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Changes in Qualitative and Quantitative analysis of *Citrus limon* L. leaves during two different seasons of Saurashtra region of Gujarat.

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ABSTRACT-

Citrus limon L.is an important medicinal fruit crop of the Rutaceae family which is originated from tropical and sub-tropical Southeast Asia. The leaves of lemon are very useful in various diseases like stomach ache, diarrhea, vomiting, carminative, excellent refrigerant drink and culinary uses. The presence of various phytochemicals is responsible for these medicinal properties. Qualitative and quantitative analysis(Total Phenol Contents and Total Tannin Contents) of Lemon leaves were done during two different seasons of Saurashtra region of Gujarat state where duration of season 1 was between Summer to Pre winter while duration of season 2 was winter to post summer. These study revealed that various phytochemical compounds were present but quantitative results showed that the production of these phytochemicals such as phenols and tannins may vary with seasonal changes due to effect of climate in different seasons of Saurashtra region and this type of study for lemon is firstly reported in this region.

KEY WORDS: *Citrus limon* L. leaves, Phenols, tannins, various seasons, saurashtra region, Gujarat.

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INTRODUCTION

A substance essential to the metabolism of a particular metabolic process is called metabolites (Kumar et al.2014)[1] . A plant cell produces two types of metabolites (a)Primary metabolites which involved directly growth and metabolism(carbohydrates, lipids and proteins).(b)Secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity. They include alkaloids, phenolics, sterols, steroids, essential oils, lignins, tannins etc. They act as defense chemicals. Their absence does not cause bad effects in the plants (Irchhaiya et al.2015)[2]. These secondary metabolites are synthesized in specialized cells at particular developmental stages making extraction and purification difficult (Kumar et al.2014)[1].Production of these phytochemicals will be particularly sensitive to climate change and it will increasing temperatures, altered rainfall patterns and climate variability will also affect the productivity of crops and their regional distribution in the next decades with severe impacts on the security. Quality and yield of any crop is only possible through its optimum climatic requirements. The changed climatic parameters affect the crop physiology, biochemistry, floral biology, biotic stresses like disease and ultimately resulted to the reduction of yield and quality of fruit crops. So it is a big challenge to the scientists of the world (Rajatiya et al.2018)[3]. Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves flowers, fruits and seeds. Levels of these phytochemicals may vary depending upon the variety, processing, cooking and growing conditions(John S. et al.2017)[4]. Lemon is an important medicinal plant of the Rutaceae family that originated in tropical and subtropical Southeast Asia (Janati S.S.F.et al.2012)[5]. The main characteristic of lemon plant is thorny branches and white flowers with purple edges. The leaves are green, elliptical-acuminate and shiny. On a lemon tree, flowers and fruits can be found at the same time (Dev, C. and Nidhi, S.R.R.S., 2016)[6].The leaves are useful for the treatment of various diseases like stomach ache, diarrhea, vomiting, carminative, excellent refrigerant drink and culinary uses(Alvarez,M.R. et al.1986)[7]. Major medicinal properties of *Citrus limon* L. include anti-cancer activity, prevent kidney stones, bring down a fewer, balance pH(Dev, C. and Nidhi, S.R.R.S., 2016)[6]. The presence of various phytochemicals is responsible for these medicinal properties. In this study, qualitative and quantitative analysis of leaves of *Citrus limon* L. for two extracts i.e. Methanol and acetone have been done during two different seasons of saurashtra region.

MATERIALS AND METHODS

Collection and Drying of selected plant part:

Leaves of *Citrus limon* L. were collected from Vadhavi (Junagadh district) Saurashtra region. The leaves of *Citrus limon* L. were collected two times During two different seasons of Saurashtra region. Duration of season 1 was between Summer to Pre winter (May 2018 to nov.2018) while duration of season 2 was between winter to post summer (Dec.2018 to June 2019). The leaves of plant collected, washed and dried at room temperature and then crushed. Dried powder stored in the air tight bottle for extract preparation.

Extract Preparation:

10gm of Citrus leaves were extracted using 100ml of organic solvent (Methanol and acetone) for 24 hours and then filtered using Whatmann filter paper No. 1. The extracted solution, (filtrate) was kept overnight to obtain dry extract while the residue after filteration was discarded. The methanol and acetone extracts of each part was stored in a cool and dry place after transferring them into vials.

