

ORIGINAL RESEARCH PAPER

Zoology

ZINC CHLORIDE INDUCED HISTOPATHOLOGY OF DART GLAND AND OVOTESTIES IN TERRESTRIAL SLUG SEMPERULA MACULATA

KEY WORDS: Zinc chloride; slug Semperula maculata; histopathology; gametogenic cells

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ABSTRACT

Now a day, the increased contamination of heavy metal due to industrial discharge. This study enlights on terrestrial Mollusc slug, Semperula maculata, against lethal concentrations of Zinc chloride (ZnCl2). Histopathological changes were observed in the cellular arrangement of dart gland and ovotestis. ZnCl₂ included alterations found in the dart gland and ovotesties. Ovotestis showed depleted number of gametocytes. Vacoulized pre-vitellogenic oocytes with early vitellogenic oocytes were noted under light microscopy. Secretory functions found altered in the ZnCl2 induced slugs. Evidence indicates that Zn produced gamete degeneration and impact over the normal function and structure of reproductive organ.

Introduction

Molluscs have more varied forms than any other animal phylum. They include snails, slugs and other gastropods, clams and other bivalves; squids and other cephalopods; and other lesser- known but similarly distinctive subgroups. The majority of species still live in the oceans, from the seashores to the abyssal zone, but some form a significant part of the freshwater fauna and the terrestrial ecosystems freshwater and terrestrial molluscs appear exceptionally vulnerable to extinction. Estimates of the numbers of many regions have not been thoroughly surveyed. There is also a shortage of specialists who can identify all the animals in any one area to species. However, in 2004 the IUCN Red List of threatened species included nearly 2000 endangered non-marine molluscs.

Along with other terrestrial fauna, invertebrate molluscs are recognized as important animals principally involved in food chain and agricultural pests. Terrestrial gastropods are sensitive to toxic chemicals producing alterations at the cellular level^{1,3}. for the mussel *Elliptio complanata*, exposure to Cu had a significant effect on the mean percentage of destabilized lysosomes for both the 7-day exposure. They also studied the exposure on gametes and embryos of oyster to environmental concentrations of pesticides and copper increased developmental abnormalities and DNA damage and reduced fertilization success and affected offspring quality significant changes in transcription of genes involved in antioxidant defence were observed for oyster larvae exposed to metalachor and metalachlor.

One effect of the extruded mucus is to form a protective barrier preventing direct contact between the toxic and the epithelia of the skin or digestive tract, so reducing the toxicity of the chemicals. Within the first 24 hr, snails in this study fed higher concentrations of 400 and 500 mg/ml began to swell around the anterior region. This agrees with, that by 30 min after ingestion of carbonate, the D. reticulatum show violent muscle convulsion, the anterior body began to swell while the posterior flattens. Most snails in this study that ingested contaminated baits ceased feeding and ceased crawling.

According to in the environment, chronic exposure conditions could have a significant effect on an entire ecosystem. Since in order to compensate for a condition of permanent chemical stress, many organisms may have to keep repair and defense mechanism continually in activities, and invest a large amount of energy into limiting cell damage, with little or no energy left for other activities . A mollusc is known to process mechanisms to deal with the bioaccumulation of several chemicals in their tissues. Several investigators reported that term strains it is and slugs displayed capacity of the coechinulation and biomagnification of heavy metric. Hence betweetrial molluscs were considered stritable to monitor the bioavailability of metallic compensations in soils as compared to other invertebrates. At exclusive concentrations, neavy metals affect numerous biological processes involved in the development and maintenance of molluscan faunt including feeding, growth, reproduction, peneral physiological activities and maturity.

Toxicity with heavy metals leads to the production of reactive oxygen species (ROS) in the biological system, which disrupts normal cellular processes. Duruibe, Ogcouegbu and Egesurugcou (2007) "reported heavy metal effects on the nervous system and the behaviour of terrestrial animals," noted behavioural changes representing it as a biomarker and correlated this to biochemical and physiological processes. "found the accumulation of copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) in the digestive gland, gills, and reproductive organs of gastropod snail Levantia hierosylima. "examined the histopathological effect of heavy metal like Cu and Pb on the hepatopancreas and ovotesties of giant land snail, Archachatina marginata (Swainson).

Swaileh and Ezzughayyar (2000) found an effect of Cd and Cu on the mortality, growth impairment, altered feeding behavior, bioconcentration, and biomagni□cation in molluscan species Helix engaddensis. ³⁰ reported depleted feeding and growth responses following exposure to Cu, Zn, Hg, and Pb toxicity in the terrestrial gastropod Arion ater (Linn). However, there seem to be insuf□cient data on the in□uence of Zinc chloride (ZnCl₂) on various physiological

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aspects of terrestrial molluscs. This study was designed to investigate histological alterations in Dart Gland of the terrestrial slug Semperula maculata after acute exposure to

MATERIALS AND METHODS

Experimental Aanimals:-

Adult herbivorous, hermaphrodite, terrestrial slugs S. maculata (Approximately of 67 cm L,11.5 cm W, 34 g wt) were collected from natural habitats of Panmala at Bedug, Miraj, district Sangli, Maharashtra, India. Animals were carried in aerated plastic bottles to the lab. Experimental animals were kept in open-air trough covered with aerated plastic lead covering to provide proper ventilation. Experimental animals were allowed to feed on fresh leaves of mulberry plant (Morus indica). All the animals were kept under controlled lab conditions of water, temperature, and fresh air for better acclimatization.

Induction and Tissue preparation:-

Experimental animals, S. maculata, were acutely exposed to previously determined mean LC50 (377.7 ppm) concentration of ZnCl₂. ³¹ Control and experimental animal were dissected after 24, 48, 72, and 96 hr, respectively, for dart gland and ovotesties 0 xed in Bouin's solution (75 ml picric acid + 25 ml formalin+5 ml acetic acid) for 6-7 hr at room temperature followed by washing with 70% ethanol for three days, dehydrated with ethanol-graded series, cleaned with xylene, and embedded in wax. Tissue blocks were prepared and sectioned with a rotary microtome at 6 mm thickness and for histological study.

Histological study:-

Hematoxyline and eosin technique (H&E):-12 Harris 1900

For histological study, tissue sections were dewaxed in xylene, hydrated in alcohol grades of 100%, 90%, 70%, 50%, and 30%, anally in distilled water. Sections were stained with aqueous hematoxyline for 7 min. Stained sections were differentiated in distilled water, again dehydrated in 30%, 50%, and 70% alcohols, respectively. All sections were treated with eosin for 45 sec. Furthermore, sections were differentiated in 70% alcohol, dehydrated in 90% absolute alcohol, cleared in xylene, and mounted in Di-N-butyl phthalate in xylene (DPX).

RESULTS

Histological study:-

Toxicity study related to the dart gland was studied by applying the standard histological techniques for slug S. maculata exposed to ZnCl2.

Dart gland:-

Histologically, the dart gland showed large peripherally arranged glandular cells with basal uninucleated circular cells, muscle Obers with lumen in it (Figure-I A). The luminal part showed several granular droplets, which exhibited pink spots stained with H&E (Figure- I A). Histological features visualized in the control section of the dart gland were similar with the observation of 23.

After the exposure to ZnCl, alterations in the internal architecture of dart glands were noted. After 24 and 48 hr, slight hypertrophic change was found in peripheralarranged glandular cells (Figure- I B,C). The lumen content was also disturbed. These alterations were more severe after the 72- and 96-hr exposure (Figure- I D, E). Peripheral glandhar cells, were lost due to their regular membrane corrent Quantitative oring of lesions of the dart glands after acute exposure is shown in Table 2. These structural alterations found were directly associated with the time of exposure and the congentration of toxicant. (Figure-IA, B, D,

Ovotesties:-

Microscopic structure of ovotesties consists of a number of follicle or acini (Figure- II A). The growing pre-vitellogenic oocytes were observed with prominent nuclei and nucleoli. The spermatocytes and mature spermatids were found scattered in the sectional view of ovotesties (Figure 7(A)). Spermatocytes I in the meiotic prophase and spermatocytes II in the rosette-forming form were observed in the control section of ovotesties. A similar type of structural architecture was described by 24.

Structure of ovotesties after 24 and 48hr remained unaltered showing the similarity to the control. Sectional view showed scattered spermatozoa along with vacuolization in growing pre-vitellogenic oocytes (Figure-IB, C). After the 72- and 96hr exposure, it was observed that spermatocytes were more disturbed and hypertrophic by their internal content. Growing pre-vitellogenic oocytes and early vitellogenic oocytes were vacuolated and some without nucleoli (Figure-I D, E). After acute exposure for long time, ovotesties exhibited major disruption in follicles of acini. The intensity of sperm degeneration was signi Cantly increased.

DISCUSSION

Toxicity mechanisms underlying ZnCl, in gastropod involved disturbances in the rate of reproduction and different aspects of behavior. In our earlier study, it was found that ZnCl2 was toxic to the neuronal and respiratory physiology of the terrestrial slug S. maculata and freshwater snail Bellamya bengalensis 20.20.27.29. In this study, the bioaccumulation of ZnC12 increased signi cantly as the time of exposure was elevated. "reported that molluscs were found to have higher capacity to accumulate metals to varying degree depending upon the concentration of exposure and the type of metal.29 studied the bioaccumulation of Pb in the intestine, prostate gland, digestive gland, ovary, albumin gland, testis, stomach, and cerebral ganglia and noted a maximal uptake in the intestine and less in the prostate gland, digestive gland ovary, and testis. 28 observed exposure dependent bioaccumulation of Hg and Zn in the nervous system and the gill tissue of freshwater snail B. bengalensis. 30 noted that Cd accumulated in the proboscis, esophagus, stomach, digestive gland, rectum, and gill of snail Babylonia areolata (spotted Babylon) and was increased with the time of exposure.

In this study, the terrestrial slug S. maculata tried to escape from trough due to acute intoxication by Zn. After intoxication, foot movements were slowed down. 31 reported altered motility in animals exposed to neurotoxic gramoxone. observed swollen foot of Limicolaria aurora after the 96-hr exposure to gramoxone. Similar type of sluggishness and swelling of the body were recorded in S. maculata after the 72hr intoxication period. 5 reported morphological changes in slug Deereoceras reticulatum induced by the sub-lethal concentration of carbamate and metaldehyde. Mucus secretion of the body and the foot of slug S. maculata were found higher in the presence of ZnCl2 after 72 and 96 hr. Extruded mucus proved to be barrier in protective mechanism and prevented contact between the toxicant and skin epithelia 3. Higher mucus production indicated toxic stress produced by ZnCl2 in trough. Other alterations, such as decreased mechanical stimuli or chemical reactions were also mediated by metaldehyde⁴. Similar results were observed in S. maculata against ZnCl.,

Similar to the digestive gland, the reproductive gland is also sensitive and may be used for the biomonitoring of heavy metal pollution. Major histopathological changes were observed in the cellular architecture of the penis and dart gland after the exposure to ZnCl2. After 96 hr, the penis and the dart gland showed changes in the structure which included the dilation of unicellular and multicellular glands,

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the degeneration in the muscular Ober, the dilation in secretory cells, and the disruption in the luminal content.

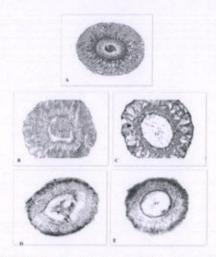


Fig-I ZnCl2 induced alteration in Dart gland of slug S. maculata at different exposure period. Fig.- A - Control group, Fig.- B- 24 hr , Fig.- C - 48 hr, Fig.- D - 72 hr, Fig.- E - 96 hr, Pgcperipheral glandular cells, Lc-luminal content.

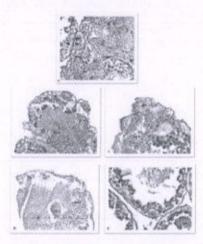


Fig-II ZnCl, induced alteration in Ovotesties of slug S. maculata at different exposure period. Fig.- A - Control group, Fig.- B- 24 hr, C - 48 hr, Fig.- D - 72 hr Fig.- E - 96 hr, Scsecretary cells, Ct-connective tissue, Lc-luminal content, ST1 -Spermatocytes I, ST2 -Spermatocytes II, PO - Pre-vitellogenic

CONCLUSION

This study reaf rms toxic effects of ZnCl, with histopathological alterations in slug S. maculata. Animal showed hyper accumulation and rapid behavioral changes to counteract ZnCl, effects. The histopathological changes represented end point contamination in the terrestrial media, which will be hazardous for the survival of the terrestrial as well as aquatic fauna. The major contaminations provide an imbalance to the ecological diversity in the region. Hence, this study was designed to create awareness to protect these animals against severe toxic pollution and to maintain ecobalance through food chain.

