International Journal for Innovative Research in Multidisciplinary Field

(ISSN: 2455-0620) (Scientific Journal Impact Factor: 6.497)

Monthly Peer-Reviewed, Refereed, Indexed Research Journal

Index Copernicus International - IC Value: 86.87

Special Issue of

National Conferences on

'Inclination of Modern Science toward Environmental Protection and Sustainable Development'

23rd January, 2019

RESEARCH CULTURE SOCIETY & PUBLICATION

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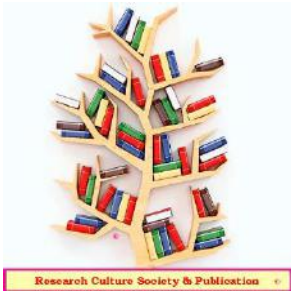

NATIONAL CONFERENCE
ON

INCLINATION OF MODERN SCIENCE TOWARDS ENVIRONMENTAL PROTECTION AND SUSTAINABLE DEVELOPMENT

23rd JANUARY, 2019

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In Collaboration with

Bahauddin Science College , Junagadh
&
Dept. of Botany, Faculty of Science,
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About the Bahauddin College:

The Bahauddin Science College, Junagadh is more than a hundred year old college (117 years) with great tradition of generating quality students and having learned faculty right from the outset. Vazir of Sorath state Shri Bahauddinbhai was a great visionary who contributed to the construction of the building. Vazir Bahauddinbhai played a vital role in the establishment of this college. There was no such educational institute other then in Bombay and Karachi at that time. The building is unique. The college officially started in 1901. College has big semi-green campus.

The original Bahauddin College consisted of arts and science faculties. From the very beginning the college has science stream with BIOLOGY, MATHEMATICS, and PHILOSOPHICAL SCIENCE as main subjects. Right from the beginning college has privilege to have very scholar and eminent faculties like Dr. A. R. Roa founder Director of VSVSC, Prof. Kapadiya, Prof. Bharucha, Prof. Naronha, and Prof. H. S. Joshi etc. College is highly reputed for producing quality students in the field of alumni like scientists, doctors, professors, industrialist etc all over the world. Till today the academic atmosphere remain intact. All most all faculties have research out comes. Few projects, patent and awards are in to the account of this college. The college was accredited by NAAC scoring grade B++ (81%).

About - Indian Science Congress Association (ISCA):

Indian Science Congress Association (ISCA) is a premier scientific organization of India with headquarters at Kolkata, West Bengal. The association started in the year 1914 in Kolkata and it meets annually in the first week of January. One of the major objectives of the ISCA is to inculcate the scientific temper among the people and to encourage young scientists to grow up steadily. 106th annual meet was held in 2019 at Lovely Professional University, Jalandhar. It was on "FUTURE INDIA – Science and Technology" It has a membership of more than 30,000 scientists. With humble support of ISCA different chapters are running in different state of India. M. S. University, Baroda is one of its prime chapter commonly known as ISCA, Baroda chapter. Baroda Chapter is actively engaged to fulfil this aim of ISCA by organising various scientific activities.

About The Department of Botany, The MSU, Baroda :

The Department of Botany**,** a front ranking division under the umbrella of The Maharaja Sayajirao University of Baroda, is actively engaged in teaching and research in both classical and cutting edge technology on various aspects of Plant Sciences. The Department has contributed immensely in Plant Taxonomy, Plant Anatomy, Plant Ecology, Mycology & Plant Pathology, Phytochemistry, Remote Sensing Technology, Plant Tissue Culture for the conservation and sustainable utilization of the plants and other plant resources of India.

About the Conference: National Conference on "Inclination of Modern Science towards Environmental Protection and Sustainable Development (IMSEPSD - 2019)"

Science is still to be explored, there are still the mysteries to be unfolded and phenomenons are still to be understood. Indian science has a great potential to achieve and meet the challenges thrown up in the national and the international arena. While maintaining competitiveness, there is a need to cast the future roadmap of science and technology considering the new challenges, opportunities and threats the future is likely to hold. The theme of FUTURE INDIA – Science and Technology will provide platform to researchers, academicians and industry champions to share their findings with the scholars eager to dig scientific findings and also to instigate budding science and technology enthusiasts. Their findings and innovations will play a vital role in environmental protection and sustainable development. There is a need for conservation of Biodiversity, protection of wetlands and prevention of environmental pollution, promotion of ecological balance enables sustainable development. This conference is a small step towards this goal.

Sub Themes of Conference:

- Modern inclination of Science.
- Science and technology for future India.
- Science & Technology in Biodiversity Assessment.
- Conservation strategies under vulnerable conditions for sustainable development.
- Potential uses of flora and fauna and understanding molecular diversity for the same.
- Carbon sequestration in curbing Climate Change.
- Ecosystem services and development.
- Causes, consequences and ethics of biodiversity.
- Biodiversity for food and agriculture.

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National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
Bahauddin Science College, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India and
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

EFFECT OF INCREASING CONCENTRATION OF SEAWATER IN SOIL ON GROWTH OF PLANTS (RGR,NAR,LAR) OF WHEAT CROP OF SAURASHTRA REGION

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Abstract: *The basic objective of the plant growth (RGR, NAR, LAR) of wheat crop in different concentration of seawater in soil is to give farmers for increasing agricultural production in semi-arid, arid regions and sea costal area of Saurashtra region. The western region of Gujarat state in India can be divided into three zones. Intensive agriculture is restricted to the central zone of Gujarat, which is characterized by semi-arid eco-climate. Plants are not incompatible to soil salinity, but most of the plants do not grow in the presence of high concentration of salts. The most common effect of salinity on plants is suppression of growth, which is associated with reduction in crop yields. Three major constraints which have been recognized on the growth of plants in saline habitats are as below (i) Water stress arising from the more negative water potential (elevated osmotic potential) of the rooting medium, (ii) Specific ion toxicity usually associated with excessive intake of chloride. In the coastal area and the saline desert, Plant growth and crop production is generally poor due to the concentration of salt in soil. Moreover in coastal area, salt concentration is increasing in ground water due to ingression of Arabian Sea. Eventually, ground water containing high salt-content is used for irrigation of crops. Present investigation is to study the responses of wheat* ***(Triticum aestivum, L. Loc 1)*** *to soil salinisation with respect to plant growth.*

Key Words: *Semi-arid regions; Arid regions; Sea costal area; Salinity; Soil type; Seawater concentration; Water potential; RGR, NAR, LAR; Plant growth.*

1. INTRODUCTION:

Today is more difficult to increase agricultural product in saline soils. Saline soils are abundant in semi-arid, arid regions and sea costal area where the amount of rainfall is insufficient for substantial leaching. Salinity problems occur in non irrigated croplands and rangelands either as a result of evaporation and transpiration of saline underground water or due to salt input from rainfall. They are particularly critical in irrigated areas. Salinity has been an important historical factor and has influenced the life spans of agricultural systems. It frequently destroyed ancient agrarian societies, and more recently large areas of the Indian subcontinent have been rendered unproductive by salt accumulation and poor water management. Plants are not incompatible to soil salinity, but most of the plants do not grow in the presence of high concentration of salts. The most common effect of salinity on plants is suppression of growth, which is associated with reduction in crop yields. Plant growth and crop production is generally poor due to the concentration of salt in soil. Moreover in coastal area, salt concentration is increasing in ground water due to ingression of Arabian Sea. Eventually, ground water containing high salt-content is used for irrigation of crops. Present investigation is to study the responses of wheat **(*Triticum aestivum*, L. Loc 1)** to soil salinisation with respect to plant growth.

2. LITERATURE REVIEW:

One of the major obstacles to increasing food production in arid and semi-arid regions is the lack of fresh water resources. Waters with salinities higher than 3 dSm^{-1} can be used to irrigate salt tolerant crops, but should be used judiciously for salt sensitive crops [1]. If some fresh water is available, it can be used to reduce the salinity of water by mixing before irrigation [1]. Crops that are relatively salt sensitive can be irrigated with saline water during the less sensitive stages of growth. Specifically fresh water should be used in early stages and saline water in later stages [3]. Salt tolerant crops are often grown with water containing salt using surface, sprinkler and drip irrigation methods [3],[1]. However, these methods usually result in increased soil salinity, soil degradation and nutrient imbalances [4],[5]. In arid areas, even fresh water irrigation could result in severe salinity problems due to the high evaporation rates at the soil surface [6].

Sub irrigation is now being emphasized as an alternative to surface irrigation methods. In this method water is supplied through the lower soil profile, less water evaporates from the soil surface. As a result, there is less accumulation of salt in the upper profile. It has been established that relatively fresh water, if present above the drains in the soil profile, could flow upward into the active root zone using saline water in a sub irrigation system [7]. Direct contact of sensitive plant tissues with irrigation water containing salt could also be prevented / delayed by sub irrigation. Sub irrigation systems have been used extensively for fresh water irrigation in humid areas [8] and to some extent to supplement crop water needs with brackish water [7]. Salt tolerance is usually assessed as the percent biomass production in saline versus control conditions over a prolonged period of time. [1]

3. STUDY OBJECTIVES:

The objectives of the present study were to assess the following characteristics of the control and salt-stressed plants in order to achieve the aim:

(1) Shoot and root elongation and leaf expansion.
(2) Dry weight accumulation in plants.
(3) Functional growth analysis (RGR, NAR and LAR).

4. MATERIALS AND 5. METHOD:

Total 200 polyethylene bags were each filled with 2 kg black-cotton soil and arranged in 10 sets so that each set contained 20 bags. Soils contained in bags for 10 different sets were then separately watered with 600 ml water containing 50%, 40%, 33%, 25%, 20%, 17%, 14%, 12%, 10% and 0% seawater. Seawater was mixed with tap water in above proportions to prepare different concentrations of seawater. Tap water without addition of seawater was treated as control or as 0% seawater content. Thus, there were a graded series of soils containing ten concentrations of seawater. Watering with 600 ml water was estimated to raise the soil moisture up to field capacity (30% moisture). Thereafter, soils were allowed to dry. After five days soil in each bag was raked with fingers and 10 seeds of wheat were sown at the depth of 8-12 mm. immediately after sowing soil was irrigated with tap water. Subsequently, soil in each bag was irrigated with tap water at the intervals of 8 days. After the germination, two seedlings that emerged first were left in each bag and others were uprooted. During growth period seedlings were watered at the intervals of 8 days to keep the soil moist. Three weeks after the emergence, seedlings in five bags at each salinity level were washed to remove the soil particles adhered with roots. Morphological characteristics of each plant were recorded. Shoot height and root length were measured. Leaf area was marked out on graph paper. Dry weight of leaves (leaf blades), stems (along with leaf sheaths) and roots were determined. At the last three harvests, i.e. at 6, 9 and 12 weeks, Values of dry weight of leaf, stem, and root components of plants together with leaf area were used to calculate RGR, NAR and LAR as follows:

$$\text{Relative growth rate(RGR)} = \frac{\log W'' - \log W'}{t'' - t'}$$, Where W" and W' are plant dry weights at time t" and t'

$$\text{Net assimilation rate (NAR)} = \frac{\text{Log } L'' - \log L' \,(W'' - W')}{(L'' - L')\,(t'' - t')}$$, Where, W', L' and W", L" are plant dry weight and leaf area, respectively at time t" and t'

$$leaf\ area\ ratio(LAR) = \frac{leaf\ area\ in\ cm^2}{Plant\ dry\ weight\ in\ mg.}$$

6. DISCUSSION:

There was a reduction in stem and root elongation and expansion of leaves for the wheat crop species with increase in soil salinity. These results are attributed to water stress. [10] reported that the plants subjected to water stress show reduction in size and dry matter production. According to Garg and Gupta [11] the most common and conspicuous effect of salinity is growth retardation. As salt concentration increases beyond a threshold level, both growth rate and ultimate size of most plant species progressively decrease. [12] Salinity also increases the leaf to stem ratio of several crops and this affects quality in the case of forage crops. Stunted growth, restricted lateral shoot development, reduction in size of leaves, fruits and grains, decrease in fresh and dry weight of different plant parts,

restricted leaf number and area and finally diminished yield are some of the features of salinisation. In case of wheat tillering is drastically reduced. Under conditions of severe salinity these tillers may die before they are able to grow and bear ears and only main shoot produces grains. The reduction in leaf area by increasing salinity is a common feature which affects plant productivity by reducing the rates of total photosynthesis by the crop canopy [13]. The salinity induced decrease in leaf area is particularly severe in salt sensitive crops like most of the legumes [14].

Dry weight of shoot, root and the whole plant of crop species decreased with increase in soil salinity. [15] Reduction in shoot growth and leaf area development of wheat in conformity with the findings of Curtis and Lauchli [16]. Ramoliya and Pandeya, found reduction in shoot and root elongation, leaf area development and dry weight of leaf, stem and root tissues of Salvadoran *oleoides* and *cardia rothii* tree species with increasing concentration of salt in soil. However, results suggested that test crop species are salt –tolerant at vegetative growth stage too.

7. ANALYSIS:

7.1 WHEAT ELONGATION OF STEM AND ROOT AND EXPANSION OF LEAF

There was a negative trend between shoot height and soil salinity according to the following expression: $Y = -2.148 + 1.12 X$ ($r = 0.299$, Ns, $df = 23$), where, Y is shoot height and X is soil salinity (dSm^{-1}).

There was a negative relationship between root length at 12-week growth stage and soil salinity according to the following expression: $Y = 19.57 - 8.385X$ ($r = -0.508$, $p<0.01$, $df = 23$), where Y is root length (cm) and X is soil salinity (dSm^{-1}).

There was a negative trend between leaf area at 12-week growth stage and soil salinity according to the following expression: $Y = 1.63 + 0.802 X$ ($r = -0.167$, Ns, $df = 23$), where Y is leaf area (cm^2) and X is soil salinity (dSm^{-1}).

7.2 WHEAT DRY MATTER ACCUMULATION

There was a negative relationship between shoot weight at 12-week growth stage and soil salinity according to the following expression: $Y = 471.04 - 30.84 X$ ($r = -0.443$, $p<0.05$, $df = 23$), where Y is shoot weight (mg) and X is soil salinity (dSm^{-1}).

There was a negative trend between root weight at 12-week growth stage and soil salinity can be expressed according to the following expression: $Y = 255.2 – 14.41 X$ ($r = - 0.112$, Ns, $df = 23$), where is Y is root weight (mg) and X is soil salinity. Plants in 15.8 dSm^{-1} soil died after 9-week.

Trend between total plant weight at 12-week growth stage and soil salinity can be expressed according to the following expression: $Y = 955.9 - 60.62 X$ ($r = - 0.341$, Ns, $df = 23$), where Y is total plant weight (mg) and X is soil salinity (dSm^{-1}).

8. FINDINGS:

According to wheat plant, elongation of stem, root and expansion of leaf dry matter accumulation findings are follows:

(1) In wheat plant negative trend between shoot height and soil salinity, negative relationship between root length at 12-week growth stage and soil salinity and negative trend between leaf area at 12-week growth stage and soil salinity

(2) In wheat plant negative relationship between shoot weight at 12-week growth stage and soil salinity, negative trend between root weight at 12-week growth stage and soil salinity.

9. RESULT:

9.1 WHEAT ELONGATION OF STEM AND ROOT AND EXPANSION OF LEAF

A consistent increase in height of wheat shoots of control plants was recorded until 9-week growth period (Fig.1A) Thereafter, shoot elongation stopped during ensuing period. Pattern of shoot elongation for salt-stressed plants over time was similar to that of control plants, but plant height decreased with increasing salt-stress.

Elongation of shoots of control and salt-stressed plants was most rapid between 3-and 6- week growth periods, whereas it was least rapid between 6-and 9-week growth period. Reduction in shoot height of salt-stressed plants as compared to shoot height of control plants was recorded since 3-week growth period. Plants grown in soil at 18.4 and 20.0 dSm^{-1} salinity did not survive after 6-week, whereas plants grown in soil at 15. 8 dSm^{-1} salinity died after 9-week growth period. Root elongation for control and salt-stressed plants was recorded until 9-week growth period (Fig.1B) and following this period root length was maintained constant. Root length for both control and salt-stressed plants was greater than their respective shoot height. Moreover, root length decreased with increasing salt-stress. Root elongation for control and salt-stressed plants was most rapid during the initial 3-week growth period.

Leaf area of control as well as of salt-stressed plants consistently increased till 9-week growth period (Fig.1C) and declined thereafter. However, leaf area decreased with increasing soil salinity. Leaf expansion was most rapid between 6-and 9-week growth periods for both control and salt-stressed plants. Leaf area of plants grown under control and saline conditions declined after 9-week growth period.

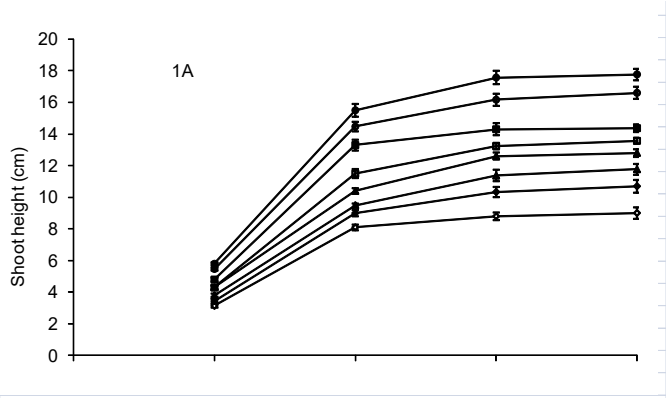

Fig.1A.Shoot height of wheat plants over time in response to soil salinity.
(●),1.2 dSm^{-1} ; (○), 6.0 dSm^{-1};(■),6.3 dSm^{-1}; (□), 8.5 dSm^{-1}; (▲), 9.5 dSm^{-1};
(△),10.2 dSm^{-1} ; (♦),13.0 $dSm^{-1}$and (◊),15.0 dSm^{-1} salinity.

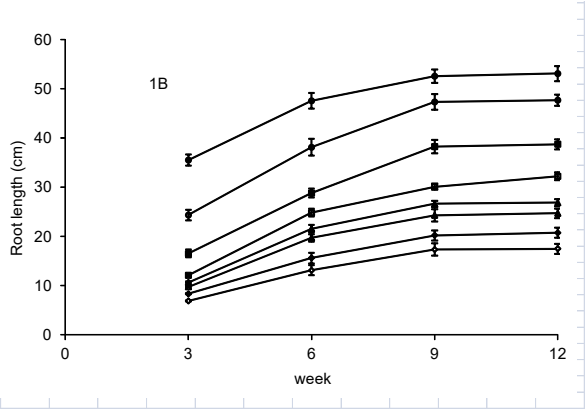

Fig.1B. root length of wheat plants over time in response to soil salinity.
(●),1.2 dSm^{-1} ; (○), 6.0 dSm^{-1};(■),6.3 dSm^{-1}; (□), 8.5 dSm^{-1}; (▲), 9.5 dSm^{-1};
(△),10.2 dSm^{-1} ; (♦),13.0 $dSm^{-1}$and (◊),15.0 dSm^{-1} salinity.

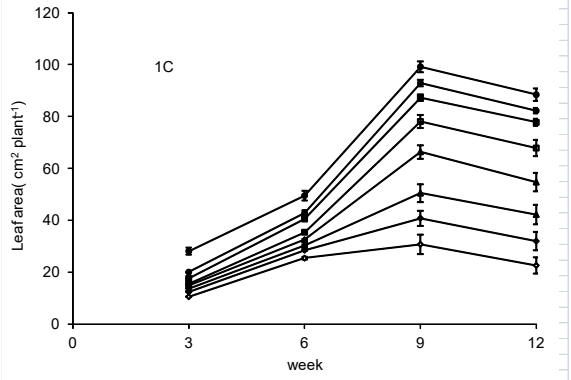

Fig.1C. Leaf area of wheat plants over time in response to soil salinity.
(●),1.2 dSm^{-1} ; (○), 6.0 dSm^{-1};(■),6.3 dSm^{-1}; (□), 8.5 dSm^{-1}; (▲), 9.5 dSm^{-1};
(△),10.2 dSm^{-1} ; (♦),13.0 $dSm^{-1}$and (◊),15.0 dSm^{-1} salinity.

9.2 WHEAT DRY MATTER ACCUMULATION

Wheat shoot weight of control plants consistently increased till 9-week and it declined slightly thereafter. Pattern of dry matter accumulation in shoots of plants grown in saline soils (soils mixed with different concentrations of seawater) was similar to that in shoots of control plants. (Fig.2A) Shoot weight was consistently greater for control plants as compared to that of salt-stressed plants. Moreover, dry weight of shoots of salt-stressed plants decreased with increase in soil salinity. Dry weight of shoots of control and salt-stressed plants differed since initial 3-week growth period. Plants grown in soils at 18.4 and 20.0 dSm^{-1} salinity did not survive after 6-week growth period.

Pattern of dry matter accumulation in plants grown in control and saline soils was similar to that in roots. (Fig.2B)

Further, pattern of dry matter accumulation in whole plant (shoot+root) was similar to that of shoot in both control and salt-stressed plants. (Fig.2C) Emergence of inflorescence was recorded on about 40% plants grown in control conditions. Further about 35%, 35%, 35%, 35%, 30%, 15% and 10 plants grown in soils at 6.0, 6.3, 8.5, 9.5, 10.2, 13.0 and 15.0 dSm^{-1} salinity produced inflorescence. Weight of inflorescence at 12-week growth stage was added to shoot weight.

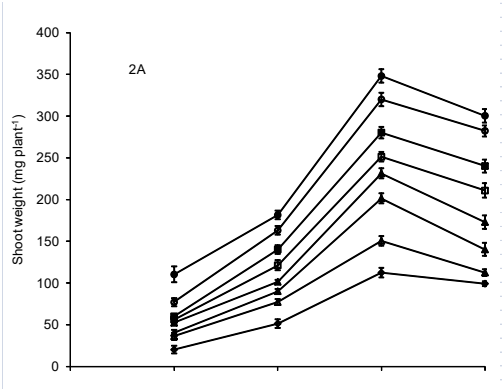

Fig.2A.Shoot weight of wheat plants over time in response to soil salinity.
(●),1.2 dSm^{-1} ; (○), 6.0 dSm^{-1};(■),6.3 dSm^{-1}; (□), 8.5 dSm^{-1}; (▲), 9.5 dSm^{-1};
(△),10.2 dSm^{-1} ; (♦),13.0 $dSm^{-1}$and (◊),15.0 dSm^{-1} salinity.

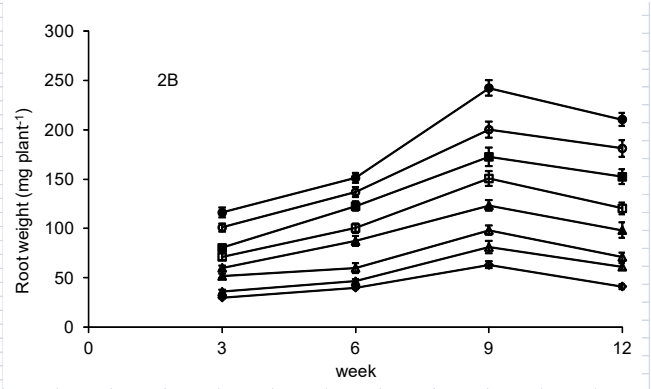

Fig.2 B.root weigth of wheat plants over time in response to soil salinity.
(●),1.2 dSm^{-1} ; (○), 6.0 dSm^{-1};(■),6.3 dSm^{-1}; (□), 8.5 dSm^{-1}; (▲), 9.5 dSm^{-1};
(△),10.2 dSm^{-1} ; (♦),13.0 $dSm^{-1}$and (◊),15.0 dSm^{-1} salinity.

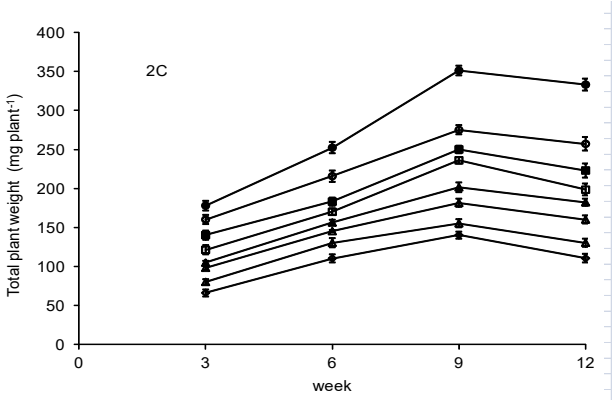

Fig.2C. Total plant weight of wheat plants over time in response to soil salinity.
(●),1.2 dSm^{-1} ; (○), 6.0 dSm^{-1};(■),6.3 dSm^{-1}; (□), 8.5 dSm^{-1}; (▲), 9.5 dSm^{-1};
(▲),10.2 dSm^{-1} ; (♦),13.0 $dSm^{-1}$and (◊),15.0 dSm^{-1} salinity.

10. RECOMMENDATIONS: For more crop production in agriculture to developed salt-tolerance crop plant.

11. CONCLUSION:

(1) There was reduction in elongation of stem and root, and expansion of leaves of wheat crop plant with increasing soil salinity. These results were primarily due to water stress induced by soil salinity.

(2) Dry matter accumulation in leaf, stems, and root tissues of salt-stressed plants decreased. In general, salinity can reduce plant growth through: osmotic effects, (ii) toxic effects of ions and (iii) imbalance of nutrients.

(3) Reduction in relative growth rate (RGR) of wheat crop plant was recorded with increase in soil salinity. It can be attributed to the reduced net assimilation rate (NAR) and not to leaf area ratio (LAR). The older leaves of salt-stressed plant were yellow in colour and these leaves might have lower photosynthesis rate and greater respiration rate. Leaf area can limit or promote plant growth may influencing an aspect of NAR, such as photosynthesis or respiration.

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National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
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A Comparative Study of Physicochemical Characteristics of Farmland Soils of Chuda and Thangadh Taluka Territory, District: Surendranagar, Gujarat State, India

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Abstract: *A Comparative physicochemical study was performed for the farmland soil samples of two different talukas of Surendranagar district namely Chuda and Thangadh covering various parameter viz. pH, electrical conductivity (EC), total organic carban, available phosphorous and available potassium. It concludes the nutrients quality of soils of both the Talukas of Surendranagar District (Gujarat - India). The present study includes study of various parameters which helps farmers to take wise decision to choose fertilizer and its quantity thereby one can achieve better crop production.*

Key Words: *Farmland soil, pH, EC, OC, Phosphorus, Potassium, and Surendranagar District.*

1. INTRODUCTION:

Soils are natural unconsolidated materials on the surface of the earth and are composed of solid, liquid and gas. They have organic and inorganic matter, which are well mixed together by natural processes. That is aggregated into a porous body that accommodates air and water [1]. The truth is that soil is a marvelous substance, a living resource of astonishing beauty, complexity and frailty. It is a complex mixture of weathered mineral materials from rock, partially decomposed organic molecules and a host of living organisms. It can be considered an ecosystem by itself. Soil is one of the most important resources of the nature. Life cycles depend on plants that grow in soil for day to day need. Soil is not only important for agriculture but also for living organisms. Soil as a component of the terrestrial ecosystem fulfills many function including those that are essential for sustaining plant growth [2]. Soil fertility and plant nutrition are two closely related subjects that emphasize the forms and availability of nutrients in soils, their movement to and their uptake by roots and the utilization of nutrients within plants. Soil fertility and plant nutrition are two closely related subjects that emphasize the forms and availability of nutrients in soils, their movement to and their uptake by roots and the utilization of nutrients within plants [3]. It is very difficult to enhance agricultural production in order to feed alarming population by avoiding soil fertility.

Any parts of earth surface that support vegetation also bears a covering of soil. The soil condition plays vital role for vegetation distribution and development [4]. Soil sampling is the most important because a very small amount of the soil mass is used for analysis and routine soil tests measure only a bit of the total lake of nutrients in the soil [5]. Soil mainly consists of 50% pore space (air and water) and 50% solid phase. The solid phase is broadly composed of 45% mineral matter and 5% organic constituents [6,7].

Available Potassium and Fertility Index of farmland soil of Danta taluka was recently reported Parmar, J. K. Total 3180 samples from 34 villages of Danta taluka was studied [8]. It reveals from literature that Physico-chemical study of soil is found significant importance in the farming practice. Soils have large amounts of nutrients essential for plant and agricultural product though only small fractions are in the form that can be uptake directly by plants. By interpretation of soil analysis data one can improve crop productivity and minimize wastage of these nutrients. Deficiencies of primary, secondary and micronutrients have been observed in severe cultivated areas [9]. Some efforts for the study of interested parameters for various areas was reported [10,11,12,13].

2. STUDY OBJECTIVES:

Present study is an effort to determine and compare the nutrient's quantity in soil of two Talukas *viz.* Chuda and Thangadh, District: Surendranagar Gujarat. With the help of this statistical information, Agriculture department recommend farmers for the usage of the quality and quantity of fertilizer to make the production economic. The objective of this paper was to evaluate the trend in pH, EC, OC, P and K status of soils of area covered under study.

2.1 EXPERIMENTAL

The soil analysis data is the best source available to assess the fertility status of soil. Two Talukas of Surendranagar District, namely Chuda and Thangadh Talukas of Gujarat State are selected. For the present study, 658 samples from 8 villages of Chuda Taluka and 274 samples from 6 villages of Thangadh Taluka are collected. Collected samples are then crushed and sieved for further analysis for different chemical parameters following standard methods. AR grade reagents and double distilled water were used for soil analysis. Results were compared with standard values [13] to find out low, medium or high nutrient's content essential for STR.

The collected soil samples were analyzed for major quality parameters *viz.* pH, Electrical Conductivity (EC), Organic Carbon (OC), Available Potassium (K) and Available Phosphorus (P). Organic matter is oxidized with chromic acid and analyzed by colorimetric method [14,15]. pH, EC, and potash measured using pH meter, conductivity meter, Flame photometer respectively while OC and Phosphorus was measured using colorimeter in the laboratory. Table 1 depicts the analytical results for these parameters along with the standard values prescribed by Anand Agriculture University, Gujarat – India.

3. RESULTS AND DISCUSSION :

Soil reaction (pH)

pH measurement of sample is very essential to measure potentiality of beneficial nutrients and toxic elements available for plants. The soil reaction or pH is used to state the acidity or alkalinity of the soil. pH was measured by preparing aqueous soil extracts (1 part of soil: 2 part of deionized water). The data reveals that most of the samples of Chuda taluka lie in neutral pH range. And almost all the samples of Thangadh Taluka lie in neutral range and is shown in Figure 1. Increased dilution of a soil/water mixture is responsible for an increase in soil pH [16].

Electrical Conductivity

The presence of soluble salts in soil is expressed by EC. The soil extract prepared for determination of EC is the same as for pH determination. The data presented in Table 1 revealed the soil EC value of the soil samples on the basis of land use system *viz.* Chuda and Thangadh, District: Surendranagar Gujarat. Most of the soils of both the Talukas are fall in normal range as shown in Figure 2. Some of the samples lie in higher range and is due to soluble salts present in the soil.

Soil organic carbon

Soil organic carbon is the seat of nitrogen in soil and its determination is often carried out as an index of nitrogen availability. In the colorimeter method [14], Organic matter is oxidized with chromic acid. More than 50% of the samples of Chuda Taluka fall in lower range and more than 90% samples of Thangadh Taluka lie in medium range. It is depicted in Table 1 and is shown in Figure 3.

Available Phosphorus

Phosphorus is one of the important essential nutrients and is utilized in the form of $H_2PO_4^-$ & HPO_4^{2-} by plant species. Colorimetric method was employed for the determination of Phosphorus. Phosphorus in form of orthophosphate plays an active role in aquatic ecosystem. Phosphorus was found in low to medium range (Table no.1) for the selected area. Figure 4 shows the graphical representation of available Phosphorous content present in the soil. Phosphorous content in soils of Chuda (29.31) is high as compared to that of soils of Thangadh (27.55).

Available Potassium

Flame Photometric Method was employed for the determination of available Potassium in terms of K_2O in soil samples. It is absorbed by the plants in large amount. It helps in building of proteins. Even a very small amount Potassium present in the soil, it plays very important role in metabolism of fresh water and is considered to be an important nutrient. Table 1 shows the high range of Potassium content found for both the Talukas.

Table 1: Soil Characteristic data for samples of selected villages of Chuda and Thangadh Taluka

Parameters	Range		No. of Sample	
			Chuda Taluka	Thangadh Taluka
pH	<6.5	Acidic	1	1

	6.5 to 8.2	Neutral	593	273
	>8.2	Basic	64	0
	Average pH Value		**7.77**	**7.47**
EC dS/cm	<1	Normal	582	262
	1 to 3	Harmful	76	12
	>3	Very Harmful	0	0
	Average EC Value		**0.53**	**0.39**
OC (%)	< 0.50	Low	376	11
	0.50 to 0.75	Medium	240	256
	> 0.75	High	42	7
	Average OC Value		**0.46**	**0.54**
Phosphorous (P_2O_5) Kg/ha	<28	Low	284	147
	28 to 56	Medium	369	127
	>56	High	5	0
	Average Phosphorous Value		**29.31**	**27.55**
Potash (K_2O) Kg/ha	<140	Low	2	0
	140-280	Medium	39	0
	>280	High	617	274
	Average Potash Value		**526.06**	**618.30**

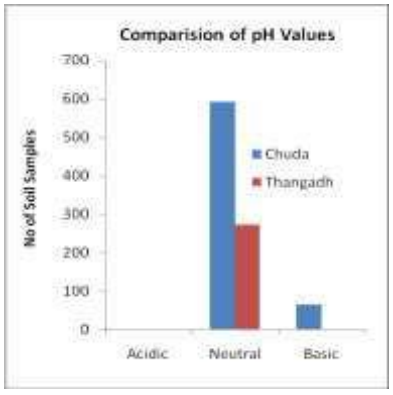

Figure 1: Comparison of pH values

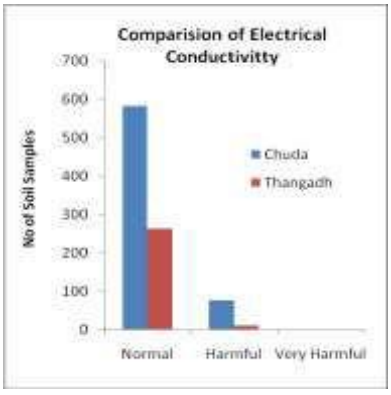

Figure 2: Comparison of EC

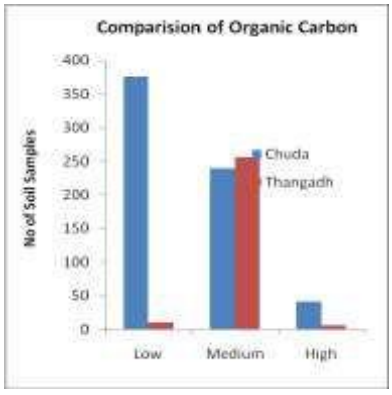

Figure 3: Comparison of Organic Carban

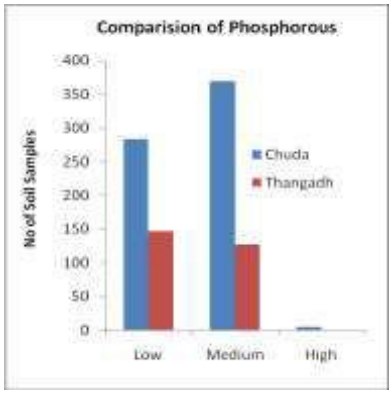

Figure 4: Comparison of Phosphorous (P_2O_5)

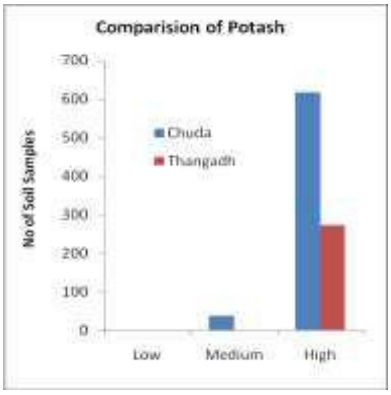

Figure 5: Comparison of Potash (K_2O)

4.CONCLUSION:

From the findings of the present comparative study, it can be concluded that the soils of the study area differ up to some extent in pH value according to type of land use system. Electrical conductivity values are found normal

for both the Talukas. Some samples are found in high range due presence of soluble salts in the farmland soils. Soils are largely deficient in Organic Carbon for Chuda taluka. Phosphorus content is also found in low to medium range. The soils of both the area found rich in Potash content. Results indicated that there is high scope of improving the soil fertility status and productivity. Thus fertility status of soil is evaluated from this study for making fertilizer recommendations. Prediction of the probable crop response to applied nutrients is possible. By identification of the type and degree of soil related problems like salinity, alkalinity and acidity etc. It is also accomplished how to find out suitability for growing crops and orchard.

ACKNOWLEDGEMENT

I am thankful to Commissioner of Higher Education, Block No. 12, second floor, Dr. Jivaraj Mehta Bhavan, Gandhinagar and Department of Agriculture, Gandhinagar for the financial assistance.

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Eco- Friendly Synthesis and Antimicrobial Screening of New Pyrazoline Derivatives

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Abstract: *Eco-friendly synthesis of organic compound is the need of modern chemistry to full fill this concept a new series of 3-(Aryl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydropyrazoline-1-carbothioamide and 3-(Aryl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydro-1-phenylpyrazoline derivatives were synthesized from various substituted 3-Methoxy-4-(2,4-dichlorophenylmethoxy)chalcones by using Eco-friendly solvent ethanol and Eco-friendly acetic acid as catalyst. Each new pyrazoline derivatives were characterized by analytical techniques like IR, NMR, Mass and Elemental analyses. Also each pyrazoline derivatives were screened for their in vitro antimicrobial activity.*

Key Words: *Eco-Friendly, Chalcone, Pyrazoline, Antimicrobial activity.*

1. INTRODUCTION:

The major cause for diseases by antimicrobial infections is due to suppressed immunity. Suppression of immunity has so many reasons such as therapeutic techniques; malignancy, immunosuppressive HIV-infection, surgeries and old age. The situation is further worsened by increasing incidence of microbial resistance to the majority of antibiotics available today in the market. Antimicrobial resistance is a major anxiety and very danger for public health and for this, the development of novel and effective antibiotics is necessary [1]. The currently available antifungal and antibacterial agents in the market are very less and majority of them have various side effects like toxicity spectrum, lack of oral formulations, few drug targets, pharmacokinetics and higher cost [2]. Heterocycles have good biological importance for their interesting biological activity. Pyrazolines are the five member heterocyclic compounds containing two nitrogen atoms adjacent to each other, have diversified biological activities such as Anticonvulsant [3], Diuretic [4], Fungicidal[5], Antitubercular[6], antinociceptive[7], Anticonvulsant[8], Anti-inflammatory[9], Antimicrobial[10,11]. The pyrazoline ring contains N–N bond which is considered to be the key factor in their biological actions but in natural compounds N–N bonds is very rare because the N-N bond is constructed with a great difficulty by living organism [12, 13]. From the literature survey interesting biological activities of different types of pyrazoline derivatives have been found therefore; our interest to synthesize new different types of pyrazoline derivatives may have good biological activity.