Phytochemical Screening:

Qualitative analysis of prepared methanol and acetone extracts were done by cold extraction method for the presence of secondary metabolites like alkaloids, flavonoids, steroids, saponins, tannins, glycosides and phenols. Quantitative estimation of Total Phenol Content and Total Tannin Content were also done.

Qualitative analysis (Mathew, B.B., et al. 2012)[8]

Phenols and tannins:

Ferric Chloride Test: To 2ml of plant extract was added 5 ml Distil water along with a few drops of 3% Ferric Chloride solution where, as the solution turned its color blue – green in color indicated the presence of phenols.

Lead Acetate Test: To 1ml of extract was added to 3 ml 10% Lead acetate solution the Bulky White precipitate formed showed the presence of phenols.

Potassium Dichromate Test: To 1ml extract was added Potassium dichromate solution precipitation was seen showing the presence of tannins and phenolic compounds.

Quantitative analysis

Total phenol content (Bharani, R.A. and Namasivayam, S.K.R., 2016)[9]

Quantitative analysis of Total Phenol contents (TPC) of *Citrus limon* L. leaves were done by folin-ciocalteau's method with some modifications. For the preparation of calibration curve, gallic acid used as standard. Gallic acid was prepared in two different solvent such as methanol and acetone with different concentrations. Aliquots of 0.5 ml of each concentration of gallic acid were mixed with 2 ml of (1:10) Folin-ciocalteau's reagent and 2 ml of 7.5% sodium carbonate solution. Then tubes were shaken vigorously and mixed well. The mixture was allowed to incubate for 30 minutes at room temperature before the absorbance was measured at 760 nm. Same as for plant extracts, 0.5 ml of all extracts (1mg/ml) were treated and absorbance was measured.

Total Tannin content (Rajan, S., et al. 2011)[10]

Quantitative analysis of total Tannin content (TTC) of *Citrus limon* L. leaves were analyzed by Folin-denis method with some modifications. Tannic acid was used as standard. Tannic acid was prepared with different concentrations of different solvents. The methanolic and acetone extract mixed with 0.3 ml of Folin-denis reagent followed by 1 ml of Na_2Co_3 (7.5%) solution and made up to 10 ml with distill water. These mixtures weer shaken well and allowed it to 30 minutes for incubation at room temperature and measured the absorbance at 755 nm using UV-visible spectrophotometer. Total tannins in extracts were expressed as equivalent to tannic acid (mg TE/g extract).

RESULTS AND DISCUSSION

Extraction of *citrus limon* L. leaves were done by using two different solvents (Methanol and acetone).

Here, duration of Season 1 Summer-pre winter (May 2018 to November 2018)

Season 2 Winter-Post summer (December 2018 to June 2019)

Table-1: Qualitative analysis of *Citrus limon* L. leaves

Secondary metabolites	Tests	Saurashtra			
		Season 1		Season 2	
		Citrus limon L. leaves (Methanol)	*Citrus limon* L. leaves (Acetone)	*Citrus limon* L.leaves (Methanol)	*Citrus limon* L. leaves (Acetone)
Alkaloids	Mayers test	+	+	+	+
	Dragondorff test	+	+	-	-
	Wagners test	-	-	-	+
Flavonoids	Alkaline reagent test	+	++	+	+
	Zn chloride test	+	+	+	+
Phenols	Ferric chloride test	++	+	++	+
	Lead acetate test	++	+	++	+
Tannins	Lead acetate test	++	+	+	++
	Potassium dichromate test	+	+		
Saponins	Frothing test	-	-	-	-
Steroids	Libermann-sterol test	-	-	-	-
Glycosides	Keller-Killani test	+	+	-	-
Carbohydrates	Molisch's test	++	+	+	+
	Fehling test	+	+	++	-
	Benedict's test	++	+++	+	+
Protein/ amino acids	Million's test	+	++	+	+
	Ninhydrine test	-	+	-	-

Here, +(present), ++ (Quite present), +++(Highly present) and –(Absent)