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Kalyan Bharati

Meliola parlensis sp. NOVA AS A NEW SPECIES OF BLACK MILDEW FROM WESTERN GHATS-II

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Abstract

An attempt has been made to survey black mildew micro fungi from Kolhapur district, Presently a new species of a black mildew fungal taxa order meliolales has been collected from sateri sacred grove. Parle near Chandgad (Dist Kolhapur) and identified as species new to science as Meliola parlensis Dopare and Patil.

Keywords: Meliola parlensis, Western Ghat, Micro fungi.

Introduction:

Parle is a small village placed in south most taluka Chandgad in Kolhapur district and situated between 15°51'56"N and 74°12'14"E. Sacred groov at parle is reserved for goddess Sateri and spread over in two hector of forest land receiving annual rainfall of about 3000mm at an elevation of 715m from MSL. Being a part of western ghat, it is surrounded with rich forest. The climatic conditions within the sacred grove at parle favors the growth of black mildew micro fungi, being commonly occurs in tropical and sub tropical and also temperate regions of the world. These are moisture loving and grow on wide range of host plants, showing high degree of host adaptation. These are predominently follicolous and tend to grow best in humid and wet conditions in shaded habitats (Toro 1952,Goos.1978), these black mildew micro fungi belongs to different taxonomic groups viz. meliolaceous, schiffnerulaceous, asterinacous and hyphomycetous fungi.

Meliolaceous fungi characterized by presence of two celled appressoria born on mycelium, mycelial setae, phialides and 3-4 septate ascospores.

During the exploration of black mildews from study area, the present species is found to be new to science and hence described as a new species on hitherto, new host.

Material and Methods:

The infected leaves of *Flaconeria insignis* Royle (Syn= *Sapium insigne*(Royle)Trimen) from angiospermic Family Euphorbiaceae were collected from sacred grove near parle, chandgad village of Kolhapur district, during winter season(2018-2019). Host plant is identified using relevant literature (Sardesai and Yadav 2002). The specimen was collected in sterilized polythene bags brought to the laboratory; dried and preserved in herbarium form for further investigation. The macro- and micro morphological characters are used for toxonomical description. The slides were prepared using technique of (Patil and Patil 2017) and stained by using cotton blue(in lactophenol). Using toxonomical

description a black mildew fungus was identified with the help of standard literature (Harsnford 1962; Hosagoudar 1996, 2000, 2008; Hosagoudar and Agarwal 2008; Bhise 2015, Lonkar 2019, Bilgrami et al 1981;1991; Jamaluddin et al. 2004)

Result:

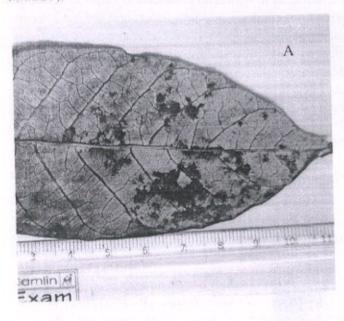
Taxanomy

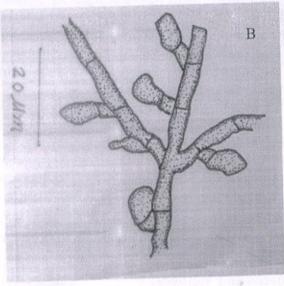
Meliola parlensis Dopare and Patil SPECIES NOVA

Colonies amphigenous, subdense scattered, confluent, rounded to irregular velvety dark black, crustose, 1cm in diameter. Hyphae substraight to undulate, opposite to alternate at acute angles, closely reticulate, 20-36X8-12um.

Appressoria two celled, alternate to unilateral, antrorse, straight 20-33X10-15um. Stalk cell cylindrical to cuneate, 7-12X7-8 um. Head cells oblong to ovate, entire, straight to curved, moderately placed, broad at apex, 17-21X10-15 um. Philalides few, pale to brownish yellow, ampulliform, mixed with appressonia, opposite to alternate, unilateral, neck long, 13-23X8-13um. Mycelial setae many, simple, straight to curved, scattere over the colony, obtuse to acute, grouped around perithecia 390-510um in length. Perithecia black, globose, verrucose, scattered over colony 96-175um in diemeter. Ascopores 4 septate, pale yellow, cylindrical, slightly constricted at septa, oblong, 26-36X12-17um.

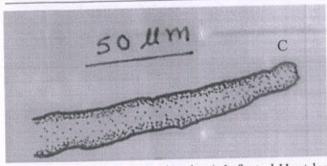
Speciman Examined: On living leaves of new host Flaconeria insignis Royle (Syn=Sapium insigne(Royle)Trimen). Family-Euphorbiaceae, Sateri Sacred groove, Parle village near Chandgad of Kolhapur district collected by Dopare Bharati 20 Jan 2019 field no. 2095 ARI order number 949(MB 830807).











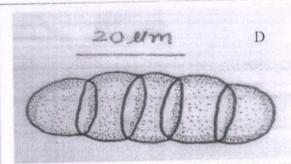


Figure A-D. Meliola Parlensis. A Infected Host leaf, B Apprissoriate Mycelium with Phialides, C Tip of Mycelial setae, D Ascospore.

Known Distribution- India as first record.

Note: The present specimen is found to be new to science on hitherto new host.

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RESEARCH ARTICLE

MASS SPECTRAL ANALYSIS (GCMS) GAS CHROMATOGRAPHY AND MASS SPECTROMETRY OF VARIOUS VOLATILE BIOACTIVE COMPOUNDS IN SONAKA SEEDLESS RAISINS

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ABSTRACT

Grape (Vitis sp.) belonging to Family Vitaceae is a commercially important fruit crop of India. Grapes are eaten as raw or they can be used for making wine, raisins, jam, and jelly, which are very nutritious and rich source of minerals like potassium, phosphorus, calcium, magnesium, other micronutrients and different vitamins. Raisins are dried fruits of certain varieties of grapevines with a high content of sugar and solid flash (Khair and Shah, 2005). Analysis of organic compounds by FT Raman spectrum of raisin. The Raman spectra of the films were recorded in the spectral range of 35–4000 cm-1 using a Raman spectrometer (Bruker Multi RAM, Germany Make). The Raman shift corresponds to the frequency of the fundamental IR absorbance band of the bond (Thygesen et al., 2003). It is known that specific chemical bonds (C-H, N-H, and C=O) generate specific peaks (Huang et al., 2010). Hence this technique proves to be a powerful technique which facilitates to determine molecular structure of spectral pattern of fingerprinting of sample.

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INTRODUCTION

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Grape (Vitis sp.) belonging to Family Vitaceae is a commercially important fruit crop of India. Grapes are eaten as raw or they can be used for making wine, raisins, jam, and jelly, which are very nutritious and rich source of minerals like potassium, phosphorus, calcium, magnesium, other micronutrients and different vitamins. The dried grapes, commonly known as raisins, have a great importance in economy of the country and considered as one of the nutritious most popular dry fruits in the world. Raisins are dried fruits of certain varieties of grapevines with a high content of sugar and solid flash (Khair and Shah, 2005). The important raisin grape varieties are Thompson seedless and their selections like Tas-A-Ganesh, Sonaka and Manikchaman. The increased production of table grapes has a great potential to produce raisins with minimum losses of fresh fruits (Telis et al., 2004). Thapar (1960) indicated that grape was introduced in India in 1300AD by the Persian invaders in North and South India (Daulatabad in Aurangabad districts of Maharashtra) during the historic event of changing the capital from Delhi to Daulatabad by King Mohammedbin-Tughlak and in South India districts Salem and Madurai by the Christian missionaries around 1832 A. D. From Daulatabad grape cultivation was spread to Hyderabad in Deccan during the Nizam's period. Nizam of Hyderabad has also introduced some grape varieties into Hyderabad from Persia in the early 20th century (Chadha and Shikhamany, 1999). There are two subgenera viz Euvitis and Muscadania. All commercially important varieties of grape belong to sub genus Euvitis, referred as true grape. More than 90% of cultivated

grape varieties belong to this species which is referred as 'old world grape' the 'European grape' or 'California grape' (Shymal and Patel, 1953 and Shetty, 1959). India is a small producer of grapes, with a world share of less than 2 percent (Barrientos and Kritzinger, 2004). The total average cultivation of grape is near about 80,000 hectares in India and 28,000 hectares in Maharashtra. The total yield in India is about 15 to 18 lakh tons and in Maharashtra, it is about 7 to 9 lakh tons. Out of this annual production, 76% is used as table grapes, 0.3% in wine industry, 3. 70% exported to Middle East and European countries as table fruit and 20% used for preparation of raisin. Recently, grape cultivation is increased more in Maharashtra and the major growing districts are Nasik, Sangli, Sholapur, Ahmednagar, Pune and Osmanabad. Near about 80 % of grape production comes from Maharashtra followed by that from Karnataka and Tamil-Nadu. The commercial production of grapes started in India only after seedless varieties were introduced in Maharashtra during the 1960s. Maharashtra accounts for 70 percent of India's total grape acreage and 63 percent of production. Varieties grown include Thompson, Sonaka, Sharad and Tas-A-Ganesh Seedless. Within Maharashtra, the grape crop comprises 12 percent of the total fruit acreage, with 42,500 acres. Sangli, Sholapur, Pune and Ahmednagar are the other locations, with more than 2,500 acres each under grape cultivation. Due to the higher water content, these fruits drastically disintegrate and are spoiled. Hence dehydration of such fruits is urgent need to avoid the spotlage. Grape is an important source of carbohydrates, minerals and vitamins but due to its low shelf life it is very difficult to fulfill the needs of the society. The preparation of raisin was started long hack, and known as "Manuka" (simply drying the grapes in open sunlight). Then after introduction of

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mutant Thomson seedless variety many grape growers turned to prepare yellowish Golden raisin and from last fifteen years many of grape growers from Sangli district are diverted towards the preparation of green raisin at Junoni [Sholapur] and nearby areas which has a good market potential in Delhi, Kanpur and other cosmopolitan cities. Nasik, Sangli and Sholapur are the leading grape producing districts of Maharashtra. The most of these regions are drought prone. So the area under grape cultivation is increasing day by day, which will create a problem of marketing of table grape. During last 19 to 20 years due to increase in yield, expanding area under cultivation and fluctuation in the market price the farmers are slowly turning towards raisin production. Hence, an attempt has been made to study some aspects of post - harvest physiology in relation to production of raisin in two verities of grape, Thompson and Sonaka seedless growing in Sangli district.

MATERIAL AND METHODS

Analysis of Bioactive Compounds (GCMS): The methods described by Anwar et al. (2006) and Sultana et al. (2008) with slight modifications were employed for the preparation of methanolic extracts. 0.5 grams of oven dried powdered raisins subjected to different chemical treatments in Sonaka seedless were taken in a 250 ml flask and mixed thoroughly with 10 ml of 100 % methanol (HPLC grade). Methanolic extracts were obtained in an electric shaker (Remi Rotary Shaker, Mumbai, India) for 48 hours in ambient conditions (shaking intensity 120 rpm). The extracts were then filtered using Whatman No. 1 filter paper. The residues obtained after the filtration were subjected to re-extraction twice with the fresh methanol and these extracts were added to previous extracts. The crude extracts so obtained were concentrated to dryness on water bath in pre weighed evaporating dishes at 45°C. After complete drying of the extracts evaporating dishes were again weighed for determination of the yield and stored in a refrigerator (-4°C), until used for further analysis. From the dried powder, known quantity was dissolved in methanol to prepare stock solution for further use in GCMS analysis. These methanolic extracts were subjected to Gas Chromatography and Mass Spectrometry for the determination volatile metabolites.