For the synthesis of pyrazoline different types of synthetic methods are listed in literature, among them best method for synthesis of pyrazolines is preparation of the pyrazolines by cyclocondensation of α,β-unsaturated compound(chalcone) and hydrazine derivatives[14-18]. Therefore we focus on the synthesis of some new pyrazolines for the first time using chalcones of vanillin analog [19] using glacial acetic acid as catalyst and methanol as a solvent media and under reflux condition.

2. MATERIAL AND METHODS:

The materials like phenyl hydrazine (Sigma Aldrich), thiosemicarbazide (Sigma Aldrich), glacial acetic acid (Renchem) and methanol (Renchem) are of analytical grade; used without further purification at work place. Melting points of all the synthesized different types of pyrazoline derivatives were taken in open capillary method and are uncorrected. Elemental analyses (% of C, H & N) of each derivative were performed on a model 2400 Perkin–Elmer

elemental analyzer. Infrared spectra (4000–400 cm^{-1}, using KBr discs) of the samples were recorded on Shimadzu-435 Spectrophotometer and ^1H NMR spectra on Bruker Advance 400MHz spectrometer with $CDCl_3$ and DMSO as a solvent and tetramethyl silane (TMS) as internal standard. The chemical shift was measured in parts per million (ppm). The antimicrobial activity of purified compounds was done by Cup-plate agar diffusion method. Progress of the reaction and purity of the compounds is checked by thin layer chromatography (TLC) plates.

2.1 EXPERIMENTAL

(A) Synthesis of 3-Methoxy-4-(2, 4-dichlorophenylmethoxy) chalcones(A)

The alcoholic solution of 3-methoxy-4-(2,4-Dichlorophenylmethoxy)benzaldehyde (3.11, 0.01mol, 25 mL)was stirred with different substituted acetophenones(0.01mol) and 20%(w/v) NaOH (5mL) for 12-17 h. Keep this reaction mixture aside for overnight, separated light green to brown colour product was filtered and recrystallized from ethanol.

(B) Synthesis of 3-(Phenyl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxy phenyl]-4,5-dihydro-1-phenylpyrazoline(1a-1k)

A mixture of 3-Methoxy-4-(2, 4-Dichlorophenylmethoxy) chalcone (4.13g, 0.01mol) and phenyl hydrazine (1.12mL, 0.012mol) was refluxed for about 8 hours in ethanol (30mL) as a solvent and glacial acetic acid as catalyst. The solid product was separated out on cooling am room temperature. The separated product was filtered and recrystallized from ethanol. M. P., 144°C. Elemental analyses, found%: C (69.32), H (4.87), N (5.52), calculated%: C (69.19), H (4.81), N (5.56).

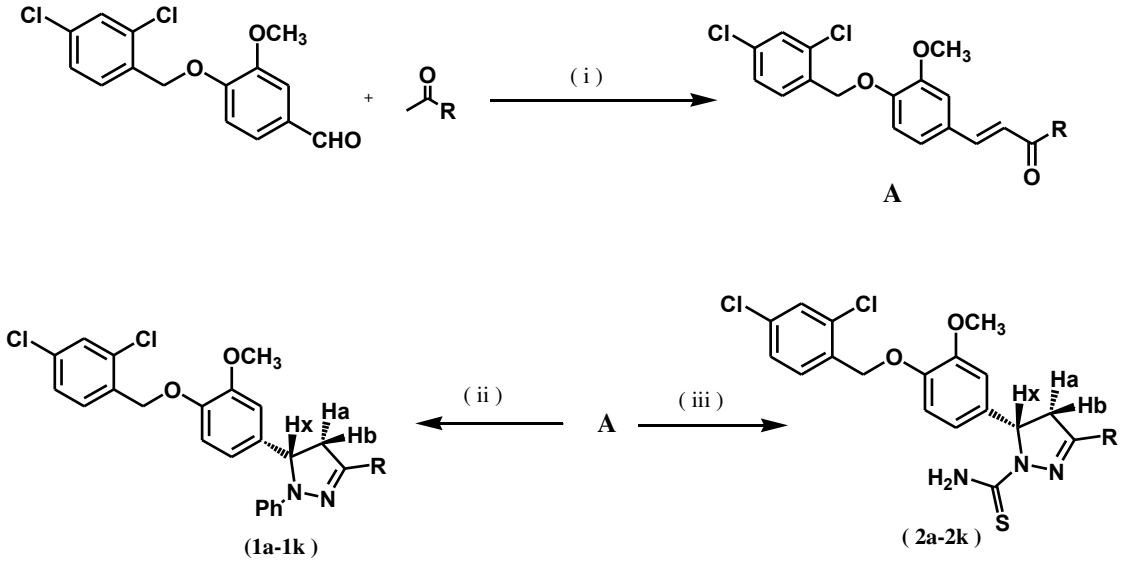
Similarly, a series of 3-(Aryl)-5-[4-(2,4-dichlorophenylmethoxy)-3-methoxy phenyl]-4,5-dihydropyrazoline-1-carbothioamides were synthesized. The analytical and physical data of all the synthesized compounds were described in Table **1.1.**

(A) Synthesis of 3-(4-Bromophenyl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydropyrazoline-1-carbothioamide(2a-2k)

A mixture of 3-Methoxy-4-(2, 4-Dichlorophenylmethoxy)-4'-bromo chalcone (4.92g, 0.01mol) and thiosemicarbazide(0.81g, 0.015mol) and few drop of KOH slurry was refluxed for about 8-10 hours in ethanol (25mL) as a solvent on oil bath. The solid product was separated out on cooling am room temperature. The separated product was filtered and recrystallized from ethanol. M. P., 116°C. Elemental analyses, found%: C (51.04), H (3.52), N(7.41), calculated%: C (50.99), H (3.57), N (7.43).

Similarly, a series of 3-(Aryl)-5-[4-(2,4-dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydro-1-phenylpyrazoline derivatives were synthesized and characterized. The analytical and physical data of all the synthesized compounds were recorded in Table **2.1.**

General Reaction Scheme for Synthesis of Different types of Pyrazoline



(i) 20% NaOH (ii) NH_2NHPh, CH_3COOH(iii) $NH_2NHCSNH_2$, CH_3COOH

SPECTRAL DATA

3-Methoxy-4-(2, 4-Dichlorophenylmethoxy)-4'-methoxy chalcone:

IR (KBr, cm^{-1}): ν 1652(-CO-CH=CH-), 1241(Ar-O-CH_2), 672(-C-Cl); ^{1}H-NMR ($CDCl_3$): δ 5.19(s, 2H, -O-CH_2-), 3.87(s, 3H, -OCH_3), 7.80-7.87 (d, 1H, 15.6Hz, =CH-Ar), 7.70-7.75(d, 1H, 16.4Hz, -CO-CH=), 7.13-8.12(m, 11H, Ar-H); Mass (m/z): 443(M^+).

3-(Phenyl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydro-1-phenylpyrazoline: IR (KBr, cm^{-1}): 1593(C=N), 692(C-Cl), 1259(Ar-O-CH_2); ^{1}H NMR (DMSO-d_6): 5.19 (s, 2H,-O-CH_2-), 3.91 (s, 3H, -OCH_3), 3.02(1H, dd, -CH_A, Pyrazoline), 3.41 (1H, dd, -CH_B, Pyrazoline), 4.92(1H, dd, -CH_X, Pyrazoline), 6.79-7.56 (15H, m, Ar-H); Mass (m/z): 502(M^+).

3-(4-Bromophenyl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydropyrazoline-1-carbothioamide: IR (KBr, cm^{-1}):1591(C=N), 692(C-Cl), 1261(Ar-O-CH_2); ^{1}H NMR (DMSO-d_6): 5.14 (s, 2H,-O-CH_2-), 3.87 (s, 3H, -OCH_3), 3.03(1H, dd, -CH_A, Pyrazoline), 3.40 (1H, dd, -CH_B, Pyrazoline), 4.94(1H, dd, -CH_X, Pyrazoline), 6.73-7.53 (12H, m, Ar-H & -NH_2); Mass (m/z): 564(M^+).

1.1. ANALYTICAL AND PHYSICAL DATA OF 3-(ARYL)-5-[4-(2,4-DICHLOROPHENYLMETHOXY)-3-METHOXYPHENYL]-4,5-DIHYDRO-1-PHENYLPYRAZOLINE

Comp.	-R	Molecular Formula	Mol. Wt.	M.P (^{0}C)	% Yield	Elemental analysis					
						Calculated (%)			Found (%)		
						C	H	N	C	H	N
1a	-C_6H_5	$C_{29}H_{24}Cl_2N_2O_2$	502	144	78	69.19	4.81	5.56	69.32	4.87	5.52
1b	-4-Br-C_6H_4	$C_{29}H_{23}BrCl_2N_2O_2$	582	234	66	59.81	3.98	4.81	59.88	4.01	4.88
1c	-4-Cl-C_6H_4	$C_{29}H_{23}Cl_3N_2O_2$	537	162	76	64.76	4.31	5.21	64.80	4.37	5.31
1d	-2-Cl-C_6H_4	$C_{29}H_{23}Cl_3N_2O_2$	537	171	76	64.76	4.31	5.21	64.74	4.41	5.19
1e	-2,4-Cl_2-C_6H_3	$C_{29}H_{22}Cl_4N_2O_2$	572	219	56	60.86	3.87	4.89	60.90	3.90	4.92
1f	-4-OH-C_6H_4	$C_{29}H_{24}Cl_2N_2O_3$	519	166	71	67.06	4.66	5.39	67.11	4.65	5.42
1g	-3-OH-C_6H_4	$C_{29}H_{24}Cl_2N_2O_3$	519	149	78	67.06	4.66	5.39	67.03	4.90	5.32
1h	-4-OCH_3-C_6H_4	$C_{30}H_{26}Cl_2N_2O_3$	533	225	72	67.55	4.91	5.25	67.67	4.94	5.20
1i	-4-NO_2-C_6H_4	$C_{29}H_{23}Cl_2N_3O_4$	548	241	61	63.51	4.23	7.66	63.59	4.33	7.63
1j	-3-NO_2-C_6H_4	$C_{29}H_{23}Cl_2N_3O_4$	548	222	58	63.51	4.23	7.66	63.60	4.25	7.61
1k	-4-NH_2-C_6H_4	$C_{29}H_{25}Cl_2N_3O_2$	518	172	71	67.19	4.86	8.11	66.11	4.80	8.19

1.2. ANTIMICROBIAL SCREENING DATA OF 3-(ARYL)-5-[4-(2,4-DICHLOROPHENYLMETHOXY)-3-METHOXYPHENYL]-4,5-DIHYDRO-1-PHENYLPYRAZOLINE

Comp.	-R	Molecular Formula	Antibacterial activity (zone of inhibition in mm)				Antifungal activity (zone of inhibition in mm)	
			S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*	*A. niger*	*C. albicans*
1a	-C_6H_5	$C_{29}H_{24}Cl_2N_2O_2$	12	15	07	13	10	12
1b	-4-Br-C_6H_4	$C_{29}H_{23}BrCl_2N_2O_2$	19	17	12	11	14	15
1c	-4-Cl-C_6H_4	$C_{29}H_{23}Cl_3N_2O_2$	17	20	13	12	15	21
1d	-2-Cl-C_6H_4	$C_{29}H_{23}Cl_3N_2O_2$	12	14	12	10	12	08
1e	-2,4-Cl_2-C_6H_3	$C_{29}H_{22}Cl_4N_2O_2$	21	22	17	13	09	11
1f	-4-OH-C_6H_4	$C_{29}H_{24}Cl_2N_2O_3$	06	15	10	05	15	12
1g	-3-OH-C_6H_4	$C_{29}H_{24}Cl_2N_2O_3$	08	09	11	07	09	16
1h	-4-OCH_3-C_6H_4	$C_{30}H_{26}Cl_2N_2O_3$	11	10	13	14	15	18
1i	-4-NO_2-C_6H_4	$C_{29}H_{23}Cl_2N_3O_4$	07	17	19	09	11	14
1j	-3-NO_2-C_6H_4	$C_{29}H_{23}Cl_2N_3O_4$	09	10	15	10	12	16
1k	-4-NH_2-C_6H_4	$C_{29}H_{25}Cl_2N_3O_2$	08	09	06	11	16	14
	Sparfloxacin		24	25	25	22	-	-
	Benzylpenicillin		18	17	16	16	-	-
	Fluconazole		-	-	-	-	22	20

2.1. ANALYTICAL AND PHYSICAL DATA OF 3-(ARYL)-5-[4-(2,4-DICHLOROPHENYLMETHOXY)-3-METHOXYPHENYL]-4,5-DIHYDRO PYRAZOLINE-1-CARBOTHIOAMIDE

Compound	-R	Molecular Formula	Mol. Wt.	M.P (^{0}C)	% Yield	Elemental analysis					
						Calculated (%)			Found (%)		
						C	H	N	C	H	N
2a	-C_6H_5	$C_{24}H_{21}Cl_2N_3O_2S$	486	123	67	59.26	4.35	8.64	59.37	4.33	8.63
2b	-4-Br-C_6H_4	$C_{24}H_{20}BrCl_2N_3O_2S$	564	116	75	50.99	3.57	7.43	51.04	3.52	7.41
2c	-4-Cl-C_6H_4	$C_{24}H_{20}Cl_3N_3O_2S$	520	174	71	55.34	3.87	8.07	55.29	3.83	8.11
2d	-2-Cl-C_6H_4	$C_{24}H_{20}Cl_3N_3O_2S$	520	202	64	55.34	3.87	8.07	55.30	3.85	8.02
2e	-2,4-Cl_2-C_6H_3	$C_{24}H_{19}Cl_4N_3O_2S$	555	188	67	51.91	3.45	7.57	52.07	3.50	7.61
2f	-4-OH-C_6H_4	$C_{24}H_{21}Cl_2N_3O_3S$	502	224	77	57.37	4.21	8.36	57.30	4.22	8.29
2g	-2-OH-C_6H_4	$C_{24}H_{21}Cl_2N_3O_3S$	502	98	72	57.37	4.21	8.36	57.22	4.25	8.32
2h	-4-OCH_3-C_6H_4	$C_{25}H_{23}Cl_2N_3O_3S$	516	143	75	58.14	4.49	8.14	58.23	4.53	8.23
2i	-2-NO_2-C_6H_4	$C_{24}H_{20}Cl_2N_4O_4S$	531	121	57	54.24	3.79	10.54	54.21	3.80	10.51
2j	-4-NO_2-C_6H_4	$C_{24}H_{20}Cl_2N_4O_4S$	531	177	56	54.24	3.79	10.54	54.32	3.82	10.57
2k	4-NH_2-C_6H_4	$C_{24}H_{22}Cl_2N_4O_2S$	501	205	76	57.49	4.42	11.17	57.53	4.38	11.25

2.2. ANTIMICROBIAL SCREENING DATA OF 3-(ARYL)-5-[4-(2,4-DICHLOROPHENYLMETHOXY)-3-METHOXYPHENYL]-4,5-DIHYDRO PYRAZOLINE-1-CARBOTHIOAMIDE

Compound	-R	Molecular Formula	Antibacterial activity (zone of inhibition in mm)				Antifungal activity (zone of inhibition in mm)	
			S. aureus	*B. subtilis*	*P.aeruginosa*	*E. coli*	*A. niger*	*C. albicans*
2a	-C_6H_5	$C_{24}H_{21}Cl_2N_3O_2S$	14	12	08	16	18	16
2b	-4-Br-C_6H_4	$C_{24}H_{20}BrCl_2N_3O_2S$	23	08	21	10	23	21
2c	-4-Cl-C_6H_4	$C_{24}H_{20}Cl_3N_3O_2S$	18	10	12	07	20	19
2d	-2-Cl-C_6H_4	$C_{24}H_{20}Cl_3N_3O_2S$	12	22	24	10	21	12
2e	-2,4-Cl_2-C_6H_3	$C_{24}H_{19}Cl_4N_3O_2S$	15	21	12	13	22	20
2f	-4-OH-C_6H_4	$C_{24}H_{21}Cl_2N_3O_3S$	12	10	10	10	19	22
2g	-2-OH-C_6H_4	$C_{24}H_{21}Cl_2N_3O_3S$	07	06	07	11	17	14
2h	-4-OCH_3-C_6H_4	$C_{25}H_{23}Cl_2N_3O_3S$	10	05	12	09	09	12
2i	-2-NO_2-C_6H_4	$C_{24}H_{20}Cl_2N_4O_4S$	14	02	13	11	16	20
2j	-4-NO_2-C_6H_4	$C_{24}H_{20}Cl_2N_4O_4S$	12	11	08	12	12	09
2k	4-NH_2-C_6H_4	$C_{24}H_{22}Cl_2N_4O_2S$	08	13	12	15	11	10
	Sparfloxacin		24	25	25	22	-	-
	Benzylpenicillin		18	17	16	16	-	-
	Fluconazole		-	-	-	-	22	20

ANTIMICROBIAL SCREENING

The samples of synthesized phenylpyrazolines **(1a-1k)** and pyrazoline-1-carbothioamide **(2a-2k)** derivatives for antimicrobial activity were prepared at concentration 40µg/ml in DMSO solvent. In case of antibacterial activity, the plates were incubated at 37°C for 24 hours and for antifungal activity the plates were incubated at 30°C for 48 hours. The antibacterial activity was checked against *Gram positive* bacteria *Staphylococcus aureus (S aureus)* and *Bacillus subtilis (B subtilis)*, *Gram negative* bacteria *Pseudomonas aeruginosa (P aeruginosa)* and *Escherichia coli (E coli)*. The antifungal activity was checked against fungi *Aspergillus niger (A niger)* and *Candida albicans (C albicans)*. The results were compared with stand drug data of Sparfloxacin, Benzyl penicillin and Fluconazole.

4. RESULT DISCUSSION:

A new series of 3-(Aryl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydropyrazoline-1-carbothioamide and 3-(Aryl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydro-1-phenylpyrazoline derivatives were synthesized by refluxing various substituted 3-Methoxy-4-(2, 4-Dichlorophenylmethoxy) chalcones with phenyl hydrazine and thiosemicarbazide results good to moderate yield. After refluxing the solvent ethanol is recovered by distillation and reused for next derivative that is cost effective and beneficial for clean environment. The catalyst acetic acid used in place of any other strong Bronsted acid is also ecofriendly. Analytical and physical data of the synthesized compounds were recorded and listed in **(Table 1.1 & 2.1).**

The FT−IR spectra of synthesized 3-(Aryl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydropyrazoline-1-carbothioamide and 3-(Aryl)-5-[4-(2,4-Dichlorophenylmethoxy)-3-methoxyphenyl]-4,5-dihydro-1-phenylpyrazoline at 1595-1597 cm^{-1} for (C=N) which is characteristic band for dihydropyrazoline moiety.

The ^{1}HNMR also support the formation of pyrazoline moiety by chemical shift of the pyrazoline ring proton, Ha, Hb & Hx, appeared as double of doublets at around 3.02-3.14, 3.40-3.79 and 4.91-5.49 δ respectively. The doublet of doublet is due to germinal and vicinal coupling in pyrazoline ring.

The molecular ion peak (m/z) is equivalent to their molecular weight of proposed compounds and the fragmentation pattern of synthesized compounds matched with the typical fragmentation pattern of the pyrazoline moiety that further confirming the structures of the compounds. The elemental analysis (% of C, H and O) data found is equivalent to their calculated value.

From antimicrobial screening data **(Table 1.2 & 2.2)** of synthesized directives show that the compounds **1b, 1e, 2b, 2c** and **1b, 1c, 1e, 2d, 2e** have good antibacterial activity against *S.aureus* and *B.subtilis* (Gram positive bacteria) respectively compare to Benzyl penicillin. The compounds **1i** and **2d** have good antibacterial activity against *P. aeruginosa* (Gram negative bacteria) compare to Benzyl penicillin. The compound **1d** has very good antifungal activity against *C. albicans* and compounds **1c, 2e, 2f** and **2i** have good antifungal activity against *A.niger* compare to Flucanazole.

5. CONCLUSION:

In the present work, a series of phenylpyrazoline and pyrazoline-1-carbothioamide derivatives have been synthesized using new chalcones and phenyl hydrazine and thiosemicarbazide with moderate to good yield. Antimicrobial activity data of synthesized compounds indicates that few synthesized compounds have good results compared to standard drugs data. Further investigation with appropriate structural modification of the above compounds may result in good antimicrobial agents. The analytical data and spectral data also support to the structure and geometry of the pyrazoline derivatives.

ACKNOWLEDGMENT:

The author is thankful to Dr. R P Bhatt, Principal, Bahauddin Science College, Junagadh and Dr. M M Chavda, Head of Chemistry Department of this college.. The author is also thankful to N F D D Center, Saurashtra University-Rajkot for providing good spectral data analysis facility.

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National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
Bahauddin Science College, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India and
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

SYNTHESIS AND BIOLOGICAL SCREENING OF *N*-((2-(4-FLUOROPHENYL)-6-METHYL*H*-IMIDAZO[1,2- α]PYRIDIN-3-YL)METHYL)-4-ARYLAMINES

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Abstract: *Synthesis of imidazo[1,2-α]pyridine derivatives that have potential in various therapeutical activities. Looking the interesting properties of imidazo[1,2-α]pyridine, with an intension to synthesize better therapeutic agents, imidazo[1,2-α]pyridine derivatives were synthesized by the selective reduction of (imine group) Schiff's bases which has been synthesized by the condensation of 2-(4-Fluorophenyl)-6-methylH-imidazo[1,2- α] pyridine-3-carbaldehyde with different aromatic amines.*

The structure of newly synthesized compounds were characterized by IR,[1]H NMR,[13]C NMR, Mass spectral Data techniques and Elemental analysis techniques. Purity of the compounds have been checked by Thin layer chromatography. The synthesized compounds were evaluated for their antibacterial activity and antifungal activity. The biological activity of the synthesized compounds have been then compared with standard drugs.

1. INTRODUCTION:

Heterocyclic rings are important constituent of all cells and thus are of all living matter like hormones, amino acids, vitamins, haemoglobin, alkaloids, and of synthesized drugs. Heterocyclic compounds have been extensively investigated by the organic chemists due to their close association with various types of biological activities[1]. Heterocyclic compounds possessing a wide spectrum of biological activities such as anticancer, antitubercular, antitumor, antiviral, antifungal, antiparasit, insecticidal, antiinflammatory, antidiabetic and analgesic compounds[2-12]. Some heterocyclic compound is also used as intermediates in the pharmaceutical and photography industries.

The study of mannich reaction appeared a great deal of attention to the chemists because it plays a vital role due to their wide range of industrial and biological applications and mannich bases are also employed as an intermediate in chemical synthesis.[13-15]

The [5,6] fused-ring moiety shown to be in top eight most frequently occurring frameworks in drugs. Among them, imidazo[1,2-α]pyridine derivatives are an essential part of pharmacologically molecules and possess especial structures that proved numerous pharmaceutical importance.[16-18] There are many drug related to imidazo[1,2-a]pyridine family such as Zolpidem, popular drug used for short-term treatment of insomnia[19], Saripidem is a sedative and anxiolytic[20], Olprinone is cardio tonic agent[21], Zolimidine is a useful drug for the treatment of peptic ulcer. It is a gastroprotective drug used for gastroesophageal reflux disease[22], Soraprazan is useful for the treatment of Stargardt's disease which is genetic disorder of the eye that leads to progressive loss of sight[23], Linaprazan is a potassium competitive acid blocker, used in the treatment of gastro esophageal reflux disease (GERD)[24], Divaplon is anxiolytic and anticonvulsant drug, GSK812397:Developed by GSK for the treatment of HIV as antiviral agent.[25]

Many research groups are working to explore towards the development new derivatives of this moiety. Many work has been done by our laboratory.[26-35]

2. LITERATURE SURVEY:

Literature survey has been carried out on heterocyclic moieties from the various source like database chemical abstracts, research papers and various organic chemistry journals, Latters in organic chemistry, Heterocyclic chemistry

journals, Progress in heterocyclic chemistry, Medicinal chemistry journals, Research Journal of Chemical Sciences, reference books etc.. Synthesis of compounds will be carried out using some name reaction like Biginelli reaction, Vilsmeier-Haack reaction, Pechmann condensation, Claisen reaction, Friedel-Crafts reactions, Perkin reaction etc. . Literature survey reveals that nitrogen containing heterocyclic compounds like imidazo[1,2-α]pyridines have received considerable attention in medicinal science due to their biological and pharmacological activities like antithyroid ,anti-inflammatory, hypnotic, sedative, herbicidal, antimicrobial, antitubercular, CNS depressant and many other therapeutic activities.

R. H. Mehta et al.[36] have synthesized coummarin Schiff's base derivatives and examined it for antibacterial activity. A. K. Khalafallah and M. E. Hassan[37] have prepared some styryl Schiff's bases spiro derivatives as potential antifungal and antibacterial activity. P. Perumal[38] have synthesized some azomethine derivatives having good antibacterial activity. E. C. Creencia and group[39] reported synthesis from ortho substituted aniline with 55 % yield.
Important drugs, poisons and medicines (both natural and synthetic) such as pyrenthrin, sulphathiazole, rotenmone, alpidem, zolpidem, fluconazole, strychnine, reserpine, certain of the antihistamines, the ergot alkaloids caffeine, cocaine, barbiturates, etc. are heterocyclic compounds.

3. STUDY OBJECTIVES:

Aminomethyl derivatives of heterocyclic compounds are associated with diverse therapeutical activities. imidazo[1,2-α]pyridine derivatives are an essential part of pharmacologically molecules and possess especial structures that proved numerous pharmaceutical importance These prompted us to synthesize some representative aminomethyl derivative. Synthetic protocol establishment of the proposed heterocyclic compound and then Synthesis of compound by different routine reactions and characterization of heterocyclic compounds by IR,^{1}H NMR,^{13}C NMR, Mass spectral Data techniques and Elemental analysis techniques. Purity of the compounds have been checked by Thin layer chromatography. The synthesized compounds were evaluated for their antibacterial activity and antifungal activity. The biological activity of the synthesized compounds then compared with standard drugs.

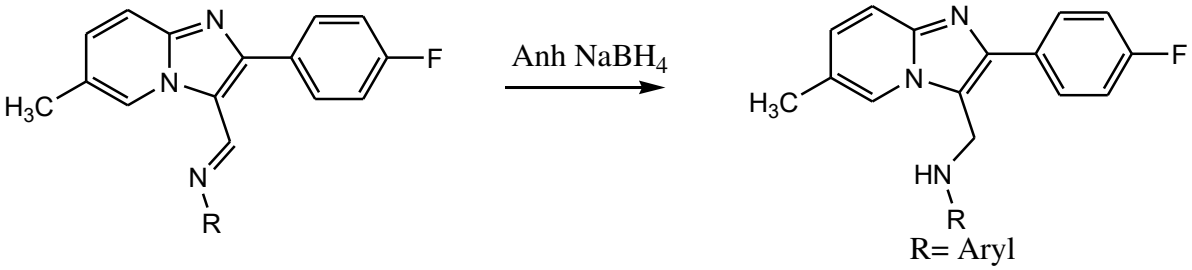
4. MATERIALS:

All chemicals were acquired from the Sigma-Aldrich Chemical Co (Sigma-Aldrich, Bangalore, India). The proton NMR and Carbon NMR were recorded using BRUKER Spectrometer (300MHz) with $CDCl_3$ and the chemical shifts are expressed in ppm. The infrared spectra (KBr) were recorded using SHIMADZU-FT-IR 8400-Spectrophotometer operating range of 4000-400 cm^{-1}. The mass spectra (EI) were recorded using Jeol JMS D-300 spectrometer. Melting points of synthesized compounds were taken in open capillary bath on controlled temperature heating mental. The crystallization of all the compounds was carried out in appropriate solvents. Reactions were monitored using Thin layer Chromatography, carried out on silica gel-G as stationary phase. 40 % Ethyl acetate in Hexane was used as a mobile phase. The visualization was achieved under UV light.

5. METHOD:

Synthesis of *N*-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)-4-methylbenzenamines.

N-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)-4-methylbenzenamines bearing imidazo[1,2-a]pyridine moiety obtained by selective reduction of (imine group) Schiff's bases (17*E*)-N-((2-(4-fluorophenyl)-6-methylh-imidazo[1,2-α]pyridin-3-yl]methylene)-4-methylbenzenamines with sodium borohydride in controlled experimental condition as shown in the reaction Scheme.



5.1 EXPERIMENTAL SECTION

[A] Synthesis of (17*E*)-N-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridin-3-yl]methylene)-4-methylbenzenamines.

First of all 2-(4-Fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridine-3-carbaldehyde prepared by taking a well stirred solution of phosphorus oxychloride (11 ml), DMF (8 ml) and chloroform (32 ml) maintained at 0-10°C was added slowly to a solution of 2-(4-Fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridine(4.52 gm, 0.02mol) in chloroform (140 ml). The mixture so obtained was refluxed for 8 hrs, then solution was evaporated to dryness in vacuum. The

residue was treated with cold water and filtered and then crystallized it in methanol. For Synthesis of (17E)-N-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridin-3-yl]methylene)-4-methylbenzenamines. The mixture of 2-(4-Fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridine-3-carbaldehyde (2.54gm, 0.01 mole) and p-toludine(1.08 gm, 0.01 mole) in methanol (20 ml) in presence of catalytic amount of gl. acetic acid was refluxed for 6 hr. The contents were cooled and product isolated was crystallized in methanol and the yield is 52%.

[B] Synthesis of *N*-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)-4-methylbenzenamines.

Sodium borohydride (0.15 mol, 0.57gm) slowly add to a methanolic solution of (17*E*)-*N*-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridin-3-yl] methylene)-4- methylbenzenamines. (0.01mol, 3.43 gm) over a period of 30 minutes at temp. 5-10°C with constant stirring. The reaction mixture was kept over night at room temp. The excess borohydride neutralized by adding water and the product was extracted with ether. The extract was washed with water untill become neutral, then dried over anhydrous Na_2SO_4 and finally the ether was evaporated to give aminomethyl derivatives. Yield, 65%, m.p. 163°C, $C_{22}H_{20}FN_3$; Found : C, 76.34; H, 5.71; N, 12.02 %; Requires : C, 76.50; H, 5.84; N, 12.17 %.

Similarly, other (17*Z*)-*N*-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-a]pyridin-3-yl]methylene)-4-arylamines. were prepared.

All the synthesized compounds(13a-13h) were characterized by IR,^{1}H NMR,^{13}C NMR and Mass spectral Data techniques and Elemental analysis techniques. Purity of the compounds have been checked by Thin layer chromatography. The synthesized compounds were evaluated for their antibacterial activity and antifungal activity. The biological activity of the synthesized compounds have been then compared with standard drugs.

6. ANALYSIS:

Spectral Characterization:

N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)benzenamine (13A)

^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 7.04 (t, 2H); 6.43 (d, 2H); 6.58 (t, 1H); 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 162.9, 147.6, 145.5, 144.1, 135.0, 134.4, 133.8, 129.6, 129.1,128.7, 122.4, 117.2, 116.0, 114.8, 113.5, 33.8, 24.7. Mass for $C_{21}H_{18}FN_3$ 331.39. IR (nujal) cm^{-1}: C-H str. (asym.) 2941, C-H str. (sym.) 2848, CH def. (asym.) 1437, C-H def. (sym.) 1390, Ar C=C str.2941, Ar C-N str 1504, C=N 1614, -NH 3356, C-F str. 705, C-N 1024.

N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)-4-methylbenzenamine (13B)

^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 6.31 (d, 2H); 6.84 (d, 2H); 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H). 2.35 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 162.9, 151.8, 146.0, 145.7, 144.4, 136.9, 134.4, 133.8, 130.4.129.1, 128.7, 122.4, 122.2, 122.0, 116.0, 114.8, 24.7, 24.3. Calculated mass for $C_{22}H_{20}FN_3$ 345.41. IR (nujal) cm^{-1}: C-H str. (asym.) 2931, C-H str. (sym.) 2847, CH def. (asym.) 1437, C-H def. (sym.) 1390, Ar C=C str.2941, Ar C-N str 1503, C=N 1614, -NH 3356, C-F str. 705, C-N 1024.

N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)-2-methylbenzenamine (13C)

^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 6.31 (d, 1H); 6.85 (t, 1H); 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H);6.46 (t, 1H); 6.84(d, 1H); 2.35 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 162.9, 146.5, 144.5, 144.1, 135.0, 134.4, 133.8, 129.9, 129.1, 128.7, 126.6, 126.2, 122.4, 117.1, 116.0, 114.8, 113.4, 34.1, 24.7, 15.5. Calculated mass for $C_{22}H_{20}FN_3$ 345.41. IR (nujal) cm^{-1}: C-H str. (asym.) 2941, C-H str. (sym.) 2848, CH def. (asym.) 1435, C-H def. (sym.) 1391, Ar C=C str.2941, Ar C-N str 1504, C=N 1614, -NH 3352, C-F str. 706, C-N 1021.

N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)-2,5-dimethylbenzenamine (13D)

^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 6.11 (s, 1H); 6.26 (d, 1H); 6.72 (d, 1H); 2.35 (s, 3H) 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H); 2.35 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 162.9, 146.4, 145.5, 144.1, 136.2,135.0, 134.4, 133.8, 129.8, 129.1, 128.7, 123.2, 122.4, 117.4, 116.0, 114.8, 113.4, 34.1, 24.7, 24.3, 15.5. Calculated mass for $C_{23}H_{22}FN_3$ 359.43. IR (nujal) cm^{-1}: C-H str. (asym.) 2941, C-H str. (sym.) 2848, CH def. (asym.) 1437, C-H def. (sym.) 1390, Ar C=C str.2941, Ar C-N str 1504, C=N 1614, -NH 3356, C-F str. 705, C-N 1024.

N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)-4-methoxybenzenamine (13E)

^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 6.32 (d, 2H); 6.55 (d, 2H); 3.73 (s, 3H); 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 162.9, 149.1, 145.5, 144.1,139.9, 135.0, 134.4, 133.8, 129.1, 128.7, 122.4, 116.0, 115.1, 114.8, 114.5, 55.9, 33.8, 24.7. Calculated mass for $C_{22}H_{20}FN_3O$ 361[1] H.41. IR (nujal) cm^{-1}: C-H str. (asym.) 2941, C-H str. (sym.) 2848, CH def. (asym.) 1437, C-H def. (sym.) 1390, Ar C=C str.2943, Ar C-N str 1504, C=N 1613, -NH 3356, C-F str. 706, C-N 1024.

2,5-dichloro-N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)benzenamine (13F)
^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 6.38 (s, 1H); 6.53 (d, 1H); 6.99 (d, 1H); 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 162.9, 145.5, 145.3, 144.1, 135.0, 134.4, 133.8, 133.2, 131.1, 129.1, 128.7, 122.4, 120.5, 118.7, 116.0, 115.3, 114.8, 33.3,24.7. Calculated mass for $C_{21}H_{16}Cl_2FN_3$ 400.28. IR (nujal) cm^{-1}: C-H str. (asym.) 2941, C-H str. (sym.) 2847, CH def. (asym.) 1437, C-H def. (sym.) 1391, Ar C=C str.2941, Ar C-N str 1504, C=N 1614, -NH 3356, C-F str. 705, C-N 1026.

4-chloro-N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)benzenamine (13G)
^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 6.37 (d, 2H); 7.05 (d, 2H); 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 162.9, 145.7, 145.5, 144.1,135.0, 134.4, 133.8, 129.7, 129.1, 128.7, 122.7, 122.4, 116.0, 114.9, 114.8, 33.8, 24.7. Calculated mass for $C_{21}H_{17}ClFN_3$ 365.83. IR (nujal) cm^{-1}: C-H str. (asym.) 2941, C-H str. (sym.) 2848, CH def. (asym.) 1437, C-H def. (sym.) 1390, Ar C=C str.2941, Ar C-N str 1503, C=N 1614, -NH 3356, C-F str. 705, C-N 1024.

4-fluoro-N-((2-(4-Flurophenyl)-6-Methyl*H*-imidazo[1,2-α]pyridin-3-yl)methyl)benzenamine (13H)
^{1}H NMR: ($CDCl_3$) δ 7.42 (d, 1H); 6.97 (d, 1H); 2.32 (d, 3H); 8.07 (s, 1H); 6.41 (d, 2H); 6.75 (d, 2H); 7.03 (d, 2H); 7.46 (d, 2H); 4.0 (t, 1H); 4.26 (d, 2H). ^{13}C NMR ($CDCl_3$) δ 162.9, 151.3, 145.5, 144.1, 143.2, 135.0, 134.4, 133.8, 129.1, 128.7, 122.4, 116.3, 116.0, 115.1, 114.8, 33.8, 24.7. Calculated mass for $C_{21}H_{17}F_2N_3$ 349.37. IR (nujal) cm^{-1}: C-H str. (asym.) 2941, C-H str. (sym.) 2848, CH def. (asym.) 1437, C-H def. (sym.) 1391, Ar C=C str.2940, Ar C-N str 1506, C=N 1614, -NH 3356, C-F str. 705, C-N 1023.

7. RESULT AND DISCUSSION:

Antimicrobial activity of N-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2-α]pyridin-3-yl]methyl)-4-arylamines.

All the compounds have been screened for vitro biological assay like antifungal activity towards Aspergillus niger, Candida albicans at a concentration of 2000/1000/ 500 µg/ml. and antibacterial activity towards Gram positive and Gram negative bacterial strains and The biological activity of the synthesized compounds have been compared with standard drugs.

All the compounds have been evaluated for antimicrobial and antifungal activity is described as under.

- **Preparing Agar Dilution plates**

1: Appropriate dilutions was done by taking 1 ml quantity of antimicrobial solution to Mueller Hinton agar (19 ml quantity) that allowed to equilibrate in a water bath for 45 to 50 °C. One part of antimicrobial solution is added to nine parts of liquid agar.

2: The agar and antimicrobial solution were mixed thoroughly and then this mixture poured quickly (for prevent from partial solidification) into Borocil glass Petri dishes.

3: Solidification of the Agar plate at room temperature, and the plates were ready to used immediately or stored at 2 to 8°C in sealed plastic bags for five days for reference work, or longer for routine tests.

4: Plates were allowed to equilibrate at room temperature before use, and assure that the agar surface was dry before inoculating the plates.