QUALITATIVE ANALYSIS

Qualitative phytochemical analysis of *Citrus limon* L.leaves have been done for two different seasons of Saurashtra region of Gujarat. Duration of first season was Summer to Pre winter (May 2018 to November 2018) while duration of season 2 was Winter to Post summer (December 2018 to June 2019). Two solvent were used for the leaves extraction: (1)Methanol (2)Acetone. Qualitative analysis of *Citrus limon* L. leaves revealed that presence of various bioactive constituents. During season 1

alkaloids, flavonoids, phenols, tannins, glycosides, sugar/carbohydrates, protein/amino acids were present while saponins and steroids absent. But during season 2 alkaloids, flavonoids, phenols, tannins, sugar/ carbohydrates, proteins/amoni acids were present while saponins, steroids and glycosides were absent. During the two different seasons, climate change showed the noticeble effects on the life cycles and distribution of phytochemicals in the various plant parts.

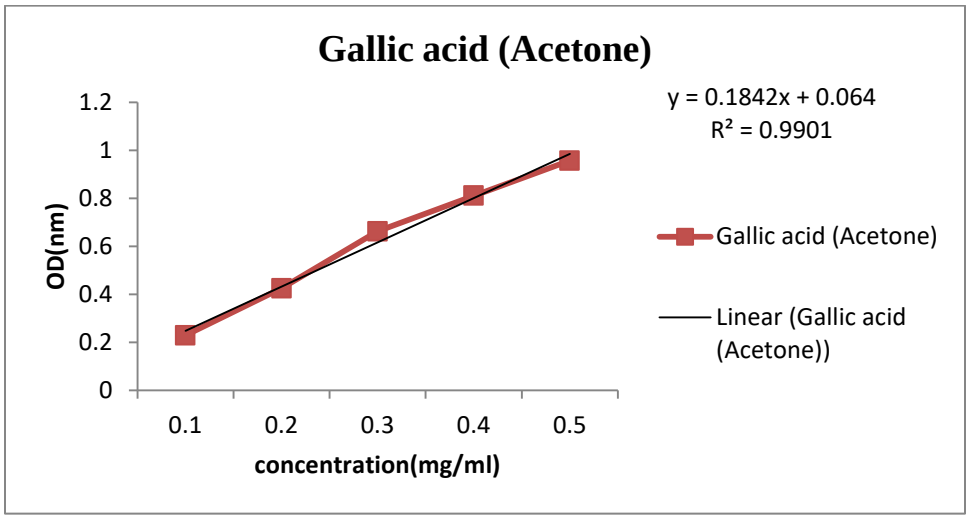

Figure 1: Standard graph of Gallic acid (Acetone)

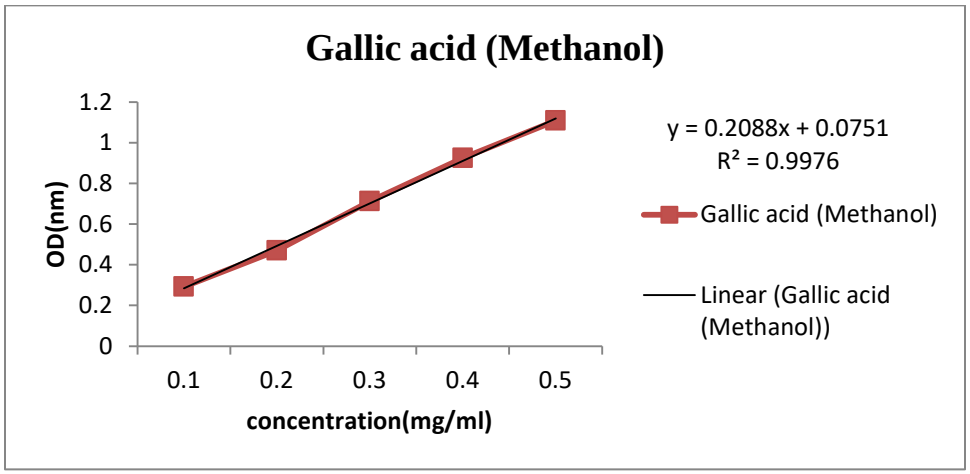

Figure 2: Standard graph of Gallic acid (Methanol)