GC-MS analysis of the samples was carried out using Shimadzu Make QP- 2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40°C and held for 3 min and the final temperature of the oven was 480°C with rate at 10°C. 2 μL sample was injected with split less mode. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 45 min. The chemical components from the methanolic extracts of raisins were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

RESULT AND DISSCUSSION

Analysis of Bioactive Compounds (GCMS: The mass spectral analysis of bioactive volatile compounds of untreated sonaka seedless raisins is shown in the Table 5 and mass fragments in the Plate 16 to 20. It is observed from the table that raisins of variety are rich in various bioactive volatile compounds. Among these compounds some represents to class of Acids, Aldehydes, Alcohols, Ketone, Alkane and Alkene. The major compound reported from the seedless untreated raisins are 2-Furancarboxaldehyde, Octacosane, Decosane, 4H-Fyran-4-one, 2, 3 dihydro-3,5 dihydoxy- 6 methyl, 1,2- Benzene dicarboxylic acid, Octane, n- Hexadecanoic acid, Heptadecane and Dibutyl phthalate. The raisins treated with K2CO3 and Sulphur fumigated shows the presences of 4 H-Pyran-4-one,2,3 dihydroxy -3, 5 dyhydroxy-6-methyl, Dibutyl phthalate, 1,2.3- Propanetriol, mono acetate, 2- Hexanone, 3-methyl-4-methylene, n-Hexdecanoic acid and Octadecanoic acid. The mass spectral analysis of bioactive volatile compounds of untreated sonaka seedless raisins is shown in the Table 5 and mass fragments in the Plate 16 to20. it is

noticed from the table and figure that the untreated Sonaka raisins shows six volatile bioactive compounds such as 1 2 -Furancarboxaldehyde, 5 (hydroxymethyl), Dibutyl phthalate, 4H- Pyran-4-one, 2, 3- dihydro-3,5 dihydroxy -6-methyl, n-Hexadecanoic acid, Octadecanoic acid and d-Glycero-d-ido-deptose. Whereas the raisins treated with K2CO3 and sulphur fumigated results in the development of fourteen various bioactive volatile compound are 9,12-Octadecadienoic acid (Z,Z) -, methyl ester, 4-Hepten-3-one, 4-methyl, n-Hexdecanoic acid, Hexadecanoic acid, methyl ester Propanoic acid, 2-methyl-, methyl ester, 4H- Pyran-4-one, 2,3dihydro-3,5-dihydroxy-6-methyl-, 9- Octadecenoic acid, methyl ester, Limonene, Oleic acid, Octadecenoic acid, methyl ester, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester, 2,6,6-Trimethyl bicycle (3.1.1)hept-Zene, Dibutyl phthalate and Petanoic acid, 4-oxo-While Zein protein coated raisins displays ten bioactive compounds are 9,12-Octadecadienoic acid, methyl ester, Propanoic acid, 3-(acetylthio)-2-methyl-, 1,2,3-Propanetriol, diacetate, Propanol, 2,3-dihydroxy, Xanthosine, Dibutyl phthalate, Hexadecanoic acid, methyl ester, Octadecenoic acid, methyl ester, 9-Octadecenoic acid, methyl ester(E) and n-Hexdecanoic acid. While the zein protein coated with mango essence exhibits eight volatile compounds are 1,2-Benzenediacarboxylic,acid, butyl 2-ethylhexyl ester, Xanthosine, Dibutyl phthalate, Squalene, Docosane, Hexadecane, 1,2-Benzenediacarboxylic, bis(2-metylpropyl) ester, Limonene and orange essence coated raisins shows ten different volatile bioactive compounds are Xanthosine, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-, 4H-Pyran-4-one,2,3-dihydroxy-3,5-hidroxy-6-methyl, Dibutyl phthalate, Benzenedia carboxylic acid, butyl 2- ethylhexyl ester, n-Hexdecanoic acid, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester, 9-Octadece namide,(2)-, 9,12-Octadecadienoic and 1,2-Benzenediacarboxylic acid, bis(2-methylpropyl) ester.

The mass spectral analysis of 5% mango essence in methanol shows ten different bioactive compounds such as 2-Propanol,1,1'-[(1methyl-1,2-ethanediyl]bis-, Benzyl alcohol, 2-Propanol,1,1'-oxybis, 2-Propanol,1,1'-[(1-methyl-1,2-ethanediyl]bis-, Diphenyl ether, 4H-2-Butanol, 3, 3-oxybis, Pyran-4-one,2-ethyl-3-hydroxy, Benzaldchyde,3-hydroxy-4-methoxy, 2(3H) Furanone, 5-hexidihydro and 4H-Pyran-4-one,2-ethyl-3-hydroxy. The 5% orange essence in methanol indicates five different volatile bioactive compounds Benzyl 2-Propanol,1,1'-oxybis, 2-Propanol,1,1'-[(1-methyl-1,2ethanediyl]bis-, 2-Propanol,1,1'-[(1-methyl-1,2-ethanediyl]bis- and Butanol,3,3'-oxybis-. While 4% rapseed oil in methanol exhibits two volatile compounds such as 1,2-Benzenediacarboxylic acid, butyl 2ethylhexyl ester and 1,2-Benzenediacarboxylic acid, methylpropyl)ester. Volatile monoterpenes shows a greater diversity of compounds. Volatiles have diverse structures and arise from the activities of several biochemical pathways. Many plants emit substantial amounts of phytogenic volatile organic compounds (PVOSs). The most common volatiles include C6 volatiles (lipoxygenase/hydroperoxide lyase-dependent pathways), indole and MeSA (the shikimic acid/tryptophane pathway), cyclic and acyclic terpenoids (isoprenoid pathway), and oximes and nitriles (derived from amino acids) (Dicke, 1999). Hanus et al., (2006) reported more than hundreds volatile compounds from the fruits of Mandragora autumnalis, which belongs to n-alkanes, branched-chain alkane, cycloxanes, alkenes, , alcohols, aldehydes, six ketones, heterocyclic compounds, thio compounds, benzene hy-drocarbons, phenols, carboxylic acids and esters of carboxylic acids.

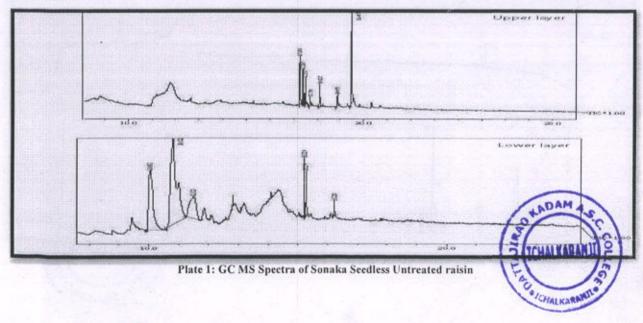
According to Ribereau-Gayon (2000), the grape fruit skin volatile compounds terpenes, C13-norisoprenoids, benzene derivatives, and aliphatic alcohols) are the main contributor to the fresh and fruity aroma to grape products Aromatic compounds are one of the most important constituent in governing the quality of grape-derived products. And play a key role affecting the quality of its products. Concentration of these volatile compounds varies according to the grape variety, cultural practices, and climatic or biological factors (Jackson and Lombard, 1993, CSIRO and Australian Bureau of Meteorology; 2012). Sixteen compounds we dischilified by Sanchez-Palomo et al. (2005) in pulp and skiny of Muscat grapes including C6-alcohols and aldehydes, terpenes and benzenic companied. Which (Linalool, geraniol, and nerol) are responsible for the product floral aroma of Muscat grapes and contribute to the arolia of their wines.

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Table 1: GCMS analysis of Sonaka raisins treated with various chemicals.

Sr. No	MolWt.	Name of compound	0	В	C	D	E	F	G	H
1.	176	1,2,3-Propanetriol, diacetate			21.49	-			CONTRACTOR OF THE PARTY OF THE	
2.	278	1,2-Benzenediacarboxylic acid, bis(2- methylpropyl) ester	-		-		0.53			17.25
3.	334	1,2-Benzenediacarboxylic acid, butyl 2- ethylhexyl ester				10.0	5.59			82.75
4.	278	1,2-Benzenediacarboxylic, bis(2-metylpropyl) ester	-			1.86	-			-
5.	170	2(3H) Furanone, 5-hexidihydro		-	A Second			2.86		
6.	162	2-Butanol,3,3-oxybis					-	6.33	-	-
7.	126	2-Furancarboxaldehyde, 5-(hydroxymethyl)-			54.45	15-24		100		-
8.	192	2-Propanol,1,1'-[(1-methyl-1,2-ethanediyl]bis-		-		-	-	58.00	12.0	
9.	144	4H- Pyran-4-one, 2, 3- dihydro-3,5 dihydroxy - 6-methyl	26.61	10.80			24.5			
10.	126	4-Hepten-3-one, 4-methyl		46.10					-	-
11.	140	4H-Pyran-4-one,2-ethyl-3-hydroxy		200	-	-		7.85		-
12.	296	9- Octadecenoic acid, methyl ester		5.12	9.29			-		
13.	294	9,12-Octadecadienoic acid, methyl ester		77.71	45.79	100	1.18			
14.	281	9-Octadecenamide,(2)-	-	-		-	1.22		-	-
15.	122	Benzaldehyde,3-hydroxy-4-methoxy			-	100	To and	5.81		
16.	108	Benzyl alcohol		-				39.10	39.1	
17.	134	Butanol,3,3'-oxybis-		100	-	4	0.7		2.30	
18.	210	d-Glycero-d-ido-deptose	13.70		-	-			-	
19.	278	Dibutyl phthalate	40.64	1.18	12,66	13.1	7.88			
20.	170	Diphenyl ether	-		-	-	-	18.12	-	
21.	310	Docosane		1		3.98			-	200
22.	226	Hexadecane	-	-	-	2.95	-	101-		
23.	270	Hexadecanoic acid, methyl ester		12.38	12,48		11745	100		
24.	136	Limonene		4.24	-	1.64	-	59:	-	
25.	256	n-Hexadecanoic acid	18.48	17.12	7.76	Stimula	5.18	36 ·	1810 · m	Hebrita.
26.	284	Octadecanoic acid	16.17	-	-	-	-		-	
27.	372	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester		2.07			4.14			
28.	298	Octadecenoic acid, methyl ester	-	3.61	12.01	-				
29.	282	Oleic acid	20.00	3.72		10.00		W. 1		000
30.	116	Petanoic acid, 4-oxo-	-	0.83	-	-			-	
31.	102	Propanoic acid, 2-methyl-, methyl ester	-	11.27		11.	Here.		0.00	
32.	162	Propanoic acid, 3-(acetylthio)-2-methyl-			29.62			1.		
33.	90	Propanol, 2,3-dihydroxy		-	18.34	-			- 1	
34.	410	Squalene		-	-	8.09	-		100	
35.	284	Xanthosine			16.42	60.9	94.4			
36.	126	1 2 -Furancarboxaldehyde, 5 (hydroxymethyl)	52.58	-	-	-			-	
37.	134	2-Propanol,1,1'-oxybis						25.97	25.9	
38.	136	2,6,6-Trimethyl bicycle (3.1.1)hept-Zene		1.33		-	-			-

O=Untreated, A=K₂CO₃+Sulphur fumigated; B=K₂CO₃+ Sulphur fumigated and Zein protein coating; C=K₂CO₃+Sulphur fumigated and Zein protein coating with mango essence; D=K₂CO₃+Sulphur fumigated and Zein protein coating with orange essence; E=5% mango essence in methanol; F=5% orange essence in methanol and G=4% Rapseed oil in methanol.



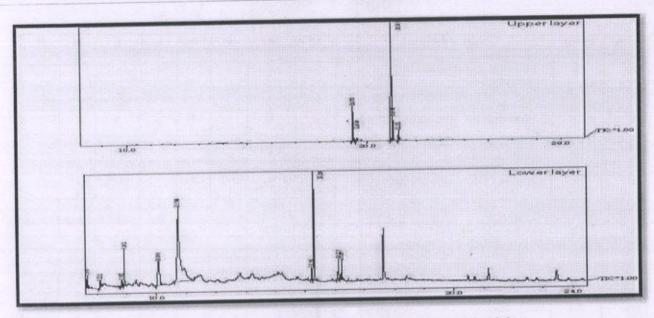


Plate 2: GC MS Spectra of Sonaka seedless raisins treated with sulphur

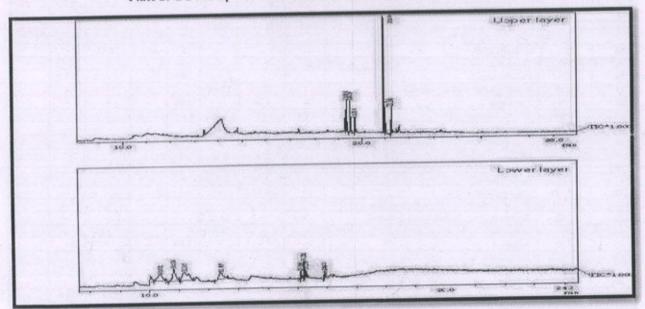


Plate 3: GC MS Spectra of Sonaka seedless treated with K2CO3+ sulphure fumigated +coated

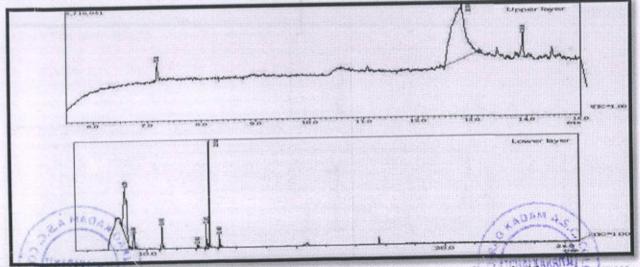


Plate 4: GC MS Spectra of Sonaka seedless treated with K2CO3+sulphure fumigated coated with mango essences

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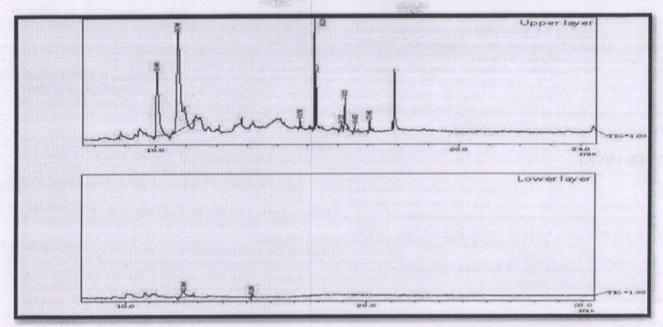


Plate 5: GC-MS Spectra of Sonaka seedless treated with K2CO3+sulphure fumigated coated with orange essences

The distribution of volatile compounds can be used for the characterisation of grape varieties and considered as important constituent of grape and wine aroma substance (Ribereau-Gayon, 2000). Genova (2012) observed that the various classes of aromatic compounds contribute to the flavor profile of grapes and grape-based products with alcohols and esters along with carbonyl compounds, terpens, organics acids, and norisoprenoids. They concluded that alcohols and aldehyde were the most represented classes, followed by terpenoids benzoic derivatives and C-13 norisoprenoids. Which are derived from carotenoids and found to be determinant in giving its characteristic flowery aroma.