Antibacterial Activity Study:

Organic compounds may be bacteriostatic or bactericidal for microbial cultures. Mueller Hinton Agar plates (showing no visible growth of bacteria), sub culturing was carried out on Nutrient Agar plates (Collins, 1967). After streaking, Nutrient Agar plates were incubated for 24 hr. at 37°C. If colonies were found, the dilution was measured as bacteriostatic and if no colonies observed, it was measured as bactericidal. Bactericidal dilutions of the organic compounds were considered as exact Minimum inhibitory concentration (MIC) for a particular organic compound.

Antifungal Activity Study:

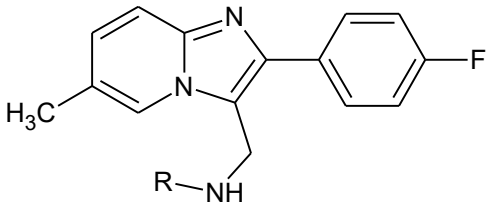
The fungal media Yeast Nitrogen base agar plate (YNBG) (Difco Make) 6.7 g and Glucose 10 g, dissolved in 100 ml of distilled water and filter. The inoculum was prepared from 3-4 days old sabouraud's Dextrose agar slants. The growth was uniformly mixed with Distilled water. The Size of inoculum prepared for inoculating YNBG agar plates was 102 –103 cfu/ml, adjusted with McFarland solution. After inoculation of properly diluted fungal solution, the plates were incubated at 37 °C for 48 hours.

Organic compounds may be bacteriostatic or bactericidal for microbial cultures. If colonies were found, the dilution was measured as bacteriostatic and if no colonies observed, it was measured as bactericidal. All the compounds have been screened for their in vitro biological assay like antibacterial activity towards Gram positive and Gram negative bacterial strains and antifungal activity towards ***Aspergillus niger, Candida albicans*** at a concentration of 2000/1000/500 µg/ml. The zones of inhibition of compounds are recorded in following table.

Table-1 Antibacterial and Antifungal activities of compound (13a-13h)

Sr. No	Code No.	Antibacterial activity												Antifungal activity					
		Gram +ve Bacteria						Gram -ve Bacteria						Uni/Multicellular Fungi					
		Staphylococcus aureus			Bacillus subtilis			Escherichia coli			Salmonella paratyphi B			Aspergillus niger			Candida albicans		
		2000	1000	500	2000	1000	500	2000	1000	500	2000	1000	500	2000	1000	500	2000	1000	500
1	13a	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-
2	13b	-	-	-	+	+	-	-	-	-	+	+	-	+	+	+	-	-	-
3	13c	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-
4	13d	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-
5	13e	-	-	-	+	+	-	+	+	-	+	+	-	-	-	-	-	-	-
6	13f	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-
7	13g	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	13h	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-

Table-2 : Physical constants of *N*-((2-(4-fluorophenyl)-6-methyl*H*-imidazo[1,2- α]pyridin-3-yl)methyl)-4-arylamines.



Sr. No.	Substitution R	Molecular Formula/ Molecular Weight	M.P. °C	Yield %	% Composition Calcd./Found		
					C	H	N
13a	C_6H_5-	$C_{21}H_{18}FN_3$ 331.38	185	58	76.11 (76.02)	5.47 (5.29)	12.68 (12.43)
13b	4-CH_3-C_6H_4-	$C_{22}H_{20}FN_3$ 345.41	163	65	76.50 (76.34)	5.84 (5.71)	12.17 (12.02)
13c	2-CH_3-C_6H_4-	$C_{22}H_{20}FN_3$ 345.41	174	75	76.50 (76.39)	5.84 (5.74)	12.17 (12.09)
13d	2,5-$(CH_3)_2$-C_6H_3-	$C_{23}H_{22}FN_3$ 359.43	160	63	76.85 (76.64)	6.17 (6.01)	11.69 (11.61)
13e	4-OCH_3-C_6H_4-	$C_{22}H_{20}FN_3O$ 361.41	198	55	73.11 (73.04)	5.58 (5.29)	11.63 (11.41)
13f	2,5-$(Cl)_2$-C_6H_3-	$C_{21}H_{16}Cl_2FN_3$ 400.26	188	45	63.01 (62.72)	4.03 (3.89)	10.50 (10.19)
13g	4-Cl-C_6H_4-	$C_{21}H_{17}ClFN_3$ 365.83	176	63	68.95 (68.65)	4.68 (4.52)	11.49 (11.32)
13h	4-F-C_6H_4-	$C_{21}H_{17}F_2N_3$ 349.37	186	49	72.19 (72.03)	4.90 (4.62)	12.10 (11.83)

8. CONCLUSION:

A heterocyclic systems namely imidazo[1,2-α]pyridine has enhanced pharmacological effect and hence they are ideally suited for further modifications to obtain numerous effective antibacterial and antifungal compounds. The structures of all the compounds were confirmed by, ^{1}H NMR & ^{13}C NMR spectroscopy, mass and FT-IR. The

manufactured compounds were tested for potential biological activities. All the compounds were found to possess reasonably good antifungal and antimicrobial activities. Investigation of antibacterial and antifungal screening data revealed that the Compounds **13a,13c,13d** were found to be most potent antimicrobial agent and **13a,13c,13d,13f,13h** were found to be most potent antifungal agent.

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National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
Bahauddin Science College, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India and
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

SYTHESIS & BIOLOGICAL DEVELOPMENT OF PERSUASIVE FLUORO WITH NITRO CONTAING PYRIMIDINES

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Abstract: An efficient Synthesis of a novel series of ***4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(substitutedphenyl)-6-(trifluoromethyl)-1,2,3,4-tetrahydro-2-thioxopyrimidine-5-carboxamide (4a-j)*** was accomplished from 4-(4-(trifluoromethyl)-2-nitrophenoxy)benzaldehyde, N-(substitutedphenyl)-4,4,4-trifluoro-3-oxobutanamide and using thiourea with few drops of Conc. HCl in CH_3OH and the product obtained was segregated. All the newly synthesized compounds were characterized by the Mass, IR, ^{1}H-NMR and mass spectroscopic techniques and by elemental analyses. The newly synthesized compounds were evaluated for their antibacterial and antifungal activity.

Key Words: N-(substitutedphenyl)-3-oxobutanamides, N-(substitutedphenyl)-4,4,4-trifluoro-3-oxobutanamide, thiourea, Conc. HCl and Methanol .

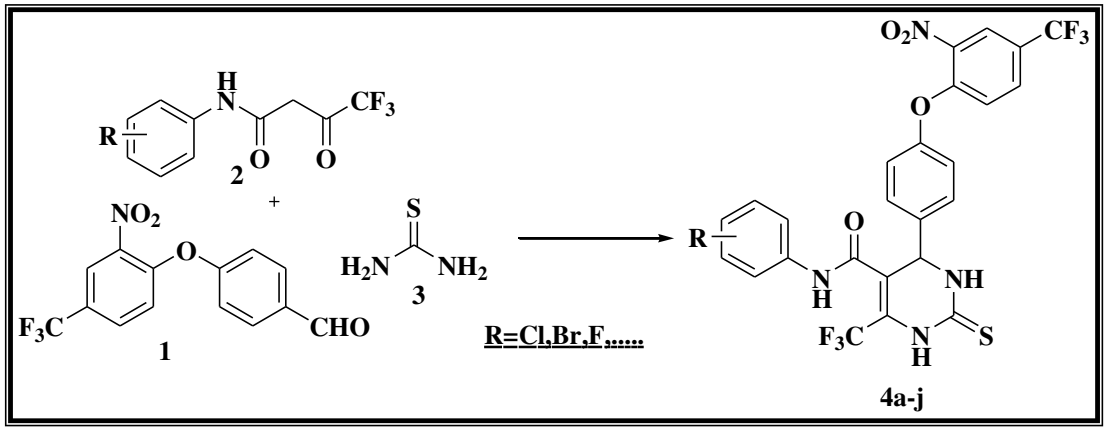
1. INTRODUCTION:

Pyrimidine is contain of hetero atom like that pyridine[1] here pyrimidine is six-membered heterocyclic's with two nitrogen atoms in the ring, pyrimidine have a two nitrogen and its position is 1 and 3 in the structure.[2] The pyrimidine ring system has extensive incidence in scenery[3], including the nucleotides, vitamin-B1 and alloxan.

Even though pyrimidine derivatives such as uric acid and alloxan be recognized in the near the beginning 19th century, a pyrimidine was not prepared by synthesis in 1879,[4] when Grimaux reported the research of barbituric acid from urea and malonic acid with $POCl_3$.[5] The systematic study of pyrimidines began[6] of Pinner,[7] who synthesized derivatives by condensing ethyl acetoacetate with amidines. Pinner first proposed the name "pyrimidin" in 1885.[8] The parent compound was first prepared by Gabriel and Colman in 1900,[9] [10] by conversion of barbituric acid to 2,4,6-trichloropyrimidine go behind by reduction using Zn-dust in hot water.

The Biginelli reaction is a two or more-component chemical reaction and this reaction was synthesis by Pietro Biginelli in 1891. The reaction can be catalyzed by Bronted acids and/or by Lewis acids such as copper(II) trifluoroacetate hydrate[11] and boron trifluoride.[12] Several solid-phase protocols utilizing different linker combinations have been published.[13][14]

Over the years, Dihydropyrimidines(DHPMs) and their derivatives 1, 2, 3, 4-Tetrahydro pyrimidines encompass displayed a charming collection in natural, synthetic, pharmacological, therapeutic and bioorganic chemistry mainly due to their wide range of molecules due to their biological properties,[15-18] Such as antiviral, antimitotic, anticarcinogenic, calcium channel blockers, antihypertensive agents, alpha-antagonists. Biginelli first reported a conformist synthesis of dihydropyrimidines (DHPM) by multi-component reaction of ethyl acetoacetate, aromatic aldehyde and urea under stalwartly acidic conditions.[19] These reactions are commonly performed in ethanol or tetrahydrofuran under strong protic acid catalyst.[20-23] For example, a integer of thienopyrimidine derivatives be asserted to possess interesting anticancer activities.[24,25] Moreover different functional groups show their new synthetic approach.



2. EXPERIMENTAL:

Typical untried procedure

A mixture of 4-(4-(trifluoromethyl)-2-nitrophenoxy)benzaldehyde, N-(substitutedphenyl)-4,4,4-trifluoro-3-oxobutanamide, thiourea and catalytic amount of conc. HCL in methanol was heated under reflux condition for 13 hrs. The reaction mixture was kept at room temperature for 20 hrs. The crystalline product obtained and recrystallized from DMF.

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(4-chlorophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4a)

Yield: 67%; mp 178ºC; elemental analysis calculator For $C_{25}H_{15}ClF_6N_4O_4S$: C, 48.67; H, 2.45; Cl, 5.75; F, 18.48; N, 9.08; O, 10.37; S, 5.20; Found: C, 48.67; H, 2.49; Cl, 5.79; F, 18.45; N, 9.05; O, 10.35; S, 5.21 %; IR (cm^{-1}): 3400 (N-H stretching of $CONH_2$), 3142 (C-H stretching of phenyl ring), 3007 (C-H asymmetrical stretching of methyl group), 2827 (C-H symmetrical stretching of methyl group), 1671 (C=O stretching of $CONH_2$), 1627 (C=O stretching of cycles) 1594 (N-H deformation of pyrimidine ring), 1570 (C=C stretching of phenyl ring), 1512 (C-NO_2 asymmetrical deformation of Nitro group), 1455 (C-H asymmetrical deformation of methyl group), 1421 (C-H symmetrical deformation of methyl group), 1330 (C-NO_2 symmetrical deformation of Nitro group), 1280 (C-N stretching), 1265 (C-O-C asymmetrical stretching), 1157, 1071 (C-H in plane deformation of aromatic ring), 1005 (C-F stretching) 826 (para-substituted); MS: *m/z* 617; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.86-7.88 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(4-bromophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4b)

Yield: 63%; mp 170ºC; elemental analysis calculator. for $C_{25}H_{15}BrF_6N_4O_4S$: C, 45.40; H, 2.29; Br, 12.08; F, 17.24; N, 8.47; O, 9.68; S, 4.85; Found: C, 45.45; H, 2.28; Br, 12.02; F, 17.28; N, 8.49; O, 9.63; S, 4.83 %; IR (cm^{-1}): 3400 (N-H stretching of $CONH_2$), 3145 (C-H stretching of phenyl ring), 3012 (C-H asymmetrical stretching of methyl group), 2825 (C-H symmetrical stretching of methyl group), 1671 (C=O stretching of $CONH_2$), 1626 (C=O stretching of cycles) 1593 (N-H deformation of pyrimidine ring), 1572 (C=C stretching of phenyl ring), 1511 (C-NO_2 asymmetrical deformation of Nitro group), 1457 (C-H asymmetrical deformation of methyl group), 1422 (C-H symmetrical deformation of methyl group), 1332 (C-NO_2 symmetrical deformation of Nitro group), 1282 (C-N stretching), 1266 (C-O-C asymmetrical stretching), 1156, 1071 (C-H in plane deformation of aromatic ring), 1003 (C-F stretching) 825 (para-substituted); MS: *m/z* 661; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.84-7.87 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(4-fluorophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4c)

Yield: 55%; mp 171ºC; elemental analysis calculator for $C_{25}H_{15}F_6N_4O_4S$: C, 50.01; H, 2.52; F, 22.15; N, 9.33; O, 10.66; S, 5.34; Found: C, 50.03; H, 2.50; F, 22.18; N, 9.30; O, 10.70; S, 5.30 %; IR (cm^{-1}): 3403 (N-H stretching of $CONH_2$), 3140 (C-H stretching of phenyl ring), 3003 (C-H asymmetrical stretching of methyl group), 2822 (C-H symmetrical stretching of methyl group), 1673 (C=O stretching of $CONH_2$), 1624 (C=O stretching of cycles) 1591 (N-H deformation of pyrimidine ring), 1576 (C=C stretching of phenyl ring), 1512 (C-NO_2 asymmetrical deformation of Nitro group), 1454 (C-H asymmetrical deformation of methyl group), 1425 (C-H symmetrical deformation of methyl group), 1329 (C-NO_2 symmetrical deformation of Nitro group), 1284 (C-N stretching), 1265 (C-O-C asymmetrical stretching), 1153, 1071 (C-H in plane deformation of aromatic ring), 1004 (C-F stretching) 826 (para-substituted);

MS: *m/z* 600; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.95-7.97 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(4-nitrophenyl)-1,2,3,4-tetrahydro-6-trifluoromethyl-2-thioxopyrimidine-5-carboxamide (4d)

Yield: 60%; mp 179ºC; elemental analysis calculator for $C_{25}H_{15}F_6N_5O_6S$: C, 47.85; H, 2.41; F, 18.17; N, 11.16; O, 15.30; S, 5.11; Found: C, 47.80; H, 2.45; F, 18.18; N, 11.10; O, 15.33; S, 5.14%; IR (cm^{-1}): 3402 (N-H stretching of $CONH_2$), 3147 (C-H stretching of phenyl ring), 3003 (C-H asymmetrical stretching of methyl group), 2825 (C-H symmetrical stretching of methyl group), 1671 (C=O stretching of $CONH_2$), 1624 (C=O stretching of cycles) 1591 (N-H deformation of pyrimidine ring), 1572 (C=C stretching of phenyl ring), 1512 (C-NO_2 asymmetrical deformation of Nitro group), 1451 (C-H asymmetrical deformation of methyl group), 1422 (C-H symmetrical deformation of methyl group), 1333 (C-NO_2 symmetrical deformation of Nitro group), 1283 (C-N stretching), 1264 (C-O-C asymmetrical stretching), 1157, 1075 (C-H in plane deformation of aromatic ring), 1005 (C-F stretching) 823 (para-substituted); MS: *m/z* 627; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.74-7.76 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(4-methylphenyl)-1,2,3,4-tetrahydro-6-trifluoromethyl-2-thioxopyrimidine-5-carboxamide (4e)

Yield: 73%; mp 177ºC; elemental analysis calculator for $C_{26}H_{18}F_6N_4O_4S$: C, 52.35; H, 3.04; F, 19.11; N, 9.39; O, 10.73; S, 5.38; Found: C, 52.37; H, 3.00; F, 19.17; N, 9.39; O, 10.70; S, 5.37%; IR (cm^{-1}): 3397 (N-H stretching of $CONH_2$), 3140 (C-H stretching of phenyl ring), 3000 (C-H asymmetrical stretching of methyl group), 2823 (C-H symmetrical stretching of methyl group), 1677 (C=O stretching of $CONH_2$), 1624 (C=O stretching of cycles) 1593 (N-H deformation of pyrimidine ring), 1572 (C=C stretching of phenyl ring), 1511 (C-NO_2 asymmetrical deformation of Nitro group), 1453 (C-H asymmetrical deformation of methyl group), 1422 (C-H symmetrical deformation of methyl group), 1334 (C-NO_2 symmetrical deformation of Nitro group), 1284 (C-N stretching), 1265 (C-O-C asymmetrical stretching), 1155, 1077 (C-H in plane deformation of aromatic ring), 1000 (C-F stretching) 821 (para-substituted); MS: *m/z* 597; ^{1}H NMR (DMSO-*d6*) δ ppm: 2.32 (s, 3H, H_m), 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.43-7.45 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(3-chlorophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4f)

Yield: 58%; mp 176ºC; elemental analysis calculator For $C_{25}H_{15}ClF_6N_4O_4S$: C, 48.67; H, 2.45; Cl, 5.75; F, 18.48; N, 9.08; O, 10.37; S, 5.20; Found: C, 48.67; H, 2.49; Cl, 5.79; F, 18.45; N, 9.08; O, 10.33; S, 5.20 %; IR (cm^{-1}): 3407 (N-H stretching of $CONH_2$), 3132 (C-H stretching of phenyl ring), 3009 (C-H asymmetrical stretching of methyl group), 2829 (C-H symmetrical stretching of methyl group), 1675 (C=O stretching of $CONH_2$), 1626 (C=O stretching of cycles) 1596 (N-H deformation of pyrimidine ring), 1571 (C=C stretching of phenyl ring), 1513 (C-NO_2 asymmetrical deformation of Nitro group), 1457 (C-H asymmetrical deformation of methyl group), 1425 (C-H symmetrical deformation of methyl group), 1331 (C-NO_2 symmetrical deformation of Nitro group), 1283 (C-N stretching), 1267 (C-O-C asymmetrical stretching), 1159, 1073 (C-H in plane deformation of aromatic ring), 1007 (C-F stretching) 828 (para-substituted); MS: *m/z* 617; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.86-7.88 (dd', 3H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(3-bromophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4g)

Yield: 60%; mp 178ºC; elemental analysis calculator for $C_{25}H_{15}BrF_6N_4O_4S$: C, 45.40; H, 2.29; Br, 12.08; F, 17.24; N, 8.47; O, 9.68; S, 4.85; Found: C, 45.45; H, 2.28; Br, 12.02; F, 17.28; N, 8.45; O, 9.60; S, 4.83%; IR (cm^{-1}): 3995 (N-H stretching of $CONH_2$), 3140 (C-H stretching of phenyl ring), 3007 (C-H asymmetrical stretching of methyl group), 2823 (C-H symmetrical stretching of methyl group), 1678 (C=O stretching of $CONH_2$), 1623 (C=O stretching of cycles) 1596 (N-H deformation of pyrimidine ring), 1573 (C=C stretching of phenyl ring), 1513 (C-NO_2 asymmetrical deformation of Nitro group), 1455 (C-H asymmetrical deformation of methyl group), 1427 (C-H symmetrical deformation of methyl group), 1334 (C-NO_2 symmetrical deformation of Nitro group), 1270 (C-N stretching), 1275 (C-O-C asymmetrical stretching), 1149, 1079 (C-H in plane deformation of aromatic ring), 1010 (C-F stretching) 825 (para-substituted); MS: *m/z* 661; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.86-7.88 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(3-fluorophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4h)

Yield: 59%; mp 170ºC; elemental analysis calculator for $C_{25}H_{15}F_6N_4O_4S$: C, 50.01; H, 2.52; F, 22.15; N, 9.33; O, 10.66; S, 5.34; Found: C, 50.03; H, 2.50; F, 22.10; N, 9.32; O, 10.73; S, 5.33 %; IR (cm^{-1}): 3295 (N-H stretching of $CONH_2$), 3149 (C-H stretching of phenyl ring), 3002 (C-H asymmetrical stretching of methyl group), 2830 (C-H symmetrical stretching of methyl group), 1676 (C=O stretching of $CONH_2$), 1623 (C=O stretching of cycles) 1594 (N-H deformation of pyrimidine ring), 1570 (C=C stretching of phenyl ring), 1517 (C-NO_2 asymmetrical deformation of Nitro group), 1458 (C-H asymmetrical deformation of methyl group), 1415 (C-H symmetrical deformation of methyl group), 1320 (C-NO_2 symmetrical deformation of Nitro group), 1287 (C-N stretching), 1260 (C-O-C asymmetrical stretching), 1159, 1074 (C-H in plane deformation of aromatic ring), 1002 (C-F stretching) 822 (para-substituted); MS: *m/z* 600; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.86-7.88 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(2-chlorophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4i)

Yield: 64%; mp 187ºC; elemental analysis calculator For $C_{25}H_{15}ClF_6N_4O_4S$: C, 50.01; H, 2.52; F, 22.15; N, 9.33; O, 10.66; S, 5.34; Found: C, 50.00; H, 2.55; F, 22.10; N, 9.30; O, 10.75; S, 5.33 %; IR (cm^{-1}): 3403 (N-H stretching of $CONH_2$), 3144 (C-H stretching of phenyl ring), 3005 (C-H asymmetrical stretching of methyl group), 2822 (C-H symmetrical stretching of methyl group), 1674 (C=O stretching of $CONH_2$), 1620 (C=O stretching of cycles) 1590 (N-H deformation of pyrimidine ring), 1576 (C=C stretching of phenyl ring), 1518 (C-NO_2 asymmetrical deformation of Nitro group), 1458 (C-H asymmetrical deformation of methyl group), 1429 (C-H symmetrical deformation of methyl group), 1329 (C-NO_2 symmetrical deformation of Nitro group), 1286 (C-N stretching), 12658 (C-O-C asymmetrical stretching), 1153, 1072 (C-H in plane deformation of aromatic ring), 1003 (C-F stretching) 828 (para-substituted); MS: *m/z* 617; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.86-7.88 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

4-(4-(4-(trifluoromethyl)-2-nitrophenoxy)phenyl)-N-(2-fluorophenyl)-1,2,3,4-tetrahydro-6- trifluoromethyl -2-thioxopyrimidine-5-carboxamide (4j)

Yield: 54%; mp 191ºC; elemental analysis calculator For $C_{25}H_{18}F_4N_4O_4S$: C, 50.01; H, 2.52; F, 22.15; N, 9.33; O, 10.66; S, 5.34; Found: C, 50.03; H, 2.50; F, 22.18; N, 9.25; O, 10.78; S, 5.8 %; IR (cm^{-1}): 3410 (N-H stretching of $CONH_2$), 3148 (C-H stretching of phenyl ring), 3009 (C-H asymmetrical stretching of methyl group), 2826 (C-H symmetrical stretching of methyl group), 1676 (C=O stretching of $CONH_2$), 1627 (C=O stretching of cycles) 1594 (N-H deformation of pyrimidine ring), 1560 (C=C stretching of phenyl ring), 1515 (C-NO_2 asymmetrical deformation of Nitro group), 1458 (C-H asymmetrical deformation of methyl group), 1424 (C-H symmetrical deformation of methyl group), 1335 (C-NO_2 symmetrical deformation of Nitro group), 1283 (C-N stretching), 1264 (C-O-C asymmetrical stretching), 1155, 1070 (C-H in plane deformation of aromatic ring), 1005 (C-F stretching) 825 (para-substituted); MS: *m/z* 600; ^{1}H NMR (DMSO-*d6*) δ ppm: 5.59 (s, 1H, H_b), 7.43-7.44 (dd', 2H, $H_{cc'}$), 7.59 (dd', 2H, $H_{dd'}$), 7.63-7.65 (s, 1H, H_e), 7.86-7.88 (dd', 4H, $H_{ff'gg'}$), 8.04-8.06 (d, 1H, H_h), 8.22 (d, 1H, H_i), 9.22 -9.31 (s, 2H, Hj_k), 9.63 (s, 1H, H_l).

3. BIOLOGICAL EVALUATION:

Antimicrobial evaluation

Total of the Prepared compounds were experienced for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method with two Gram-positive bacteria **Staphylococcus aureus** MTCC-96, **Streptococcus pyogenes** MTCC 443, two Gram-negative bacteria **Escherichia coli** MTCC 442, **Pseudomonas aeruginosa** MTCC 441 and three fungal strains **Candida albicans** MTCC 227, **Aspergillus Niger** MTCC 282, **Aspergillus clavatus** MTCC 1323 taking **gentamycin, chloramphenicol, norfloxacin, nystatin and greseofulvin** as regular drugs.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, specified as the humble concentration of the compound preventing the observable growth, were determined by using micro dilution broth method according to NCCLS(National Committee for Clinical Laboratory Standards) standards.

Minimal Inhibition Concentration [MIC]:-

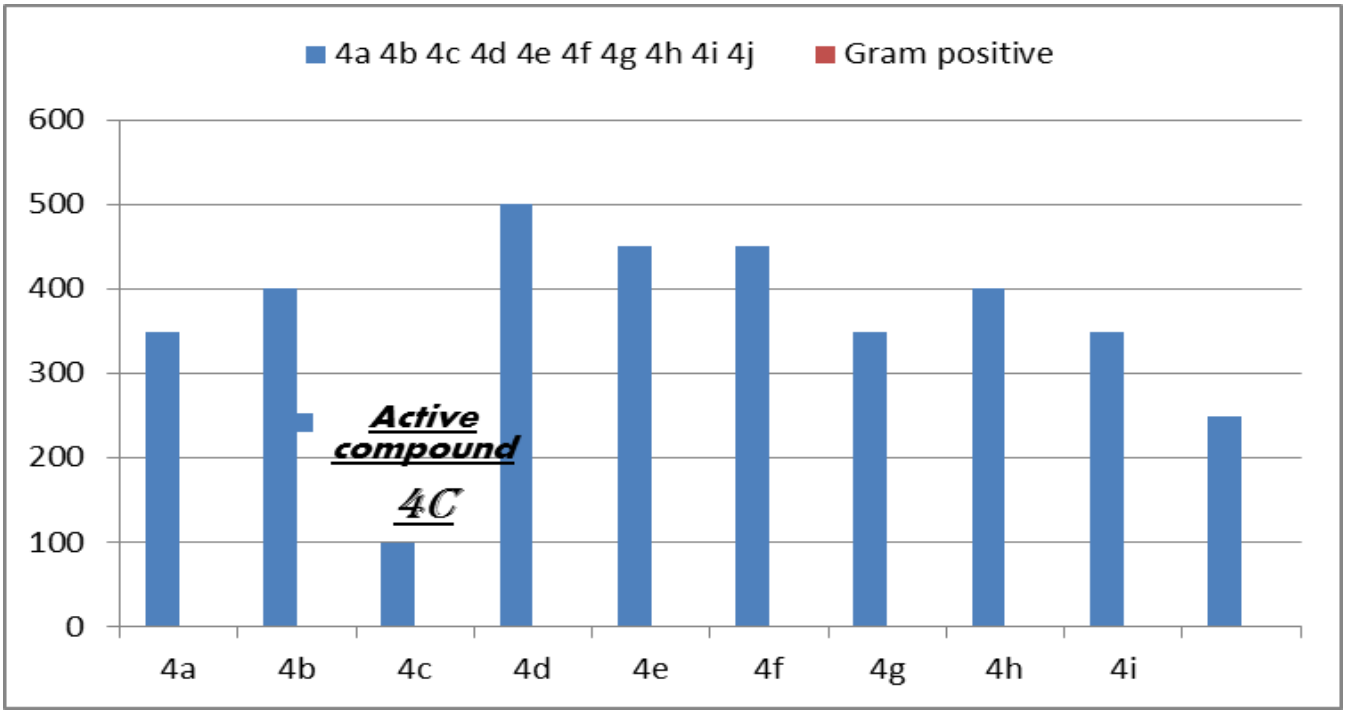
The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well.

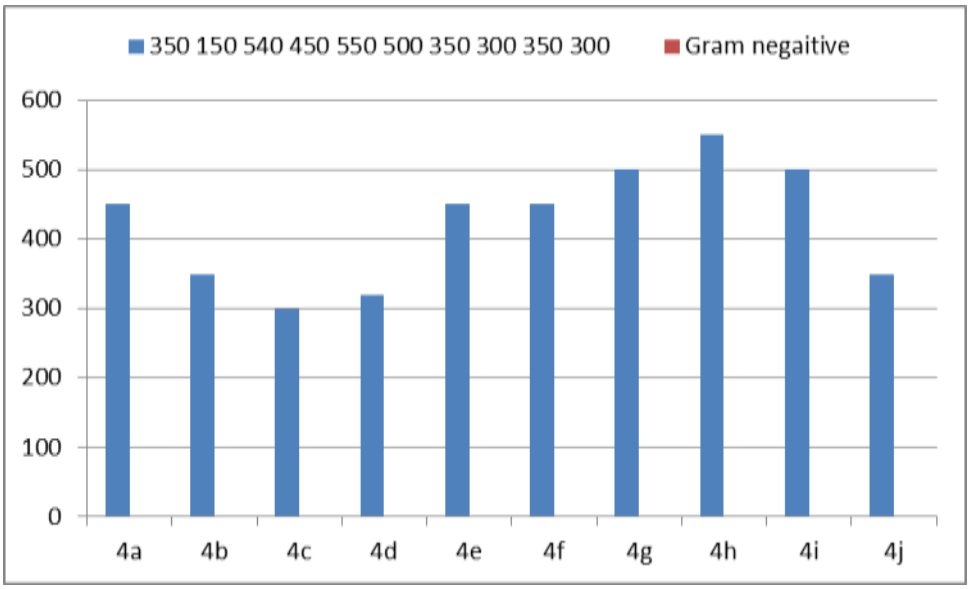
- Serial dilutions were prepared in primary and secondary screening..

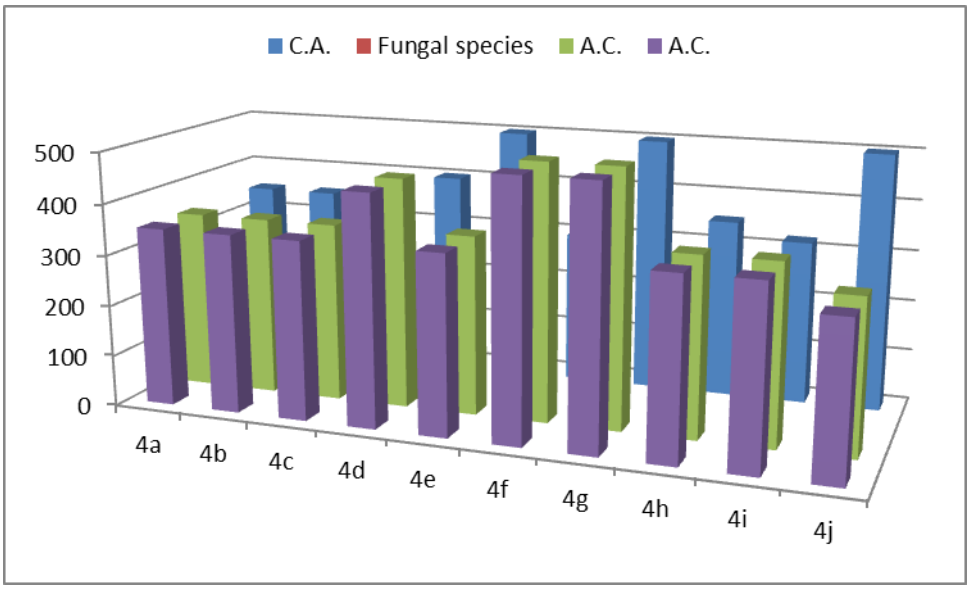
- The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 35 ^{0}C overnight.
- The MIC of the control organism is read to check the accuracy of the drug concentrations.
- The lowest concentration inhibiting growth of the organism is recorded as the MIC.
- The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

Table-1:- ***in vitro Antimicrobial Screening Results for*** **(4a-j)**

Code	Minimal inhibition concentration (µg mL^{-1})						
	Gram-positive		Gram-negative		Fungal species		
	S.a.	*S. p.*	*E.c.*	*P.a.*	*C. a.*	*A. n.*	*A.c.*
4a	350	550	350	450	350	550	350
4b	400	500	150	350	350	400	350
4c	100	350	540	300	350	320	350
4d	500	300	450	320	400	400	450
4e	450	450	550	450	500	450	350
4f	450	400	500	450	300	450	500
4g	350	450	350	500	500	500	500
4h	400	350	300	550	350	450	350
4i	350	500	350	500	320	450	350
4j	250	400	300	350	500	120	300
Ampicillin	250	100	100	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	450	100	100







4. CONCLUSION:

In construct, we acquire in make of imaginative pyrimidine derivatives using devoid of any plight and appropriate process. By method produces these products in high-quality yield and trouble-free work on. Product is isolated by effortless filtration. The isolated products are much uncontaminated and do not require any another purification. Here few compound active but normal activity not high active compound.

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National Conference on
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Potential use of Aloe Vera soil microflora (Actinomycetes) for the development of antimicrobial compound

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Abstract: *Amongst various major group of microorganisms found in soil Actinomycetes are most important due to ability of producing antibiotics from them. Microorganisms became resistance against current available antibiotics hence we need to look into novel antimicrobial compound which have capability to kill various pathogens. Present research we explore new ecological region near Aloe Vera plant soil. Three districts Aloe Vera soil selected from saurashtra Gujarat. Treated with serial dilution and make diluted aliquots for scattered colony growth of microbes. Various media used for Actinomycete growth. Morphological and microscopical methods were used for mycelium study. Agar well diffusion method used for check antimicrobial activity along with different biochemical tests of isolates. From Gir Somnath observed three types of isolates GAV1, GAV2 and GAV3, from Rajkot observed three types of isolates RAV1, RAV2 and RAV3 by using Junagadh observed three types of isolates JAV1, JAV2 and JAV3. 66.67 % of isolates arial mycelium white color and in vegetative mycelium 55.55 % yellow color was observed. 8 isolates showed antimicrobial activity against 8 different pathogens except JAV3. RAV2 and JAV3 showed > 20 mm ZOI (Zone of Inhibition) against Staphylococcus aureus. < 5 mm or in some isolates no ZOI were observed against Pseudomonas aeruginosa, Salmonella typhi and Enterobacter aerogenes. Against Bacillus subtilis 78 % isolates, against Staphylococcus aureus, Proteus vulgaris and Escherichia coli 67 % isolates & against Klebsiella aerogenes 56 % isolates showed activity. Actinomycetes of soil near Aloe Vera have potential to develop antimicrobial compound.*

Key Words: *Actinomycetes; Ecological; Mycelium; Isolates; Antimicrobial.*

1. INTRODUCTION:

Biodiversity in both ways above and below the ground depends on soil. Biodiversity wealth in under the surface of soil is due to millions of small living microorganisms and that are the base of soil life and plays important role in ecosystem of earth.(1) Microflora is vital part of soil composition, consist various living or dead microbial cells with their residues. Organic matter quantity as well as quality depends on nature and kind of microflora. Bacteria, fungi, algae and actinomycetes are major groups in soil microflora. There are thousands of species and genera of microbes available in this part of soil. (2) Most useful group is small living organism bacteria in microflora. More than 300 millions of actinomycetes occur in one gram of soil part makes second most useful microbes in soil part. Heterogeneous group of microbes present in microflora that is fungal, algae found in all soil have cultivated area with millions of numbers in per gram of soil. Biological fertility of soil depends on microorganisms in microflora part of soil.(3) There are many ecological area still not studied in respect to their microflora part for healthcare purpose. Microbes in soil also plays important role in nutrient cycle of planet. There are many research aspects are available on the soil microflora in terms of development of novel medicinal important compounds, various metabolites, enzymes, in agricultural and many more. Microorganism plays important role in decomposition and breakdown of organic matter, recycle the nutriens of soil, make humus and soil structure, nitrogen fixation, plant growth promotion, control pests and various disease.(4)

There are many microbes are developing resistant against available antibiotics in market and due to these available antibiotics became less effective with those resistant microbes. So there are need to develop new antibiotics against resistant microbes are very necessary in regarding health of patients. Also in present study we select soil from near Aloe Vera plant to correlate activity of soil actinomycetes.(5) Many times "Wonder plant" term was used for Aloe Vera plant due to its use in various ways. Ingredients in Aloe Vera plant have activity and power for soothe life of human and health myriad. It contain antibacterial, antiseptic, anti ageing, use in lymphatic and circulation of blood, against various liver problems, in cancer, in diabetes etc to make human life protected against various un healthy conditions.(6) In recent years many research are made on this plant with different aspects with their therapeutic properties but soil near this plant area and diversified area are not much explored till today.

2. LITERATURE REVIEW:

According to R. A. Mothana and V. Linclequist (2005) medicinal plants found in all over the world 20 % of them submitted for biological and pharmacological tests, and many antibiotics introduced in market obtained from naturally available sources. A. O. Azaghani, I. Williams, D. B. Holiday and A. R. Johnson (1995) stats that gel of Aloe Vera was antibacterial activity against *Pseudomonas aeruginosa*. Growth of candida albicans fungus was inhibited by aloe Vera gel was reported by L. M. Cera, J. P. Heggers, M C. Robson and W. J. Hagstorm (1980). O. O. Agarry, M. T. Olaleye and C. O. Bello Michael (2005) studied comparation of antimicrobial activity of Aloe Vera plant. Sudha Sri Kesavan S et al (2015) reported some antimicrobial activity from soil of Sathyabama University, chhenai with its isolation and characterization of some isolates. There are many methods for pretreatment of soil part and actinomycetes study reported by various researchers Solingen VP et al (2001), Kim CM et al (1994). Talbot GH et al (2006) reported antimicrobial availability and task force of diseases society of America; drugs need by bad bugs an update. Oskay M et al (2004) reported some antimicrobial activity of isolates from various farming soil of turkey.

3. STUDY OBJECTIVES:

Objective of present work is finding various sites near Aloe Vera plant, isolation of microflora of soil with actinomycetes microbe's colonies, and screened by using three regions Rajkot, Junagadh, Gir somnath. Study antimicrobial activity of those isolates from different ecological sites. Characterization of isolates produced from those sites of soil nearby Aloe Vera plant. To study many parameters like morphological, microscopically, TLC analysis, bioautography and chemical tests of those actinomycetes isolate.

4. MATERIALS AND METHOD:

4.1 Sampling sites

Gir somnath, Rajkot and Junagadh district of Gujarat selected. Different places like herbal garden of RK university, girnar forest and nursery in gir somnath selected randomly.