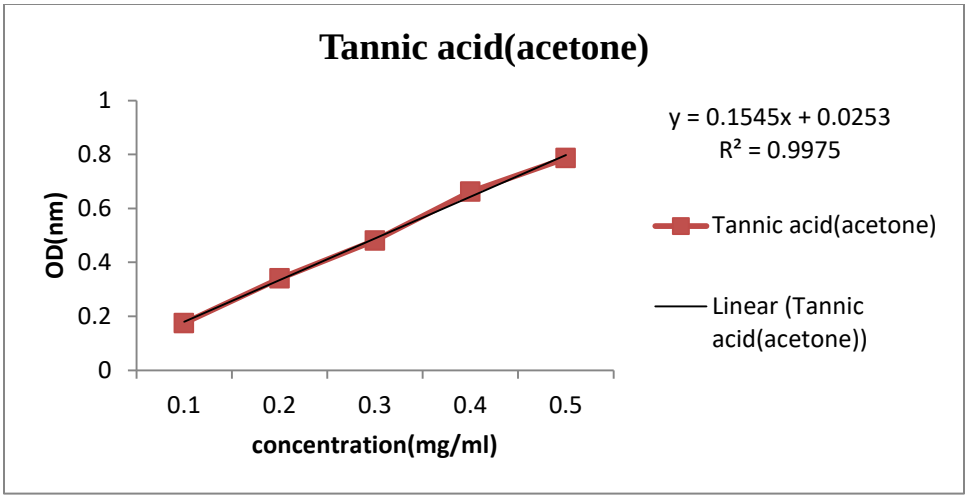

Figure 3: Standard graph of Tannic acid (Acetone)

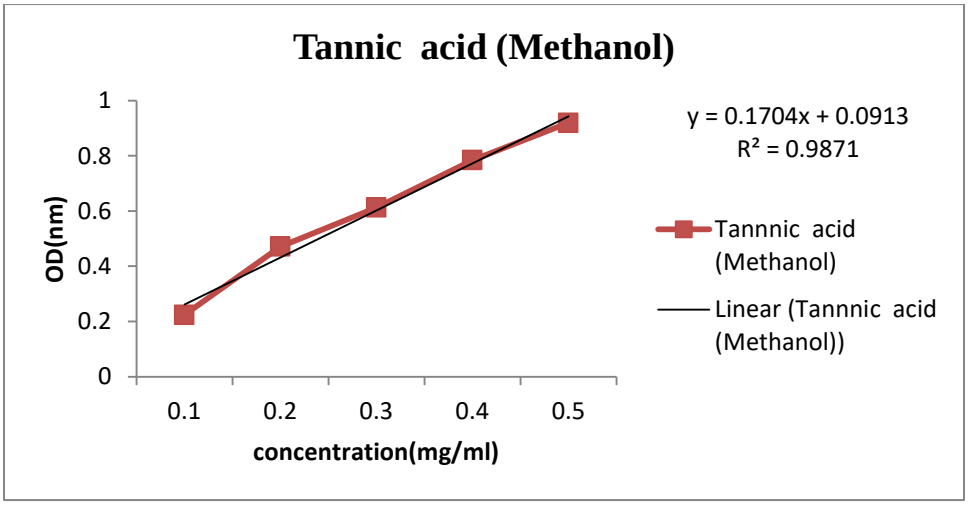

Figure 4: Standard graph of Tannic acid (Methanol)