The biological activity of volatile compounds is dependent on the synergistic or additive effects of the constituent types present at different concentrations. Volatile compounds from aromatic plants can cause a number of positive or negative interactions (Vokou et al., 2003) Like isoprene, some monoterpenes and sesquiterpenes have the potential to combine with various reactive oxygen species (Bonn and Moortgat, 2003), and can protect against internal oxidative damage (Loreto et al., 2004). Kubo and Kubo (1995) studied the antimicrobial activity of the constituents (E) 2-Heprenal; (E) 2octenal; (E) 2-nonenal; (E) 2-decenal; (E) 2-undecenal; (E) (E) 2, 4decadienal; 3-methyl-2-butenal; hexanoic acid; octanoic acid; hexanal) from the dried flowers of a Brazilian medicinal plant, Tanaatum balsamita against Bacillus subtilis, Brevibacterium ammoniagenes, Staphylococcus aureus, Staphylococcus mutans, Propionibacterium acnes, Pseudomonas aeruginosa, Enterobactor aerogenes, Escherichia coli, Proteus vulgaris, Pitorosporum ovale; penicillium chrysogenum and Trichophyton mentagrophyte. Hexanal is viewed as indicator of oxidative state in a number of foodstuffs (Sanches-Silva et al., 2004). Hexanal odour activated hypothalamic nuclei, which control maternal and emotional behavior (Hamaguchi-Hamada et al., 2004). Hence, the presence of some of the important bioactive volatile compounds in the raisins of sonaka seedless varieties will certainly prove the use of dry fruits for the preparation of various antimicrobial products and designing of drug against various microbial pathogenic diseases. In case of raisins of sonaka seedlees variety seventeen new volatile bioactive compounds were reported (1,2,3-Propanetriol, diacetate, 1,2-Benzenediacarboxylic acid, bis(2-methylpropyl) ester, 1,2-Benzenediacarboxylic acid, 2-Furancarboxaldehyde, ethylhexyl ester, (hydroxymethyl)- -3-one, 4-4-Hepten methyl, 9-Octadecenoic acid, methyl ester, 9,12-Octadecadienoic acid, methyl ester, 9-Octadecenamide,(2)-, Docosane, Hexadecane, n-Hexadecanoic acid, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester, Octadecenoic

acid, methyl ester, Oleic acid, Petanoic acid, 4-oxo-, Propanoic acid, 2-methyl-, methyl ester, Squalene and Squalene). Other then the untreated raisins . Thus the accumulation of various forms of bioactive compounds which willcertainly improves the flavour, aroma and taste of raisins due to pretreatment as well as zein coating with mango essence. This will improves the market potential and consumer acceptability of the raisins followed by improvement in the shelf-life of raisins during postharvest handling.

SUMMARY AND CONCLUSION

Analysis of Bioactive Compound by GCMS: Raisins of both the varieties are rich in various bioactive volatile compounds. Among these compounds some represents to class of Acids, Aldehydes, Alcohols, Ketone, Alkane and Alkene. The major compound reported from the seedless untreated raisins were 2-Furancarboxaldehyde, Octacosane, Decosane, 4H-Fyran-4-one, 2, 3 dihydro-3,5 dihydoxy- 6 methyl, 1,2-Benzene dicarboxylic acid. The raisins treated with K_2CO_3 and Sulphur fumigated showed the presence of 4 H-Pyran-4-one,2,3 dihydroxy -3, 5 dyhydroxy-6-methyl, Dibutyl phthalate. The untreated Sonaka raisins showed six volatile bioactive compounds such as 1 2 -Furancarboxaldehyde, 5 (hydroxymethyl), Dibutyl phthalate, 4H- Pyran-4-one, 2, 3- dihydro-3,5 dihydroxy -6-methyl. Whereas the raisins treated with K2CO3 and sulphur fumigated results in the development of bioactive volatile compound as 9,12-Octadecadienoic acid (Z,Z) -, methyl ester, 4-Hepten-3-one, 4-methyl. While Zein protein coated raisins displayed bioactive compounds are 9,12-Octadecadienoic acid, methyl este. Propanoic acid, 3-(acetylthio)-2-methyl-. The zein protein coated with essence exhibits volatile compounds 1 2. mango 98 Benzenediacarboxylic,acid, butyl 2-ethylhexyl ester, Xanthosine, Squalene.

The mass spectral analysis of 5% mango essence in methanol showed major different bioactive compounds such as 2-Propanol,1,1'-[(1-methyl-1,2-ethanediyl]bis-, Benzyl alcohol, 2-Propanol,1,1'-oxybis, 2-Propanol,1,1'-[(1-methyl-1,2-ethanediyl]bis-. The 5% orange essence in methanol showed different volatile bioactive compounds Benzyl alcohol, 2-Propanol, 1, 1'-oxybis, 2-Propanol, 1, 1'-[(1-methyl-1, 2-ethanediyl]bis. In study of Sonaka seedless variety seventeen new volatile bioactive compounds were reported (1,2,3-1 ropanetriol, diacetate, 1,2-Benzenediacarboxylic acid, butyl 2- ethylbexyl ester, 1,2-Benzenediacarboxylic acid, butyl 2- ethylbexyl ester, 2-Furancarboxaldehyde, 5-(liydabyymethyl) 3 tone 31-1-Hepten methyl, 9 Octadecenoic acid, methyl ester, 9,12-Octadecadionoic acid, methyl ester,

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9-Octadecenamide,(2)-, Docosane, Hexadecane, n-Hexadecanoic acid, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester, Octadecenoic acid, methyl ester, Oleic acid, Petanoic acid, 4-oxo-, Propanoic acid, 2-methyl-, methyl ester, Squalene and Squalene). Other then the untreated raisins . Thus the accumulation of various forms of bioactive compounds which will certainly improves the flavour, aroma and taste of raisins due to pretreatment as well as zein coating with mango essence. This will improves the market potential and consumer acceptability of the raisins followed by improvement in the shelf life of raisins during postharvest handling.

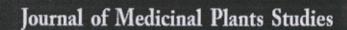
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Qualitative analysis of phytochemical in Eulophia nuda using LCMS

VR Dawande and RV Gurav

Abstract

The beneficial medicinal effects of plant material typically result from combination of secondary products present in plant. The identification and quantification of secondary metabolites plays an important role in exploiting the medicinal potential of plant. Liquid chromatography coupled with mass spectrometry can identify several compounds from plant extract. LC MS analysis of methanolic extract of *E. nuda* confirmed presence of phenolics, flavonoids, and other bioactive compounds such as alkaloids, toxins and antibiotics.

Keywords: Eulophia nuda, LC-MS, Bioactive compounds

Introduction

The genus *Eulophia*, commonly known as Amarkand, belonging to most interesting plant family i.e. orchidaceae is well known for its potential in ethnic medicines. *Eulophia* species are used in herbal medicines and food (rhizome) by many different tribes. Out of 30 species distributed throughout India, 6 species namely; *E. nuda*, *E. ochreata*, *E. herbacea*, *E. graminea*, *E. epidendrea* and *E. ramentacea* are reported from Maharashtra. Of these 6 species, *E. nuda* have shown high biological activity as revealed from its TPC, TFC and antioxidant activity [1]. Qualitative phytochemical analysis of *Eulophia nuda* revealed that it stores many phytochemically active ingredients like Alkaloids, flavonoids, saponins, Cardiac glycosides, Tepenoids and Steroids [2]. *Eulophia nuda* is exploited as antidote for snake bite, as antihelmintic, against tumors, cases of bronchitis, scrofulous affection of the glands of the neck and in disease of the blood [3]. The plant is also claimed to be useful in tuberculosis [4]. Earlier phytochemical studies showed presence of essential minerals, polyphenols, saponins, alkaloids, phytic acid etc. in *E. ochreata* and *E. nuda*. Nine different phenanthrenes, a rather uncommon class of aromatic metabolites, have been reported from *E. nuda* till date [5].

The present study was carried out in search of bioactive compounds from E. nuda with the help of LC-MS.

Materials and methods

Plant material

Eulophia nuda, collected from different parts of Western Ghats of Maharashtra was maintained in Botanical Garden, Dept. of Botany, Shivaji university, Kolhapur (MS), The species was identified by Dr. R. V. Gurav, and herbarium specimen deposited in Dept. of Botany, Shivaji University, Kolhapur (MS), India.

Extraction of plant material

Rhizomes of *Eulophia nuda*, were chopped, shade dried and ground to fine powder with mixer. 500 mg of fine powder of each species extracted with 20 ml methanol at room temperature for 24 hrs. The extract was filtered using Büchner funnel and stored at 4⁰C till further use.

The methanolic extract of *E.nuda* was analyzed by reverse phase HPLC on an Agilent 1200 series HPLC system fitted with microchip technology column (Agilent, G1316A). The HPLC conditions were as follows: flow rate, 0.4 µL/min; solvent A,100% water; solvent B, 100% methanol; gradient, 20-100% over 5 min and kept at 100% for 5 min. Then 2 µL of the extract dissolved in methanol-water (80:20, v/v), was analyzed by ESI in positive mode using at Agilent G6540B time-of-flight (TOF) mass spectrophotometer.

"GWILKARRAND"

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Mass spectral data were acquired in the range m/z 100-1700, with an acquisition rate of 1spectra/s, averaging 10,000 transients. The source parameters were adjusted as follows: drying gas temperature 250 °Cdrying gas flow rate 5 L/min, nebulizer pressure 45 psi, and fragmentor voltage 150 V. Data acquisitions and processing were done using Agilent Mass Hunter Workstation Acquisition v. B.02.00 software.

Results and Discussions

The methanolic extract of *E. nuda* was subjected to liquid chromatography coupled with Mass Spectrometry. The results indicated the presence of number of metabolites including flavonoids, phenolics, toxins and certain antibiotics (Table 1). The presence of antibiotics in extract indicates that may be several endophytic bacteria are symbiotically associated with plant rhizome.

Flavonoids

Flavonoids are polyphenolic plant secondary metabolites. They are synthesized by the polypropanoid pathway with phenylalanine as startup molecule. Flavonoids consist of a large group of polyphenolic compounds having a benzo-ypyrone structure and are ubiquitously present in plants. Flavones, Flavonols, Flavanones, Flavanonol, and Isoflavone are subgroups of plant flavonoids. As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capacity both in vivo and in vitro systems. Flavonoids have ability to induce human protective enzyme systems. The number of studies has suggested protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers and other age-related diseases [6] In present study methanolic extract of E. nuda showed presence of six flavonones 5,6,3'trimethoxyflavone, 5,3'dihydroxy 4,5-dimethoxy-6,3,7methylene dioxyisoflavone, 5,7,2'trihydroxy,3,6,4'5' tetramethoxy flavones, Demethyltorosoflavone Dionflavone and Isopongoflavone (Table 1). The study also presence of flavonol; quercetin3-(21 galoylrutinoside) and two flavonoids; fulvinervin B and Gancaonin J. Amongst this quercetin was isolated from leaves of E. epidendrea [7].

Phenolics

Phenolic compounds are amongst the major compounds of plants'secondary metabolites, playing an important role in plant defense mechanism. In this study, methanolic extract of *E. nuda* indicated presence of two phenolic compounds namely, 3-ethyl catechol and Orchinol. 3-ethyl catechol is catechol bearing an ethyl substituent at position 3. Catechol is one of the allelochemicals which belong to phenolic compounds synthesized in plants. Orchinol is one of the phytoalexin accumulated in plant system having antimicrobial effect ^[5]. Orchinol was first isolated from orchid *Orchis militaris* by Gaumann and Kern in 1959 ^[8] possessing antifungal activity. Orchinol is also reported from several other orchid species such as *Agrostophyllum callosum* ^[5].