4.2 Treatment of sample

Near aloe Vera plant soil from three different places collected. Use sterile articles to store soil samples in sterile glass tubes. 25 cm depth of microflora part of soil selected within 1 to 2 ft area surrounding plant. Air dried samples and make serial dilution of samples up to 10^{-5} for colonies formation different media like starch casein agar, nutrient agar and actinomycetes agar used for growth of microbes. Antifungal agent nystatin 50 µg/ml added to prevent growth of fungus. Use 0.1 ml of diluents sample and spread on media and incubated for 7 to 8 days at 37°C in BOD incubator. (7)

4.3 Colony study

Colonies were observed with morphological and microscopical methods. Identify actinomycetes then make pure colony in same media and preserved in 20 % glycerol in media. (8)

4.4 Antimicrobial activity

Primary screening was done by cross streak method by various test pathogens. In MHA (Muller Hinton Agar) media and incubated at 37°C for 2 to 3 days. Fermentation method used for those isolates showed activity in primary screening. Crude extract was prepared from them and used in further screening method. Secondary screening of antimicrobial activity was done by agar well diffusion method.(9) 8 mm diameter well prepared and using test pathogens measure Zone of Inhibition in mm surrounding crude extract obtained from isolates.

4.5 MIC (Minimum inhibitory concentration)

MIC was calculated by assay of isolate crude extract in microplates against defined concentration of samples by using various test pathogens. 3 mg of extract added in bore in media with broth dilution methods.(10)

4.6 TLC (Thin layer Chromatography) & Bioautography

Ethyl acetate was used for isolation of crude extract and prepared TLC slides and measure spot of active compound under UV light and RF value was obtained. Ready prepared silica gel slides TLC 65F 256 used in this study. Bioautography performed of crude extract with TLC slides with *Staphylococcus Aureus* pathogens in agar media. [11-13] Incubation conditions at 37°C and observed growth of bacteria.

Various biochemical tests were performed for characterization of isolates. [14-16]

4.7 Chemicals and Reagents

All cultural media, chemicals, reagents, diagnostic kit used from himedia laboratories, Mumbai, India.

5. RESULTS AND DISCUSSION:

5.1 Actinomycetes Isolates: From three different places samples total 9 actinomycetes isolates were obtained as mentioned in table 1 with characters of colonies, color of arial mycelium and vegetative mycelium.

Table 1: Isolates of Actinomycetes from *Aloe Vera* soil samples

Sr. No.	District	Colony			Code	No. of isolated samples
		Characters	Aerial mycelium color	Vegitative mycelium color		
1	Gir Somnath	Rough	White	Yellow	GAI1	1
		Leathery	Cream	Yellow	GAI2	2
		Powdery	White	Brown	GAI3	3
2	Rajkot	Rough	White	Yellow	RAI1	4
		Powdery	White	Cream	RAI2	5
		Rough	Yellow	Brown	RAI3	6
3	Junagadh	Rough	White	Yellow	JAI1	7
		Powdery	White	Yellow	JAI2	8
		Wrinkled	Yellow	Gray	JAI3	9
Total						**9**

5.2 Microscopy: By using electron microscopy as shown in figure 1 total 56 % isolates belongs to streptomyces, 22 % belongs to micromonospora, 11 % each belongs to intrasporangium and saccharopolyspora genus.

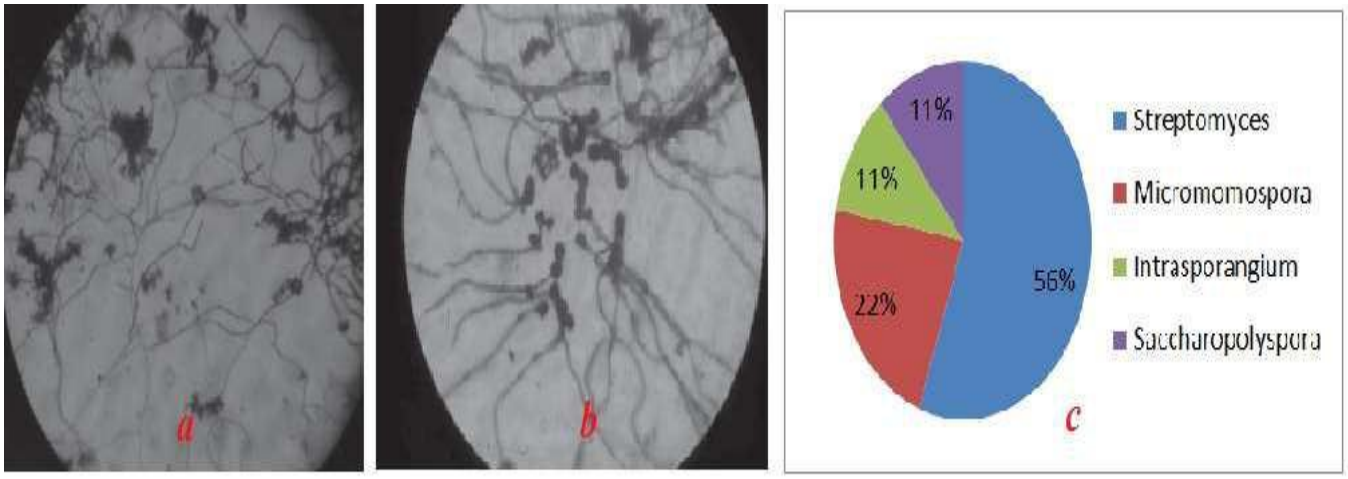

Figure 1: Electron Microscopy *1000 (a) streptomyces genus, (b) micromonospora genus and (c) percentage distribution of isolates based on genus.

5.3 Antimicrobial activity:

By using cross streak method it was found that except JAV3 all isolates showed some antimicrobial activity against test pathogens (Bs, Sa, Pv, Ec, Ka, Pa, St, Et). Remaining 8 isolates were studied in secondary screening method. Secondary screening data by agar well diffusion method was shown in table 2 and figure 2.

Table 2: Distribution of antimicrobial activity by agar well diffusion method

Isolates		Test Pathogens							
		Bs	***Sa***	***Pv***	***Ec***	***Ka***	***Pa***	***St***	***Et***
GAV1	Mean	18.33	20.33	14.67	17.33	16.33	5.33	0	0
	±SD	0.58	0.58	1.15	0.58	0.58	1.15	0	0
GAV2	Mean	17.00	18.00	13.33	17.33	17.33	0	0	4.33
	±SD	0.00	1.73	1.00	1.08	0.53	0	0	1.15
GAV3	Mean	0.00	7.33	0	7.00	0	5.00	0	0
	±SD	0.00	1.53	0	1.00	0	1.00	0	0
RAV1	Mean	4.33	0	3.33	0	0	0	4.33	0
	±SD	0.58	0	1.53	0	0	0	0.57	0
RAV2	Mean	18.33	21.33	17.67	17.33	16.00	0	0	0
	±SD	1.53	2.03	0.58	0.58	1.00	0	0	0
RAV3	Mean	11.33	0	0	0	0	0	0	2.67
	±SD	1.15	0	0	0	0	0	0	0.58
JAV1	Mean	20.00	20.33	18.67	18.33	14.33	0	4.00	0
	±SD	2.65	1.15	1.15	0.57	0.58	0	1.00	0
JAV2	Mean	18.00	21.00	17.33	17.33	17.33	0	0	0
	±SD	1.00	1.00	1.15	1.53	1.15	0	0	0
JAV3	Mean	0	0	0	0	0	0	0	0
	±SD	0	0	0	0	0	0	0	0

Data of three replicates

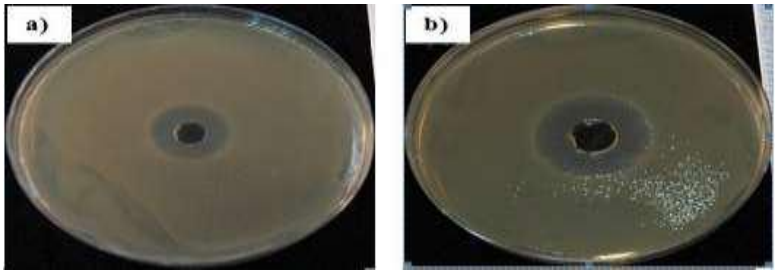

Figure 2: (a) GAV1 showed Zone of inhibition against Bs (b) GAV2 showed Zone of Inhibition against Sa

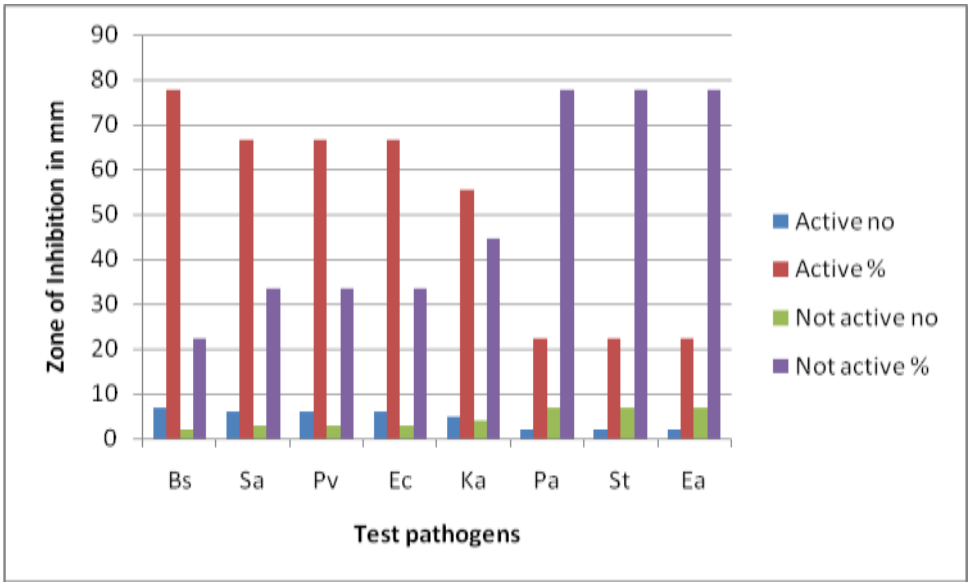

Figure 3: Distribution of isolates in number and percentages for antimicrobial activity against test pathogens

It was found that GAV1, GAV2, GAV3, RAV1, RAV2, JAV1, JAV2 and JAV3 showed activity against 6, 6, 4, 3, 5, 6, 5 and 0 number of test pathogens respectively. It was also found that 7 (78 %) isolates showed activity against Bs, 6 (67 %) isolates showed activity against Sa, Pv and Ec, 5 (56 %) isolates showed activity against Ka, 2 (22 %) isolates showed activity against Pa, St and Ea test pathogens.

Table 3: Distribution of antimicrobial activity with different range of zone of inhibition

Zone of Inhibition in mm		Test Pathogens							
		Bs	Sa	Pv	Ea	Ka	Pa	St	Ea
No active	no	2	3	3	3	4	7	7	7
	%	22.22	33.33	33.33	33.33	44.44	77.78	77.78	77.78
>20	no	0	2	0	0	0	0	0	0
	%	0.00	22.22	0.00	0.00	0.00	0.00	0.00	0.00
15 to 20	no	5	3	4	5	4	0	0	0
	%	55.56	33.33	44.44	55.56	44.44	0.00	0.00	0.00
10 to 15	no	1	0	1	0	1	0	0	0
	%	11.11	0.00	11.11	0.00	11.11	0.00	0.00	0.00
<10	no	1	1	1	1	0	2	2	2
	%	11.11	11.11	11.11	11.11	0.00	22.22	22.22	22.22

It was found that 2 isolates showed more than 20 mm ZOI against Sa. 5 isolates between 15 to 20 mm ZOI ranges against Bs 5 isolates, against Sa 3 isolates, against Pv 4 isolates, against Ea 5 isolates, against Ka 4 isolates showed activity. Between 10 to 15 mm ZOI range against Ba, Pv and Ka 1 isolate showed their activity. Less than 10 mm ZOI against Bs, Sa, Pv and Ea 1 isolates showed their activity, against Pa, St and Ea 2 isolates showed antimicrobial activity.

By TLC study of ethyl acetate extract of isolates range of Rf factor was between 0.51 to 0.67 and single spot were observed under UV light. And same TLC silica slide was used for autobiography to further conform antimicrobial activity of isolates against two test pathogens Sa and Bs. It was observed that clear zone of inhibition in agar plates in which TLC slide was incubated.

Below table represents finding of biochemical parameters of various actinomycetes isolates.

Table 4: Different biochemical tests results of isolates

Gram's staining								
Isolates	GAV1	GAV2	GAV3	RAV1	RAV2	RAV3	JAV1	JAV2
Result	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Negative
Growth on media								
Growth on media	GAV1	GAV2	GAV3	RAV1	RAV2	RAV3	JAV1	JAV2
ISP2	Good	Moderate	Good	Good	Moderate	Good	Moderate	Good
ISP3	Good	Good	Good	Moderate	Good	Good	Good	Moderate
ISP4	Poor	Poor	Moderate	Poor	Moderate	Poor	Poor	Poor
ISP5	Moderate	Good	Good	Good	Moderate	Good	Moderate	Moderate
AIA	Good	Good	Good	Moderate	Good	Good	Good	Moderate
ISP7	Moderate	Good	Moderate	Moderate	Moderate	Good	Moderate	Moderate
Ability of isolates to utilize sugars								
Sugars	GAV1	GAV2	GAV3	RAV1	RAV2	RAV3	JAV1	JAV2
D-glucose	+++	+++	++	+++	++	+++	++	+++
sucrose	+++	+++	+++	++	+++	+++	++	+++
Lactose	+	--	++	+	++	++	++	+
Dextrose	+++	++	+++	++	+++	++	+++	+++
D-Mannitol	++	+	--	++	--	+	--	--
D-xylose	--	+	++	--	--	+	++	--
Raffinose	--	--	--	--	--	--	--	--
L-Rhamnose	+	--	+	--	+	--	+	--
L-Arabinose	+	+	++	+	+	++	+	+
Maltose	+++	++	++	++	++	++	--	++
+++ = Very good growth, ++ = moderate growth, + = Poor growth, -- = no growth								
	GAV1	GAV2	GAV3	RAV1	RAV2	RAV3	JAV1	JAV2
Indol	--	--	--	--	+	--	--	+
Methyl red	+	+	+	+	+	+	--	--
Voges–	--	--	--	--	--	--	--	--

Proskauer								
Citrate	--	+	+	+	--	--	--	--
Hydrogen Sulphide Production	+	+	+	+	--	+	+	+
+ = positive, -- = Negative								
Enzymatic Activity								
Protease	+	+	+	+	+	+	+	+
Gelatinase	+	+	+	--	+	--	+	+
Amylase	+	+	+	+	--	--	+	+
Lecithinase	--	--	+	+	+	+	--	+
Cellulose	--	--	+	+	+	--	--	--
Urease	--	--	--	--	--	--	--	--
+ = positive, -- = Negative								

6. CONCLUTION :

Present study concluded that from microflora part of soil near Aloe Vera plant area rich source of actinomycetes with various specied and genus of organisms. Out of 9 actinomycetes isolates from different locations seven isolates GAV1, GAV2, GAV3, RAV1, RAV2, RAV3, JAV1 and JAV2 obtained from gir somnath, Rajkot and Junagadh site of Gujarat were contain antimicrobial activity against different pathogens amongst them some have developed resistance against available antibiotics. By further purification and detailed analysis of those active isolates we might get industrially important compound and other metabolites also. Soil actinomycetes near “wonder plant” Aloe vera have capability of producing potent antimicrobial compound from actinomycetes of that region in microflora part. Compounds can use in future to cure disease condition and protect society against some resistant producing microorganism.

7. ABBREVIATIONS:

EC: *Escherichia Coli*, ZOI: Zone of Inhibition, *SA*: *Staphylococcus Aureus*, *Bs*: *Bacillus Subtilis*, *KA*: *Klebsiella Aerogenes*, *PV*: *Proteus Vulgaris*, *ST*: *Salmonella Typhi, ET*: *Enterobacter Aerogenes, PA*: *Pseudomonas Aeruginosa*, AIA: Actinomycetes isolates agar

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National Conference on
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Li-Fi Technology: Transmission of Data through Light

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Abstract: *Considering recent scenario, the necessity and utilization of the internet is growing exponentially because of the digitization offered by the technologies currently in use such as Internet of Things (IoT), Smart phones, Cloud computing, etc. Light Emitting Diodes (LED) are used in different areas of routine life. The advantage of using this device is that in addition to their lightening capabilities, it can be used for data transmissions as well.This paper introduces the concept of Li-Fi technology, where Li-Fi stands for Light-Fidelity, which is wireless technology to provide connectivity within high dense area where there are no obstacles. The technology is evolving recently and was proposed by the German physicist Harald Haas in 2011. Li-Fi facilitates transmission of data through illuminati-on by sending data through an LED light bulb that varies in intensity faster than human eye can follow[2]. Li-Fi (Light Fidelity) refers to 5G Visible Light Communication systems which uses light-emitting diodes as the medium to transmit data.This paper primarily focuses on what is actually Li-Fi, how it works, applications, comparisons with existing technologies Limitations and future enhancements.*

Key Words: *Light Fidelity, Transmission, Wi-Fi, LED, VLC, Li-F, LED.*

1. INTRODUCTION:

The term Li-Fi stands for Light-Fidelity. Li-Fi is an emerging technology in the domain of the internet where light will be used as medium to transport data. Now-a-days, internet has become a major utility like water and electricity where individuals are in search of Wi-Fi hot-spots in public places like hotels, hostels, café, restaurants, tea post etc. Keeping into mind today's scenario, as the number of individuals increases in wireless network, its speed decreases and as a consequences capacity goes down. Technologies are evolved so faster which may raise the questions will there be an enough bandwidth for all the systems or devices. Wireless radio frequencies are getting higher, as a result of which RF interferences increases continuously. As the no of devices increases, then what will be the network traffic condition? All these limitations can be tackled by this upcoming technology called Li-Fi. Although Wi-Fi provides us speed, it is still insufficient to accommodate no of desired users. To overcome the limitation of Wi-Fi, Concept of Li-Fi Technology was introduced. Li-Fi is high speed bi-directional network and mobile communication of data using light which uses LED and VLC.

The technology is just an emerging technology which was proposed by the German physicist Harald Haas in 2011, at the University of Edinburgh. Li-Fi provides transmission of data and information through illumination by sending information through an LED light bulb that varies in intensity faster than human eye can follow [3]. Li-Fi is a wireless light based networking technology that uses light emitting diodes (LEDs) for transmission of data and information. Li-Fi utilizes visible light communication (VLC) technology, as medium to deliver high-speed communication in a manner similar to Wi-Fi.

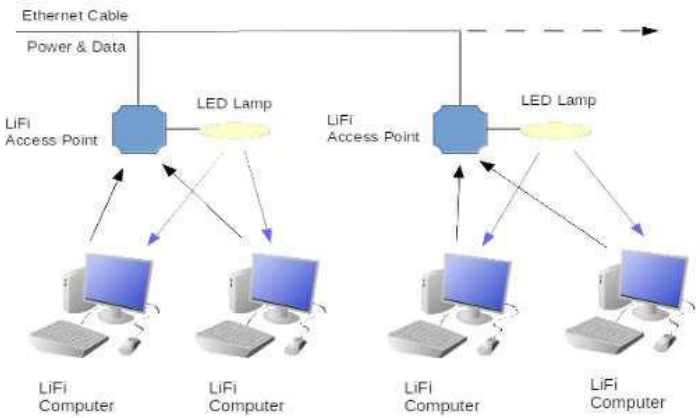

Figure 1 Li-Fi Wireless Access Point Could Work Using Room Lighting

Li-Fi provides improved and better bandwidth, efficiency, availability and security than Wi-Fi. Li-Fi can be regarded as light-based Wi-Fi, i.e. instead of radio and infrared waves it uses light to transmit data [1]. By utilizing the low-cost nature of LEDs and Lighting bulbs, there are lots of opportunities to exploit this medium.

2. Visible Light Communication Usage

Alternative of radio waves is visible light spectrum. Gama rays can't be used as they could be dangerous. X-rays have similar health issues. Ultraviolet light is good for place without people, but otherwise dangerous for the human body. Infrared, due to eye safety regulation, can only be used with low power. Hence we are left with the only visible-light spectrum [9].

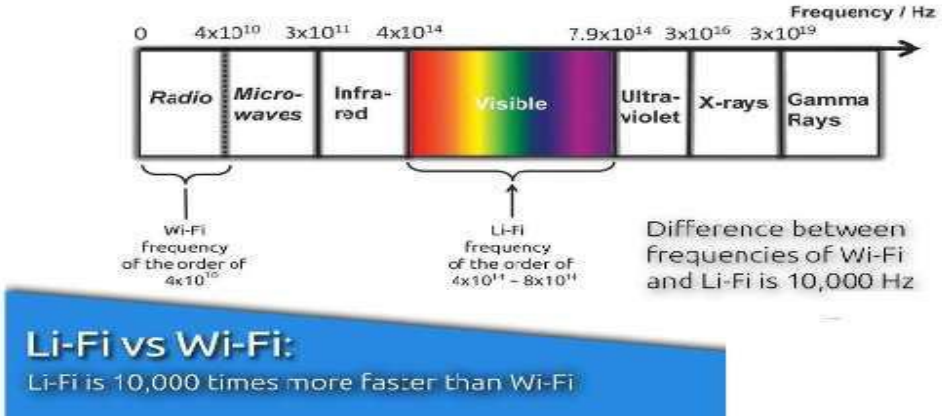

Figure 2: The Electromagnetic Spectrum of Li-Fi and Wi-Fi.

Frank Deicke, who leads Li-Fi development at Fraunhofer Institute for Photonic Microsystems in Dresden, Germany, has said that Li-Fi can achieve the same data rates as USB cables which is challenging for wireless technologies such as Bluetooth and Wi-Fi. He also cites another advantage of Li-Fi being that the latency of Li-Fi is in the order of microseconds where as that of Wi-Fi is in the order of milliseconds [2].

Figure 3: Data transmission from source to destination by Visible Light Communication.

Li-Fi utilizes a Visible Light Communications (VLC) System for data transmission, which basically works on the principle of rapid pulses of light to transmit information wirelessly. Simplifying this VLC System must have two primary components: (1) At least one device with a photo-diode capable of receiving light signals and (2) Signal processing unit which contains light source.

A VLC light source may contains a fluorescent or light emitting diode (LED) bulb. For implementing Li-Fi, LED bulbs are most suitable and ideal, because any reliable and robust Li-Fi system requires extremely high rates of light output.

3. Design of Li-Fi Technology

Li-Fi architecture contains couple of LED bulbs or lamps which includes several wireless devices such as smart phones, Laptops and PDA. The following criteria should be taken into account while designing Li-Fi technology:

- Presence of light.
- Controller that cods data into those LEDs.

Figure 4: The main component of Li-Fi system: LEDs, Photodiode and Image sensor.

- Use fluorescent light and LEDs, for better and improved performance to transmit data.
- A photo detector to receive data, which can be an image sensor.

4. Implementation of Li-Fi

The Li-Fi Works on simple but efficient principle. Data and Information to be transmitted is fed into an LED light bulb with signal processing technology. It then sends data embedded in its beam at rapid speeds to the photo-detector. When LED light bulb receives constant current source, stream of photons is constantly emitted from the bulb which is observed as illumination. When current alters slowly, the bulb dims up and down.

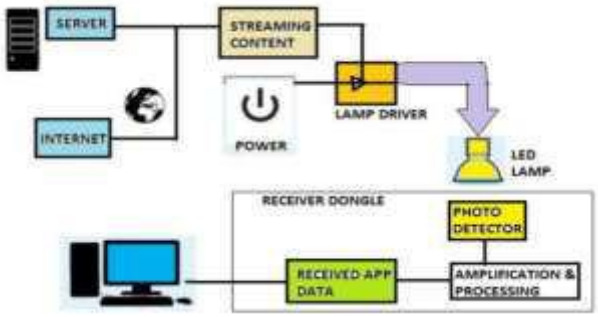

Figure 5: Architecture of Li-Fi System

LED bulbs are type of semiconductor devices, which simply means the current and optical output can be modulated at significantly high speeds which can be recognized by the photo-detector device and it can be transformed back to electrical current signal. The electrical signal is then converted back into streams of binary data that could be identified as graphics, web applications, video and audio applications that run on devices which posses internet facility. Using this technique high-speed information can be transmitted from an LED light bulb.

Therefore, LED cells can modulate thousands of signals and can be switched on and off faster than the human eye can detect since LEDs operate at speed of less than 1 μs, thereby causing the light source to emerge continuously on. This invisible on-off activity enables data transmission using binary codes. Switching on an LED is binary '1', switching it off is binary '0'[7].

Figure: 6 visible light wireless data transmission

In turn, the alterations in light intensity from the LED light source are interpreted and converted as electrical signal by the receiving photodiode device. Once the electronic signal is received, it is extracted from the carrier wave and then, it is converted into a continuous stream of binary data constituting of graphics, audio, video, web, and application information to be absorbed by any Internet-enabled device.

5. Key Issues of Wi-Fi which are addressed by Li-Fi:

1) Capacity: Wi-Fi uses radio waves to transmit data which are expensive and limited. It has insufficient spectrum for increasing data, when number of user's increases. Li-Fi addresses issue by using the visible light spectrum which is 10,000 times wider than the spectrum of radio waves. In addition, the light sources are already installed. Hence Li-Fi has greater bandwidth and equipment which is already available.
2) Efficiency: - Large number of base stations consume huge amount of energy for transmitting the radio waves and to cool the base station cabins. In fact, the efficiency of such stations is only 5%. Li-Fi tackles this issue by using LED lights which consume less energy and are highly efficient.
3) Availability: Wi-Fi is available within the range of Base stations which is limited and it is unavailable in all environments, particularly in airplanes, chemical and power plants and in hospitals. Li-Fi addresses this issue by using light sources which are available in every corner of the universe. The billions of light bulbs available in worldwide needs to be replaced by LEDs.
4) Security: Radio waves can penetrate through walls. This leads to many security concerns as they can be easily intercepted.
Li-Fi uses light which doesn't penetrate through walls and thus data transmission using light waves is more secure.

6. Limitations of Li-Fi:

1) Light Source Reliance*:* - Without Light sources, internet could not be accessed, which would ultimately limit the locations and situations in which Li-Fi technology can be utilized.
2) Limited Range: - As it utilizes visible light, light cannot penetrate through walls, so physical barriers limit the signal range.
3) Interference: - Other light sources may cause interference with the signal. One of the major drawbacks is the interception of signal outdoors. Sunlight can cause interfering in the signal, resulting in the interrupted internet.

7. Applications of Li-Fi

1) Medical Applications: - Li-Fi Technology can be used to control medical equipments, which can be suitable for conducting robotic surgeries and procedures.
2) Underwater Explorations and Communications: - Submarines could use their headlamps to communicate with each other, operate data autonomously.
3) Airlines: - Since airlines contain multiple lights, so Li-Fi can be used for data transmission.
4) Traffic Management :- In traffic signals Li-Fi technology can be utilized to communicate with passing vehicles through the LED lights of the cars, other vehicles etc which can help in supervising the traffic in a better method resulting into smooth flow of traffic , which in turn helps in reducing the accident numbers.
***5) Mobile Connectivity**: - Laptops, tablets, smart phones and several other devices can interconnect with each other using Li-Fi. Usage of short range links results in providing high data rates as well as it increases security.
6) Power plants and Hazardous Environments - Li-Fi technology can be used in petrochemical and mines plants, to provide safety communication as it does not cause electromagnetic interference. Li-Fi can also be utilized in petroleum or chemical plants where other frequencies could be hazardous. This technology also enables us to control plants and their growth without direct presence.
7) Smart Lighting: - Street lamps can be used in future to provide Li-Fi hotspots and can also be used to control and monitor lighting and data

8. Comparison of Li-Fi Vs Wi-Fi

No	Parameter	LI-FI	WI-FI
(1)	Speed	1-3.5 Gbps	54-250 Mbps
(2)	Range	10 meters	20-100 meters
(3)	Spectrum	10,000 times broader than that of Wi-Fi	Radio spectrum range
(4)	Data density	High	Low
(5)	Security	Highly secured due to non-penetration	Less secure due to

		of light through walls.	transparency.
(6)	Bandwidth	High due to broad spectrum	Low
(7)	Data Transfer Medium	Light as carrier	Radio Spectrum
(8)	Frequency Band	100 times of THz	2.4 Ghz
(9)	Interferences of obstacles	High	Low
(10)	Latency	In the order of microseconds	In the order of milliseconds
(11)	IEEE Standard	802.15.7	802.11b
(12)	Network Topology	Point-to-Point	Point-to-Multi Point

Table: 1 Comparison of Li-Fi vs. Wi-Fi

9. CONCLUSION:

From the above presented review, it can be analyzed and concluded that, in the future information can be transmitted from devices, smart phones through the medium of light using Li-Fi, where each and every bulb can be used as hotspot to transmit data with security as light cannot penetrate through walls. The upcoming technology also ensures the availability in air planes, under water areas etc. Li-Fi technology ensures us to address the issues such as shortage of radio frequency bandwidth as it provides the range 10,000 faster than Wi-Fi. Researcher are developing micron sized LED which are able to flicker on and off around 1000 times quicker than larger than LED. They offer the faster data transfer and take up less space so we could save space or add more LED‟s to further boost the channel of communication [9]. With the demand for the internet which is exponentially growing, the technology has proven to be milestone in communication systems. With the use of LED light bulbs the information can be transmitted at very high rates by simply turning on and off the LEDs. The future of LI-FI is GI-FI. GI-FI or gigabit wireless refers to wireless communication at a data rate of more than one billion bits (gigabit) per second[5].

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National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
Bahauddin Science College, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India and
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Linear models for leaf area measurement from small leaflets of multipurpose trees of arid to semi-arid area

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Abstract: *Leaf area is a determinant of plant productivity, transpiration, development rate. It is also useful for the analysis of canopy architecture. That's why accurate measurement of leaf area required for the specific research. In this study, tiny leaves of A. nilotica, L. leucocephala, P. juliflora were measured with the Leaf Area Meter software. This method is accurate and rapid. Comparisons between conventional method and LAM software method is done by using the leaves of D. latifolia. Then, growth analysis was done.*

Key Words: *leaf area, LAM, A. nilotica, L. leucocephala, P. juliflora*

1. INTRODUCTION:

Leaf area is a key variable in study of physiology, horticulture and crop science (1) (2). It is a one of the major parameters in plant modeling studies and thus the most important parameter to develop physiological model in plant breeding (3). It plays an important role to determine productivity, development rate, yield potential, radiation use efficiency and water as well as nutrient takes up (4). Rates of canopy, gas exchange and energy balance are also affected by leaf area (5) (6). The leaf area measurement of the leaves of three tree species *A. nilotica*, *L. leucocephala and P. juliflora* is very difficult due to their small leaf size, however, they are multipurpose trees and of great economic importance.

2. LITERATURE REVIEW:

Leaf area is a core element of ecological field and modeling studies (7). Many workers have demonstrated the importance of it in various studies. In plant pathology to compare the effect of different diseases on plant types may use leaf area as an indicator of treatment effectiveness (8). It is also required to determine evapotranspiration of forest (9). It is used in the monitoring of phytomass production and grain yield of cereal crops (10). To understand rates of energy and materials exchange between forest and atmosphere, leaf area is a key variable. Change in the leaf area may be useful for estimating crop growth.

It also useful for analysis of canopy architecture as it allows the determination of leaf area. Thus accurate measurements of leaf area are essential for understanding the interaction between crop growth and its environment. Many methods have been developed to measure the leaf area which includes drawing, blueprinting, photographing, and image analysis (11). These processes are time consuming and facilities are generally expensive for the measurements. Therefore, another simple, inexpensive, rapid, reliable and non-destructive method of estimating leaf area is required for the experiments (3).

Estimation of leaf area from equations using simple measurement of leaf dimension is an alternative to serve the purpose (12).

3. STUDY OBJECTIVES:

In this study, four species from Fabaceae family are taken; viz. *Acacia nilotica, Leuceana leucocephala, Prosopis juliflora* and *Dalbergia sissoo* for the leaf area measurements. The main objective of this study is to find out regression equation for leaf area of these species, using non - destructive method.

4. MATERIALS:

Material collection:

In this study, four plant species were studied for leaf area measurement and to find out regression growth constant of the leaf. 1) *Acacia nilotica*, 2) *Leucaena leucocephala*, 3) *Prosopis juliflora* and *4) Dalbergia latifolia* plant species are studied. Leaves were collected from the botanical garden of Department of Biosciences, Saurashtra University, Rajkot. In this study, 60 leaves of different sizes were taken from each species.

5. METHOD:

Measurement of leaf area:

Leaves were washed thoroughly under running tap water. Then they are bloated on filter paper. Small leaflets separated from the petiole and midrib were scanned together in 72×72 psi resolution and saved as .bmp file. Individual leaf area was measured by using Leaf Area Meter software.

6. DISCUSSION:

Estimation of leaf area from the plant species gives more insight it to understand the physiology and ecology of the plant, canopy structure and function and the plant system as a whole. In this study, the experiment is divided into two parts. In the first part of the study an attempt is made to measure leaf area from plants such as *A. nilotica, L. leucocephala, P. juliflora* which are technically difficult using standard conventional method. Even leaf area meter also become limiting in a number of the occasions when these plants demonstrate it sleeping movement/folding movement. However, we could do it accurately and precisely due to the high sensitivity of the software at a pixel level. In the second part of the experiment, a plant species i.e. *D. latifolia* is used. The leaves of this plant can be drawn on graph paper for length and width dimensions. To avoid personal bias, two volunteers are also requested to judge the efficiency of the software and to compare and contrast time taken, sensitivity and accuracy of the software.

7. ANALYSIS:

Growth analysis:

Fresh weight was taken of individual leaf. All leaves were kept in oven at 65ºC for 48 hrs and dry weight was measured. The difference of fresh weight and dry weight was considered as water content.

Statistical analysis:

Leaf area obtained using LAM software is calculated to derive constant against leaf dry weight.

8. RESULT:

Acacia nilotica:

In *A. nilotica*, plant leaf area ranged from 2.659 cm^2 to 63.598 (Table 2) cm^2 from 60 leaves. Fresh weight data ranged from 31.5 mg/leaf to 411.6 mg/leaf (Table 2) and water content ranged from 11.8 mg/leaf to 213.1 mg/leaf (Table 2) The $R^2 = 0.823$ reported for dry weight (Fig. 1) The regression equation, $y = 5.309x + 18.17$ was used to find out leaf area (Fig. 1) Similarly, data on water content derived as difference between fresh weight and dry weight showed $R^2 = 0.742$ (Fig. 1) The regression equation, $y = 3.342x+30.75$ was obtained (Fig. 2) The R^2 value of dry weight was more significant than water content.

Leucaena leucocephala:

In *L. leucocephala*, plant leaf area ranged from 4.624 cm^2 to 75.472 cm^2 from 60 leaves. Fresh weight data ranged from 45.8 mg/leaf to 1366.1 mg/leaf and water content ranged from 30.3 mg/leaf to 1014.9 mg/leaf (Table 3). The $R^2 = 0.810$ reported for dry weight. The regression equation, $y = 5.439x + 9.416$ was used to find out leaf area (Fig. 3). The $R^2 = 0.809$ reported for water content. The regression equation, $y = 8.408x – 6.104$ was obtained (Fig. 4).

Prosopis juliflora:

In *P. juliflora* plant leaf area ranged from 12.886 cm^2 to 43.874 cm^2 from 60 leaves. Fresh weight data ranged from 181.8 mg/leaf to 587.7 mg/leaf and water content ranged from 85.9 mg/leaf to 365.4 mg/leaf (Table 4). The $R^2 = 0.805$ reported for dry weight. The regression equation, $y = 5.0x + 31.54$ was used to find leaf area (Fig. 5). The $R^2 = 0.805$ reported for water content. The regression equation, $y = 8.233x + 15.48$ was obtained (Fig. 6).

Dalbergia latifolia:

In *D. latifolia* plant leaf area ranged from 4.573 cm^2 to 41.891 cm^2 from 60 leaves. Fresh weight data ranged from 21.1 mg/leaf to 703.8 mg/leaf and water content ranged from 9.5 mg/leaf to 413 mg/leaf (Table 5). The $R^2 = 0.936$ reported for dry weight. The regression equation, $y = 6.367x + 0.611$ was used to find leaf area (Fig. 7). The $R^2 = 0.909$ reported for water content. The regression equation, $y = 10.01x – 30.14$ was obtained (Fig. 8).

11. CONCLUSION:

In this study, dry weight and water content showed statistically significant relationship with leaf area. Overall data suggest that the software measurement is more accurate and rapid. The data can archive in the computer and can be studied as and when needed. Water content and dry weight are good parameters for linear model development.