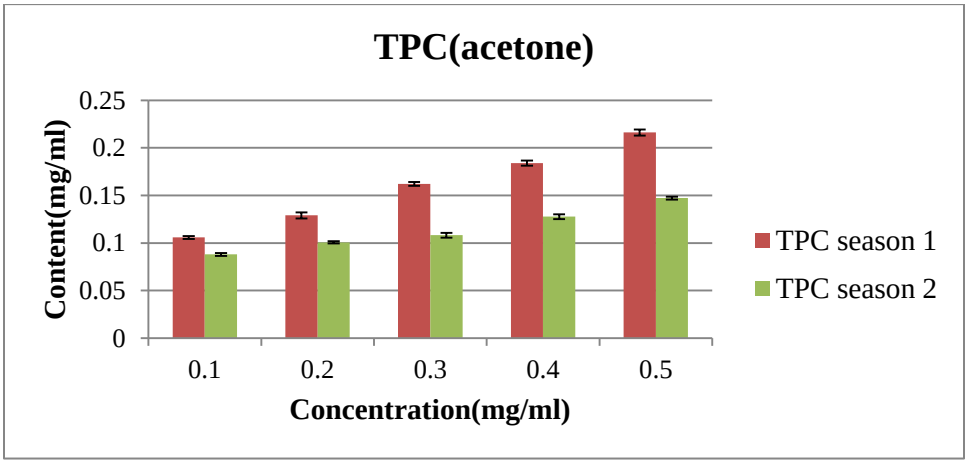

Figure 5: Total phenol content of AE of *Citrus limon* L.leaves from saurashtra region (Season 1:Summer to Pre winter and Season 2:Winter to Post summer)

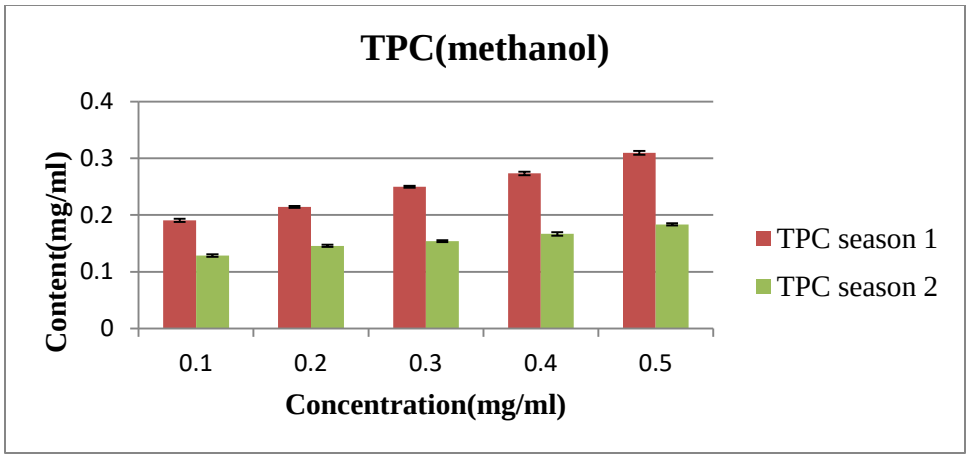

Figure 6: Total phenol content of ME of *Citrus limon* L. leaves from saurashtra region

(Season 1:Summer to Pre winter and Season 2:Winter to Post summer)

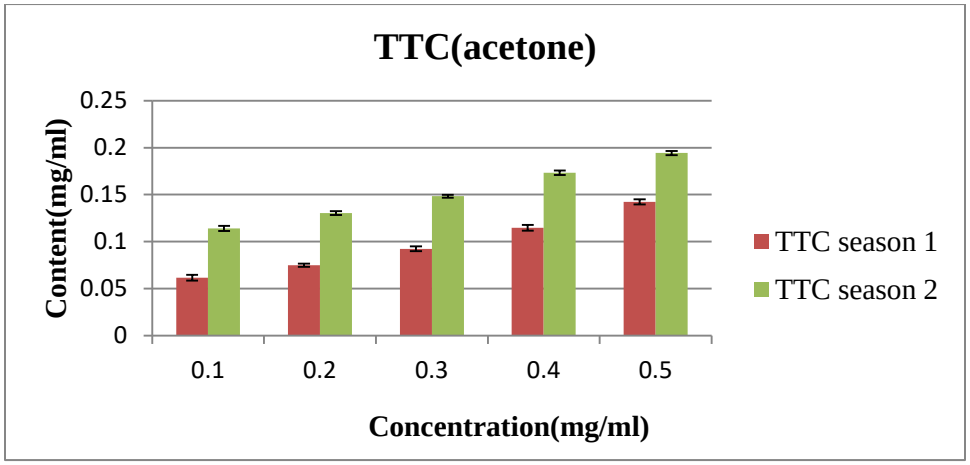

Figure 7: Total tannin content of AE of *Citrus limon* L. leaves from saurashtra region

(Season 1:Summer to Pre winter and Season 2:Winter to Post summer)