Coumarin

The present investigation shows the presence of 8-Cinnamoyl 3,4 dihydro 5, 7 dihydroxy 4 phenylcoumarin (Table 1) in methanolic extract of *E. nuda*. Coumarins (1, 2-benzopyrones) are ubiquitously found in higher plants where they originate from the phenylpropanoid pathway. They contribute essentially to the persistence of plants being

involved in processes such as defense against phytopathogens, response to abiotic stresses, regulation of oxidative stress, and probably hormonal regulation [9].

Alkaloids

Alkaloids are a class of nitrogenous organic compounds of plant origin having immense physiological activities in human. In this study three alkaloids namely protopine, Quadrigemine A and Paclitaxel were detected in methanolic extract of *E. nuda* (Table 1).

Protopine is a benzylisoquinoline alkaloid occurring in opium poppy, *Corydalis* tubers and other plants of the family papaveraceae, like *Fumaria officinalis*. It has been found to inhibit histamine H1 receptors and platelet aggregation, and acts as an analgesic ^[10]. The cytotoxic activity of Quadrigemine A, on cultured rat hepatoma cells (HTC line) is previously reported ^[11]. Paclitaxel is an anti-cancer (antineoplastic or cytotoxic) chemotherapy drug, isolated from *Taxus brevifolia* for the first time ^[12].

Antibiotics

The methanolic extract of E. nuda showed presence of five antibiotics as HygromycinA, Penicillin V, Penicillin K, Rifamycin Z and Spiramycin 3, indicating some of the bacteria may be present symbiotically in rhizomes of E. nuda. Hygromycin A, an antibiotic produced by Streptomyces hygroscopicus is active against grampositive bacteria including mycobacteria and actinomycetes, as well as against endomoeba, leptospira and pleuropneumonia-like organisms. Hygromycin A is a specific inhibitor of the peptide bond formation step of protein synthesis. The action of hygromycin A on peptidyl transfer is similar to that of chloramphenicol, an antibiotic that shares some common structural features with hygromycin A. Both antibiotics inhibit the binding of C-A-C-C-A-Leu to the acceptor site of peptidyl transferase and stimulate that of C-A-C-C-A-LeuAc to the donor site of the enzyme. Moreover, hygromycin A blocks the binding of chloramphenicol to ribosomes, indicating that the binding sites of the antibiotics may be closely related. Hygromycin A is a more potent agent than chloramphenicol and binds quite strongly to ribosomes [13]. Phenoxymethylpenicillin (Penicillin V) and Penicillin K are narrow spectrum antibiotics used to treat mild to moderate infections caused by susceptible bacteria. It is natural penicillin antibiotic that is administered orally. Penicillin V may also be used in some cases as prophylaxis against susceptible organisms (www.drugbank.ca/drugs/DB00417). Rifamycin Z is a member of group rifamycins, a group of antibiotics that are synthesized either naturally by the bacterium Amycolatopsis mediterranei. They are a subclass of the larger family of ansamycins. Rifamycins are particularly effective against mycobacteria, and are therefore used to treat tuberculosis, leprosy, and mycobacterium avium complex (MAC) infections [14]. Spiramycin 3 is a macrolide antibiotic and antiparasitic. It is used to treat toxoplasmosis and various other infections of soft tissues.

Toxins

These are toxic secondary metabolites naturally occurring in plants with high chemical diversity. In *E. nuda* four toxins were detected namely, Gonyautoxin 6, Gonyautoxin 8, Phaseolotoxin and vomitoxin. Gonyautoxins (GTX) are a few similar toxic molecules that are naturally produced by algae. They are part of the group of sactoxins, a argument of the group of neurotoxins along with a molecule that is also retred to as

saxitoxin (STX), neosaxitoxin (NSTX) and decarbamoyl saxitoxin (dcSTX). Currently eight molecules are assigned to the group of gonyautoxins, known as gonyautoxin 1 (GTX-1) to gonyautoxin 8 (GTX-8). Ingestion of gonyautoxins through consumption of mollusks contaminated by toxic algae can cause a human illness called paralytic shellfish poisoning (PSP) [15]. Phaseolotoxin is a modified tripeptide [Nô-(N'-sulfodiaminophosphinyl)-ornithyl-alanylhomoarginine]

produced by certains strains of Pseudomonas syringae pv. phaseolicola, Pseudomonas syringae pv. actinidiae and strain Pseudomonas syringae pv. syringae CFBP 3388 [16]. Phaseolotoxin is a reversible inhibitor of the enzyme ornithine carbamoyltransferase (OCTase; EC 2.1.3.3), which catalyzes of citrulline from ornithine formation carbamoylphosphate in the arginine biosynthetic pathway. Phaseolotoxin is an effective inhibitor of OCTase activity from plant, mammalian, and bacterial sources and causes a phenotypic requirement for arginine. Additionally, phaseolotoxin inhibits the enzyme ornithine decarboxylase (EC 4.1.1.17), which is involved in the biosynthesis of polyamines [17]. Vomitoxin also known as deoxynivalenol (DON) is a type B trichothecene, an epoxysesquiterpenoid. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye and maize, and less often in rice, sorghum, and triticale. The occurrence of deoxynivalenol is associated primarily with Fusarium graminearum (Gibberella zeae) and F. culmorum, both of which are important plant pathogens which cause fusarium head blight in wheat and gibberella or fusarium ear blight in maize [18]. Besides, two more compounds were detected as Picrasin G and phylloquinone in present study. Phylloquinone is often called vitamin K1. It is a fat-soluble vitamin that is stable to air and

moisture but decomposes in sunlight. It is found naturally in a wide variety of green plants.

Phylloquinone is also an antidote for coumatetralyl. Vitamin K is needed for the posttranslational modification of certain proteins, mostly required for blood coagulation (http://www.drugbank.ca/drugs/DB01022). Picrasin G is one of the members of Quassinoids which are known to have various biological activities, including anti-inflammatory, anti-malarial, amoebicidal, antifeedant, insecticidal, and herbicidal [19].

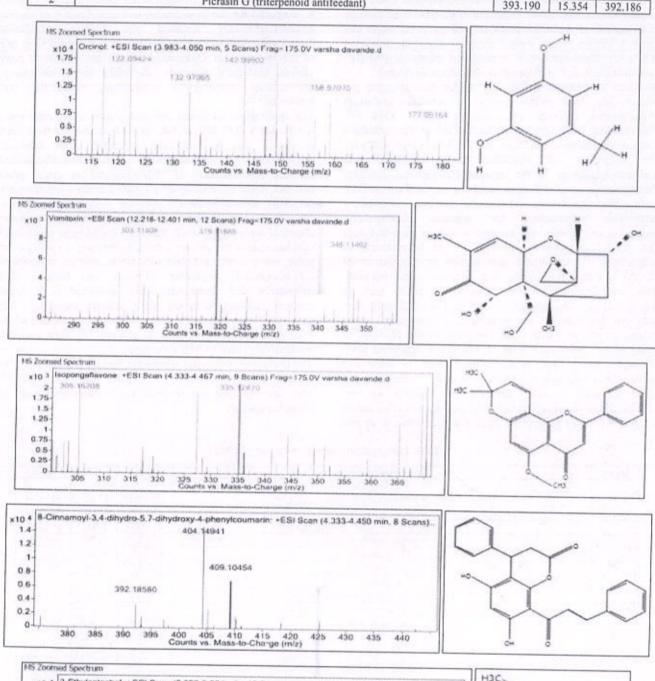
The applications of liquid chromatography along with mass spectrometry (LC-MS) in natural product analysis have been increasing due to improved separation and detection capabilities of LC-MS instruments. Among the methods used for the determination of phytochemical in crude plant extracts, liquid chromatography coupled to mass spectrometry (LC/MS) with atmospheric pressure ionization techniques, i.e., electro spray ionization (ESI) or atmospheric pressure chemical ionization (APCI), has been found to be a powerful tool because of the soft ionization, facilitating the analysis of polar, non-volatile, and thermally labile class of compounds. 60 compounds, including cinnamic and benzoic acid derivatives and flavonoids, were identified from apple residues resulting from the juice industry using LC- MS analysis [20]. Phytochemical from various plant species such as Potentilla anserine [21], Ocimum sanctum [22] were analyzed qualitatively using LCMS techniques.

The present investigation represents first report of qualitative analysis of phytochemical in *Eulophia nuda* using LCMS. The data obtained can be used for metabolomic study in *Eulophia* species.

Table 1: Phytochemicals in E. nuda using LCMS

Sr. no.	Name of Compound	m/z	RT	Mass
	Flavonones	1 3		
1	5,6,3'trimethoxyflavone	335.08	12.277	312.090
2	5,3'dihydroxy 4,5-dimethoxy-6,3,7methylene dioxyisoflavone	359.075	10.947	358.068
3	5,7,2'trihydroxy,3,6,4'5' tetramethoxy flavones	413.084	9.937	390.09
4	Demethyltorosoflavone D	357.059	10.235	356.05
5	Dionflavone	645.170	4.901	622.18
6	Isopongoflavone	335.128	4.423	334.12
	Flavonol			in alternatives
1	Quercetin3-(2"galoylrutinoside)	763.17	14.004	762.16
	Flavonoids			
1	Fulvinervin B	387.159	15.809	386.15
2	Gancaonin J	417.202	15.722	394.21
	Phenolics			
1	3-ethyl catechool	161.056	5.945	138.06
2	Orchinol	147.041	4.021	124.05
	Caumarin		3 " - 5N	West of
1	8,Cinnamoyl 3,4dihydro 5,7 dihydroxy 4 phenylcoumarin	409.104	4.383	386.114
	Alkaloids			
1	Protopine	354.134	16.081	353.12
2	Quadrangemine A	691.419	14.313	650.41
3	Paclitaxel	876.317	10.399	853.321
	Antibiotics		souther received	
1	Epithienamycin E	393.040	5.067	392.03
2	Hygromcin A	512.174	13.639	511,16
3	Penicillin V	351.101	4.674	350.093
4	Penicillin k	343.169	7.405	342.16
5	Rifamycin Z	674.256	6.589	651.26
6	Spiramycin 3	450.275	14.731	898.528
	Toxins			T.V. ST.
1	Gonyautoxin 6	390 (194	15.352	395.086
2	Gonyautoxin 8	0476.048	13.59	475.042
3	Phaseolotoxin	532,206	15 589	531.196

4	Vomitoxin	319.116	12.282	296.127
	Others		12000	270.127
1	Phylloquinone (vitamine K)	226,183	14.79	450.351
2	Picrasin G (triterpenoid antifeedant)	393.190		



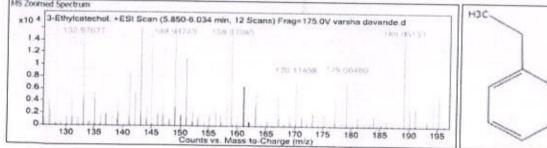


Fig 1: Zoom spectrum and structure of some of compounds identified from methanolic extract of E nuda in LCMS analysis

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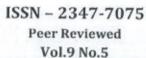
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COMPETENCE IN USING 'POST-PREDICATOR' SENTENCE STRUCTURES OF TEACHERS TEACHING IN PROFESSIONAL COLLEGES

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Abstract:

There have been many studies conducted on teacher talk and classroom situations. Research in Teacher Talk is particularly concerned with language teaching. These works are concerned with English language teaching in second language classrooms, teaching methods, and students' feedback. They were involved in teacher classroom behaviour and pupil classroom behaviour. The ungrammatical modification should not occur in the teacher's language because teacher talk should be the model for students to imitate. There have been various studies carried out in the field of linguistic on isolated elements of sentences. These studies were especially intended to find out the characteristics of Indian English. They were based on either corpus or written texts of Indian English. The use of proper sentence structure or syntax is one of the indicators that shows the competence of the person in using the language. It has been assumed that the teachers teaching in professional colleges have problems in using the post-predicator sentence structure elements. This paper brings forth some problems of the content subject teachers in using the English language as the language of instruction by analysing the recorded data.

Keywords: English Communication, Competence, Teachers teaching in English at professional Colleges and their use of sentence structures, the problems.

Introduction:

In today's world, competence in English language is essential. An appropriate use of language can make a positive change in the attitude of the listener. So, it is vital to know what problems hinder English language proficiency. The speakers who don't have this understanding produce problem sentences. The part of the sentence which becomes problematic is the post predicator structure that may be a word, a phrase or a dependent clause, only technical and job-related skills are not enough to reach the higher positions. It is the language involved in the linguistic globalization. Syntax and vocabulary are essential to be able to communicate and interact successfully. The professional communication needs proper grammar. English skills have become a basic requirement for almost any professional dealing with the globalized business world. In fact, English in business is increasingly being used as a shared language among speakers of different mother tongues, a so-called lingua frança. A lack of language skills becomes an important barrier to the expansion of global markets. The growing trend of the recruiters in engineering

today is to look for skilled/global engineers who possess excellent English communication and presentation skills.