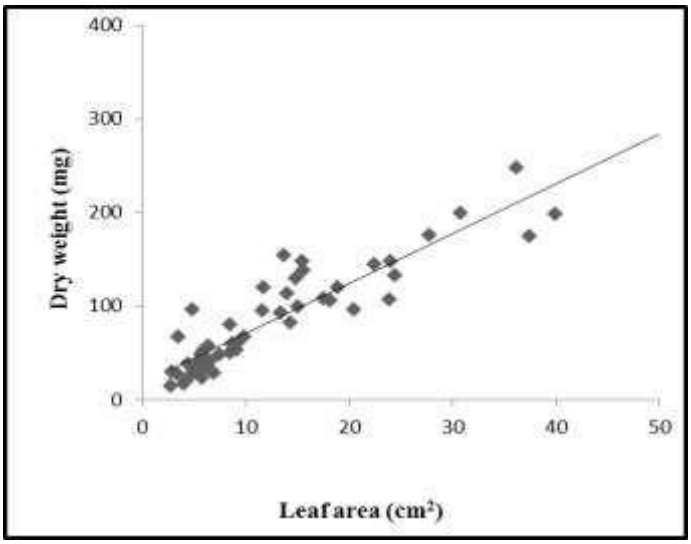

Fig.1 Relationship between dry matter and leaf area in *Acacia nilotica* (N = 60, Y=5.309x + 18.47, R^2 = 0.823, P<0.001)

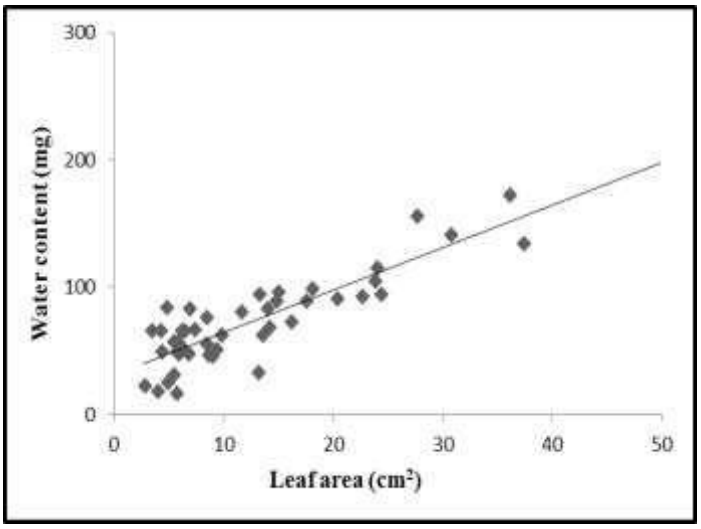

Fig.2 Relationship between water content and leaf area in *A. nilotica* (N=60, Y=3.342x + 30.75, R^2 = 0.742, P<0.001)

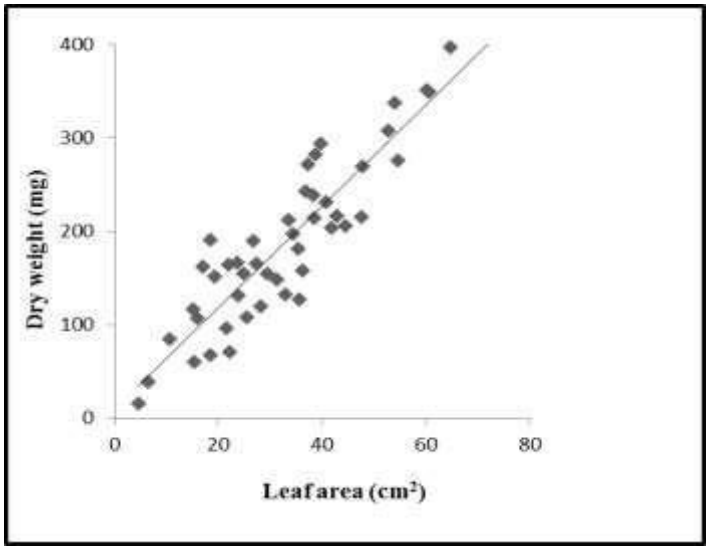

Fig.3 Relationship between dry weight and leaf area in *Leucaena leucocephala* (N=60,Y== 5.439x + 9.416,R^2 = 0.810,P<0.001)

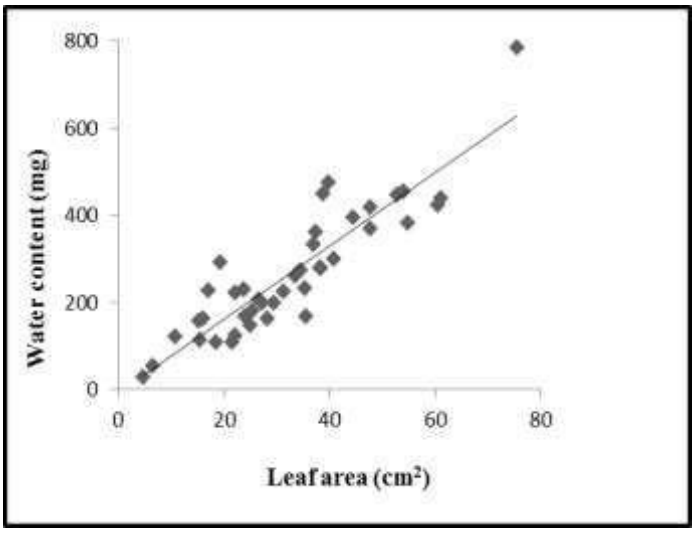

Fig.4 Relationship between water content and leaf area in *Leucaena leucocephala* (N = 60, Y = 8.408x – 6.104, R^2 = 0.809, P<0.001)

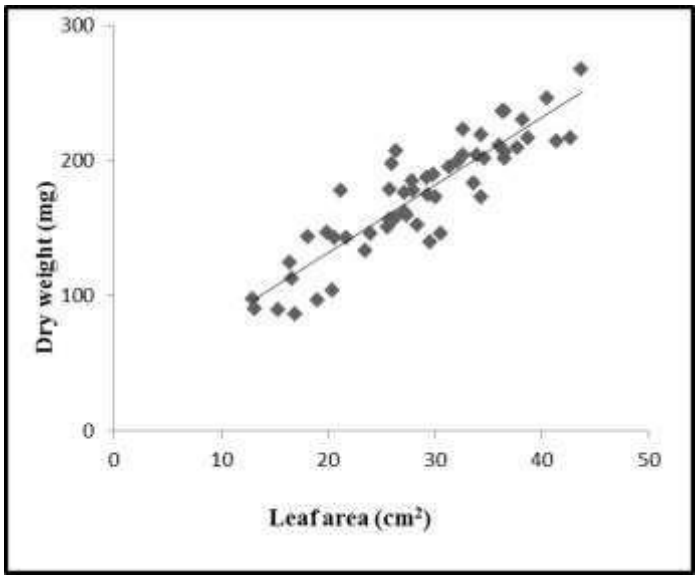

Fig.5 Relationship between Dry weight and Leaf area in *Prosopis juliflora* (N=60,Y=5.000x + 31.54,R^2 = 0.805,P<0.001)

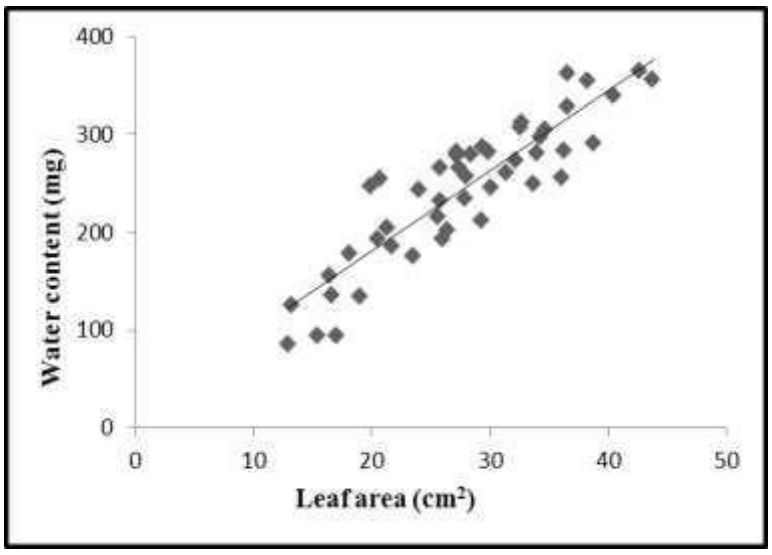

Fig.6 Relationship between Water content and Leaf area in *Prosopis juliflora* (N=60,Y=8.233x + 15.48,R^2 = 0.805,P<0.001)

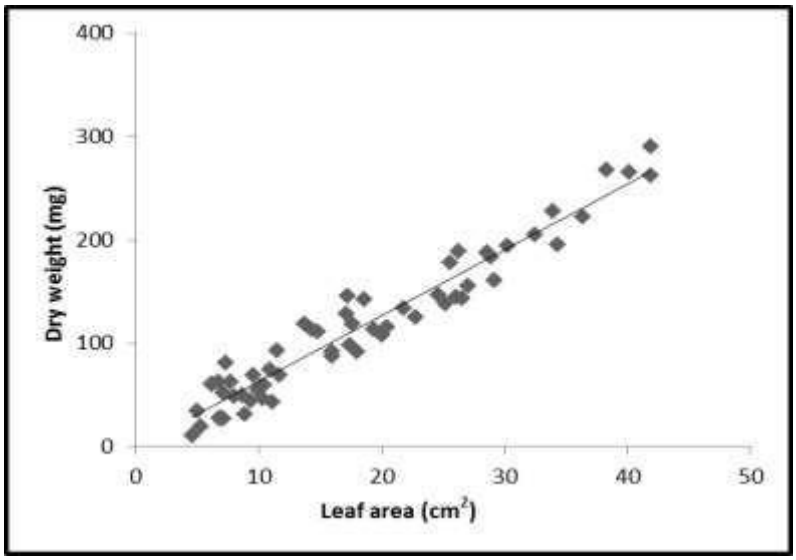

Fig.7 Relationship between Dry weight and leaf area in *Dalbergia latifoia* (N = 60, Y = 6.367x – 0.611, R^2=0.936)

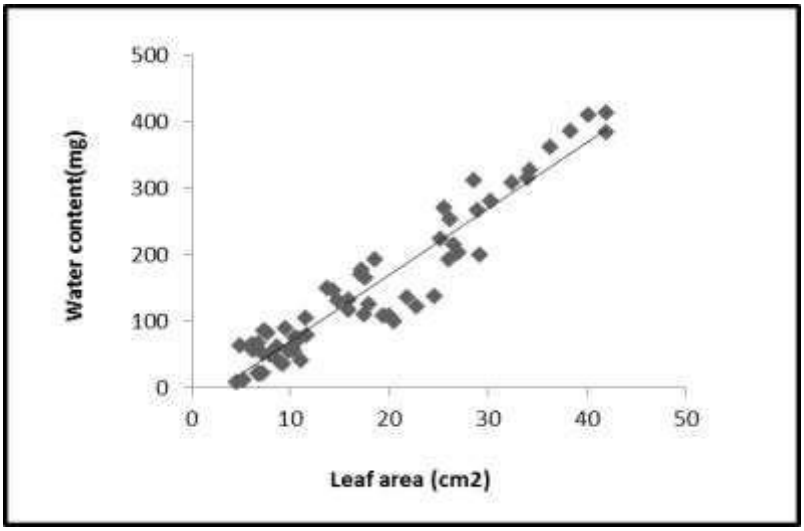

Fig.8 Relationship between water content and leaf area in *Dalbergia latifoia* (N = 60, Y = 10.01x – 30.14, R^2 = 0.909)

Table: 1 Data obtained for leaf area of *D. latifoia* by conventional method and LAM method

No. Of leaves	Leaf area measurement by conventional method(cm^2)	Leaf area measurement by software(cm^2)	Time is consumed in conventional method (minute)
1	3.97	2.86623	2
2	4.49	5.15995	3
3	3.93	3.97762	3
4	3.48	4.51403	2
5	6.28	6.51404	4
6	3.32	2.67705	2
7	5.14	4.86375	3
8	5.65	4.97327	3
9	6.29	5.62417	4
10	7.31	6.73183	5
11	4.92	5.06163	3
12	5.5	6.63227	3
13	8.78	8.18299	4
14	7.24	12.37592	6
15	7.49	11.91917	6

Table: 2 Data obtained for leaf area of *A. nilotica* by LAM software

No.	Fresh weight (mg)	Dry weight (mg)	Water content	Leaf area
1	411.6	198.5	213.1	39.92554
2	44.1	22.4	21.7	13.57568
3	263	148.2	114.8	23.98768
4	370.5	194.2	176.3	63.59835
5	180.7	96.9	83.8	4.76667
6	60	29.7	30.3	35.40529
7	73.4	42.2	31.2	5.47483
8	64.1	31.2	32.9	13.17617
9	35.9	17.5	18.4	3.97513
10	216.4	154.4	62	13.59684
11	82.7	33.1	49.6	5.71378
12	184.7	92.5	92.2	22.64853
13	267	138	129	15.54582
14	212.3	107.2	105.1	23.80598
15	196.5	113.5	83	13.96523
16	93.7	36.3	57.4	5.42007
17	107	41.6	65.4	6.47048
18	104.1	38.1	66	6.20663
19	31.5	19.7	11.8	13.65533
20	78	30.4	47.6	6.81522
21	331.8	175.8	156	27.70519
22	186.9	96.1	90.8	20.41828
23	127	50.8	76.2	8.44435
24	308.7	174.8	133.9	37.39908
25	96.7	52.1	44.6	43.76501
26	419.8	247.8	172	36.11718
27	340.3	199.6	140.7	30.7581
28	207.3	144.6	62.7	22.39838
29	227.7	133.3	94.4	24.35607
30	219.8	130.4	89.4	14.80406
31	137.1	120.7	16.4	11.62172
32	204.5	105.8	98.7	18.10091
33	99.6	53.9	45.7	9.03676
34	196.3	100.1	96.2	14.97083
35	197.2	108.4	88.8	17.52592
36	106.4	59.5	46.9	8.64224
37	114.4	63.3	51.1	9.3367
38	295.4	148.2	147.2	15.40394
39	114.4	48.1	66.3	7.3815
40	187.7	93.6	94.1	13.29565
41	138.1	65.2	72.9	16.21788
42	175.6	95	80.6	11.60678
43	252.5	120.2	132.3	18.86756
44	89.7	42.1	47.6	5.9266
45	129.3	67.2	62.1	9.78972
46	51.8	29.4	22.4	2.83511
47	150.7	82.7	68	14.23032
48	55.3	30.4	24.9	4.89859
49	39.6	23.6	16	5.73867
50	109.3	58.2	51.1	6.37216
51	136	80.6	55.4	8.42444
52	78.6	38.8	49.6	4.37961
53	53.1	27.5	92.2	3.2844
54	94.3	48.2	129	5.59182

55	110.4	56.2	105.1	6.09711
56	103.6	29	83	6.92101
57	131.8	52.3	57.4	5.85068
58	56.8	67.4	65.4	3.47357
59	53	21.8	66	4.24396
60	44.4	14.9	11.8	2.65963

Table: 3 Data obtained for leaf area of *L. leucocephala* by LAM software

No.	Fresh weight(mg)	Dry weight(mg)	Water content	Leaf area
1	60.5	24.4	36.1	27.49642
2	461.9	187.3	274.6	13.41513
3	206.8	85.2	121.6	10.67087
4	274.3	116.2	158.1	15.22348
5	271.1	107.5	163.6	16.03369
6	313.6	132.5	181.1	32.90995
7	93.4	39.1	54.3	6.46799
8	354.7	154.9	199.8	29.46002
9	386.8	164.9	221.9	22.05986
10	634.9	272.3	362.6	37.34058
11	473.3	212.2	261.1	33.53969
12	578	243.2	334.8	36.90997
13	206.3	96	110.3	21.48114
14	690.2	270	420.2	47.77872
15	792	337.3	454.7	54.07247
16	302.6	154.7	147.9	24.93728
17	414.4	181.2	233.2	35.36049
18	1366.1	351.2	1014.9	60.31395
19	959.3	173.1	786.2	75.47271
20	667	227.8	439.2	61.12914
21	586.3	215	371.3	47.70529
22	769.8	294.1	475.7	39.68161
23	407.4	291	116.4	30.89002
24	301.5	132	169.5	23.91674
25	127.3	39.5	87.8	29.85579
26	397.5	190	207.5	26.63238
27	175	59.8	115.2	15.33176
28	496.9	190.6	306.3	18.45187
29	45.8	15.5	30.3	4.62479
30	293.3	108.4	184.9	37.46877
31	289.2	107.7	181.5	25.44009
32	601.3	205.7	395.6	44.45948
33	443.2	151.9	291.3	19.22101
34	212.8	87.3	125.5	32.92612
35	194.2	70.4	123.8	22.18431
36	390.5	162.4	228.1	17.07788
37	184.4	61.5	122.9	30.69338
38	839	286.8	552.2	15.65285
39	497.8	174.6	323.2	48.07991
40	804.5	315.1	489.4	37.36672
41	533.9	214.6	319.3	38.43704
42	375.6	158	217.6	36.30511
43	834.7	349.7	485	60.54046
44	331.3	127.3	204	35.51979
45	465.5	203.5	262	41.79363
46	572.6	231.9	340.7	40.88261
47	406.4	148.9	257.5	31.28704

48	875	396.9	478.1	64.84913
49	196.6	67.9	128.7	18.45187
50	392.6	165.6	227	27.24968
51	713.4	275.6	437.8	54.69724
52	421.1	166.9	254.2	23.6977
53	518.3	177.5	340.8	51.02454
54	770.6	282.7	487.9	38.73698
55	807.3	308.3	499	52.77066
56	310.5	119.5	191	28.25155
57	709	257.9	451.1	64.38367
58	555.8	238.5	317.3	38.21178
59	506.5	197.3	309.2	34.43951
60	642.8	216.6	426.2	42.83781

Table: 4 Data obtained for leaf area of *P. juliflora* by LAM software

No.	Fresh weight(mg)	Dry weight(mg)	Water content	Leaf area (cm^2)
1	435.3	178.01	257.29	27.9317
2	473.4	190.2	283.2	29.83214
3	455.1	176.6	278.5	27.1282
4	517.1	204.2	312.9	32.59009
5	232.1	97	135.1	18.97708
6	585.7	230.5	355.2	38.18066
7	367.5	151.2	216.3	25.53343
8	472.9	199.4	273.5	32.10471
9	309.6	133.3	176.3	23.4799
10	509	216.9	292.1	38.67476
11	420.3	173.6	246.7	30.06488
12	624.2	267.8	356.4	43.69656
13	486	204.1	281.9	33.95413
14	411.9	178.8	233.1	25.77736
15	457.5	195.6	261.9	31.32313
16	426	159.6	266.4	27.30444
17	564.7	202.2	362.5	36.52789
18	582.5	217.1	365.4	42.62002
19	432.3	152.3	280	28.29387
20	445	162.4	282.6	27.18372
21	298	104.5	193.5	20.4021
22	463	174.7	288.3	29.30818
23	390	146.7	243.3	23.98146
24	471.6	173.4	298.2	34.27772
25	398.8	143.5	255.3	20.60372
26	373.3	148.5	224.8	34.0985
27	422.9	156.3	266.6	25.72882
28	429.4	160.6	268.8	43.7787
29	394.3	147.3	247	19.89059
30	441	163.7	277.3	40.14832
31	587.7	246.8	340.9	40.4345
32	530.6	223.1	307.5	32.56645
33	567.2	237.4	329.8	36.48059
34	216	90.4	125.6	13.16746
35	507.3	201.9	305.4	34.58886
36	433.4	183.8	249.6	33.62183
37	367.5	154.5	213	36.22172
38	480.9	219.6	261.3	34.33248
39	376.1	167.2	208.9	43.87453
40	447.5	210	237.5	37.6679

41	521	236.8	284.2	36.25408
42	299.3	146.2	153.1	30.50794
43	308.4	157.5	150.9	26.24532
44	400.1	188	212.1	29.25093
45	295.8	140.1	155.7	29.48616
46	328.5	143	185.5	21.63422
47	322.7	144.3	178.4	18.06606
48	467.3	211.3	256	35.99521
49	429.7	299.6	130.1	30.42331
50	210.5	98	85.9	12.88619
51	383.3	178.3	205	21.2148
52	280.5	124.6	155.9	16.40208
53	391.9	197.8	194.1	25.93667
54	420.5	185.2	235.3	27.79977
55	181.8	86.8	95	16.92355
56	433.6	207	226.6	36.45819
57	446	215	231	41.39039
58	184.5	89.6	94.9	15.33549
59	248.1	112.5	135.6	16.57881
60	409.2	207.2	202	26.35235

Table: 5 Data obtained for leaf area of *D. latifoia* by LAM software

No.	Fresh weight(mg)	Dry weight(mg)	Water content	Leaf area(cm^2)
1	21.1	11.6	9.5	4.57376
2	33.3	20.2	13.1	5.23712
3	50.6	28.1	22.5	6.74926
4	47.9	26.9	21	6.809
5	146.4	62.8	83.6	7.67148
6	71.2	31.7	39.5	8.82021
7	99.9	35.1	64.8	4.93842
8	118.4	60.8	57.6	6.14813
9	127.1	60.5	66.6	6.14067
10	131.4	63.3	68.1	6.65592
11	99.3	48.8	50.5	7.94653
12	49.6	27.4	22.2	7.11267
13	84.9	43	41.9	11.03179
14	103.8	47.1	56.7	10.29501
15	104.5	52.4	52.1	7.11267
16	81.5	44.6	36.9	9.27696
17	167.9	81	86.9	7.24086
18	159.5	69.4	90.1	9.49103
19	148.5	74.5	74	10.86129
20	149.3	69.8	79.5	11.67026
21	113.1	50.1	63	8.59992
22	119.7	58.5	61.2	10.0934
23	136.4	59.8	76.6	10.41449
24	111.1	55.3	55.8	9.86191
25	195.1	92.1	133	15.91794
26	206.1	88.3	117.8	15.91172
27	243.4	111.6	131.8	14.77793
28	260.7	114.1	146.6	14.2913
29	199	93.7	105.3	11.48357
30	269	118.6	150.4	13.68271
31	217	108.3	108.7	19.99637
32	222.6	113.7	108.9	19.36289
33	210.5	98.8	111.7	17.37657

34	300.6	129.3	171.3	17.08783
35	323.4	145.8	177.6	17.20855
36	247.7	125.1	122.6	22.69458
37	270.5	134.1	136.4	21.75618
38	335.7	142.6	193.1	18.50165
39	217	116	101	20.40459
40	364.1	138.7	225.4	25.18122
41	361.3	160.9	200.4	29.13768
42	360	156.1	203.9	26.95223
43	337.9	144.9	193	26.00263
44	476.8	194.8	282	30.24534
45	500.6	188.2	312.4	28.5378
46	676.5	265.6	410.9	40.15703
47	655	267.8	387.2	38.25534
48	703.8	290.1	413.7	41.8907
49	648.6	262.8	385.8	41.89195
50	544.3	227.5	316.8	33.90684
51	586	222.6	363.4	36.30635
52	450.1	178.4	271.7	25.52347
53	524.6	195.6	329	34.26154
54	284.6	118.6	166	17.60557
55	218.3	91.7	126.6	17.92044
56	451.8	183.6	268.2	28.83649
57	442.6	188.8	253.8	26.17687
58	514.4	205.6	308.8	32.40714
59	284.7	146.8	137.9	24.52035
60	359.6	143.7	215.9	26.51165

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National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
Bahauddin Science College, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India and
Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Potential use of DNA barcoding for the identification of some medicinally important plants from Dang forest, Gujarat, India

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Abstract: *Short gene sequence from a standardized region of the genome as a tool for species identification known as 'DNA Barcode' is helpful in biodiversity studies. Medicinal plants are very important to cure the disease. In case of adulteration, DNA barcoding is very effective way to identify the medicinal plants. Besides this DNA barcoding provides insights in to species level taxonomy and useful for conservation of endangered plants. In present study phylogenetic method was also used in addition to DNA barcoding for identification of plants to confer their evolutionary relationship with the closely related species. In present study rbcL shows standard marker with high amplification and used as a standard barcode for rapid and accurate identification of plants.*

Key Words: *Medicinal plants, Adulteration, DNA barcode, rbcL, Phylogeny.*

1. INTRODUCTION:

Traditional herbal medicines are gaining popularity worldwide and about 80% of the world's population utilizes traditional medicines for well-being and healthcare [1]. Generally information like local name, medicinal uses and origin are provided by the herbalist, but this information are insufficient to identify the species because herbalist do not possess knowledge of medicinal plants in the wild [2]. Some medicinal products have multiple synonyms and same vernacular name is applied to multiple plant species [3]. The identity and medical implications of the plants can become conservational because increasing demands for medicinal products may lead to overharvesting of that species. If plant species are not correctly identified then this may lead to introduction of toxic or unsuitable plants into the market and with the risk of adverse health effects [4, 5]. Identification of endemic plants is also important because it infers the relationships among the other taxa within the same genus [6]. Authentication using DNA barcodes overcomes these problems.

The term "DNA barcoding" has been coined to describe the use of a short gene sequence from a standardized region of the genome as a tool for species identification [7, 8]. In plants, several candidate DNA barcodes have attached the attention of many researchers such as *trnH-psbA, rbcL, matK, rpoB, rpoC1, ndhJ, accD, YCF5* in the plastid genome and *rDNA ITS* in the nuclear genome to classify the medicinal plants [9,10]

2. MATERIALS & METHODS:

2.1 Collection of Plant material

Medicinal plants were collected from different forest regions of Dang district, Gujarat, India. Fresh leaves samples were collected in sterile polyethylene bag for further taxonomic study in laboratory and also used for genomic DNA isolation for molecular identification using DNA barcoding.

2.2 DNA isolation, PCR and sequencing

Genomic DNA were extracted from fresh leaves samples using a Plant/Fungi DNA isolation kit (Sigma Cat# E5038) and subsequently PCR reactions were carried out using Veriti® thermal cycler (Applied

BioSystems) under the following conditions: 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 1:30 min, with a final extension at 72 °C for 10 min. The PCR reactions were performed in a 20µl volume containing 1x final concentration of DreamTaq Green PCR Master mix (Cat# K1081), 50 ng of genomic DNA and 10 pmol of both primers rbcLaF and rbcLaR [11,12]. The PCR products were visualized on 2% agarose gel and amplified PCR product was purified using PurelinkTM Quick PCR Purification kit (Cat# K310001). Purified PCR products were usedt for DNA sequencing at Biodiversity Gene Bank, Gujarat State Biotechnolgy Mission, Gandhinagar, Gujarat. The obtained sequences were compared with sequences available in the NCBI database using the Basic Local Alignment Search Tool. BOLD data system was used to generate DNA barcodes for nucleotides sequences.

2.3 Phylogenetic analyses of sequence data

The phylogenetic tree was generated by using rbcL sequences data of *medicnal plants* in present study and other related species obtained from GenBank. Sequence ambiguities were edited by aligning the forward and reverse sequence in Bioedit 7.2.5 [13]. Multiple sequence alignment was carried out by using Clustal X [14]. Nucleotide substitution models that best fit for phylogenetic analyse were determined by using jModelTest [15] with models selected based on the Akaike Information Criterion (AIC). The same model was selected for maximum parsimony analysis.Combine genes phylogenetic tree was obtained based on maximum parsimony analysis in PAUP* ver.4.0 [16] with GTR+I+G model of nucleotide substitution using a heuristic algorithm with random addition of sequences (10 replicates) and tree-bisection reconnection branch swapping (TBR). The same settings were used for bootstrap analysis with 1000 replicates. All positions containing gaps and missing data were eliminated during construction of phylogenetic tree.

3. RESULTS:

3.1 Molecular identification, DNA barcoding and phylogenetic analyses

The generated nucleotide sequence was used for BLAST search in the GenBank database (www.ncbi.nlm.nih.gov) for identification of the species. Identification was done by 99% base pair match of the sequence obtained to the closest available reference sequences. After molecular identification, morpho-taxonomic characteristics of the identified fungal species were compared with the available literature for the further confirmation of the identity of species. The phylogenetic tree was generated by using rbcL sequences data of medicinal plants in present study for identification of plants to confer their accurate species identification and evolutionary relationship with the closely related species (Figure 1). Nucleotide sequences were submitted to BOLD Data system to generate DNA barcodes (Table 1).

Figure 1: Maximum likelihood cladogram generated from rbcL dataset for DNA barcoded medicinal plants of present study and other species from Genbank with accession numbers. Bootstrap values are indicated on the tree branches. (Figure 1, Given at last page)

Table 1: DNA barcoding details of medicinal plants collected from Dang forest in present study. (Given at last)

The morphological features and ethnomedicinal importance of collected plants were discussed individually:

a. ***Cardiospermum grandiflorum* Sw.**
Taxonomic position: Tracheophyta; Magnoliopsida; Sapindales; Sapindaceae
Common names: Balloon vine, grand balloon vine, heart pea, heart seed, heart seed vine,showy balloon vine.
Medicinal uses: Root derivatives of the plant have been shown to offer laxative, emetic and diuretic effects. The leaves of the plant have been used to alleviate swelling, oedema and pulmonary complications [17] and may have antibacterial activity [18].

b. ***Colocasia esculenta* (L.) Schott**
Taxonomic position: Tracheophyta; Liliopsida; Alismatales Araceae
Common names: Taro, cocoyam, green taro, aivi, dasheen.
Medicinal uses: Methanol extracts of C. esculenta possess broad spectrum antimicrobial and antioxidant activity. Also Extracts exhibited significant anti-inflammatory activity [19].
The pressed juice of the petiole is stypic, and may be used to arrest arterial haemorrhage. Leaf juice is stimulant, expectorant, astringent, appetizer, and otalgia. Decoction of the peel is given as a folk medicine to cure diarrhea. Juice of the corm is used in cases of alopecia [20].

c. ***Curculigo orchioides* Gaertn.**
Taxonomic position: Tracheophyta; Liliopsida; Asparagales; Hypoxidaceae
Common names: Black musli or Golden eye grass

Medicinal uses: It has been prescribed in various combinations and dozes by tribal and traditional vaidyas for a number of ailments and disorders as acidity, blood cancer, diabetes, epilepsy, hernia, paralysis, rheumatism, ring worm, worm infection, wounds and for cooling of stomach, dizziness in cattle and poison removal [21].

d. ***Flueggea virosa*** **(Roxb. ex Willd.) Royle**
Taxonomic position: Tracheophyta; Magnoliopsida; Malpighiales; Phyllanthaceae
Common names: Common bushweed
Medicinal uses: The plant has been used for the treatment of fever, malaria, sexual dysfunction, pain, diabetes, epilepsy, snakebite, venereal disease, rheumatism, arrhythmia, rash, diarrhea, pneumonia, cough, hepatitis, and HIV-related illness, and as a contraceptive. The methanol and water extracts of the plant have been reported to possess a number of biological activities such as antiplasmodial, trypanocidal, and antiarrhythmic [22].

e. ***Homonoia riparia*** **Lour.**
Taxonomic position: Tracheophyta; Magnoliopsida; Malpighiales; Euphorbiaceae
Common names: Willow-Leaved Water Croton
Medicinal uses: Different parts of the plant are active against various ailments like roots for its laxative, diuretic and emetic property. Leaf juice regarded as depurative. The leaves are used to treat wounds caused by aquatic fish bites like scorpion fish. Pounded leaves and fruits are used as a poultice for skin diseases. The leaves were used for cuts and wounds, used in piles, for blood clotting and muscle fracture, also for skin diseases. Fifty per cent ethanolic extract of the plant (excluding root) was found to be inactive when tested for antibacterial, antifungal, antiprotozoal, antiviral and diuretic activities [23].

f. ***Manilkara hexandra*** **(Roxb.) Dubard**
Taxonomic position: Tracheophyta; Equisetopsida; Ericales; Sapotaceae
Common names: Ceylon Iron Wood or Khirni
Medicinal uses: The plant has been known for its curative properties and has been utilized for treatment of various diseases such as ulcer, bronchitis, jaundice, ulitis, fever, hyper dyspepsia, arthritis and alimentary disorders. Plant extracts and metabolites from this plant possess pharmacological properties such as anti–inflammatory, antiulcer, aphrodisiac, alexipharmic, anthelmintic and antibacterial activity [24].

g. ***Salvadora oleoides*** **Decne.**
Taxonomic position: Tracheophyta; Magnoliopsida; Brassicales; Salvadoraceae
Common names: Bada Peelu or Meetha jal
Medicinal uses: Used in the treatment of various ailments like piles, tumors, bronchitis, cough rheumatism, fever, conjunctivitis, carminative and alexipharmic etc. The plant is reported to possess anti hypoglycemic, hypolipidemic, analgesic, and antimicrobial activity [25].

h. ***Strychnos nux-vomica*** **L.**
Taxonomic position: Tracheophyta; Equisetopsida; Gentianales; Loganiaceae
Common names: Poison Nut
Medicinal uses: Its dried seeds are used for the treatment of neuro disorders, arthritis, vomiting and also used for treatment of various disorders. Pharmacologically it has been validated for its effect on inflammation, microbial infections, gastrointestinal problem, nervous system, bones cells, cardiovascular systems, cancer and blood glucose level. It also has antioxidant activity and antifeedant activity [26].

i. ***Tylophora asthmatica*** **(L. f.) Wight & Arn.**
Taxonomic position: Tracheophyta; Equisetopsida; Gentianales; Apocynaceae
Common names: Indian Ipecac, Ananthamul, Damabuti
Medicinal uses: Has been traditionally used for the treatment of bronchial asthma, jaundice and inflammation. It has antitumor, immunomodulatory, antioxidant, antiasthmatic, muscle relaxant. Although the leaf and root of this plant are widely used for treating jaundice. Also have activities include immune-modulatory activity, anti-inflammatory activity, anticancer activity, antihistaminic and antirehumatic [27].

j. ***Cyanthillium cinereum*** **(L.) H. Rob.**
Taxonomic position: Tracheophyta; Equisetopsida; Asterales; Asteraceae
Common names: Little ironweed or purple fleabane
Medicinal uses: Traditionally plant decoction is used to treat urinary tract infections and fevers. Cyanthillium cinereum has therapeutic potentials against dysentery, diarrhea, cough, cholera, asthma and cancer [28].

4. Discussion

Molecular barcoding methods are reliable tools for the identification of medicinal plants, their substitutes and adulterants at the genus and species level. The methods discussed provide consistent and reliable results regarding plant identification. Genomic rbcL region for DNA barcoding could be more useful for medicinal plant material identification [29]. Increasing demand for herbal remedies, authentication of the medicinal plant material is very important; therefore DNA barcoding provide extensive database for easy identification of medicinal plants. Present study indicates that this DNA barcoding and phylogenetic analyses based on rbcL sequence database providing efficiently and accurate species identification.

Acknowledgment

Authors gratefully acknowledge Biodiversity Gene Bank, Gujarat State Biotechnology Mission, Gandhinagar, Gujarat, India for providing research facility for the entire work and Mr. Akash G. Vanzara, Department of Botany, The M. S. University of Baroda, Vadodara for helping in morphological identification.

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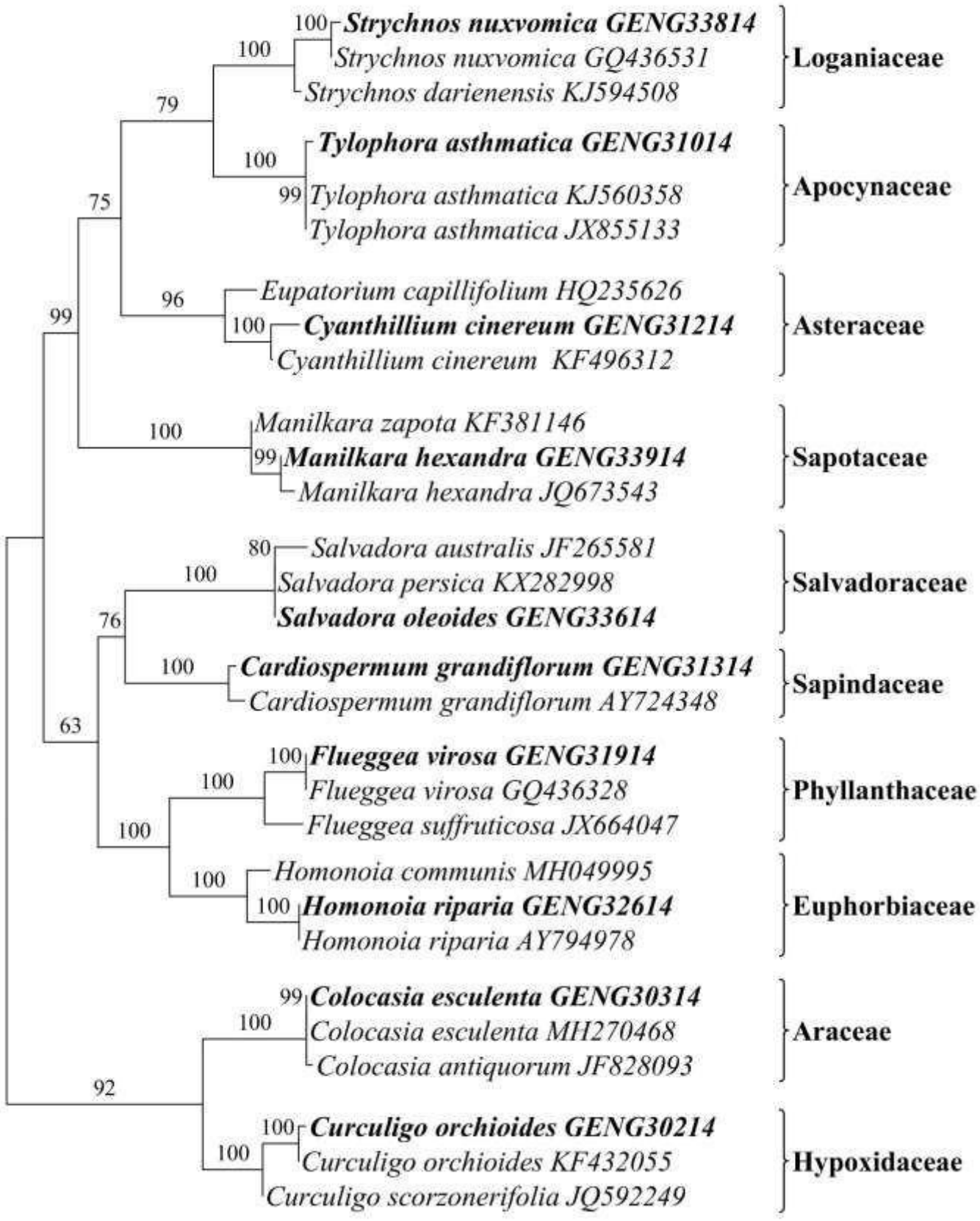
ANNEXTURES:

Table 1: DNA barcoding details of medicinal plants collected from Dang forest in present study

Sr. No.	Specimen ID	Molecular identification using DNA barcoding	Sample ID	Process ID
1	S1	*Cardiospermum grandiflorum* Sw.	DNAFR000294	GENG313-14
2	S2	*Colocasia esculenta* (L.) Schott	DNAFR000284	GENG303-14
3	S3	*Curculigo orchioides* Gaertn.	DNAFR000283	GENG302-14
4	S4	*Flueggea virosa* (Roxb. ex Willd.) Royle	DNAFR000324	GENG319-14
5	S5	*Homonoia riparia* Lour	DNAFR000331	GENG326-14
6	S6	*Manilkara hexandra* (Roxb.) Dubard	DNAFR000344	GENG339-14
7	S7	*Salvadora oleoides* Decne.	DNAFR000341	GENG336-14
8	S8	*Strychnos nux-vomica* L.	DNAFR000343	GENG338-14
9	S9	*Tylophora asthmatica* (L. f.) Wight & Arn.	DNAFR000291	GENG310-14
10	S10	*Cyanthillium cinereum* (L.) H. Rob.	DNAFR000293	GENG312-14

Figure 1:


Strychnos nuxvomica GENG33814
Strychnos nuxvomica GQ436531
Strychnos darienensis KJ594508
Loganiaceae
Tylophora asthmatica GENG31014
Tylophora asthmatica KJ560358
Tylophora asthmatica JX855133
Apocynaceae
Eupatorium capillifolium HQ235626
Cyanthillium cinereum GENG31214
Cyanthillium cinereum KF496312
Asteraceae
Manilkara zapota KF381146
Manilkara hexandra GENG33914
Manilkara hexandra JQ673543
Sapotaceae
Salvadora australis JF265581
Salvadora persica KX282998
Salvadora oleoides GENG33614
Salvadoraceae
Cardiospermum grandiflorum GENG31314
Cardiospermum grandiflorum AY724348
Sapindaceae
Flueggea virosa GENG31914
Flueggea virosa GQ436328
Flueggea suffruticosa JX664047
Phyllanthaceae
Homonoia communis MH049995
Homonoia riparia GENG32614
Homonoia riparia AY794978
Euphorbiaceae
Colocasia esculenta GENG30314
Colocasia esculenta MH270468
Colocasia antiquorum JF828093
Araceae
Curculigo orchioides GENG30214
Curculigo orchioides KF432055
Curculigo scorzonerifolia JQ592249
Hypoxidaceae

National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
Bahauddin Science College, Junagadh, Gujarat, India, Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India and Indian Science Congress Association, Baroda chapter.

