□□	1000-1500
19.	Sulphate	mg/l	520 □□□□□□	380 □□□□□□	320 □□□□□□	200-400
20.	Zinc	mg/l	1.12 □□□□□□	1.00 □□□□□□	1.00 □□□□□□	5.0
21.	Chromium	mg/l	4.38 □□□□□□	2.16 □□□□□□	1.80 □□□□□□	0.1
22.	Manganese	mg/l	1.24 □□□□□□	1.12 □□□□□□	1.10 □□□□□□	2.0
23.	Copper	mg/l	1.26 □□□□□□	0.10 □□□□□□	0.10 □□□□□□	0.2
24.	Lead	mg/l	0.80 □□□□□□	0.15 □□□□□□	0.10 □□□□□□	0.10
25.	Iron	mg/l	2.14 □□□□□□	1.82 □□□□□□	1.80 □□□□□□	4.5

Figure-1. SEM Pictures of Bacteria



a,a1) Bacillus subtilis (NCBT-012)
b,b1) Pseudomonas fluorescens (NCBT-046)
c,c1) Enterobacter aerogens (NCBT-024)
d,d1) Clostridium butyricum (NCBT-017)

Figure-2. SEM Pictures of Fungi



a,a1) Aspergillus erythrocephalus (NCBT-124)
b,b1) Aspergillus fumigatus (NCBT-126)
c,c1) Cladosporium herbarum (NCBT-142)
d,d1) Fusarium oxysporum (NCBT-156)

and fungi before and after degradation of textile dye effluent revealed different morphology. The bacteria have undergone remarkable physical disintegradation, reduction population. The cell wall matrix layer of bacterial degradation samples have shown shrinkage and sticky nature[42]. The fungi also undergone cell wall matrix shrinkage, surface erosion, cracks, folding, dumping, loss in conidial or spore production. Volke-Sepulveda (2002)[43], Limon-Gonzalez and Favela-Torres (2004)[44] and Sahebnazar et al. (2010)[45] reported that these morphological changes is mycelium, conidin, conidine or spore structure are due to the extracellular metabolites and enzymes released by the fungi in response to degradation stress.

DISCUSSION

The PCR amplification yielded product at 393 bp (Figure 1) as expected for *KAP* 3.2 gene. Similarly, Mahajan *et al*. (2015) and Wang *et al.* (2010) also obtained products at 393 bp whereas McLaren *et al.* (1997) observed product at 424 bp.

PCR amplicons were subjected to SSCP analysis to detect the polymorphic patterns of *KAP* 3.2 gene in three different genetic groups of sheep. PCR-SSCP analysis of *KAP* 3.2 gene (Figure-2) revealed *AA*, *AB* and *BB* genotypes in the two breeds with predominance of *AA* genotype. The genotype frequencies of *AA, AB* and *BB* were in the order of 0.84, 0.16 and 0.0 in Sandyno breed and 0.86, 0.12 and 0.02 in Nilagiri breed respectively. The *A* and *B* allele frequencies were 0.92 and 0.08 respectively in both

Sandyno and Nilagiri breeds of sheep (Table-1). Wang *et al*. (2011) observed similar type of polymorphism in *KAP* 3.2 gene with three genotypes (*AA, AB* and *BB*) in Tibetan sheep. Similarly, Itenge-Mweza, (2012) in Merino sheep and Mahajan *et al.* (2015) in Rambouillet sheep observed three genotypes by PCR-SSCP analysis. Contrary to the present findings, Mahajan *et al.* (2015) in Rambouillet sheep observed the genotypic frequency for *KAP* 3.2 gene as 0.46 , 0.40 and 0.14 for *AA, AB* and *BB* genotypes respectively. Whereas, the gene frequencies were 0.66 and 0.34 for *A* and *B* alleles, respectively in Rambouillet sheep. The present populations were consistent with Hardy-Weinberg equilibrium and had no significant difference (P > 0.05) in *KAP* 3.2 gene for both Sandyno and Nilagiri breeds.

The heterozygosity value (0.1591) in Sandyno breed was almost similar to the expected heterozygosity (0.1481) for *KAP 3.2* gene (Table-2). In Nilagiri breed of sheep, the heterozygosity value (0.1176) was less than the expected heterozygosity (0.1460) suggesting a high degree of homozygosity (0.8824). However, Mahajan *et al*. (2015) reported expected heterozygosity (He) value of 0.45 in Rambouillet sheep and Wang *et al.* (2010) for Tibetan sheep (0.50) and Wang *et al.* (2011) for Tibetan (0.50), Oula (0.47) and Qiaoke (0.29) sheep.

The effective number of alleles (N_e) for *KAP 3.2* gene was 1.1716 and 1.1690 in Sandyno and Nilagiri breeds of sheep respectively (Table-2). Mahajan *et al*. (2015) observed almost similar value of 1.81 in Rambouillet sheep. The results obtained in this study were not in agreement with those reported by Wang *et al.* (2010) for Tibetan sheep (2.00) and Wang *et al.* (2011) in Tibetan (2.00), Oula (1.87) and Qiaoke (1.40) sheep.

The PIC values for *KAP* 3.2 gene was 0.1356 and 0.1341 in Sandyno and Nilagiri sheep respectively (Table 2). However, Mahajan *et al*. (2015) estimated polymorphic information content (PIC) values with medium polymorphism as 0.35 in Rambouillet sheep. The result is deviated from the findings of Wang *et al.* (2010) for Tibetan sheep (0.38) and Wang *et al.* (2011) in Tibetan (0.38), Oula (0.36) and Qiaoke (0.24) sheep.

The F_{IS} values were negative (– 0.0864) in Sandyno breed and were positive (0.1862) in Nilagiri breed for *KAP* 3.2 gene (Table 2). However, Mahajan *et al*. (2015) observed Fixation index (F_{IS}) value of 0.11 in Rambouillet sheep. Deviation from the reported studies at *KAP* 3.2 gene may be due to breed differences and selective breeding practices. However, presence of few alleles at the *KAP* 3.2 loci in Sandyno and Nilagiri breeds of sheep indicates monomorphic situation.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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