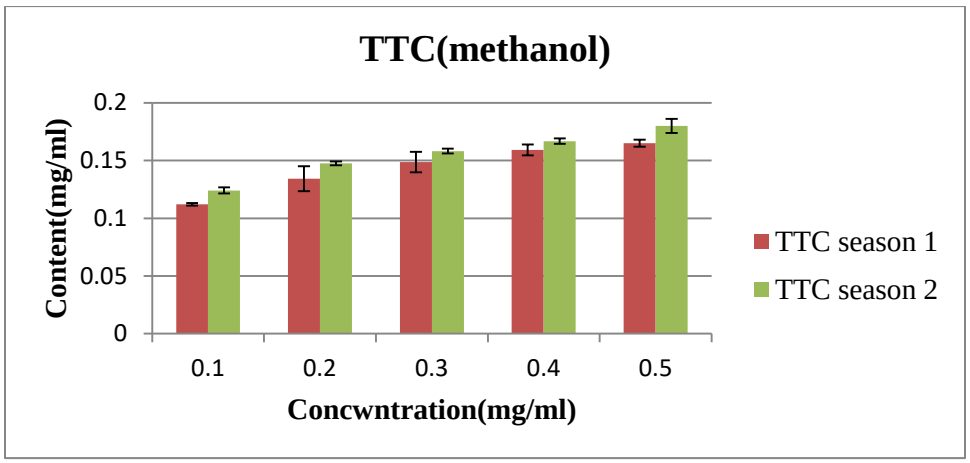

Figure 8: Total tannin content of ME of *Citrus limon* L. leaves from saurashtra region
(Season 1:Summer to Pre winter and Season 2:Winter to Post summer)

QUANTITATIVE ANALYSIS

Total phenol content of *Citrus limon* L. leaves were estimated by Folin- ciocalteau's method for both season of Saurashtra region of Gujarat. TPC was calculated for methanol extract from standard calibration curve of Gallic acid($y=0.2088x+0.0751$, $R^2=0.9976$) and for acetone extract from standard calibration curve of Gallic acid($y=0.1842x+0.064$, $R^2=0.9901$) in terms of mg/ml Gallic acid equivalent of sample. In first season of *Citrus limon* L. leaves, the values of TPC were found between 0.190mg to 0.309 mg for methanol extract but for acetone extract TPC were found between 0.105 mg to 0.216 mg/ml of Gallic acid equivalent(GAE) while during second season of *Citrus limon* L. leaves, the values of TPC were found between 0.128 mg to 0.183 mg for methanol extract but for acetone extract TPC were found between 0.087 mg to 0.147 mg/ml Gallic acid equivalents(GAE).Among these two season of *Citrus limon* L. leaves, it was concluded that season 1 recorded the best result in quantity of phenol content than season 2.

Total tannin content of *Citrus limon* L. leaves was calculated for methanol extract from standard calibration curve of Tannic acid($y=0.1704x+0.0913$, $R^2=0.9871$) and for acetone extract from standard calibration curve of Tannic acid($y=0.1545x+0.0253$, $R^2=0.9975$) in terms of mg/ml Tannic acid equivalent of sample. In first season of *Citrus limon* L. leaves, the values of TTC were found between 0.112mg to 0.164 mg for methanol extract but for acetone extract TTC were found between 0.061 mg to 0.142 mg/ml of tannic acid equivalent while during second season of *Citrus limon* L. leaves, the values of TTC were found between 0.124 mg to 0.179 mg for methanol extract but for acetone extract TTC were found between 0.114 mg to 0.194 mg/ml tannic acid equivalents. From this study it was concluded that methanol extract showed higher tannin content in season 1 but in season 2 acetone extract showed higher tannin content compare to season 1.

CONCLUSION

Present investigation revealed that leaves of *Citrus limon* L. contain significant amount of various phytochemicals such as alkaloids, phenols, flavonoids, tannis, saponins, sugar/carbohydrates but quantitative results showed that the production of these phytochemicals such as phenols and tannins may vary with seasonal changes due to effect of climate in different seasons of saurashtra region. They are unique source for pharmaceuticals, food additives, flavours and also shown potential in treating various ailments. Therefore, an improved knowledge of factors responsible for such changes requires an

intensive and continuos studies on growth of plant and secondary metabolite production (Mishra, T., 2016)[11].This type of study for *Citrus limon* L. were reported firstly in this Saurashtra region.

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