DNA Barcoding analysis and phylogenetic relationship between some species of Acanthaceae family in Girnar region

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Abstract: The Acanthaceae is a large and economically important family of flowering plants. A phylogeny of Acanthaceae is presented based on the chloroplast DNA regions rbcL with 13 genera and 17 species. In present investigation we have been focused on Acanthaceae family. With the help of DNA barcode and bioinformatics tools we have been established phylogenetic relationship among these plants and we had also observed morphological characters of the some plants. Some selected plants of the Acanthaceae family were first morphologically identify and then genomic DNA was isolated and amplification of *rbcL* (*Ribulose bisphosphate carboxylase* gene 1) unit with two set of primer *rbcLa*F and *rbcLa*R. These set of primer showed high similarities more than 95% to 100% of the sequences with the relative species. All samples were amplified with 500 bp to 600 bp. These sequences are submitted in barcode of life database (BOLD) system and then all sample are create framework phylogeny for this prevailing family.

Key Words: *DNA, rbcL,* BOLD.

1. INTRODUCTION:

Specimens are collected from the Girnar wild life sanctuary, Junagadh (Gujarat, India). Girnar forest is located at the periphery of Junagadh city and is spread over 181.3 square km area. Girnar forest lies between parallel of latitude 21.25'' N Latitude and meridian of 70.30'' and 70.40'' E Longitude. The climate of Girnar is sub-tropical monsoonal and can be eco-climately classified as semi-arid type. The area is significantly affected by the Southwestern monsoon. The three distinct seasons that can be observed here are summer (March to mid-June), Monsoon (mid-June to mid-September) and Winter (Mid of November to February). So, it is having maximum plant diversity are present. It is famous for its biodiversity and Asiatic lion.

Here we discuss only one family. The Acanthaceae family is a dicotyledonous flowering plants family. In the order of Lamiales, containing almost 250 genera and about 2500 species. They are mostly tropical herbs, shrubs, or twining vines or epiphytes, with only a few species distributed in temperate regions. Leaves are usually opposite and without stipules. Flowers are bisexual, zygomorphic to sub actinomorphic, usually arranged to terminal or axillary spikes, and racemes or panicles. Calyx is usually 4 or 5 lobed. Corolla is sympetalous, and limbs are usually 5. Stamens are epipetalous, two or four, and didynamous. Ovary are superior, 2-loculed, and placentation axile. Fruit is a capsule, and usually loculicidal [1]. Seeds are usually compressed. Total 13 genera and 17 species of family Acanthaceae are molecularly and morphologically identified and find out the relationship between each other through phylogenetic analysis. Among in these total 8 plants species of *Barleria cristata* (White flower), B. *cristata* (purple flower), B. *prionitis, Andrographis paniculata, Haplanthodes verticillastus, Peristrophe paniculata, Justicia betonica* and *Ruellia tuberosa* has been selected for DNA barcoding, rest of other plants (*Eranthemum roseum, Adhatoda vasica, Rungia pectinata, Hygrophila auriculata, Neuracanthus sphaerostachys* and *Hemigraphis latebrosa*) are already submitted by GSBTM, and the other 2 plants (B. *cuspidate* and *Staurogyne zeylanica*) species has not submitted in BOLD. So, We used reference sequence for phylogenetic analysis from NCBI.

DNA Barcoding generate a unique identification tag for individual species. DNA barcoding, a term first proposed by Hebert in 2003 [2], has developed as a rapid and reliable technology to identify species based on variation in the sequence of short standard DNA region(s). The *rbcL* gene was one of the first plant genes to be sequenced and is still among the most frequently sequenced segments of plant DNA. This gene has been used widely in systematic studies of land plants most of angiosperms. The advantages of this gene are that it is easy to amplify, sequence and align in most land plants. It is a good DNA barcoding region for plants at the family and genus levels. Chloroplast DNA can serve as a main source of phylogenetic analysis [3] at different systematic level in various genera of plant kingdom.

2. STUDY OBJECTIVES:

Study the important medicinal plant diversity & creating digital databank for easier identification & for establishment of phylogenetic relationship between the species. It also helpful for identification of adulteration in herbal mixture.

3. MATERIALS:

Materials required for plant collection

- Plant cutter, forceps
- Field note book, pencil, marker pen
- Flora book
- Newspaper, blotting paper, tissue paper
- Herbarium pressure, plastic bags
- Digital camera, Garmin GPS reader
- Silica beads, garden kit tools, first aid box

Materials required for voucher preparation

- Magic tap or glue
- 2% $HgCl_2$
- Herbarium sheet
- Herbarium pressure
- Plastic bag

Reagents required for DNA isolation

- Freshly prepared PVP
- 1% (v/v) β-mercaptoethanol
- Extraction buffer:
  - 250 mM NaCl
  - 25 mM EDTA
  - 0.5% SDS
  - 200 mM Tris HCl (pH 8.0)
- 7.5 M ammonium acetate
- Isopropanol (IPA)
- TE buffer (10 mM Tris–HCl pH 8.0, 0.1mM EDTA pH 8.0)
- RNase (10 mg/ml)
- Chloroform-Isoamyl alcohol (24:1)
- 80% ethanol

Materials and reagents required for agarose gel electrophoresis

- Agarose gel
- TBE buffer (pH - 8)
- EtBr (Conc. 10 mg/ml)
- Gel loading dye (0.25%Bromophenol blue, 50% Sucrose)
- Gel casting tray
- Combs

Reagents required for PCR

- Taq PCR reaction mix
- Forward and reverse rbcL primers
- DNA tamplets
- Milli Q water

4. METHOD:

Field collection:

Plant samples were collected from the different site of Girnar wild life sanctuary, Gujarat, India. We have selected these areas for the survey of plants because it is having maximum diversity of plants. Field work are selected on the basis of the available information on the areas to be visited and the people to be studied taking aid of maps, floras, icons, literature discussion with persons and with guide.

Sample identification:

Samples were identified using the morphological key according to characters of plants and it was compared with the previously identified plant floras. We have used various volumes of floras like flora of Gujarat [4], flora of Maharashtra [5] and other online international floras for more information of plants. Identified plant name was the check in www.theplantlist.org website for accepted name and synonyms.

DNA extraction:

Approximately, 800 mg of leaf material were grinded in liquid nitrogen along with 5 µl of 1% (v/v) β-mercaptoethanol and adding 300 µl of freshly prepared and preheated extraction buffer (250 mM NaCl, 25mM EDTA, 0.5% SDS, 200mM Tris HCL (pH 8.0)) is added to the homogenate, and the tube is flicked at the bottom occasionally to keep the extract mixed. The homogenate is incubated at room temperature for 1 h. freshly prepared PVP (soluble PVP, Sigma, MW 10 000) (6% of final volume) and one half volume of 7.5 M ammonium acetate are added separately. The mixture is incubated on ice for 30 min and centrifuged for 10 min in a microcentrifuge (10 000 g at 4°C). The supernatant is transferred to a fresh tube to which is added 1 vol isopropanol, and left at –20°C for 30 min to precipitate the DNA. After centrifugation at 10 000 g for 10 min, the supernatant is discarded and the DNA pellet is vacuum-dried. The DNA pellet is resuspended in 500 µl TE buffer (10 mM Tris–HCl pH 8.0, 0.1 mM EDTA pH 8.0) or distilled water. Two microliters of RNase (1 mg/ml) was added to the solution and incubated at 37°C for 15 min. One vol of chloroform–isoamyl alcohol (24:1) is added and emulsified by inverted shaking to remove both RNase and plant pigments. The procedure is repeated once again. After centrifugation (10 000 g at 4°C) for 5 min, the supernatant is transferred to a fresh tube to which 1 vol of isopropanol is added and left at –20°C for 10 min. After centrifugation at 10 000 g for 10 min, the pellet is washed with 1 ml 80% ethanol and vacuum-dried. The DNA pellet is redissolved in 30 µl TE or distilled water.

Agarose gel electrophoresis of DNA:

Agarose gel electrophoresis is one of the most common tools of molecular biology. Gel electrophoresis separates DNA molecules based on size. However, the concentration of agarose used in the gel is an important factor that must be taken into consideration when dealing with DNA of specific sizes. Mainly some types of concentration are prepared: 0.5%, 0.8%, 1.0%, and 2.0% w/v [6]. Here purity and concentration of DNA was checked on 0.8% agarose gel electrophoresis. Ethidium bromide is often used to stain the DNA molecules for subsequent visualization under UV light.

Polymerase Chain Reaction (PCR):

A total volume of 20µl PCR master mixture contained the following: 10µl Top Taq buffer, 1µl 10pmol Forward primer, 1µl 10pmol Reverse primer. 1µl of genomic DNA template, 1µl BSA (Bovine Serum Albumin) and 6µl of Milli Q water. The primer pairs rbcLF (5'_TGTAAAACGACGGCCAGTATGTCACCACAA ACA GAG ACT AAA GC_3') and rbcLR (5'_CAGGAAACAGCTATGACGTAAAATCAAGTC CACCRCG_3') were used for the PCR. The PCR was performed with a 2720 thermal cycler (Applied Biosystem) as follows: 96°C for 5 minutes, followed by 35 cycles of 96°C for 30 seconds, 55°C for 1 min and 72°C for 1:30 minute, followed by an elongation step at 72°C for 5:55 minutes. 2% agarose gel using 1X TAE buffer containing 1µl (Conc. 10mg/ mL) EtBr (Ethidium Bromide) was used for PCR product electrophoresis. Gel images with UV camera were obtained using BIO–RAD Gel Doc. The PCR product sizes were determined using 5µL of Gene Ruler 1kb DNA Ladder.

PCR Purification:

The purpose of PCR cleanup is to remove salts, extra nucleotides, unamplified DNA, and primers before sequencing. PCR products were cleaned using the ExoSap® (Affymatrix) using the manufacturer's recommended protocol (2 µL reagent per 5µL amplified DNA product). ExoSAP-IT™ reagent was added to an aliquot of the post-amplification reaction and incubated in a Veriti (96 Well Fast 10Thermal Cycler), Applied Biosystem, USA for 15 min at 37°C followed by an additional incubation for 15 min at 80°C.

Cycle sequencing:

Sequencing of DNA was done using 3500XL Genetic Analyzer (Applied Biosystem). It was carried out using BigDye® Terminator v 3.1 Cycle sequencing kit. The Bigdye Terminator v3.1 Cycle sequencing Kit provides the

required reagent components for the sequencing reaction in a ready reaction, pre- mixed format. Cycle sequencing was performed by the 10µl volume. Reaction mixture contained the following: 2µl Terminator ready reaction mixture, 1 µl Bigdye Sequencing buffer, 1µl Template, 1µl 10 pmole Primer and 5µl Deionized water. Two reaction tubes were prepared for forward sequencing primers and reverse sequencing primers. In this experiment rbcLa gene amplification primers (rbcLa F and rbcLa R) served as sequencing primers. Amplification was carried out in thermal cycler (Applied Biosystem veriti®). Reaction was amplified through 35 cycles with the following condition: 96°C for 4 min., followed by 25 cycle for 96°C for 10 seconds, 55°C for 5 seconds and 60°C for 4 minutes. In the results of sequencing, complete elimination of unincorporated dye terminators before performing capillary electrophoresis is necessary. Purification was done using Big Dye Terminator ® purification kit. In the kit solution total 45 µl SAMTM solution and10µl BigDye X Terminator used per reaction. Sample were allowed to mix till 30 min on vortex and further centrifuged at 1500 rpm for 2 min before performing capillary gel electrophoresis.

BLAST

For comparative analysis we used the NCBI website to perform a BLAST search with the derived sequences. This BLAST will provide closest, if not identical *rbc*L sequences stored in the GenBank. We considered a successful match if there is a query identity score of >95% involved a single genus.

BOLD Systems

The Barcode of Life Data Systems is designed to support the generation and application of DNA barcode data. It accepts sequences *rbc*L and Maturase K genes (matK) and returns a species-level identification when possible (http://www.boldsystems.org/index.php/IDS_Open Id Engine). For BOLD submission various data are required like voucher information, taxonomy detail, specimen detail and collection details. This sheet was uploaded in BOLD database in GENG project of Gujarat Biodiversity Gene bank. After getting the conformation upload sequence file in ab1. Format, field photographs and voucher photographs.

Pairwise alignment

Pairwise sequence alignment was done by using BioEdit methods. BioEdit is a biological sequence alignment editor. This is used to find the best-matching piecewise local or global alignments of two query sequences. BioEdit is not powerful sequence analysis program, but offers many quick and easy functions for sequence editing, annotation as well as a few links to external sequence analysis programs. Here comparisons between Barleria species sequence for rbcl gene.

Phylogenetic Tree

Phylogenetics is the area of research concerned with finding the genetic relationships between species. The basic idea is to compare specific characters (features) of the species, under the natural assumption that similar species (i.e., species with similar characters) are genetically close. CLC main workbench software is simple to use and dedicated to reconstructing and analysing phylogenetic relationships between molecular sequences.

5. RESULT:

Total 17 plants of the acanthaceae family are collected from the Girnar region (Junagadh, Gujarat). Vouchers were prepared and submitted to Gujarat Biodiversity Gene Bank, Gandhinagar, Gujarat. All the details such as collection date, collection sites, taxonomy, local name, location, GPS, botanical name were recorded and photographs were also capture during collection. All the plants photos are shown here (Images – 1).

Total genomic DNA was isolated from all plants samples using the C. S. Kim et al. (1997) [7]. DNA was succesfully isolated and eventually loaded on 0.8% agarose gel (Figure – 1). Clear and sharp bands was obtain. In the figure 1 to 8 number was indicates the genomic DNA of *Barleria cristata* (White flower), B. *cristata* (purple flower), B. *prionitis, Andrographis paniculata, Haplanthodes verticillastus, Peristrophe paniculata, Justicia betonica* and *Ruellia tuberosa* species. Sometimes the isolation of DNA were not successful because due to buffers which used in DNA isolation were not properly prepared or exactly suited for the process, so it is one of the responsible factor for unsuccessful isolation of DNA from the plant.

Images – 1 collected plants

Amplified DNA which was eventually loaded on 1% agarose gel along with a molecular weight standard (100 bp to 1000 bp) ladder. Clear and sharp bands obtain between ~500bp to 700bp (Figure-2). For appropriate amplification of gene, a template DNA must be of high purity. If any impurity remains in the genomic DNA sample, it will interfere in PCR. In this the isolated DNA was good enough to proceed further downstream experiment.

The sequences obtained using 3500XL Genetic Analyzer (Applied Biosystem). Data Collection Software were further analyzed using ABI Sequencing analysis Software v5.1 (Figure-3). The sequences are obtained from all the 17 plants for rbcL genes. These sequences were ranged from 550bpbp to 600bp.

Figure – 1 Agarose gel electrophoresis of genomic DNA isolation

Figure – 2 Agarose gel electrophoresis of amplified DNA

Figure – 3 sequencing result from ABI 3500XL sequencing analyzer (Chromatogram)

Figure – 4 Phylogenetic tree using CLC main workbench software

Local alignments are more useful for dissimilar sequences that are suspected to contain regions of similarity or similar sequence motifs within their larger sequence context. Maximum identity show in Barleria prionitis and Barleria cristata is 0.96%, in Barleria cuspidata and Barleria criatata is 0.78% and minimum identity show in Barleria prionitis and Barleria cuspidata is 0.77% (Table-1).

Table-1 Pairwise (local) alignment using Bioedit

Phylogenetic tree was established using neighbor-joining algorithm method. Here show more variation in our 16 Acanthaceae plants (Figure-4).

6. DISCUSSION:

Plant samples were collected from different sites of north region of Junagadh. Total 17 plants were selected from Acanthaceae family. All the details such as collection date, collection sites, taxonomy, local name, location, GPS, botanical name were recorded and photographs were also capture during collection. Here we show two plants are morphologically different but there scientific name was same. Like one species of *Barleria cristata* have white flower and one species of B. *cristata* have purple flower. Same as in the *Hygrophila auriculata*, one species have spike and other one have no spike. Both plants are morphologically differ but molecularly same plants.

The present study is carried out by focusing on molecularly identifying of the maximum Acanthaceae plants in Girnar region through DNA barcoding. Many techniques were used for identification of plants. Mainly plants were identified on the basis of morphological characters and molecular techniques. In the present study, primary morphological identification of these plants. Morphological characters like leaf shape, leaf size, flower structure, flower colour, plant size, fruit and etc.

Molecular determination of the species

Total genomic DNA was isolated from all plants samples using the C. S. Kim et al. (1997) protocol. This method was suitable for various plant materials and the show high quality DNA. The purity achieved by the protocol and sharp band was observed on agarose gel (Figure-1). The low quality of DNA may be due to their cell wall is not properly lyses. High quality DNA was further used for PCR amplification. *RbcL* gene was amplified from the genomic DNA, of each of the samples using gene specific primers. *RbcL* is most common plastid locus for plant. *The rbcL* is widely used in phylogenetic surveys with over 50000 sequences available in genebank. The advantages of this gene are that it is easy to amplify, sequence and align in most land plants. It is a good DNA barcoding region for plants at the family and genus levels [8]. In plant systematics for phylogenetic purposes is *rbcL*, followed by the *trnL-F* intergenic spacer, *matK*, *ndhF*, and *atpB* [9], [10]. The *rbcL* has been suggested as a candidate for plant barcoding [11].

PCR is an importance to research in molecular biology and genetics, and the major advances in DNA sequencing technology such as development of automated DNA sequencing easier. Three normal steps are performed before automated DNA sequencing. The first step is purification of PCR products. A second step is cycle sequencing of purified PCR products. The third step is purification of cycle sequenced products and subjected to automated DNA sequencer. PCR purification is required prior to sequencing and hence the samples obtained as PCR products were purified. Advantage of purifying PCR product for sequencing includes removal of primers, nucleotides, buffer components, non-targeted amplification products. Here the amplified DNA product purified by ExoSAP-IT™ reagent (USB Corporation, Cleveland, OH) was subjected to cycle sequencing. Cleanup remove salts, extra nucleotides and primers successfully. After purification PCR products are subjected to cycle sequencing. In PCR either forward or reverse primer was added in each reaction and cycle sequencing was performed.

The all sequences obtained using the sequencing facility, were subjected to various Bioinformatics analysis like NCBI BLAST, pairwise alignment using Bioedit and phylogenetic analysis using CLC Main Workbench software from the all sequences. The NCBI BLAST tool was used to confirm the % identity with relative gene for all sequences

in database. It was found that identity range from 90%-100%. Pairwise sequence alignment was done by Bioedit software. A sequence alignment is a way of arranging the sequences of DNA, RNA or protein to identify regions of similarity that may be a consequence of functional, structural, or evolutionary relationships between the sequences [12]. Here comparisons between three species of Barleria genus. A phylogenetic analysis was done by using CLC Main Workbench software. Recent molecular techniques have been introduced to provide more objective criteria. Phylogenetic tree indicated the relationship between genetic distances of strains.

7. ANALYSIS:

Morphologically different plants species are genetically same.

- All plants have same family but one plants have different clade.

8. FINDINGS:

DNA was succesfully isolated from the all plants and the one species, *Heplanthodes vertivillasters* is a first time submitted in BOLD by us. During the all molecular analysis two morphologically different plants species are genetically similar. The phylogenetic analysis is a corelation between the all species which one is belong to same family. The species *Barleria cuspida* is a more advance to *Barleria cristata*. These are the novel approach of the application of DNA barcode for establishment of phylogenetic relationship among the different plants of family.

9. RECOMMENDATIONS:

Some alternate cost effective methodology is required for easily molecular identification and phylogenetic relationship among the plants.

10. CONCLUSION:

DNA barcoding can be effectively used for an identification of medicinal plants. It provides information about adulteration in any plants or medicinal mixture. Our studies will also helpful for reference database to other interested researchers for identification. In traditional taxonomy, we are generally identifying plants according to phenotypic expression which are express in form of different characters. But, sometime there are some genotypically changes even in same genus and species. In this research, we have tried to find out that weather evolutionary path is of the same plant family.

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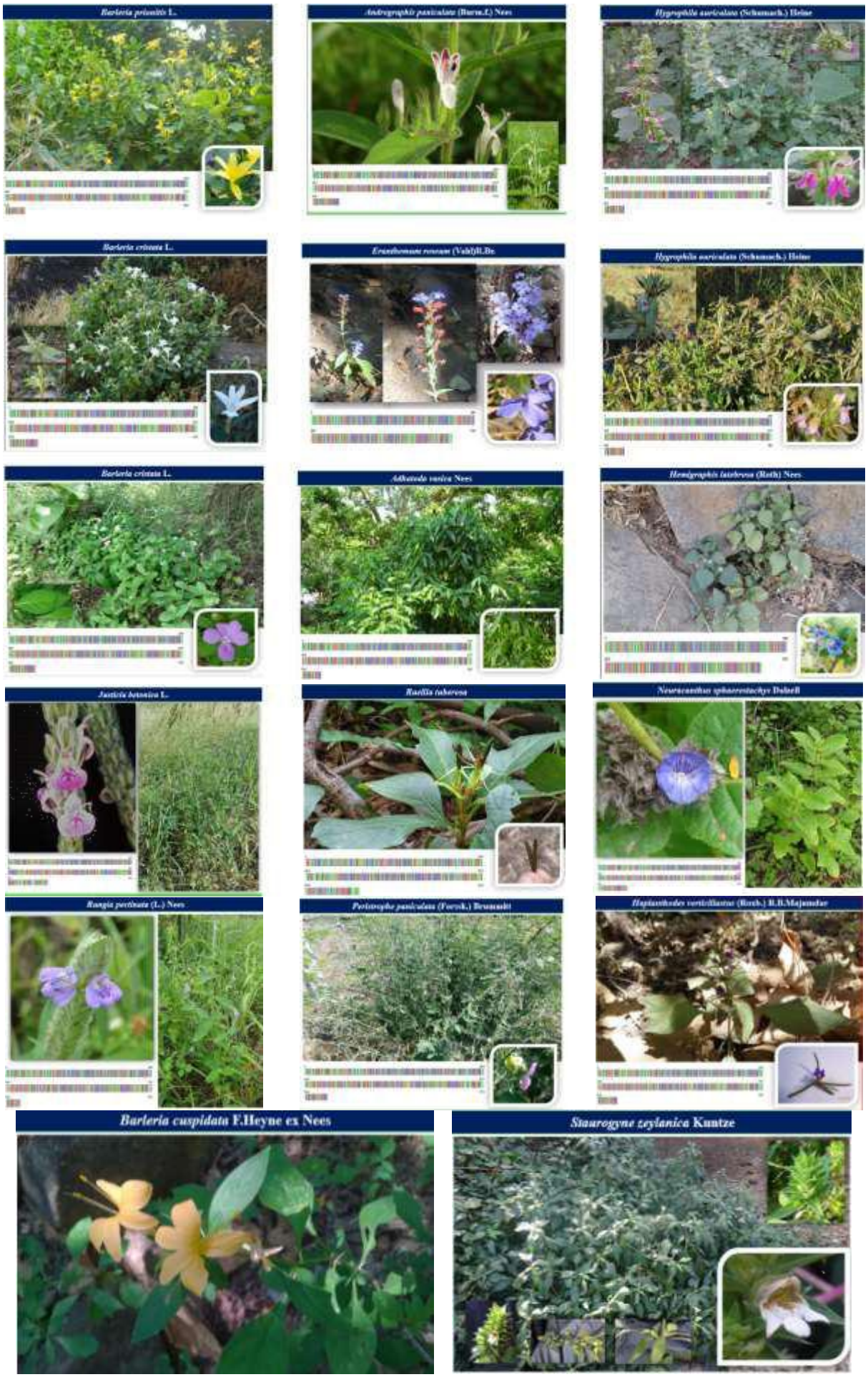

Images – 1 collected plants

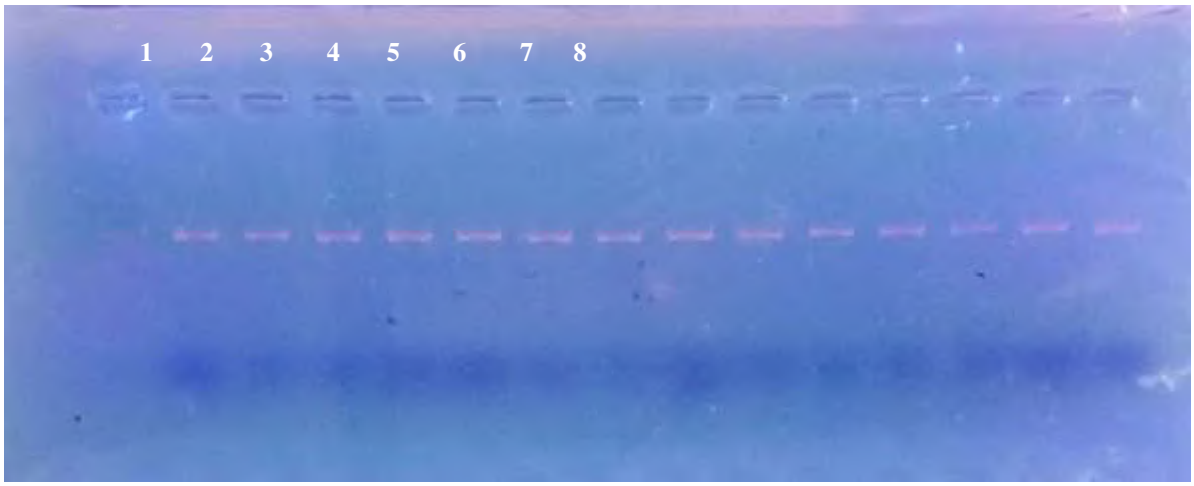

Figure – 1 Agarose gel electrophoresis of genomic DNA isolation

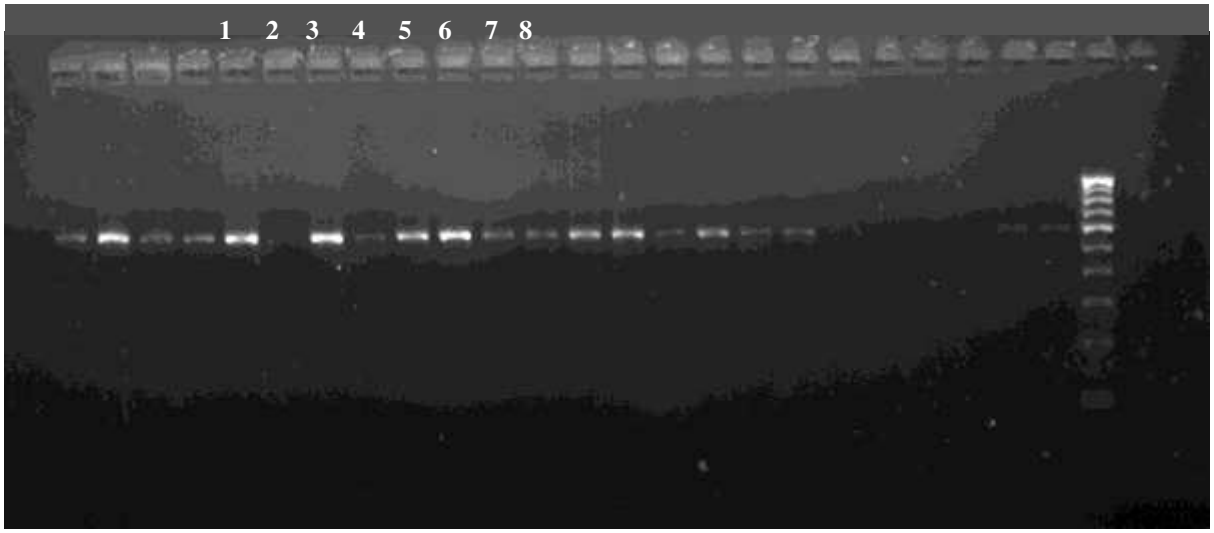

Figure – 2 Agarose gel electrophoresis of amplified DNA

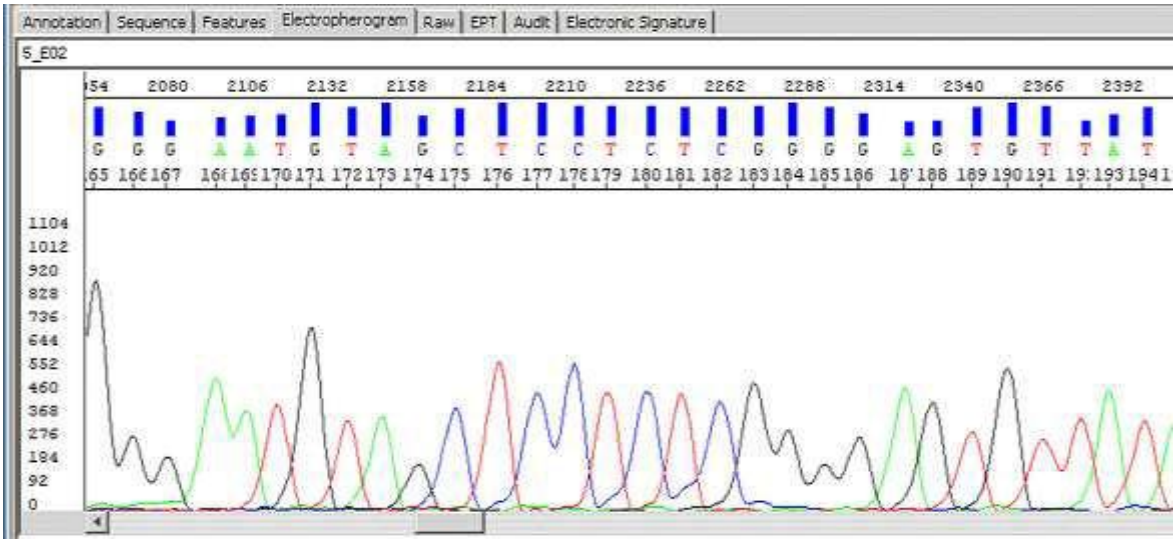

Figure – 3 sequencing result from ABI 3500XL sequencing analyzer (Chromatogram)

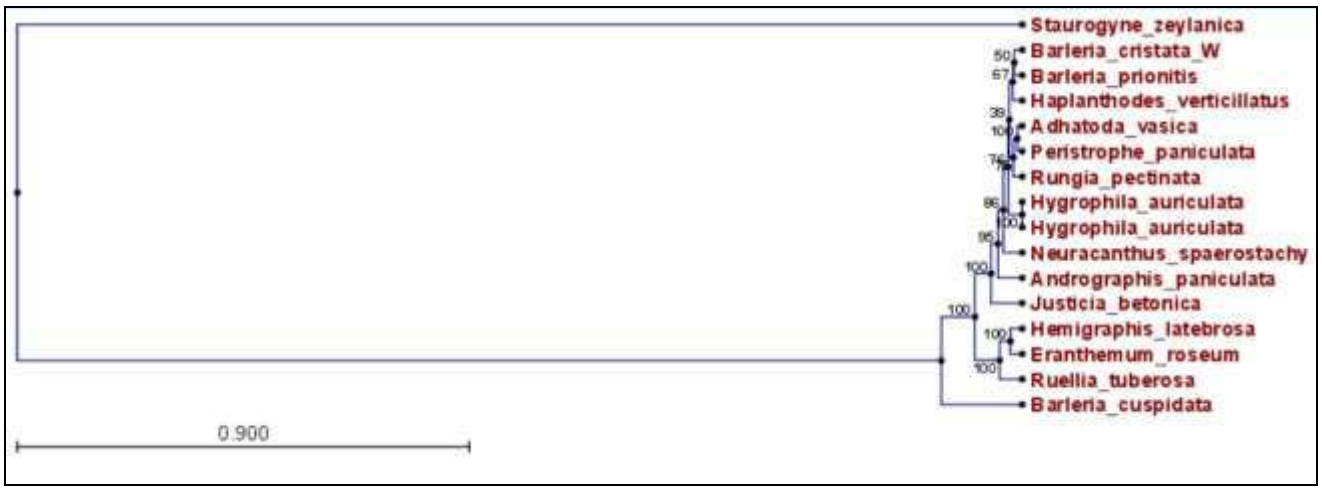

Figure – 4 Phylogenetic tree using CLC main workbench software

Sr. No.	Species Name	Identity	Location
1	Barleria prionitis and Barleria cristata	0.96%	chloroplast
2	Barleria cuspidata and Barleria criatata	0.78%	chloroplast
3	Barleria prionitis and Barleria cuspidata	0.77%	chloroplast

Table-1 Pairwise (local) alignment using Bioedit

National Conference on
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A Comparative study of physicochemical distinctiveness of Farmland soils of Dhangadhra and Chotila taluka territory, District: Surendranagar, Gujarat State, India

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Abstract: *A Comparative physicochemical study was performed for the agricultural land samples of two different talukas of Surendranagar district namely Dhangadhra and Chotila covering various parameter viz. pH, electrical conductivity (EC), total organic carban, available phosphorous and available potassium. It concludes the nutrients quality of soils of Dhangadhra and Chotila taluka territory, District: Surendranagar (Gujarat - India). As the present study includes various parameters, it helps farmers to take wise choice of fertilizer and its quantity thereby one achieve better crop production.*

Key Words: *Farmland soil, pH, EC, OC, Phosphorus, Potassium, and Surendranagar District.*

1. INTRODUCTION:

Soils are natural unconsolidated materials on the surface of the earth and are composed of solid, liquid and gas. They have organic and inorganic matter, which are well mixed together by natural processes. That is aggregated into a porous body that accommodates air and water [1]. Soil is one of the most important resources of the nature. Life cycles depend on plants that grow in soil for day to day need. Soil is not only important for agriculture but also for living organisms. Soil as a component of the terrestrial ecosystem fulfills many function including those that are essential for sustaining plant growth [2]. Soil fertility and plant nutrition are two closely related subjects that emphasize the forms and availability of nutrients in soils, their movement to and their uptake by roots and the utilization of nutrients within plants [3]. It is very difficult to enhance agricultural production in order to feed alarming population by avoiding soil fertility.

Any parts of earth surface that support vegetation also bears a covering of soil. The soil condition plays vital role for vegetation distribution and development [4]. Soil sampling is the most important because a very small amount of the soil mass is used for analysis and routine soil tests measure only a bit of the total lake of nutrients in the soil [5]. Soil mainly consists of 50% pore space (air and water) and 50% solid phase. The solid phase is broadly composed of 45% mineral matter and 5% organic constituents [6,7].

Available Potassium and Fertility Index of farmland soil of Danta taluka was recently reported Parmar, J. K. Total 3180 samples from 34 villages of Danta taluka was studied [8]. It reveals from literature that Physico-chemical study of soil is found significant importance in the farming practice. Soils have large amounts of nutrients essential for plant and agricultural product though only small fractions are in the form that can be uptake directly by plants. By interpretation of soil analysis data one can improve crop productivity and minimize wastage of these nutrients. Deficiencies of primary, secondary and micronutrients have been observed in severe cultivated areas [9]. Some efforts for the study of interested parameters for various areas was reported [10,11,12,13].

2, STUDY OBJECTIVES:

Present study is an effort to determine and compare the nutrient's quantity in soil of two Talukas *viz.* Dhangadhra and Chotila, District: Surendranagar Gujarat. With the help of this statistical information, Agriculture department recommend farmers for the usage of the quality and quantity of fertilizer to make the production

economic. The objective of this paper was to evaluate the trend in pH, EC, OC, P and K status of soils of area covered under study.

2.1 EXPERIMENTAL

The soil analysis data is the best source available to evaluate the fertility status of soil. Two Talukas of Surendranagar District, namely Dhangadhra and Chotila Taluka of Gujarat State are selected. For the present study, 4490 samples from 29 villages of Dhangadhra Taluka and 2173 samples from 51 villages of Chotila Taluka are collected. Collected samples are then crushed and sieved for further analysis for different chemical parameters following standard methods. AR grade reagents and double distilled water were used for soil analysis. Results were compared with standard values [13] to find out low, medium or high nutrient's content essential for STR.

The collected soil samples were analyzed for major quality parameters *viz.* pH, Electrical Conductivity (EC), Organic Carbon (OC), Available Potassium (K) and Available Phosphorus (P). Organic matter is oxidized with chromic acid and analyzed by colorimetric method [14,15]. pH, EC, and potash measured using pH meter, conductivity meter, Flame photometer respectively and OC and Phosphorus was measured using colorimeter in the laboratory. Table 1 depicts the analytical results for these parameters along with the standard values prescribed by Anand Agriculture University, Gujarat – India.

3. RESULTS AND DISCUSSION:

Soil reaction (pH)

The measurement soil pH is very essential to assess potentiality of beneficial nutrients and toxic elements available for plants. The soil reaction or pH is used to state the acidity or alkalinity of the soil. pH was measured by preparing aqueous soil extracts (1 part of soil: 2 part of deionized water). The data reveals that most of the samples of Chotila taluka lie in neutral pH range. But almost 30% of samples of Dhangadhra Taluka lie in alkaline range and is shown in Figure 1. Increased dilution of a soil/water mixture is responsible for an increase in soil pH [16].

Electrical Conductivity

EC express the presence soluble salts in soil. The soil extract prepared for determination of EC is the same as for pH determination. The data presented in Table 1 revealed the soil EC value of the soil samples on the basis of land use system *viz*. Dhangadhra and Chotila, District: Surendranagar Gujarat. Most of the soils of both the Talukas are fall in normal range as shown in Figure 2. Some of the samples lie in higher range and is due to soluble salts present in the soil.

Soil organic carbon

Soil organic carbon is the seat of nitrogen in soil and its determination is often carried out as an index of nitrogen availability. In the colorimeter method [14], Organic matter is oxidized with chromic acid. It reveals from the data depicted in Table 1 that percentage of average Organic Carbon value is same for both the region covered under study and the value is 0.42. It is shown in Figure 3.

Available Phosphorus

Phosphorus is one of the important essential nutrients and is utilized in the form of $H_2PO_4^-$ & HPO_4^{2-} by plant species. Colorimetric method was employed for the determination of Phosphorus. Phosphorus in form of orthophosphate plays an active role in aquatic ecosystem. Phosphorus was found in low to medium range (Table no.1) for the selected area. Figure 4 shows the graphical representation of available Phosphorous content present in the soil. Phosphorous content in soils of Dhangadhra (27.63) is less as compared to that of soils of Chotila (29.04).

Available Potassium

Flame Photometric Method was employed for the determination of available Potassium in terms of K_2O in soil samples. It is absorbed by the plants in large amount. It helps in building of proteins. Even a very small amount Potassium present in the soil, it plays very important role in metabolism of fresh water and is considered to be an important nutrient. Table 1 shows the medium to high range of Potassium content found for both the Talukas.