English Communication and Competence

The syntax is the study of the rules and patterns used to form a number of new sentences. The focus of the present paper is related to the problems in using syntactic structures. The study of sentence structures especially sentence structures used by teachers in their lectures while they are teaching in English. English skills have become a basic requirement for almost any professional dealing with the globalized business world. In fact, English in business is increasingly being used as a shared language among speakers of different mother tongues, a so-called lingua franca. A lack of language skills becomes an important barrier to the expansion of global markets. The growing trend of the recruiters today is to look for skilled/global employee who possess excellent English communication and presentation skills.

Chomsky's concept of 'competence' is concerned with the grammaticality and the concept of 'performance' is concerned with the use of competence in actual situation i.e.



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'language use'. Communicative competence is defined by Wikipedia as "a language user's grammatical knowledge of syntax, morphology, phonology and the like, as well as social knowledge about how and when to use utterances appropriately." The concept of communicative competence has been developed Dell Hymns (1972). He communicative competence not only as an inherent grammatical competence but also as the ability to use grammatical competence in a variety of communicative situations and thus brought socio-linguistic perspective.

Canale and Swain (1980) and Canale (1983) define communicative competence as a synthesis of an underlying system of knowledge and skill needed for communication. According to them, communicative competence can be classified into four types: Grammatical Competence. Sociolinguistic competence, Discourse competence Strategic and competence. They Grammatical explain competence as the acquisition of phonological rules, morphological rules, syntactic rules, semantic rules and lexical items. Today, it is usually called linguistic competence. (Yano n.d.) Many theorists like Savignon (1972), Taylor (1988), and Bachman (1990) have tried to replace communicative competence proficiency.

English used by the faculty in the professional colleges is as important as the subject content in the process of education. There has been much more debate among the scholars that whether grammar rules are necessary or not necessary to make the practical use of language. Most of the teachers teaching other subjects in English at professional colleges seems to be reluctant about grammar or prescribed rules of language. It may be assumed by them that it is enough for them to comprehend the content of the subject instead of using correct grammatical sentence structures. It seems that the relation between the teaching of technical English and teaching of technical subjects in English haven't been taken as an important component. It's not only English language teaching but also teaching other content subjects in English should be considered for the enhancement of proficiency in English communication skills. Even if there may be a little communication failure due to non-standard constructions while teaching; it indirectly creates the wrong impression on the student's mind and promotes incorrect use of English.

The function classes which come after the predicator are regarded as 'Post-predicator'

Dr. Sunita J. Velhal



elements by the researcher. These postpredicator elements can be O (Object), C (Complement) or A (Adverbial). Such function elements can be a phrase or a clause; finite or non-finite. The complexity of clauses is marked on the basis of subordination clauses used at PPSSEs (Post-Predicator Sentence Structure Elements). After this, the accuracy in the PPSSEs is tested with error analysis of phrases and clauses. For this purpose, the method of grammatical analysis introduced in 'English Grammar for Today: A New Introduction' by Leech et al. (1982) is used.

The 'Predicator' here is only a verb or verb phrase. The term 'Element' is used for function classes such as S (subject), P (predicator), O (object), C (Complement), and A (Adverbial). These function classes which come after the predicator are regarded as 'Postpredicator' elements by the researcher. These post- predicator elements can be O (Object), C (Complement) or A (Adverbial). Such function elements can be a phrase or a clause; finite or non-finite. There are eight major clause patterns given by Leech et al. (1982, Table 5.3: 85). These patterns are used for analyzing the data. The elements which are the focus points of this study. The focus of the study was the language used by the teachers teaching the subjects other than English. The selected sample teachers were working in different rural and urban institutes.

The data was obtained from the selected professional colleges which include mainly Engineering, Architecture, Pharmacy, Law, Management, and Medicine for the purpose of research. The audio files were processed into transcripts to make them suitable for the analysis of syntax. An attempt is made to analyze the data collected with the purpose to explore the quantitative and qualitative aspects of the language used by them. The purpose of the error analysis is to decide an accuracy standard of English used by the samples.

Complexity of Structures Used as PPSSEs

The analysis will throw light on the level of complexity of language used by the teachers teaching in the professional colleges. The samples selected are from six different urban and rural professional college faculties mainly Engineering, Architecture, Pharmacy, Law, Management and Medicine. For this purpose, 1822 sentences have been analyzed and total 2274 post-predicator structures have been studied.

The total phrases among these structures used as PPSSEs are 1908 whereas the subordinate clauses used as PPSSEs are 366. So, the ratio of these phrases and clauses is **0.83**: **0.16**. It shows the number of phrases used is higher than the clauses. Again, the phrases without subordination are 1312 i.e., 68 %. The total phrases with direct subordination are 384 i.e., 20 % and phrases with indirect subordination are 212 i.e. only 11 %. The above data shows that the teachers have better proficiency in using phrases as PPSSEs but show less proficiency in using clauses as PPSSEs. The average ratio shows that the ratio of phrases used by all faculties as PPSSEs is higher than the ratio of clauses used as PPSSEs. There is little difference in the ratio of the use of total PPSSEs by all the faculties.

The ratio of the phrases with the structure ({M}H{M}) i.e. phrases without subordination is higher than the ratio of phrases with direct subordination (H(M)) and indirect subordination (H[M]). The percentage of phrases with direct subordination is more than the percentage of the phrases with indirect subordination. It shows that the competence in using PPSSEs structures by these samples seems to be similar on basis of the construct of complexity. The diagram below shows that these teachers have the competence in using the simple structures than the complex structures.

Table-1



Error Analysis of the Samples

The post-predicator structures used by them exhibit the problems of lack or improper use of articles, improper preposition phrases, improper pronouns, the problems of lexical collocations as well as errors in clauses which include errors in word order, in clauses/ Questions/Tag Questions, errors in Subordination, and improper Structures. Here are some examples of errors found in the data collected. (Own data 2019)

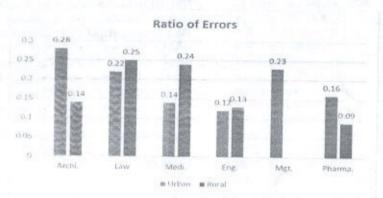
- These are the trusses and they are stronger on their one direction and they very bigger on the other direction. (18) Use of improper PP)
- This is an instance of wrong collocation of preposition 'on' with noun 'direction' that requires 'in' as well as there is no use of predicator after 'they'.
- ...if we are knowing the strength of this material..(186) (requires 'If we know...')

- It is an example of the use of progressive participle for the verb of perception.
- ..these observations is regarding the debates..(are related to)(42) (are)
- They were having good background. (had)
 (44)
- 7. Errors in Subordination:
- 8. who are agreeing mutually (agree)
- It will move the muscular process <u>nearly</u>, <u>nearly</u> means it will come near you, <u>isn't it?</u>
 (162) (means to say 'closure' but has used the word 'nearly' and incorrect tag question 'isn't,it? instead of 'won't it?)
- There is either incorrect use of articles or lack of them in noun phrases before nouns or
- enumerators e.g.
- 12. a (an) allopathic,
- on (the) fifth chromosome, (the) second molecule (82)

Dr. Sunita J. Velhal



Table-2



The diagram above shows that the ratio of errors of Engineering and pharmacy faculty is less than other faculty samples. The error ratio of law and medical samples is considerable. There is little difference in the error ratio of management and medical faculty. The error ratio of architecture faculty is highest.

Conclusions:

The analysis shows that there are problems in the area of post-predicator sentence structure elements used by these teachers teaching in the professional colleges. Engineering and pharmacy faculty have better competence in using English language. The paper is based on the detailed study made by the researcher in this area.

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The Impact of Natural Disasters on Human Life: A Study with John Steinbeck's Novel, 'The Grapes of Wrath'

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Abstract:

A natural disaster is a major adverse event resulting from natural process of the earth. It can cause loss of life or damage property and typically leaves some social and economic damage in its wake. An adverse event can have disastrous consequences and leave lasting damage, which can take years to repair. It also impacts the mental health of the affected communities often leading to post traumatic symptoms. It creates social disorder and sometimes strengthens social evils. The present research paper aims to analyse the direct and indirect impact of natural disasters on human life through John Steinbeck's realistic novel, 'The Grapes of Wrath'

Key Words: Natural disaster, social disorder, social evil, property, human life

Introduction:

John Steinbeck's realistic novel 'The Grapes of Wrath' is a classic peace of literature that captures the plight of the poor workers affected by a devastated economy during a time of natural disaster. The novel has background of the Great Depression of the 1930s in America and it focuses on the family of share croppers. They are driven from their Oklahoma home by drought, economic hardships and the change in the agriculture industry. They set out for California along with thousands of others in search of land, jobs and dignity. The root cause of their displacement is the Dust Bowl and its consequences. All were making their way to the promised land of California where they felt they would get good jobs and wages. The novelist also describes the collective action by lower classes against the individualistic self interest represented by corporate bank elites who have sole aim of maximizing their profits even at the cost of starvation of the poor. All this was the result of natural disaster, which is 'Dust Bowl'.

Discussion:

The novel has the background of the Great Depression of America in 1930s. It begins with the description of the Dust Bowl in which the poor farmers in Oklahoma had got their crops ruined. The Joads are the representatives of them. Through the description of the family, the novelist picturises the impact of natural disaster that is the 'Dust Bowl' on the lives of farmers in Oklahoma. Tom Joad, the central character in the novel is released on parole from prison. In the hot summer, he starts walking to the farm where his father works as a share cropper. Unfortunately, the Dust Bowl had made the land untenable for the small farmers and many of them were deserting their unprofitable land.

The Joads had deserted their house and land. They were making preparations to leave Oklahoma for California. They loaded the truck to its limits. All members were eager to move to California. But, Grampa was not ready to leave and uproot himself from the land. Finally, the family members decided to give him medicine that made him unconscious and they took him with them. His refusal to leave highlights the fact that how important the land was for these people. In this way, the drought and dust storm forces the Joads and other to leave the house and village.

The migrants were traveling to California. It was a big city but not big enough to accommodate and give promising work to their hands. All migrants were hopeful about their dream land, California. But Jim Casy, the religious icon in the novel believed that something more devilish than the devil itself was awaiting for them against which they needed to fight. At the beginning of the journey, the family dog of the Joads met with an accident and died. After that it was the turn of Grampa who suffered from stroke and died. After loosing their land, they lost their first family member.

During the journey, the Joads met the Willsons, a family from Kansas; going to California. They began to help each other in their journey. Day by day, the working class people began to come together for their collective interests. These changes were a threat to the selfish owners. The migrants' problems had moved from 'I' to 'We', which had become the major concern for the owners. Here, the disaster helped the poor to come together for their benefits. During the day their vehicles and cars were separate but at night all families were coming together. In a real sense, the sense of word 'We' started growing.

"And the little screaming fact that sounds through all history: repression works only to strengthen and knit the repressed"....



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It is clear that every disaster brings human beings together. It may be the only positive impact of the disaster.

When the Joads reach Arizona, a border guard stopped them but eventually they reached the deserts of California. Some migrant workers told them how California hates migrant workers. During that time Granma was suffering from delusion. Finally, she died as they reached in California. This was another loss for the family. Unfortunately, they could not afford last rites. It is clear that the natural disaster leaded not only the Joads but also many migrant workers to social and economical crisis. The worst conditions in California disturbed many families like the Joads. Connic Rivers, Rose of Sharon's husband and the son in law of the Joads left his pregnant wife. It was a big emotional jolt to the family.

The Joads got shelter in the government camp which was a little comfortable. At this time Tom was in search of the job. Finally he could succeed to find the job in a farm but the owners had cut down the wages in which they just afford grocery. Their condition at the Hooper Ranch was so bad. The strike by the migrants had proved adverse in raising the prices of the items they needed to live on. The owners also made the counter attack through inflation. It was another tragedy for the Joads that Jim Casy was killed while protesting in the strike. In a fit of anger, Tom killed the man who had killed Jim. So the police was behind Tom. But the family members hide him somehow. Finally, they had to leave Hooper Ranch to escape capture from the police. In this way, the family was scattering day by day.

The Dust Bowl in Oklahoma had started the plight which was never ending. The natural disaster led the Joads and others to social and economical disasters which are manmade but the root cause of all this was the natural disaster. At the end of the novel, it was raining heavily. It had damaged cars and tents of the migrants. The greatest terror than this had arrived that no work would be available for next three months. As a result of all this, there was no work, no food. Many were begging and dying on the road. In such conditions, there was no government relief. So, life in the region had become worst than animals.