Table 1: Soil Characteristic data for samples of selected villages of Dhangadhra and Chotila Taluka

Parameters	Range		No. of Sample	
			Dhangadhra Taluka	Chotila Taluka
pH	<6.5	Acidic	20	17
	6.5 to 8.2	Neutral	2955	2154
	>8.2	Basic	1515	2
	Average pH Value		**7.84**	**7.10**

EC dS/cm	<1	Normal	3969	1843
	1 to 3	Harmful	419	301
	>3	Very Harmful	102	29
	Average EC Value		0.58	0.64
OC (%)	< 0.50	Low	2865	1530
	0.50 to 0.75	Medium	1582	631
	> 0.75	High	43	12
	Average OC Value		0.42	0.42
Phosphorous (P_2O_5) Kg/ha	<28	Low	2303	964
	28 to 56	Medium	2186	1209
	>56	High	1	0
	Average Phosphorous Value		27.63	29.04
Potash (K_2O) Kg/ha	<140	Low	2	4
	140-280	Medium	575	255
	>280	High	3913	1914
	Average Potash Value		415.61	505.72

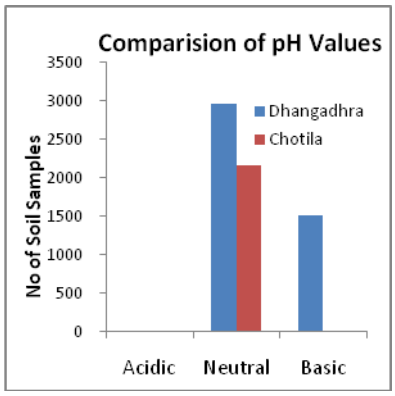

Figure 1: Comparison of pH values

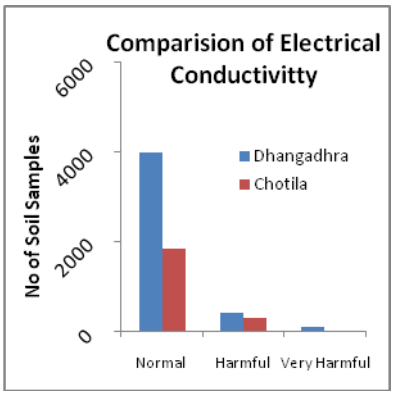

Figure 2: Comparison of EC

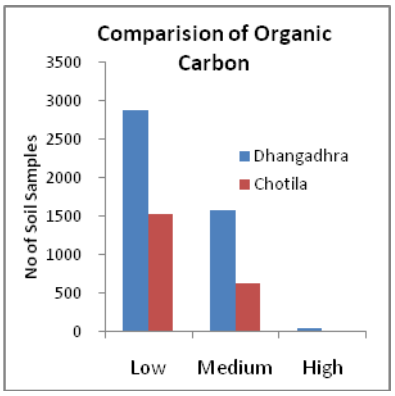

Figure 3: Comparison of Organic Carban

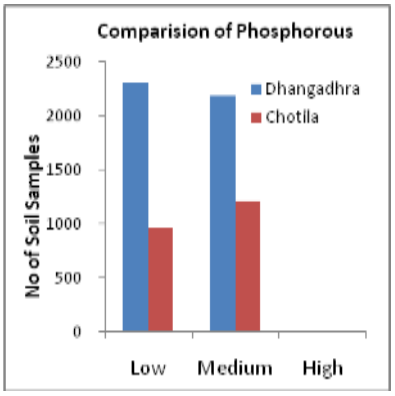

Figure 4: Comparison of Phosphorous (P_2O_5)

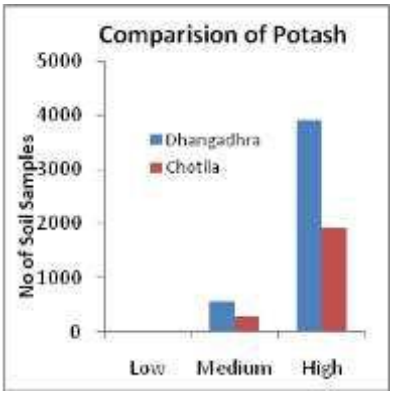

Figure 5: Comparison of Potash (K_2O)

4. CONCLUSION:

From the findings of the present comparative study, it can be concluded that the soils of the study area differ up to some extent in pH value according to type of land use system. Electrical conductivity values are found normal for both the Talukas. Soils are largely deficient in Organic Carbon for both the region. Phosphorus content is also found in low to medium range. The soils of both area found rich for Potash content. Results indicated that there is high

scope of improving the soil fertility status and productivity. Thus fertility status of soil is evaluated from this study for making fertilizer recommendations. Prediction of the probable crop response to applied nutrients is possible. By identification of the type and degree of soil related problems like salinity, alkalinity and acidity etc. It is also accomplished how to find out suitability for growing crops and orchard.

ACKNOWLEDGEMENT

I am thankful to Commissioner of Higher Education, Block No. 12, second floor, Dr. Jivaraj Mehta Bhavan, Gandhinagar and Department of Agriculture, Gandhinagar for the financial assistance.

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Characterization of Presence Available Potassium and Fertility Index of Indian Farmland Soil of Dhangadhra Taluka Territory, District: Surendranagar (Gujarat)

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Abstract: *Feature of land for agriculture purpose is based on analytical results of various parameters like pH, electrical conductivity (EC), total organic carbon, available nitrogen (N), available phosphorus (P2O5) and available potassium (K2O). The present study was performed during year 2017-18 under the flagship programme namely "soil health card (SHC)" of Government of India. This study leads us to the conclusion of available Potassium content in fertile soil of Dhangadhra Taluka territory of Surendranagar District, Gujarat (India). Almost all the sample of 29 different villages of Dhangadhra Taluka covered under the study fall into medium and high potassium content. The average fertility index for available potassium for the samples of this Taluka is 4.45. From these analytical data, one can decide which type of fertilizer and its quantity should be used for better crop production.*

Key Words: *Quality of farmland soil, pH, EC, OC, Phosphorus, Potassium, and Dhangadhra Taluka.*

1. INTRODUCTION:

Soils represent vibrant ecosystems, making it suitable to sense about them in terms such as health, liveliness and bioproductivity. Soils are the resources that supply humans with more than 90% of all the food we eat. To retain soils in sustainable manner is a challenge as it serves our needs in future. Use and location of the soil play vital role in measurement of soil parameters and properties. When estimating soil health, it is therefore common to investigate a range of soil physical, chemical, and biological properties (Brevik E. C.)[1]. Soil mainly consists of half of the pore space (i.e. air and water) and half of the solid phase. The solid phase is broadly composed of 45% mineral matter and 5% organic constituents (Gupta P. K., 2000; Kaur H., 2013)[2], [3]. Any parts of earth surface that support vegetation also bears a jacket of soil. Vegetation distribution and development largely depends on the soil condition [4]. Soil sampling is possibly the most crucial step for any soil analysis. Because a very small fraction of the soil is used for analysis, routine soil tests measure only a morsel of the total lake of nutrients in the soil [5].

Fertility of soil and plant nutrition are two closely related issues that accentuate the forms and ease of use of nutrients in soils, their movement to and their uptake by roots and the consumption of nutrients within plants [6]. Without maintaining soil fertility, it is hard to enhance agricultural production for feeding the alarming population. Hence, in order to get optimum crop production the soil fertility need to be maintained. The loss of soil nutrients is owed to cultivation practices. The flaming of plant residues as practiced under the traditional system of crop production or the annual burning of vegetation on grazing land are major contributors to the loss of nutrients [7], while the use of chemical fertilizer is also minimal.

Patel and Dabhi reported physicochemical studies of soils of ten different villages of Dahod, Gujarat, India. They collected total 90 samples with three depths of same site. The physicochemical properties such as moisture content, specific gravity, pH measurement, total organic carban, potash content and estimations of Mg^{+2}, Na^{+}, K^{+} and Cl^{-}, HCO^{-3}, PO_4^{-3}, NO_3^{-} % of soil were well studied [8].

Patel D. H. and Lakdawala M. M. reported their work which deals with quality of soil of Kalol and Godhra Taluka. Soil samples were collected from nineteen different villages of Kalol, Godhra Taluka. Physicochemical study

of soil is based on various parameters like pH, electrical conductivity, total organic carbon, available nitrogen, available phosphorus and available potassium were determined as per standard methods [9].

Recommendation of fertilizer addition is done on the basis of STR (Soil Test Recommendation). STR content determination major nutrients (N, P, K) following standard methods. Their values suggest quality of soil in terms of its nutrients contents i.e. high, medium or low nutrients. With the help of these data, farmer is recommended for quality and quantity of fertilizer addition to soil (Miller R. W. and Donahue R. L., 1995) [10]. One of the communication deals with quality of soil of Dahegam Taluka, Gujarat, India. Soil samples were collected from forty different villages of Dahegam Taluka. Physicochemical parameters of soil such as pH, Electrical Conductivity (EC), Calcium, Magnesium, Bicarbonate, Chloride, Total Organic Carbon, Available Nitrogen (N), Available Phosphorus and Available Potassium were determined as per standard methods. Results show that 20% soils are deficient in organic carbon whereas 95% soils are deficient in available potassium (Shah M. et al. 2011) [11].

2. STUDY OBJECTIVES:

Current study is an effort to find out the available potassium quantity in soils of Dhangadhra Taluka territory of Surendranagar District, Gujarat (India). This information will help farmers to decide the quantity of fertilizer to be noshed in soil to make the production economic. The objective of this paper was to assess the status of potassium in selected soil samples using the standard Flame photometric method for the determination of potassium. Thereby it is elaborated the trend in fertility status of farmland soils of Dhangadhra Taluka territory of Surendranagar District, Gujarat (India).

3. MATERIALS AND METHOD:

In present study, total 4490 soil samples from 29 villages of Dhangadhra Taluka, Dist: Surendranagar, Gujarat-India were analyzed for available potassium content. Soil sampling is the most crucial step for any soil analysis. It becomes tremendously essential to get a truly representative soil sample of the field. This is for the reason that a very small fraction of the huge soil mass is used for analysis. Hence, standard quadric procedure for sampling of soil samples was followed and samples taken in polythene bags. The soil samples were dried and crushed and then sieved with 2 mm sieve for the removal of stones, sand and other unwanted materials. In laboratory these samples were analyzed for available potassium content following standard method (Jackson M. L., 1967) [13]. For the depicted analysis, AR grade reagents and double distilled water were used. Results were compared with standard values [12] to find out low, medium or high nutrient's content essential for Soil Testing and Recommendation. Soil sample is shaken with 1N ammonium acetate (pH 7). Potassium ions absorbed on the soil colloids is replaced by ammonium ion during the extraction process.

$$\text{Colloid } K^+ + CH_3COONH_4 \longrightarrow \text{Colloid } NH_4^+ + CH_3COOK$$

K^+ is more effectively replaced by NH_4^+ as being the almost similar ionic radii. The extract is then filtered and potassium is determined with flame photometer.

5 gm of sample was added with 50 ml of 1 N ammonium acetate (pH 7) and was shaken for 30 minutes on a horizontal shaker. The resultant solution was then allowed to filter through 11 µm pore sized filter paper. Filtrate was then aspired to the flame photometer and output of the instrument was recorded. The available K_2O value can be calculated from this photometric reading by following standard mathematical formula. Based on the soil analysis result data for available Potassium, soil samples are generally categorized into three main categories i.e. low, medium and high nutrient's content and is revealed in Table 1. Using these fertility indexes was also calculated [12].

RESULTS AND DISCUSSION

Anand Agriculture University, Gujarat State of India shows permissible standards for available potash in the farmland soil which is shown in Table 1. Thereby soil samples selected in present study is categorized for low, medium or high content of potassium.

Table 1: Range of Low, Medium and High category of Available Potassium in the form of K_2O

Category	*Total Available potassium*
Low	<140 kg K_2O/ Ha
Medium	140-280 kg K_2O/ Ha
High	>280 kg K_2O/ Ha

The collected soil samples of Dhangadhra Taluka were analyzed for available potassium content and experimental values with their fertility index are depicted in the Table 2. This table describes the number of samples lies in three categories i.e. Low, Medium and High potassium content. This table also represents the calculated fertility index values for available K_2O of the soil for the villages selected in the current study.

Table 2: Study of Presence of available K_2O Content in the soil of selected villages of Dhangadhra taluka, District - Surendranagar

Sr. No	Name of village	No. of Sample	No of samples in falls in LMH Potassium content			Fertility Index
			L	M	H	
1	Kuda	149	0	38	111	4.09
2	Kondh	750	0	12	738	22.38
3	Koparani	59	0	35	24	1.42
4	Khambhada	142	0	82	60	3.44
5	Ganjela	137	1	1	135	4.08
6	Gala	97	0	0	97	2.91
7	Gopalgadh	38	1	25	12	0.87
8	Juna Ghanshyamgadh	80	0	15	65	2.25
9	Dudapar	103	0	23	80	2.86
10	Dumana	110	0	0	110	3.30
11	Dhangadra	251	0	3	248	7.50
12	Dholi	173	0	101	72	4.18
13	Narichana	250	0	2	248	7.48
14	Nimaknagar	12	0	9	3	0.27
15	Bavali	228	0	30	198	6.54
16	Bharada	263	0	32	231	7.57
17	Bhechada	113	0	47	66	2.92
18	Manpur	73	0	8	65	2.11
19	Malvan	291	0	2	289	8.71
20	Methan	299	0	0	299	8.97
21	Rajcharadi	208	0	4	204	6.20
22	Rajpar	73	0	59	14	1.60
23	Ravaliyavadar	149	0	21	128	4.26
24	Vasadava	85	0	7	78	2.48
25	Vaghagadh	84	0	9	75	2.43
26	Satapar	40	0	4	36	1.16
27	Sultanpur	78	0	1	77	2.33
28	Haripar	100	0	0	100	3.00
29	Hirapur	55	0	5	50	1.60
Total		4490	2	575	3913	---
Percentage (%)			0.04	12.81	87.15	---

For the farmland soils of selected villages, 3913 samples contain higher potassium content and 575 samples lie in medium range while only 2 samples found in low potash content. The analytical data is depicted in Table 2. The samples having high range of potassium content might be due to the unnecessary use of fertilizers. Rest of the samples falls in medium range is a sign of good quality of soil advocate sufficient amount of presence of available potassium and hence there is not any need to feed the soil with nutrient supplements. Results are in harmony with farming practices pursued by farmers of this region. Most of the farmer's are using chemical fertilizers like potash since last 30 to 35 years which contains concentrated amount of potassium, nitrogen, organic carbon and phosphorus. Phosphatic fertilizers are less preferred due to higher cost and rare availability. With the help of these results, farmers are advised through proper channel to use integrated nutrient management practice in order to maintain optimum concentration of all the essential nutrients for plants. They are also recommended for use of biofertilizers containing organic carbon and nitrogen solubilising bacteria. The graphical representation of the data plainly confirms the recent status of all 29 villages for the presence of potassium in their soil.

The village wise category for number of samples fall in low, medium and high content of available potassium is represented in Figure 1. This apparent from the Figure 1 that number of samples collected from the village and the status of available potassium level in that sample whether it has low, medium or high potassium content range. LMH data is further processed for fertility Index calculated as per the following equation.

$$Fertility\ Index = \frac{[(NL \times 1) + (NM \times 2) + (NH \times 3)]}{100}$$

Where, NL, NM and NH are number of samples fall in low, medium and high range of potassium content. Figure 2 shows the fertility index for available potassium and is finally used for recommendation of fertilizers and crop selection for farming.

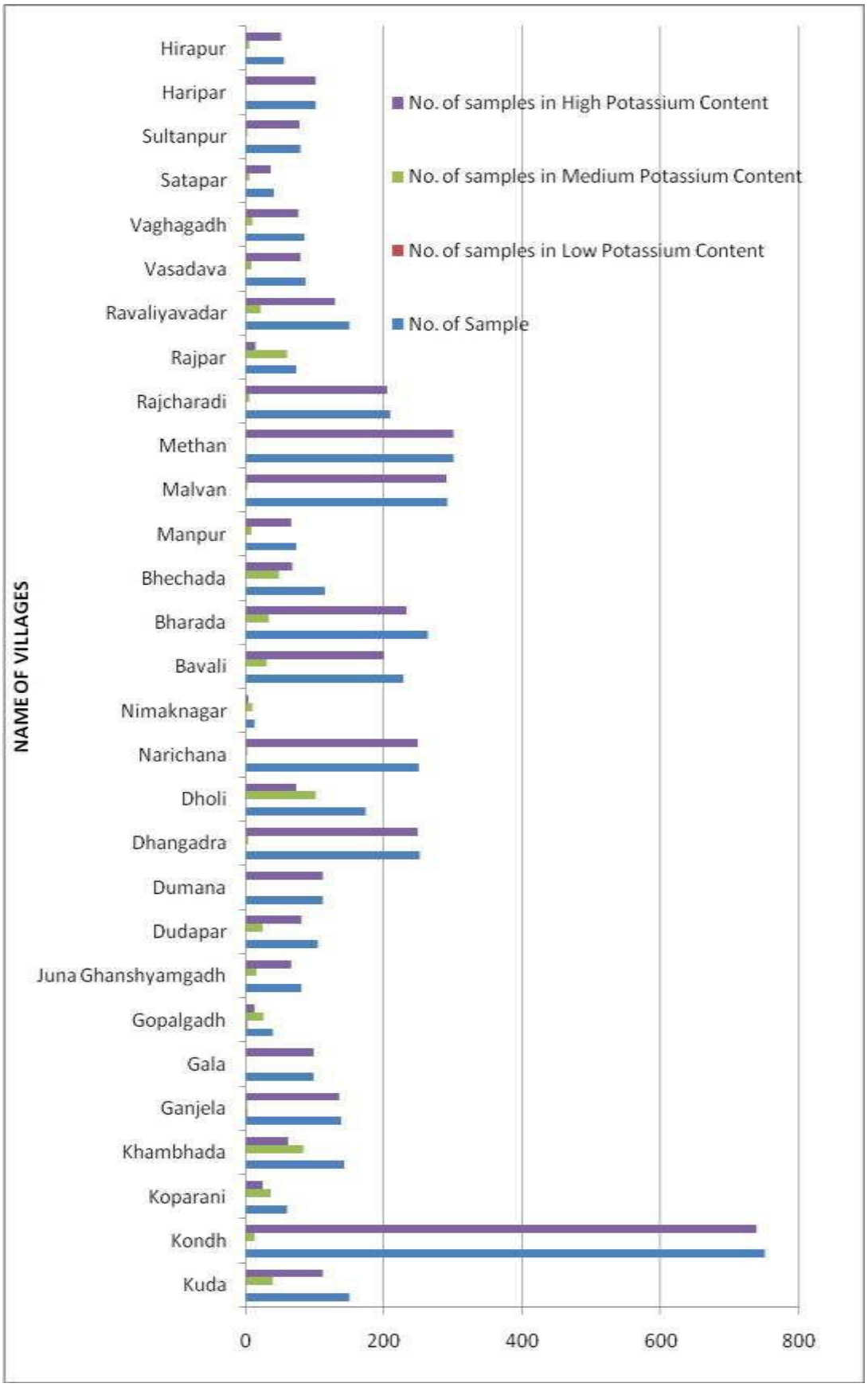

Figure 1: Numbers of samples of Dhangadhra Taluka lies in Low, Medium and High available potassium content range

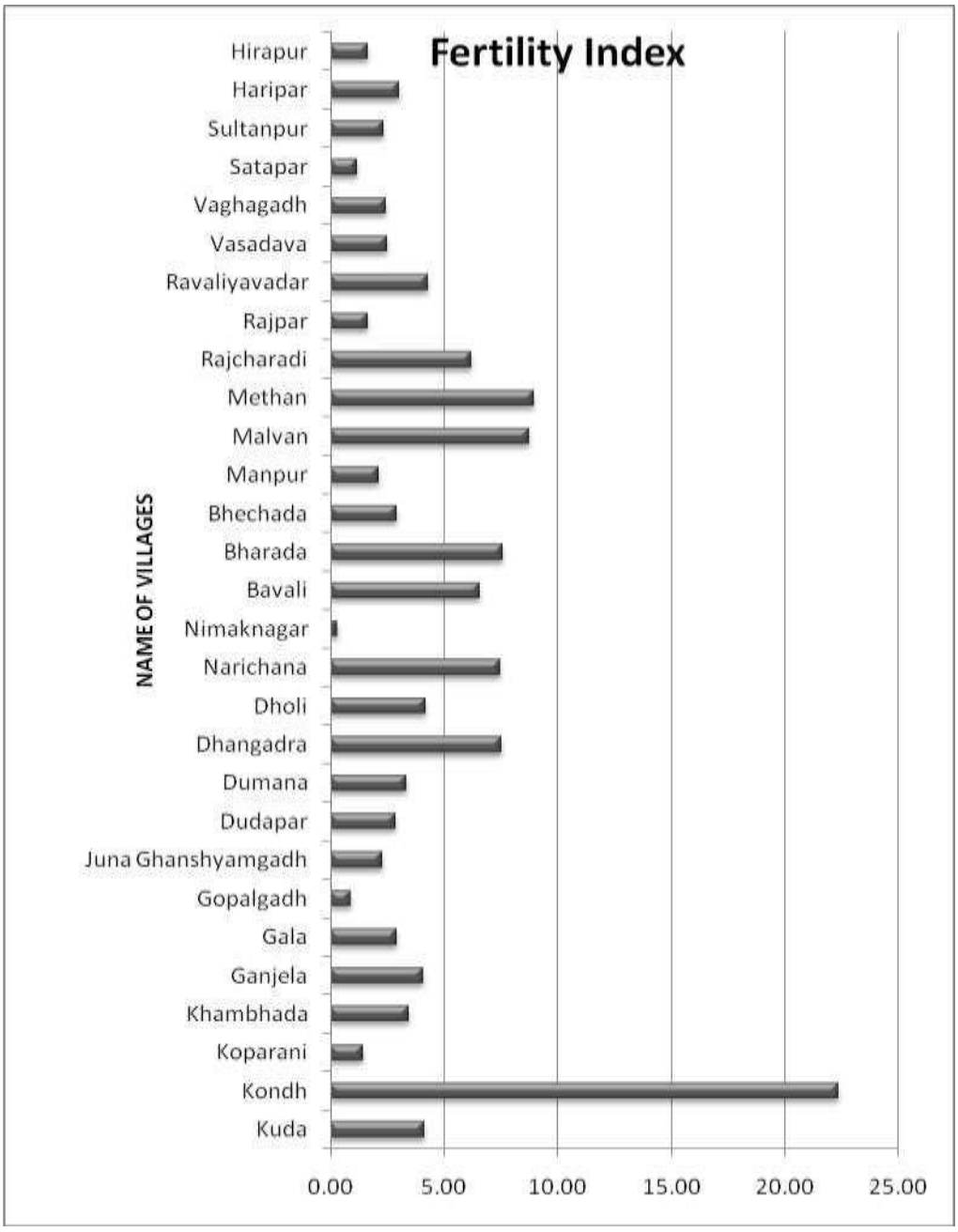

Figure 2: Fertility index for Potassium content of Dhangadhra Taluka territory of Surendranagar District

CONCLUSION:

Maintaining the soil chemical fertility is a key requirement to sustaining crop productivity and the soil organic matter depends on the quantity of organic material added to the soil either by natural returns through roots, stubble etc. or by chemical fertilizers. It can be concluded from the findings of current study that almost all farmland soils selected for the study were medium to high potassium content. The soil falls in medium range indicates good quality for farming practice. On the other hand, soils lie in high range of potash advocating the intensive use of unnecessary potash containing fertilizer. Results recommends for stopping the usage of potash rich fertilizer.

ACKNOWLEDGEMENT

I am thankful to Commissioner of Higher Education, Block No. 12, second floor, Dr. Jivaraj Mehta Bhavan, Gandhinagar and Department of Agriculture, Gandhinagar for the financial assistance.

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A Characteristic study of fertile soils by means of available potash and Fertility Index of some villages of Chotila taluka territory, District: Surendranagar, Gujarat (India)

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Abstract: *Fertility of farmland soils depends on its texture, temperature, chemical composition etc. Chemic composition of soil is very important as it fulfill needs of desire crop. The present work deals with the characteris study of available potassium (K_2O) in soils of fifty one (51) villages selected from Chotila taluka, Distri Surendranagar (Gujarat - India). We found high potassium content in soils of more than 80% farmers. The fertil index study was also carried out for these samples and the mean value is 1.23. The resultant data depict the necess of potash supplement in form of fertilizer for desire crop production.*

Key Words: *Quality of farmland soil, pH, EC, OC, Phosphorus, Potassium, and Chotila Taluka.*

1. INTRODUCTION:

Soil is a composed of minerals, organic matters, gases, liquids and countless organisms that supports life. Soil is an unconsolidated mineral and organic mineral on the immediate surface of the earth that contains living and non-living matter and serves as a natural medium for the growth of plants. Soil is an important matter for farming practice. Soil supplies many necessary nutrients required for healthy growth of a crop. Soil fertility is the inborn capacity of the soil to provide the essential nutrients for plant in sufficient amount and in proper proportion [1]. Soil is one of the most important resources of the nature. Soils are medium in which crops grow to feed and clothe. It is not only important for agriculture but also for living organisms. Soil as a component of the terrestrial ecosystem accomplished many functions including those that are essential for sustaining plant growth [2]. Vegetating earth surface bears a covering of soil. Distribution and development of vegetation depends largely on the soil condition [3]. Soil sampling is of very much important step for the soil analysis as it requires a very tiny proportion of the soil for analysis, from very huge part of the total lake of nutrients in the soil [4]. Soil mainly consists of 50% pore space (air and water) and 50% solid phase. The solid phase is broadly composed of 45% mineral matter and 5% organic constituents [5,6].
Available Potassium and Fertility Index of farmland soil of Danta taluka was recently reported Parmar, J. K. [7]. Literature exposes that physicochemical study of soil is found noteworthy importance in the farming practice. Soils have large amounts of plant essential nutrients but only small fractions are in readily available forms which can be absorbed directly by plants. Soil analysis can improve crop productivity by feeding required supplement to soil. Deficiencies of primary, secondary and micronutrients have been observed in exhaustive cultivated areas [8]. The study of major essential nutrients for various area was reported earlier to sensitize about soil health [9,10,11,12].

2. STUDY OBJECTIVES:

Present study is the investigation of Potassium content in the farmland soils of Chotila Taluka territory of Surendranagar District, Gujarat – India. This information is helpful to agriculture experts and officers to recommend farmers for the requirement of quality and quantity of fertilizer to be feed in soil to make the production economic. The objective of this paper was to assess the status of potassium in selected soil samples using the Flame photometric method for the determination of potassium. It is also studied the trend in fertility status of soils of Chotila Taluka.

3. MATERIALS AND METHOD:

The soil analysis data is the best source available to assess soil fertility status. All 51 villages from Chotila Taluka covering all four sides were selected for the present study. A representative soil samples were collected from each village which represent soils of 4 to 10 farm's depending upon area of village. Representative soil samples were collected by following standard quadric procedure and taken in polythene bags. The soil samples were then dried, crushed and processed to pass through 2 mm sieve. The samples were then analyzed for available Potassium content following standard method (Jackson M. L., 1967) [13]. All reagents employed for the analysis are of AR grade and double distilled water is used for soil analysis. Results were compared with standard values [7] to find out low, medium or high nutrient's content. Soil sample is shaken with neutral 1 N ammonium acetate. During the extraction process, ammonium ion replaces potassium ion absorbed on the soil colloids.

$$\text{Colloid } K^+ + CH_3COONH_4 \longrightarrow \text{Colloid } NH^{4+} + CH_3COOK$$

Because of similar ionic radii, K^+ is more effectively replaced by NH_4^+ ion. The extract is then filtered and potassium is determined with flame photometer.

3.1 EXPERIMENTAL SETUP

About 5 gm soil was scooped and added with 50 ml of 1 N neutral ammonium acetate solution and shaken for half an hour on a horizontal shaker. The content was filtered through whatman (Grade 1) filter paper. Filtrate was then aspirated in the flame photometer and reading was recorded. Blank reading was also taken. The available K2O value can be calculated with the help of mathematical equation given in standard method. Based on the soil analysis result data for available Potassium, soil samples are generally categorized into three categories i.e. low, medium and high and is depicted in Table 1. Fertility index was calculated on this basis [7].

4. RESULTS AND DISCUSSION:

Table 1 represents the range of low, medium and high potassium content as per standards of soil analysis; it is the permissible standard according to Anand Agriculture University. Soil sample were categorized into low, medium or high content of potassium by following these standard values.

Table 1: Range of Low, Medium and High category of Available Potassium in the form of K_2O

Category	*Total Available potassium*
Low	<140 kg K_2O/ Ha
Medium	140-280 kg K_2O/ Ha
High	>280 kg K_2O/ Ha

Table 2: Study of Presence of Potassium Content in the soil of Chotila Taluka territory of Surendranagar District

Sr. No	Name of village	No. of Sample	No of samples in LMH Potassium content			Fertility Index
			L	M	H	
1	Akadiya	33	0	0	33	0.99
2	Akala	57	0	56	1	1.15
3	Anandpur (Bha)	50	0	0	50	1.50
4	Bamanbor	37	0	0	37	1.11
5	Bhetasuda	27	0	0	27	0.81
6	Bhimgadh	22	0	0	22	0.66
7	Bhimora	28	0	0	28	0.84
8	Bhojapara	12	0	1	11	0.35
9	Bhojapari	30	0	0	30	0.9
10	Chanapaa	31	0	9	22	0.84
11	Chiroda	23	0	0	23	0.69
12	Chiroda (Thango)	31	0	7	24	0.86
13	Chobari	59	0	1	58	1.76
14	Chotila	115	0	44	71	3.01
15	Devapara (Aa)	48	0	0	48	1.44
16	Dhokadava	189	1	0	188	5.65
17	Dudheli	33	0	0	33	0.99
18	Golida	59	0	0	59	1.77
19	Janivadla	45	0	34	11	1.01

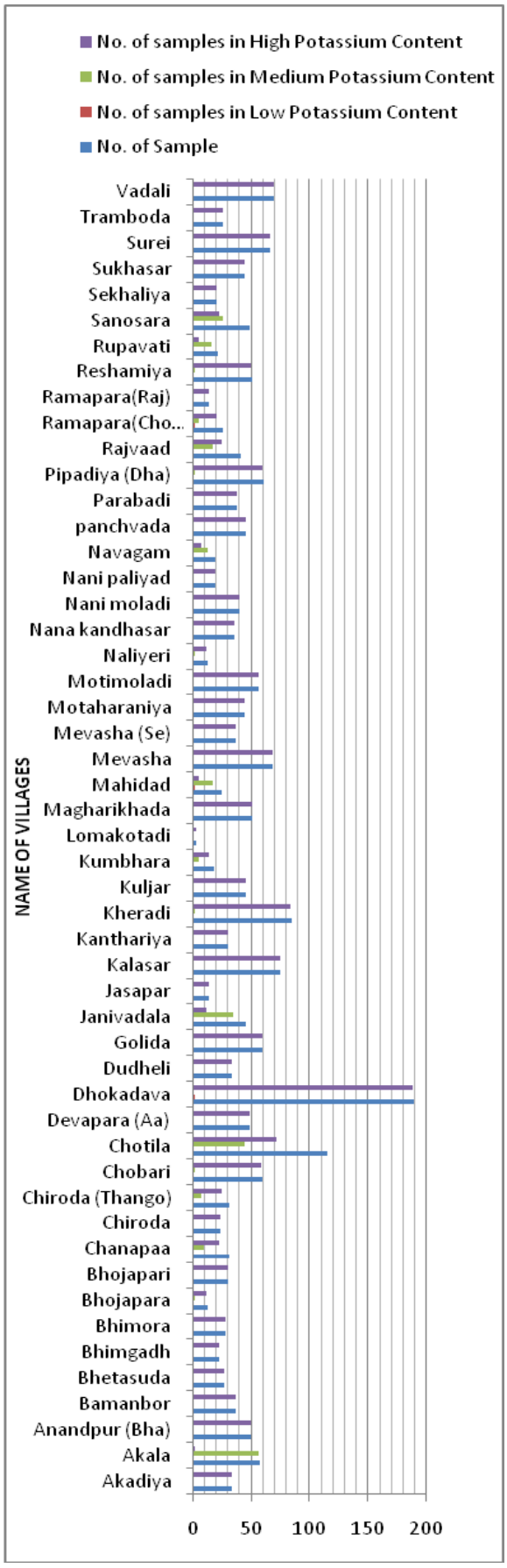
20	Jasapar	13	0	0	13	0.39
21	Kalasar	75	0	0	75	2.25
22	Kanthariya	30	0	0	30	0.90
23	Kheradi	85	0	1	84	2.54
24	Kuljar	45	0	0	45	1.35
25	Kumbhara	18	0	5	13	0.49
26	Lomakotadi	3	0	0	3	0.09
27	Magharikhada	51	0	0	51	1.53
28	Mahidad	24	2	17	5	0.51
29	Mevasha	68	0	0	68	2.04
30	Mevasha (Se)	37	0	0	37	1.11
31	Motaharaniya	44	0	0	44	1.32
32	Motimoladi	56	0	0	56	1.68
33	Naliyeri	12	0	1	11	0.35
34	Nana kandhasar	35	0	0	35	1.05
35	Nani moladi	40	0	0	40	1.20
36	Nani paliyad	19	0	0	19	0.57
37	Navagam	19	0	12	7	0.45
38	panchvada	45	0	0	45	1.35
39	Parabadi	38	0	0	38	1.14
40	Pipadiya (Dha)	61	0	2	59	1.81
41	Rajvaad	41	0	17	24	1.06
42	Ramapara(Chobari)	26	1	5	20	0.71
43	Ramapara(Raj)	14	0	0	14	0.42
44	Reshamiya	51	0	1	50	1.52
45	Rupavati	21	0	16	5	0.47
46	Sanosara	48	0	26	22	1.18
47	Sekhaliya	20	0	0	20	0.6
48	Sukhasar	44	0	0	44	1.32
49	Surei	66	0	0	66	1.98
50	Tramboda	26	0	0	26	0.78
51	Vadali	69	0	0	69	2.07
	Total	2173	4	255	1914	---
	Percentage		0.18	11.73	88.08	---

The data represented in Table 2 reveals the experimental values of available potassium of soil of the Chotila Taluka with their fertility index. The data describes the number of samples lies in Low, Medium and High potassium content. The same table represents the calculated values of fertility index for available K2O of the soil for all the villages covered in the study.

The soils of most of the village covered in the present study contain high potassium that might be due to the excessive use of fertilizers. Some villages *viz,* Akala, Janivadla, Mahidad, Navagam, Rupavati, Sanosara etc. lie in medium range indicates sufficient amount of available potassium in farmland soil and hence there is not any need to feed the soil with nutrient supplements. Results are in tune with farming practices followed by farmers of this region. Most of the farmer's are using chemical fertilizers like potash since last two to three decades which contains concentrated amount of potassium and nitrogen, organic carbon and phosphorus. On the basis of these results farmers are advised to use integrated nutrient management practice to sustain farmland soil for plants. Farmers are also advised to add biofertilizers containing organic carbon and nitrogen solubilising bacteria. The graphical representation (Figure 1 and 2) clearly confirms the recent status of all 51 villages for the presence of potassium in their soil. Figure 1 shows the village wise number of samples lie in low, medium and high content of available potassium. Graphical representation shown in Figure 1 disclosed that how many samples were collected from the village and what is the status of available potassium level in that sample *viz.,* low, medium or high Potassium content. Figure 2 shows the fertility index for available potassium. The LMH data is further processed for the calculation of fertility index as per the following equation.

$$\text{Fertility Index} = \frac{[(\text{NL} \times 1) + (\text{NM} \times 2) + (\text{NH} \times 3)]}{100}$$

Where, NL, NM and NH are number of samples fall in low, medium and high range of potassium content.



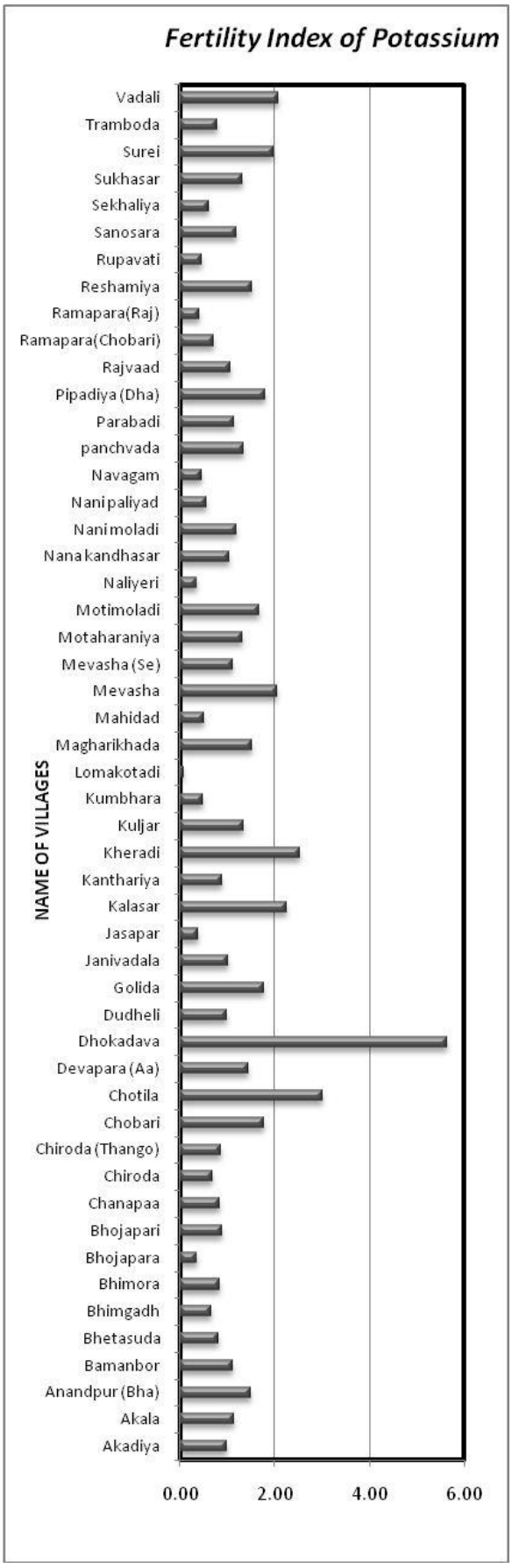


Figure 1: Numbers of samples of Chotila Taluka lies in Low, Medium and High available potassium content range

Figure 2: *Fertility index for Potassium content of Chotila Taluka territory of Surendranagar District*

5. CONCLUSION:

Farmland soil is sustained if one has knowledge about nutrient requirement by crop to be cultivated and the same nutrient available in soil for that crop. To enhance agriculture production, it is utmost demand by farmland soil and the vegetation to use fertilizer wisely rather than old farming practice. This study is helpful for recommendation of fertilizer in terms of quality as well as quantity to achieve better crop production and to sustain farmland soils by extensive cultivation. Soils lie in high range of potash is alarming the wide use of potash containing fertilizer which is more than enough. Results recommends for stopping the usage of potash rich fertilizer.