After three days of heavy rain, they decided to go on. Suddenly, Rose of Sharon went on labour pains. That's why, the Joads could not leave the box car. The men in the camp built up embankment to prevent the flooding. When they reached at the car, they found Rose of Sharon had delivered a dead baby. Uncle John placed the body in an apple box and floated into the stream. When the family was in the barn to get shelter, on the other side there was a starving old man and a boy. Rose of Sharon and her mother realised what to be done. Finally, Rose of Sharon fed the old man. The flood had washed out everything but humanity was alive in a corner. The worst flood had affected the migrants but it brought them together to concern for each other. On the other hand, the flood and drought had also brought the owners together to exploit migrants. Here, the impact of natural disaster can be seen on the lives of the migrants and owners. It also strengthened the Great Depression of 1930s in America

Conclusion:

In the novel, John Steinbeck has depicted the impact of natural disaster on the lives of people in some parts of America. Generally, every disaster leaves positive and negative impact on human life. In the present novel, the only positive impact of the natural disaster is the unity of migrants against the cruelty of the owners. Otherwise, it leaves worst impact on human life. The disaster ruined the Joads who are the representatives of the tenants and the poor. It ruined three generations of the Joads. It shows that any disaster can directly ruin the present generation and indirectly hits upcoming generations. There is one more notable thing that a natural disaster aids the devils in the society. At the beginning in the novel, the Dust Bowl helped the banks and the rich to exploit tenants. At the end, the heavy rain stopped the harvesting season early. It left the tenants jobless and helped to cut wages. Moreover, it washed away what the tenants had to survive. All these helped social and economical devils in the society to grow up. The most powerful devil of that time, the Great Depression of 1930s in America got boosts from such natural disasters and ruined economical base of America and the world.

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Advantage & Disadvantage of Rainwater Harvesting

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Abstract:

Rainwater harvesting is the process of Rainwater harvesting collecting rainwater and putting it to good use. There are different ways in which this task can be accomplished. Rainwater harvesting refers to the trapping and storing of rainwater so that it can be used at a later time when the need arises. As the rain falls, water is directed to a suitable collection point. It can also mean collecting rainwater before it infiltrates into the ground and becomes underground water. Harvesting mainly entails gathering something from its natural source. Rainwater harvesting, from the common definition of harvesting, is a process that involves collecting rainwater and increasing its value by eliminating impurities or directing it to places where its use is highly required. It's a practice that has been around for a long time.

Rainwater harvesting is beneficial because it provides a source of water for domestic use. Industries can also for use in some of their processes. Many areas experience water shortages during summer due to lack of rain and as a result of the high rate of evaporation. This saves you money by cutting down your monthly expenditure on water bills. Harvesting rainwater plays a key role in mitigate. Collected rainwater can be used for building and construction, Helps In Preventing Water Pollution, Irrigation etc. Disadvantages of Harvesting Rainwater Treating rainwater to make it fit for human consumption will see you incurring additional expenses. Huge Efforts and Resources Required. Limited Storage, Dependent on Rainfall, Risk of Contamination Cleaning and Maintenance Acidic Rain etc.

Key words

- 1. Mitigates: Reduces the Impacts of Floods
- 2. Trenches: When it rains, the water is directed to the farm using trenches. It is one of the traditional methods of rainwater harvesting.

Introduction:

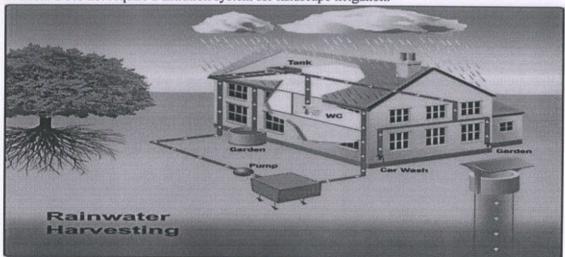
Living creatures of the universe are made of five basic elements, viz., Earth, Water, Fire, Air and Sky, Obviously, water is one of the most important elements and no creature can survive without it. Despite having a great regard for water, we seem to have failed to address this sector seriously. Human being could not save and conserve water and it sources, probably because of its availability in abundance. But this irresponsible attitude resulted in deterioration of water bodies with respect to quantity and quality both. Now, situation has arrived when even a single drop of water matters. However "better late than never", we have not realized the seriousness of this issue and initiated efforts to overcome those problems.

Rainwater harvesting as the name suggests is the harvesting of rainwater in the sense it is a process involving collection and the storage of rain water using the help of artificially designed systems that runs off natural or man-made catchment areas like the roof top, compounds, rock surface, hill slopes, artificially repaired impervious or semi-pervious land surface. Quite obviously a number of factors play a vital role in the amount of water harvested, some of these factors are the frequency and the quantity of rainfall, catchments characteristics, water demands and the quantum of runoff and above all else the speed and ease with which the rainwater percolates through the subsoil to recharge the ground water.

Need for rainwater harvesting:

It makes use of natural resources and reduces flooding, storm water runoff, erosion and contamination of surface water with pesticides, sediment, metals and fertilizers. Reduces the need for imported water. Happens to be an excellent source of water for landscape irrigation with no chemicals such as fluoride and chlorine and no dissolved salts and minerals from the soil. Home systems can be relatively

simple to install and operate; it could very well reduce your water bill. Promotes both water and energy conservation. Does not require a filtration system for landscape irrigation.



Methods of Rainwater Harvesting

There are many ways in which rainwater can be harvested. Some of these methods are very effective and can aid in the collection of a lot of water even for commercial activities while others are only suitable for harvesting water meant for domestic use. Every system has its merits and demerits. These are the common methods of rainwater harvesting:

- 1. Surface Water Collection Systems: Surface water is simply water that accumulates on the ground's surface. When rainwater falls on the surface of the earth, it usually flows down slopes as it moves towards a point of depression where the moving water can collect. Surface water collection systems enable the collection of ground surface rainwater before it flows to other locations. Examples of such systems include rivers, ponds, and wells.
- 2. Rooftop system: These can also be used to harvest rainwater. They can be used to direct rainwater that falls on the roof of a building into containers or tanks. These tanks are usually elevated so that when the tap is opened, water flows at a high pressure. This method of rainwater harvesting is good because the accumulated water is mostly clean and usually requires no further treatment to make it fit for human use.
- 3. Dams: These are barriers that are designed to trap water. Rainwater can accumulate directly in them or drainage systems can be created to direct water into them. Water collected in dams is mostly used for irrigation purposes or treated and then distributed for domestic use. They can also be used to harvest a lot of water because of the way in which they are modeled. Unlike ponds, measures are put in place to reduce the amount of water draining into the ground.
- 4. Underground Tanks: These are also ideal for collecting rainwater. They are constructed by digging into the ground and creating a space which is then cemented to reduce water infiltration. The top is also sealed and water is obtained through pipes directed into the tank. To get water out, pumps are used. Underground tanks are wonderful for harvesting rainwater because the rate of evaporation is reduced since they are located underground where sunlight does not really penetrate.
- 5. Rain saucer: Sometimes one can decide to collect rainwater directly as it falls from the sky by using a rain-saucer. These look like upside down umbrellas or big funnels. Some are usually attached to a pipe so that the collected water is directed elsewhere. Some people also do a little improvisation by placing the collecting container underground with only the rain-saucer above the ground. It is a simple method yet effective.
- 6. Water Collection Reservoirs: Water collected through this method is not really clean and may be contaminated. However, it can still be used for crop irrigation. such rainwater is harvested from roads and pavements.

DO TCHALK

- 7. Barrage: A barrage is a dam that has several openings which can be closed or opened to control the quantity of water that passes through it. It is usually large and can be used to collect a lot of water.
- 8. Slopes: Rainwater tends to collect at the bottom of slopes when it flows on the ground. When it rains heavily, water levels can rise to the hill top. This is a simple and natural way to harvest rainwater.

Advantages of Rainwater Harvesting

- 1. Water for Domestic Use: Rainwater harvesting is beneficial because it provides a source of water for domestic use. The collected water can be used for house cleaning purposes, washing laundry and for cooking. When treated, rainwater is good for drinking. It is an easy way of obtaining water for use in the home.
- 2. Water for Industrial Use: Industries can also harvest rainwater for use in some of their processes. Rainwater meant for industrial use is normally harvested in large scale. Such companies can construct their own dams or have underground tanks to store rainwater.
- 3. Supplementary Water Source: Many areas experience water shortages during summer due to lack of rain and as a result of the high rate of evaporation. It can be difficult to get a reliable source of water during these periods. Those who sell water may also increase their prices because of the high demand and short supply. Harvesting rainwater is therefore seen as a way of preparing for the sunny days when water is scarce.
- 4. Cost Effective: We basically harvest rainwater for free because it is naturally occurring. If you store enough water during the rainy season, you may never have to pay for water services again because you'll have enough supply to last you through the summer. This saves you money by cutting down your monthly expenditure on water bills.
- 5. Reliable Flow of Harvested Water: Even though harvesting of rainwater depends on rainfall, once stored, the supply of the available quantity is guaranteed. You can have an uninterrupted flow of water from the place of storage as long as the amount harvested has not been exhausted. The same cannot be said when you depend on an outside source to supply your water. There is also the benefit of location-suitability because the source of water is in your place of stay.
- 6. Mitigates/Reduces the Impacts of Floods: Harvesting rainwater plays a key role in mitigating or reducing the impacts of floods. When rainwater is directed to farms through trenches or collected in dams, its movement is controlled. This prevents the accumulation of water in one area, something that often causes flooding. Rivers can also overflow and cause flooding in the adjacent areas. The negative impacts of floods are too many and costly. Harvesting rainwater is, therefore, an effective way of reducing the impacts of this natural disaster.
- 7. Building and Construction: Collected rainwater can be used for building and construction. The process of building a house requires a lot of water. Harvesting rainwater would thus avail water for this activity.
- 8. Helps in Preventing Water Pollution: Rainwater flowing on the ground surface can carry with it a lot of impurities and toxic substances. When it drains into water bodies, it pollutes them because of these impurities. Harvesting rainwater, therefore, prevents pollution of water bodies.
- 9. Irrigation: Rainwater is good for farming because once harvested, it can be used for irrigation especially during the summer. One can, therefore, have a thriving farm and realize a bumper harvest.

Disadvantages of Harvesting Rainwater

- 1. Additional Expenditure: Treating rainwater to make it fit for human consumption will see you incurring additional expenses. This would not happen when you use water supplied to you by the local council because it's already treated.
- 2. Huge Efforts and Resources Required: Constructing a dam or an underground tank is no mean fete. Before you begin harvesting rainwater, you'll have spent a considerable amount of resources. There are other cheap means but then you'll not collect a reasonable amount of water.

- 3. Dependent on Rainfall: You can't harvest rainwater when it does not rain. This process is therefore solely dependent on the availability of rain which can sometimes be very unreliable. What then happens when the dry spell is prolonged and you don't have an alternative source of water?
- **4. Limited Storage:** Even if it rains for three months straight, you cannot harvest all that water even if you wanted to. This is because there is limited storage to keep the rainwater.
- 5. Risk of Contamination: If not preserved with care, rainwater can be contaminated. This can cause several health problems especially when the water is used without first being treated. Waterborne diseases are so many and treating them is very costly.
- **6.** Cleaning and Maintenance: The storage facilities have to be occasionally cleaned and maintained. Cleaning an underground water tank is not easy and maintaining a dam is very expensive. This makes the thought of harvesting rainwater unattractive.
- 7. Dual Cost: You'll incur expenses twice because of paying your normal water bills and installing and maintaining the rainwater harvesting system. This will set you back financially in a way.
- 8. Roof Tops That Contain Chemicals: Some rooftops contain chemicals and impurities that mix with the rainwater. When consumed, this water can affect human health by causing illnesses and other health conditions.
- 9. Acidic Rain: Due to pollution, sometimes the rain that falls is acidic. Harvesting this type of rainwater is dangerous because of the chemicals contained. Using acidic rain for irrigation can also cause the death of crops because it erodes the quality of soil and creates conditions that are not conducive for plant germination. When the soil has a high pH, plants do not grow properly.
- 10. Lack of Water for Wildlife: Wild animals get their drinking water from natural sources such as seasonal streams and rivers. They also use them for cooling in the hot weather. Harvesting rainwater reduces the amount of water that flows into these streams and rivers.

Conclusion

Rainfall is a very important weather phenomenon. It is a source of water and is very critical for the growth of crops and farming. Harvesting rainwater is a practice that has been going on for a while. Many people actually engage in it without even realizing that they are doing so. Because of weather changes, water sources can dry up and in the process impact animal, human, and plant life negatively. Rainwater harvesting is a smart way of preparing for such times because even when the conventional water sources dry up, we can still use the stored water for many purposes. The best part is that this type of water is naturally occurring. Some of the setbacks can be avoided by taking precautionary measures. When we employ innovation and technology, we can come up with better ways of rainwater harvesting and increase the storage capacity.

Living creatures of the universe are made of five basic elements, viz., Earth, Water, Fire, Air and Sky, Obviously, water is one of the most important elements and no creature can survive without it. Despite having a great regard for water, we seem to have failed to address this sector seriously. Human being could not save and conserve water and it sources, probably because of its availability in abundance. But this irresponsible attitude resulted in deterioration of water bodies with respect to quantity and quality both.

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IMPORTANT FACTORS INFLUENCING CONSUMER BEHAVIOR

Dr. Varsha Raghunath Shinde, Assist.Prof. of D.K.A.S.C.College, Ichalkaranji.

Abstract:

Consumer behavior entails "all activities associated with the purchase, use and disposal of goods and services, including the consumer's emotional, mental and behavioral responses that precede or follow these activities." The term, consumer can refer to individual consumers as well as organizational consumers, and more specifically, "an end user, and not necessarily a purchaser, in the distribution chain of a good or service." Consumer behavior is the study of individuals and organizations and how they select and use products and services. It is mainly concerned with psychology, motivations, and behavior. Some of the most important factors influencing consumer behavior. Marketing Mix Factors, Personal Factors, Psychological Factors, Social Factors, Cultural Factors. The study of consumer behavior indicates how individuals, groups and organizations select, buy, use and dispose goods, services, ideas, or experiences to satisfy their needs and desires.

Consumer behavior is concerned with, the purchase of goods or services; how consumers acquire products and services, and all the activities leading up to a purchase decision, including information search, evaluating goods and services and payment methods including the purchase experience, use or consumption activities concerns the who, where, when and how of consumption and the usage experience, including the symbolic associations and the way that goods are distributed within families or consumption units. Disposal activities concerns the way that consumers dispose of products and packaging; may also include reselling activities such as e Buy and second-hand markets.

Keywords: Consumer Buying Behavior, Marketing Mix Factors.

Important Factors Influencing Consumer Behavior. Introduction

Consumer behavior is the study of consumers and the processes they use to choose, use (consume), and dispose of products and services, including consumers' emotional, mental responses. Consumer behavior incorporates ideas from several sciences including psychology, biology, chemistry, and economics. Consumer behavior is the study of how individual customers, groups or organizations select, buy, use, and dispose ideas, goods, and services to satisfy their needs and wants. It refers to the actions of the consumers in the marketplace and the underlying motives for those actions. Consumer behavior is the study of individuals and organizations and how they select and use products and services. It is mainly concerned with psychology, motivations, and behavior.

How consumers think and feel about different alternatives (brands, products, services, and retailers) how consumers reason and select between different alternatives. The behavior of consumers while researching and shopping. How consumer behavior is influenced by their environment (peers, culture, media) how marketing campaigns can be adapted and improved to more effectively influence the consumer. Consumer responses may be emotional responses: refer to emotions such as feelings or moods, mental responses: refer to the consumer's thought processes, their behavioral responses: refer to the consumer's observable responses in relation to the purchase and disposal of goods or services.

Objectives of the Study

1. To understand what consumer behavior is and the different types of consumers.

2. To understand the relationship between consumer behavior and the marketing concept, as well as segmentation, targeting and positioning.

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- 3. To understand the relationship between consumer behavior and customer value, satisfaction,
- 4. To understand how new technologies are enabling marketers to better satisfy the needs and trust and retention.
- 5. To understand how marketers are increasingly able to reach consumers wherever consumers wants of the consumers.
- 6. To understand how the world's economic condition is leading to consumption instability wish to be reached.
- 1. According to Engel, Blackwell, and Mansard, 'consumer behaviour is the actions and decision Definitions of consumer behavior processes of people who purchase goods and services for personal consumption'.
- 2. According to Louden and Bitta, 'consumer behaviour is the decision process and physical activity, which individuals engage in when evaluating, acquiring, using or disposing of goods and services'.

Some of the most important factors influencing consumer behavior are as follows: A. Marketing Important Factors Influencing Consumer Behavior Mix Factors B. Personal Factors C. Psychological Factors D. Social Factors E. Cultural Factors. The study of consumer behavior indicates how individuals, groups and organizations select, buy, use and dispose goods, services, ideas, or experiences to satisfy their needs and desires. Consumer behavior is affected by several factors. Marketers need to have a good knowledge of the factors affecting the

In general, the factors that affect consumer behavior are discussed in the following sections:

Each component of the market mix product, pricing, promotion and place of distribution—has a direct or indirect impact on the buying process of the consumers.

The special characteristics of the product, the physical appearance and the packaging can influence the buying decision of a consumer.

The price charged on the product or services consumed by the consumer affect the buying behaviour of the consumers. Marketers must consider the price sensitivity of the target customers while fixing prices.

The variables of promotion mix such as advertising, publicity, public relations, personal selling and sales promotion affect the buying behaviour of the consumers. Marketers select the promotion mix after considering the nature of the target audience.

The channels of distribution and the place of distribution affect the buying behaviour of the consumers, The marketers makes an attempt to select the right channel and distribute the products at the right place.

The personal factors such as age, occupation, lifestyle, social and economic status and the gender of consumer may affect the buying decisions of the consumers individually or collectively.

The age factor greatly influences the buying behaviour. For example, teenagers prefer trendy clother whereas office executives prefer sober and formal clothing. colours such

The consumer behaviour varies across gender. For example, girls prefer certain femili pink, purple and peach, whereas boys go for blue, black and brown.

Highly educated persons may spend on books, personal care products, and so on. B or no education may spend less on books and more on personal grooming products.

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4. Income level:

Normally, the higher the income level, the higher is the level of spending and vice versa. But this may not be the case in developing countries, especially in the rural areas.

5. Status in the society:

Persons enjoying higher status in the society spend a good amount of money on luxury items such as luxury cars, luxury watches, premium brands of clothing, jewellery and perfumes.

C. Psychological Factors:

A person's buying behaviour is influenced by the psychological factors such as the following:

It refers to changes in individual behaviour that are caused by information and experience. For example, when a customer buys a new brand of apparels, and is satisfied by its use, then they are more likely to buy the same brand the next time. Through learning, people acquire beliefs and attitudes, which in turn influence the buying behaviour.

2. Attitude:

It is human tendency to respond in a given manner to a particular situation or object or idea. Consumers may develop a positive, or a negative, or a neutral attitude towards certain products or brands, which in turn affects their buying behaviour.

3. Motives:

A motive is the inner drive that motivates a person to act or behave in a certain manner. A marketer must identify the buying motives of the target customers and influence them to act positively towards the marketed products.

4. Beliefs:

A belief is a descriptive thought that a person holds about certain things. It may be based on knowledge, opinion, faith, trust and confidence. People may hold certain beliefs of certain brands/products. Beliefs develop brand images, which in turn can affect the buying behaviour.

D. Social Factors:

The social factors such as reference groups family, and social status affects the buying behaviour. Social factors in turn reflect a constant and dynamic influx through which individuals learn different meanings of consumption.

1. Reference groups:

A reference group is a small group of people such as colleagues at workplace, club members, friends circle, neighbors, family members, and so on.

The reference groups influence the members in following manner:

- a. They influence members' values and attitudes.
- b. They expose members to new behaviors and lifestyles.
- c. They create pressure to choose certain products or brands.

2. Family:

The family is the main reference group that may influence the consumer behavior. Nowadays children are well informed about goods and services through media or friends circle, and other sources Therefore, they influence considerably in the decisions of buying both fast moving consumer goods an

3. Roles and status:

A person performs certain roles in a particular group such as family, club, organization, and L on. For example, a person may perform the role of a vice president in a firm and another person may Perform the role of a marketing manager. The vice president may enjoy higher status in the organization as compared to the marketing manager. People may purchase the products that conform to their roles and status, especially in the case of branded clothes, luxury watches, luxury cars, and so on.

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E. Cultural Factors:

There is a subtle influence of cultural factors on a consumer's decision process. Consumers live in a complex social and cultural environment. The types of products and services they buy can be influenced by the overall cultural context in which they grow up to become individuals. Cultural factors includes race and religion, tradition, caste and moral values. Culture also includes subcultures, subcastes, religious sects and languages.

1. Culture:

It influences consumer behaviour to a great extent. Cultural values and elements are passed from one generation to another through family, educational institutions, religious bodies and social environment. The cultural diversity influences food habits, clothing, customs and traditions. For example, consuming alcohol and meat in certain religious communities is not restricted, but in certain communities, consumption of alcohol and meat is prohibited.

2. Subculture:

Each culture consists of smaller subcultures that provide specific identity to its members. Subcultures include sub-castes, religious sects (Roman Catholics, Syrian Catholics, Protestant Christians, etc.), geographic regions (South Indians, North Indianss) and language (Marathi, Malayali, Gujarati). The behaviour of people belonging to various subcultures is different. Therefore, marketers may adopt multicultural marketing approaches, that is, designing and marketing goods and services that cater to the tastes and preferences of the consumers belonging to different subcultures.

Changes in Food Buying Behavior:

Changes in various aspects of food consumption behavior were measured which are presented in Table for different groups of people based on their regional shift.

Changes in Food Buying Behavior for Different Categories of Respondents

Changes in Food Buyin Dimensions of Food Buying Behavior	Not moved	Moved during the last 5yrs	Moved during the last 5-10 yrs	Total	F	Sig
There is definitely some changes in the way we purchase food items now	3.35	3.33	3.50	3.38	0.622	0.537
We now use more ready-to-eat food items than earlier	3.30	2.93	3.23	3.20	2.663	0.071
We now buy most food items from organized retails of shops/shop	3.42	3.25	3.50	3.40	1.167	0.313
We now less frequent local grocery is to buy	2.61	3.09	2.85	2.77	7.1123	0.001
I look ore number of food ducts options than earlier	3.86	3.94	3.96	3.90	0.353	0.703



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want to buy my products at the place	3.40	3.36	3.41	3.39	0.064	0.938
where the display is better			-12 CM-10 & DF 1950			
I want to buy my food products where I may get all the items at one place	3.56	3.64	3.60	3.59	0.193	0.825
now buy food items for more number of days than earlier	3.24	2.86	3.04	3.11	4.003	0.019
Now our purchase decisions for food products are heavily dependent on advertisements	2.74	2.61	2.53	2.66	1.382	0.253
Price is not an important criteriafor buying food items now compared to earlier period	2.77	2.64	2.65	2.71	0.576	0.563
Now-a-days both husband and wife are taking decisions about the type of food items to be purchased	3.84	3.71	3.74	3.79	0.792	0.454
Children influence the food buying decisions to a great extent	3.53	3.29	3.50	3.47	2.256	0.106

Note: 1 indicates "strongly disagree" and 5 indicates as "strongly agree"

Changes in various aspects of food consumption behavior were measured (on 1-5 scale, where 1 indicates strongly-disagree and 5 indicates strongly-agree), which are presented in Table for different groups of people based on their regional shift. Looking at total number of responses, it is clear that people strongly agreed that shifting to a new city affects their food habit(mean score 3.44). They now prefer more healthy foods (4.25) and have started eating new dishes (3.75) compared to earlier. They also agree that they take their dinner together in the family (3.64), learn cooking and eating new food items after relocation (3.47). They also agreed the influence of children on the type of food items that they eat. Respondents disagreed with statements like, they have started eating out at restaurants more frequently (2.61) and they stopped eating some of their traditional food items after shifting to a region (2.65). Most of the changes are common to all the three categories of respondents as their mean

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scores are not significantly different (at level of significance as 0.05). However, people who have shifted to new location indicated less preference for organic food and also for eating out at restaurants compared to people who have not moved to a new region.

Conclusion

The present study explored the consumer behavior in India from different perspectives. Cleanliness of the product, free from pesticides, freshness, good for health, and clean place of sale are some of the most important attributes, which are rated very highly by people in India while buying food products. Value for money, overall quality, taste, availability of variety of products at same place, seasonality for the product, flavor, good display of products, nearby availability and good ambience are some other important parameters. Promotional offer does not have much impact on the sale of food products and people did not rate food products from other country very highly for purchase decision. People rate various parameters differently for different product groups. For highly perishable items, freshness, cleanliness and good for health are the most important parameters but for products like food grains-pulses, cleanliness and free from pesticides are the most important criteria. Based on factor analysis, it appeared that store quality, marketing mix and taste-flavor explained the maximum variance in the purchase decision for fruit and vegetables. Although quality of food products is one of the most important parameters for food product purchase decision, people do not see much improvement in the quality related parameters for food items.

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