ACKNOWLEDGEMENT:

I am thankful to Commissioner of Higher Education, Block No. 12, second floor, Dr. Jivaraj Mehta Bhavan, Gandhinagar and Department of Agriculture, Gandhinagar for the financial assistance.

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"COMPARATIVE STUDY OF THE EFFECT OF CHEMICAL FERTILIZERS AND ORGANIC FERTILIZERS ON *EISENIA FOETIDA*"

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Abstract: *Today fertilizer has become essential to modern agriculture to feed the growing population. Chemical fertilizers are used extensively in modern agriculture, in order to improve crop yield. Urea is the most popular and widely used dry N fertilizer. The objective of the present study is to characterize the effect of fertilizers on the earthworm. The effects of soil fertilization with inorganic and organic fertilizers on earthworm rearing (populations, biomass, number of cocoons, juveniles etc.) were studied under different doses of the fertilizers for 60 days. When compared, marked changes were observed in the activity of Eisenia foetida in both type of fertilizers introduced. The present work indicates towards the deleterious effect of inorganic fertilizers on the survival of earthworm community in soil.*

Keywords: *Earthworms, organic fertilizers, chemical fertilizers, cocoons, biomass.*

1. INTRODUCTION:

Increased crop production largely relies on the type of fertilizers used to supplement essential nutrients for plants. Fertilizer application is required to replace crop land nutrients that have been consumed by previous plant growth with the ultimate goal of maximizing productivity and economic returns. Now a day, there is increased emphasis on the impact on soil environment due to continuous use of chemical fertilizers. The impact of chemical fertilizer application on agricultural land is seen not only in terms of the soil quality but also on the survival of soil organisms dwelling there in. Earthworms are major component of soil fauna in a wide variety of soils and climates and are involved directly or indirectly in biodegradation, stabilization through humus formation and various soil processes ([1] and [2]). Earthworms represent the greater fraction of biomass of invertebrate in the soil as soil macro fauna and play a vital role in structuring and enhancing plant nutrients and hence they can be successfully used as bio indicators for the evaluation of toxic risks of xenobiotic in terrestrial ecosystems [3]. Earthworm populations are influenced by various factors (soil, temperature, moisture, and pH) and the availability of organic matter for food, which may come from plant residues and animal or human waste applied to the land [4]. The abundance of earthworms in soils represents the health of soil ecosystems and the level of environmental safety ([5], [6] and [7]).
In literature, some researchers have concluded the chemical fertilizers to be harmful for soil organisms but on the contradictory they have been supported too to be beneficial as far as their food supply is concern. The acute toxicity of urea on *E. foetida* by using a simple paper contact method was studied, where the relative toxicity grade of urea was categorized as ―very toxic‖ to *E. foetida* [8]. According to reference [9], the inorganic fertilizers may also contribute indirectly to an increase in earthworm populations by increasing the quantity of crop residues returned to the soils, although the long-term use of inorganic nitrogen fertilizers may sometimes cause a decrease in earthworm abundance and biomass, particularly if it is ammonia-based [10]. Other study which measured earthworm activity in mineral fertilizer by considering casting frequency of the worm found higher levels of casting activity in plots with inorganic fertilizers alone and with combination with organic matter than the control plots [11].

The general recommendation for urea is 120kg/hectare in agricultural lands as per the Indian soil testing manual released in 2011 by the Department of Agriculture, Ministry of Agriculture, India. But Indian farmers overuse urea to achieve more productivity ignoring the negative effects on soil organisms particularly the earthworms. Our

study was a short term investigation aimed to find out whether there is some difference in the effect of inorganic and organic fertilizers on earthworms, which are counted among the vital soil organisms favorable for maintaining fertility of soil. Urea was selected as the inorganic fertilizer and an organic fertilizer namely = Kala Sona‘ in the local market was used for this purpose.

2. MATERIAL & METHODS:

Experimental Model: Earthworms (*E. foetida*) were procured from the vermicomposting unit of Rajasthan College of Agriculture, Udaipur. They were maintained in the laboratory conditions and acclimatized for 15 days. The worms used in the experiment were approximately same body weight and body length.

CHEMICALS USED:

1) **UREA (46% N):** The inorganic fertilizer used in the experiment was Urea which was purchased from the local market. Once applied to the soil, urea is converted to ammonia, which reacts with water to form ammonium ions within two to three days (faster under warm conditions).
2) **KALA SONA (Humic Acid 95%):** Kala Sona is a unique soil conditioner, a naturally occurring organic substance consisting primarily of humic acid and minor levels of minerals, gypsum and clays. It eases organic material incorporation to the soil, accelerating its decomposition and nutrient utilization and eventually increases the carbon content of the soil.

Preparations of soil beds: The experiment was conducted as per method given in reference [11]. Plastic tubs were used for preparations of soil beds. Dried soil (from nearby farmland) was crushed and filtered through a fine mesh sieve. One kg of fine soil was then poured in each plastic tub and then water was added to moistened the soil then 250gm dried powdered (3 week old) cow dung was also added to each plastic tub to avoid starvation.

Addition of Urea: The Urea dose being practically applied in the local agricultural lands for the *Kharif* crop was found to be 174 kg/ hectare of land area. Here, in our experimental set up the soil bed contained 1 kg of soil and cow dung mixture made in the ratio of 1:1. Therefore, the calculated value of Urea for the soil bed was 3.48gm/ kg of soil. In addition to the dose being practiced by the farmers i.e. 3.48gm/ kg, three more doses of Urea were set viz. 0.75gm/ kg, 1.5gm/ kg, and 2.25gm/ kg.

Addition of Kala Sona: The dose of Kala Sona being used by the farmers was 4.5 kg/ hectare and so for our experimental set up the calculated dose of this organic fertilizer was 0.45gm/ kg of soil. One more dose of Kala Sona 0.9gm/ kg was set as the experimental dose.

Experimental set-up: 20 mature earthworms (same age group) were added to each plastic tub. The tubs were covered with wet muslin cloth so that the moisture level needed by the worms is maintained and also it will prevent them to crawl out of the tub. Thus one control set and five experimental set were prepared. 3 replicates were used for each set. To maintain up-to 70 percent moisture level water was supplied regularly. After 15, 30, 45 and 60 days the changes were observed in activity, morphology, growth of earthworms as well as the number of cocoons and juveniles were also counted.

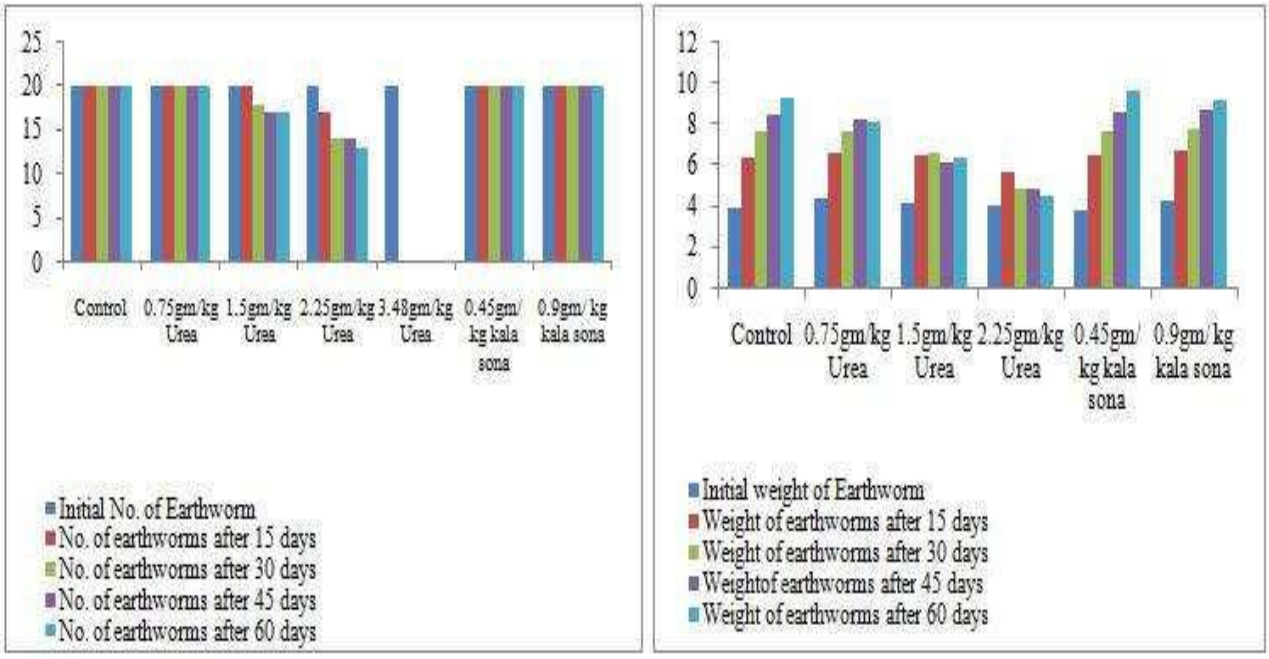
3. OBSERVATION:

The chemical fertilizer urea was found to be fatal for the earthworm population when the dose reached more than 1.5gm/kg soil. A parallel control experiment set was also set to compare the changes in the behavior and morphology. The morphological change observed in the different sets of our experiment has been discussed below. The initial number of earthworms in all the set up along with the control set was 20. At the end of 60th day, all the 20 earthworms were alive in the control set up and in both the organic fertilizer soil bed. In addition, under the dose of 0.75gm/kg urea, the earthworms were also safe. But, as the dose increased from 0.75gm/kg to 1.5gm/kg and 2.25gm/kg, mortality among the test animal was seen.

		Control	0.75gm/kg	1.5gm/kg	2.25gm/kg	3.48gm/kg	0.45gm/ kg	0.9gm/ kg
Days	**Parameters**	**set**	**Urea**	**Urea**	**Urea**	**Urea**	**Kala Sona**	**Kala Sona**
On 1st day	Number	20	20	20	20	20	20	20
	Biomass of alive							
	worms (gm)	3.96	4.44	4.21	4.07	4.19	3.88	4.31
	Biomass/							
	individual (gm)	0.19	0.22	0.21	0.20	0.20	0.19	0.21
								20
On 15th day	Number	20	20	20	17	0	20	
								6.72
	Biomass (gm)	6.41	6.64	6.53	6.74	NA	6.48	
	Biomass/							
	individual (gm)	0.32	0.33	0.32	0.39	NA	0.32	0.33
								7
	Cocoons	8	10	7	8	NA	6	

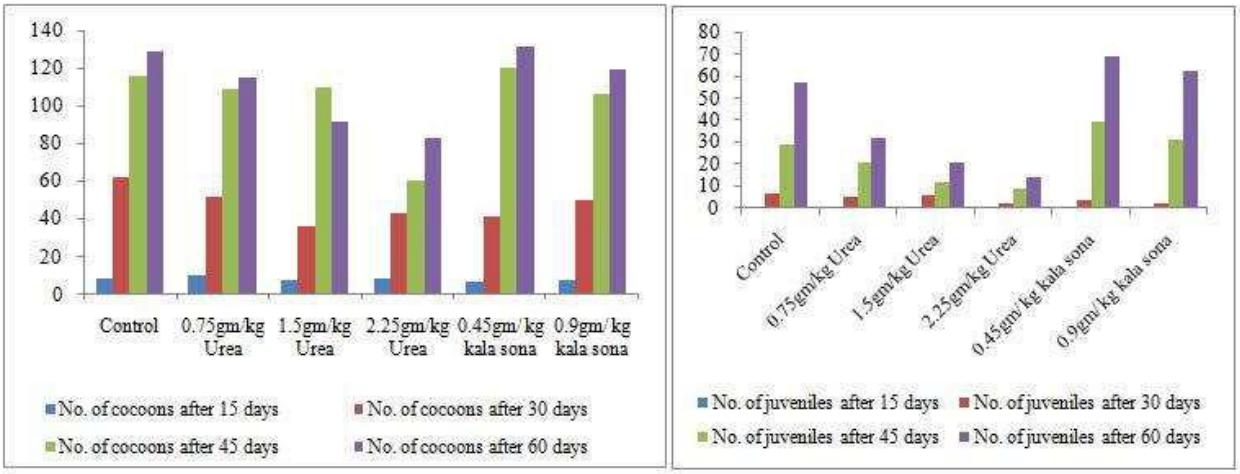
On 30th day	Number	20	20	17	14	0	20	20
	Biomass (gm)	7.74	7.66	6.61	4.95	NA	7.72	7.80
	Biomass/ individual (gm)	0.38	0.38	0.39	0.35	NA	0.38	0.39
	Cocoons	62	51	36	43	NA	41	50
	Juveniles	7	5	6		NA	4	2
On 45th day	Number	20	20	17	14	0	20	20
	Biomass (gm)	8.56	8.33	6.12	4.87	NA	8.67	8.71
	Biomass/ individual (gm)	0.42	0.41	0.36	0.34	NA	0.43	0.44
	Cocoons	116	109	110	60	NA	120	106
	Juveniles	29	21	12	9	NA	39	31
On 60th day	Number	20	20	17	13	0	20	20
	Biomass	9.31	8.18	6.45	4.55	NA	9.63	9.20
	Biomass/ individual	0.46	0.40	0.37	0.35	NA	0.48	0.46
	Cocoons	129	115	91	83	NA	131	119
	Juveniles	57	32	21	14	NA	69	62

Apart from the observed mortality among the test animal, the changes in weight was noticed in the worms which were able to survive throughout the study period under the higher doses of urea than 0.75gm/kg. The weight of the earthworms was found to be increasing in the starting days of the experiment but after one month a steady pattern of weight gain was observed in urea dose of 2.25gm/kg and also the earthworms in this set was seen weakened in the later days.The initial increased weight of earthworms in this set from rest of the sets can be due to swelling of earthworm‘s body. On the other hand, there was found appreciable weight gain of the worms under both the organic fertilizer _Kala Sona‘ set during the whole study than the control set.



When the weight gain by earthworm on the 60th day was taken into consideration, highest weight was noted at the end of experiment in the organic fertilizer set of dose 0.45gm/kg and least in 2.25gm/kg urea set. The variation in the weight change was then analyzed on the per individual basis which is shown in table-1. The number of cocoons was highest in the control set and least in urea dose of 2.25gm/kg. Next to the control set the cocoons were found greater in the Kala Sona organic fertilizer dose of 0.45gm/kg. The control set had the highest number of juveniles

compared to the rest of experimental set up. In the Urea dose of 2.25gm/kg the numbers of juveniles were only 14 which was the least among all the set up this can be attributed to the least number of cocoons in this set of urea dose.



At the urea dose of 3.48gm/kg soil, the entire worms were found dead within 24hrs which is shown in the photograph. Shrinking of earthworm body and rupturing of the epidermis and cuticle with the secretion of yellowish fluid was observed in this dose of urea. Least number of earthworms i.e. 13 were left in the soil bed treated with urea dose of 2.25gm/kg at the end of experiment. Although, these number of worms were alive in this experimental set up but the worm‘s body was found weakened with less body weight. The worms in this set dose were found trying to escape out from the tub; this has also been in fig.-3(c).

At the end of our experiment, the healthiest earthworms were seen in the experimental set up with the organic fertilizer _Kala Sona‘ and the weakened were the chemical fertilizer urea treated worms. Healthy numbers of cocoon and juveniles were counted in the organic fertilizer treated sets.

4. RESULTS AND DISCUSSION:

Urea is the most commonly used nitrogen fertilizer worldwide. The overused urea in agricultural fields may affect the soil organisms especially, the earthworms which are known well for their role in soil fertility [17].The experiment conducted during this investigation revealed many interesting facts. The chemical fertilizer Urea was found to be quite toxic to the earthworms. Different doses of Urea was administered to the soil and simultaneously one organic fertilizer = Kala Sona‘ was also used at two doses to conduct a perfect comparison of the two types fertilizer on the earthworm activity. A control set up was run parallel to the experimental set up. There were significant changes in the mortality and weight of tested earthworms after exposure to Urea.

There was a positive correlation between earthworm mortality and the concentration of Urea added to soil. The mortality reached 100% when the dose of Urea reached 3.48gm/kg which is the actual dose being practiced by the farmers in the agricultural land. The weight of earthworms exposed to Urea decreased steadily with the increase in the dose of Urea. The loss in body weight changed with increased exposure time. The sharp decrease in weight of earthworms revealed that the high concentration of urea was very toxic to the worms or it could be lethal for the total population. However, at the low concentration of urea there was no significant change in morphology except reduction in body weight.

The major effects on the earthworms could be seen in terms of the number of adult worms, their biomass and cocoon production which clearly indicates the general health of earthworms. The counting of the number of cocoons and juveniles was also done to estimate the harmful effects on their reproductive activity in the two types of fertilizers. The Juvenile and immature worms were also found to be influenced by urea application. Other studies recorded a drastic decrease in earthworm populations and biomass in grassland soils treated only with nitrogenous fertilizers [18]. Healthy earthworms in the organic fertilizer set up can be attributed to the fact that the organic fertilizers probably provide food directly for the earthworms and this might be the reason for the higher earthworm populations in the pots treated with organic matter [19]. Other researchers found similar or different results on this topic. Studies showed that

the applications of fertilizers with nitrogen and phosphorous caused significant increases in earthworm number and bio-mass in an oxisoil from India [20].

Similar results were obtained by reference ([21], [22] and [23]). On the contrary, some studies found a significant reduction of earthworm population in certain conditions; it was reported that organic N had a greater effect on earthworm populations than inorganic N ([24], [25] and [26]). In reference [27], a negative impact with ammonium nitrate on grasslands has been reported. On the other hand, study in reference [28] found that urea had a beneficial effect on earthworm activity. Studies indicate, however, that the form and amount of mineral fertilizer can have negative effects on earthworm populations. For example, ammonium sulfate and sulfur coated urea, which at high doses can lead to soil acidification which decreases earthworm populations. Fertilizers with nitrogen create acidic conditions in soil, which is fatal for earthworm ([29], [30] and [7]).

In our experiment, mortality of all the 20 earthworms were seen in the dose of urea which is practically been applied in the agricultural land by the farmers i.e. 3.48gm/kg soil. The deleterious effects of urea on earthworm were found under this dose within 24hrs in the form of all dead worms with lesions and separation of the posterior body parts. Urea had a strong toxic effect on the earthworm *Eisenia foetida*, this can be as it exerted its toxic action by way of skin infiltration.

In rest of the doses variation in number, biomass of worms and the number of cocoons and juveniles were observed which indicates that the inorganic fertilizer had a sound effect on the reproductive potential of the earthworms. On the other hand, the organic fertilizer ‗Kala Sona' was found to have positive effect on all the parameters studied of the worms.

5. CONCLUSION:

This work is thus to examine the effects of mineral fertilizer urea and organic fertilizer ‗Kala Sona' on earthworm *Eisenia foetida*. The application of environmentally realistic doses of urea revealed the possible harmful effects on earthworms when applied in the laboratory. The results of the present investigation clearly demonstrate that treatment with inorganic fertilizer urea is very harmful for *Eisenia foetida*. Whereas, the organic fertilizer Kala Sona was found to have a favorable effect all over. This study may be useful to evaluate ecological risk from agricultural activities such as the application of agrochemicals, and to avoid ecological damage from inappropriate application of chemical fertilizers. Therefore, it is advised that the use of nitrogenous fertilizer like urea should be within ecologically safe limits.

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National Conference on
'Inclination of Modern Science toward Environmental Protection and Sustainable Development'
23rd January, 2019
Jointly organized by
Bahauddin Science College, Bhakta Kavi Narsinh Mehta University, Junagadh, Gujarat, India and
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"Self Electricity Generation and Energy Saving By Solar Using Programmable System on Chip (PSOC)"

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Abstract: *New technological development efficiency of solar cell is increasing everyday and it is becoming cheap al for use in regular day today production of energy. Along with this government is also providing help and subsidies set up of new solar power plant. This all things together provide a great new platform for setup and generation new solar power plant for individual customer. This paper will try to bring all component and aspects of generati energy from solar cell easy, convenient and in user friendly way for non technical person, by using lat development in the field of electrical and electronics.*

Keywords: *Psoc. MPPT (Maximum Power Point Tracking), Solar Panel.*

1. INTRODUCTION:

Basically from solar cell & wind mill gives the DC voltage output store in the battery & through inverter circuit it gives 230v AC output which can use for building surrounding lights & stair case lights & as well as main load. Under current acute power shortage scenario with increasing cost of natural gas, coal and other power generator turbine fuel, there is a very urgent and great need of finding alternate source of energy to generate electricity. Of all the renewable energy sources, solar energy received the greatest attention in the decade of the 1970's and has been the rub of much emotion and pleasure. Many regarded it is as the solution for reducing the use of fossil and nuclear fuels and for a linear environment solar energy as a result has been the object of inflated. Overly optimistic predictions ranging from largely supplementing to eventually replacing all the current means of production of both electric power and thermal energy requirements. Solar energy, in sheer six does have the potential to supply voltage energy needs Electric, thermal chemical even transportation fuels .it is however, very diffuse cyclic and often undependable. Although solar energy may be used in many markets such as in active and passive space heating and cooling industrial process heating ,desalination and in electric generation.After investments by federal and agencies amounting to several hundred million dollars in the 1970's and all these technologies, only one is in commercial use today. Namely flat plate collectors for water heating

All other remain in various stages of research and development .It now appears that solar electric systems are not expected to make engineering and economic sense as central generating plants of hundreds of mega watts capacities in the foreseeable future. Solar panel & wind mill are the main source. Basically from solar cell & wind mill gives the DC voltage output store in the battery & through inverter circuit it gives 230v AC output which can use for building surrounding lights & stair case lights as well as main load.The system was designed and implemented with the following goals to be completely different from traditional electricity labs and to be fresh and interesting.To be intimately related to real world industrial power issues such as power quality. To show a complex, interrelated system that is closer to the "real world" than the usual simple systems covered in educational labs.

To motivate learning by introducing such elements as environmental and economic concerns of practical interest to the students. This paper describes a new PSoC microcontroller based PV (photovoltaic) system. Because the energy from the sun, fluctuates with climate conditions, the impedance of the PV system must be adjusted to match the change in lighting condition. To do this, we employ a PSoC microcontroller which can handle both analog and digital circuits, to reduce the need for additional circuit elements. The PSoC controller is used as a programmable Maximum Power Point Tracking (MPPT) controller. The intermittent nature of solar and wind power can be effectively mitigated by using a solar and wind hybrid system. Energy storage on-site (batteries) ensures that power is

available when the sun isn't shining or the wind isn't blowing. Pairing solar and wind collection systems at one site can provide diversity protection against the variable natures of both energy sources.

2. LITERATURE REVIEW:

The literature in the subject areas of this is very extensive. An excellent textbook for instructional use is Wind and Solar Power Systems by Patel (1999) that covers the specific issues in this project in a style appropriate for Industrial Technology students. Sabin (1999) and coworkers have summarized the various standards and benchmarks used in large-scale power quality, and Koval (1999) and coworkers have presented similar finding for rural (small-scale) power quality problems. Taylor (1987) is responsible for some of the early practical work on power quality measurements in wind generation.

Kariniotakis and Stavrakakis (1995) have written extensively on simulation problems in wind generator and power grid interactions. Finally, many papers have been written on the electronics regulation/ control aspects of the problem including a recent study by Neris and co-workers (1999) proposing an IGBT (Integrated-Base-Bipolar-Transistor) based regulator [3].

Many articles have appeared on the impact of new electronics technologies on power quality management, for example Poisson (1999) and coworkers have described the impact of DSP chips on the problem.

Numerous technologies hold promise for harnessing and utilizing the sun's energy. They include: agricultural and architectural technologies, solar lighting, solar thermal, HVAC, solar cookers, electricity generation, solar to petroleum, transportation, and satellites to mention a few.A number of well-known large corporations have launched initiatives to generate their own energy for a variety of reasons, such as reducing energy price volatility, increasing security of supply, decreasing costs or meeting carbon objectives. Example - companies with their own renewable energy generation include IKEA, Google, Toyota, Toshiba, Hertz, FedEx, AT&T, BMW, Renault, VW, Audi and PepsiCo. VW, for example, is investing €1b in offshore wind projects to meet renewable energy objectives and provide a natural hedge against volatile energy prices. That said, a third of respondents expect to meet a greater share of their energy needs through self-generation over the next five years [12].

Now a days the big problem of electricity. There is no alternate source available in emergency. Thus we are trying to reduce the problems of electric energy by using the natu ral source like solar power & wind power.

In order to obtain the maximum power from the PV cell as shown in Fig. 1, we need to find and control the maximum power point voltage (VMPP) which may vary depending on the sunlight conditions. To obtain the maximum power, we apply the constant voltage method to find MPPV from the open-circuit voltage (V_{oc}).

3. PROBLEM STATEMENT:

The proposed system is to reduce the problems of electric energy by using the natural source like solar energy. The solar cell give the DC voltage output to store in the battery & through inverter circuit , it gives 230V AC output which can use for building surrounding lights, stair case lights as well as main load. The actual proposed system designed by using solar installation.

This proposed system uses PSOC microcontroller based (photovoltaic) because the energy from the sun fluctuates with climate conditions; the impedance of the PV system must be adjusted to match the change in climate condition. In this system, PSOC microcontroller can handle both analog and digital circuits, to reduce the need for additional circuit elements. PSOC is an application related review of programmable array systems & the system-on-chip.

4. WORKING PRINCIPAL:

AS people are much concerned with the fossil fuel exhaustion and the environmental problems caused by the conventional power generation, renewable energy sources and among them photovoltaic panels and wind-generators are now widely used. In this we are using solar panel & wind mill are the main source. Basically from solar cell & wind mill gives the DC voltage output store in the battery & through inverter circuit it gives 230v AC output which can use for building surrounding lights & stair case lights & as well as main load.

5. METHOD:

- **Photovoltaic technology:** Photovoltaic is the field of technology and research related to the devices which directly convert sunlight into electricity. The solar cell is the elementary building block of the photovoltaic technology. Solar cells are made of semiconductor materials, such as silicon. One of the properties of semiconductors that makes them most useful is that their conductivity may easily be modified by introducing impurities into their crystal lattice.

The intermittent nature of solar or wind power can be effectively mitigated by using a solar or wind system. Energy storage on-site (batteries) ensures that power is available when the sun isn't shining or the wind isn't blowing.

Pairing solar and wind collection systems at one site can provide diversity protection against the variable natures of both energy sources.

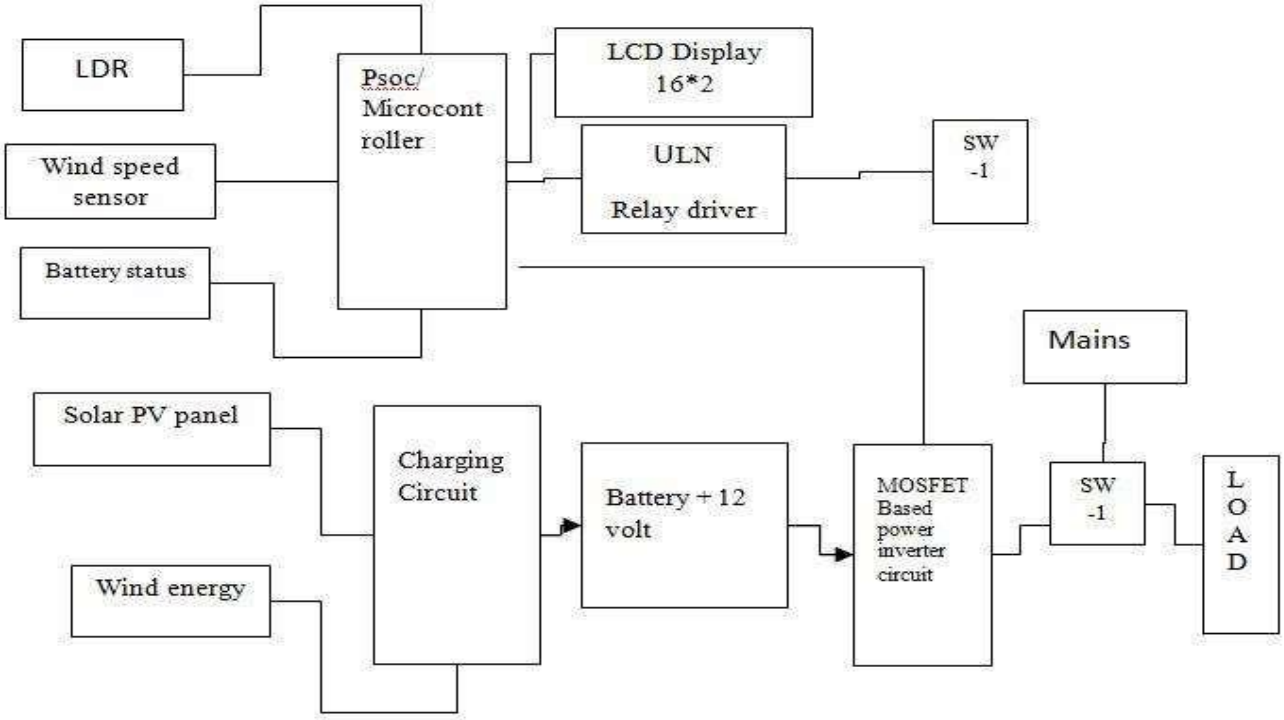

Fig 1 -Basic Block Diagram Of Self electricity Generation using Psoc

- **Wind energy-** Wind power is the conversion of wind energy into a useful form of energy. All renewable energy (except tidal and geothermal power), ultimately comes from the sun . About one or 2 percent of this energy is converted to wind energy (which is about 50-100 times more than the energy converted to biomass by all plants on earth.
- **Battery:** An electrical battery is one or more electrochemical cells that convert stored chemical energy into electrica energy. Here are two types of batteries: primary batteries which are designed to be used once and discarded when they are exhausted, and secondary batteries which are designed to be recharged and used multiple times. The CY8CKIT-030 PSoC 3 Development Kit is based on the PSoC 3 family of devices. PSoC 3 is a Programmable System-on-Chip™ platform for 8- and 16-bit applications. It combines precision analog and digital logic with a high-performance CPU.
- **PSoC Creator:** "PSoC" is an application related review of programmable array systems, the system-on-chip. Cypress's PSoC Creator software is a state-of-the-art, easy-to-use integrated development environment (IDE) that introduces a hardware and software design environment based on classic schematic entry and revolutionary embedded design.
- **LCD display:** A liquid crystal display (LCD) is a thin, flat electronic visual display that uses the light modulating properties of liquid crystals (LCs). LCs does not emit light directly.
- **LDR:**The main purpose of a light dependent resistor is to change the brightness of a light in different weather conditions. This can easily be explained with the use of a watch. It is the light dependent resistor that allows the watch to know when it has gotten dark, and change the emissions level of the light at that time.

6. EXPERIMENTAL RESULT:

TABLE I-solar panel output voltage of proposed system variable with time

Time	Output voltage of solar panel
7 AM	15.3 V
9 AM	17.6 V
11 AM	18.10 V
12 PM	19.9 V
1.30 PM	17.3 V
3 PM	16.47 V
5 PM	14.4V

Table I gives the information about solar panel output voltage with respect to time. Table shows output voltage in day time at the afternoon solar panel voltage get exceeds as time goes increasing solar panel voltage diminishes.

7. CONCLUSION:

In this case we are following all component & aspects of generating energy from solar cell easy, convenient & in user friendly way for non technical person, by using latest development of electrical and electronics. It is useful for providing grid quality, reliable power in rural areas where the line voltage is low and insufficient to connected load. In other places, other power sources could be used. For example hybrid combinations of wind power, solar power, geothermal power, hydroelectric power, tidal power, biomass generated power, power from incineration of solid wastes, and many other technologies could be considered depending on local interests and resources. PSoC is an industrial tool, aiming shorter design to market cycle. It targets professional applications and university projects with limited budget, as well. The key elements of this test bed concept presented in this paper are two or more renewable power sources connected to a power grid with complex electrical interactions. The Govt. of India is planning to electrify 18,000 villages by year 2012 through renewable energy systems especially by solar PV systems. The various MPPT (Maximum Power Point Tracking) methods are also applicable for control mechanism

The cost of the electricity produced by photovoltaic technology is higher than that from conventional fuels. This is a major obstacle for sustained long-term growth of solar technologies, and currently requires massive governmental support to create artificial markets for solar electricity

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Study on effect of increasing concentration of NaCl on germination of seeds of cotton plant

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Abstract: *Salt suppression of growth occurs in all plants but their tolerance levels and rate of growth reduction at high salt concentrations vary widely among different plant species. (Cixin He et al, 2007). A study was conducted to assess the effect of increasing concentration of NaCl salt on the germination of seeds of Cotton (Vikram Bt Cotton seed). Soil salinity at 0%, 1%, 2%, 3%, 5% was maintained by adding NaCl to soil contained in petriplates. Seed germination started on 2nd day after sowing and 100 % seeds germinated over a period of three days in control (Blank). Seedling emergence was recorded on the 3rd, 4th, 5th and up to 15 days after sowing in soils with salinities of 1%, 2%, 3%, 5% respectively. However, seed germination decreased with increase in soil salinity. Effect of salinization of soil on certain physiological attributes of Cotton plants has been checked.*

Key Words: *Cotton plant, Soil salinity, seed germination, Relative Water Content.*

1. INTRODUCTION:

Salinity is one of the most serious factors that limiting crop production, especially for the sensitive species. Salinity in topsoil and subsoil is one of the major abiotic environmental stresses to crop production. Worldwide, soil salinity is becoming a serious threat to agricultural productivity. About 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity. Plants show variable capacity to salinity tolerance that could be range from negligible effect to plant death.

Various plant growths and development processes viz. seed germination, seedling growth, flowering and fruiting are adversely affected by salinity, resulting in reduced yield and quality. Salt suppression of growth occurs in all plants, but their tolerance levels and rate of growth reduction at high salt concentrations vary widely among different plant species. (Cixin He *et al*, 2007)

Salinity of irrigation water is also a problem and is becoming an increasingly serious one as water of less and less desirable quality is exploited for irrigation and as greater intensity of water use leads to degradation.

In some cases, salinity induces nutritional imbalances or deficiencies causing decreased growth and plant injury. Salt-affected plants usually appear normal, although they are stunted with plant parts such as leaves, stems and fruits usually smaller than normal, and may have darker green leaves which, in some cases, are thicker and more succulent. As salt concentrations increase above a threshold level, both the growth rate and ultimate size of most plant species progressively decrease. Not all plant parts are affected equally. Top growth is often suppressed more than root growth.

Cotton plant:
Scientific name: *Gossypium hirsutum*
Family: Malvaceae (Kapok, Mallow, Cola)

Cotton is an important cash crop worldwide. Although cotton is classified as a salt tolerant crop, it is often adversely affected by soil salinity especially during emergence and seedling growth. A salt- tolerant species such as sugarbeet might have a reduction of only 20% in dry weight, whereas, a moderately tolerant species such as cotton might have a 60% reduction, and a sensitive species such as soybean might die the adverse effect of the salt solution on germination was increased with an increase in salt concentration. Increasing salinity decreased fresh and dry leaf and root weights. Leaf K+/Na+ ratio was decreased as increasing salinity levels in all tested varieties.

Different strategies commonly used by plants to improve salt stress tolerance: To maintain desirable K+/Na+ ratio in the cytosole, regulation of K+ uptake and prevention of Na+ entry, efflux of Na+ from cell or compartmentalize Na+ into vacuole.

2. MATERIALS AND METHODS:

Cotton seed (vikram Bt cotten seed), Nacl (solid), Soil, petri plates are required for experiments.

3. METHOD:

An experiment was carried out to investigate the germination of cotton seed in the laboratory. Take a 100 gm soil sample in each 5 plate. 1st is control plate and not add Nacl concentration to it. In 2nd plate 0.01 mm/gm Nacl added, in 3rd 0.02 mm/gm Nacl added, In 4th 0.03 Nacl added, In 5th 0.05 Nacl added. Now, Mix that Nacl and Soil properly in the plate.

The collected seeds were initially stored a room temperature until transport to field. The seeds were stored at -18°C to avoid germination loss and germination experiments were carried out within 2 months after the maturation of the seeds. (K. Tobe *et al*.)

The seeds were soaked in distilled H_2O for 24 h and then planted in pots filled with a 1/3:2/3(v/v) mixture of perlite : peat mosse. Germination was carried out in the greenhouse at temperature of 18°C, 12 h photoperiod and relative humidity of 80%. Seedlings were allowed to grow in the greenhouse under controlled conditions (temperature of 25°C, 12 h photoperiod and relative humidity of 80%) (Basel Saleh, 2011).

Vibality test

Take a bicker with water and put the few cotton seeds in it. A floating seed which none viable and the bottom part seed is viable. So, take a bottom part seed and put three seed in each plate. And now, give the water to each plate. Give proper water to each plate after one or two days. Now, Count the germinated seed in every twelve hours and do it for 15 days.

Fig.1 Viability test in Cotton seeds

Step-1 check the seed viability
Step-2 put the viable seeds in the filter paper for two-three days.
Step-3 shift the proper seeds into the petri-plate.
Step-4 there are 5 petri plates,put 3 seeds in each plate. These plates were filled with soil. Measured amount of Nacl was also added into them.
Step-5 add water regularly in a day, till your 15 days observation is done. Note down every day's result of growth on the seeds.

4. RESULT:

To check the effect of Nacl concentration cotton plant in the lab. Cotton seed germination in presence of Nacl.

Day	Control	1% Nacl	2% Nacl	3% Nacl	5% Nacl
0	0	0	0	0	0
1	0	0	0	0	0
2	2	0	0	0	0
3	3	1	1	1	0
4	3	1	2	2	1
5	3	1	2	2	1
6	3	2	3	2	2
7	3	2	3	2	2
8	3	3	3	2	2
9	3	3	3	2	2
10	3	3	3	2	2
11	3	3	3	2	2
12	3	3	3	2	2
13	3	3	3	2	2
14	3	3	3	2	2
15	3	3	3	2	2

Fig.2 Initial day of germination

Fig. 3 Shows seed germingation 5%, 3%, 2%, 1% and control from left side

5. DISCUSSION:

Maximum plants shows grow in presence of Nacl. Generally it grows in 2% as well as 1% Nacl concentration maximum plant tolerance occurs in 2% Nacl concentration. 5% Nacl presence is harmful for germination of plants. On the control plate seed germination was fast and proper. On 1%, 2% and 3% the growth was in the increasing manner. But on the 5% plate the germination was slowest.

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