CHAPTER 4

The survey for the occurrence of burl disorder on the stems and main branches of different germplasms of the *Mangifera indica* L. (Anacardiaceae) was conducted during the year 2017-2019. Several morphological characteristics were recorded for the prevalence of burl disease in different germplasms. The field survey was conducted throughout India, from which nearly 473 germplasm/varieties were screened against the burl disease. Based on this survey, it was noticed that about 34 germplasms/varieties (Table 5) were more susceptible to this disease. When compared with Gujarat state, out of 167 germplasms/varieties under cultivation, 20 germplasms/varieties (Tables 6) showed the highest incidence and susceptibility towards it.

All the germplasms/varieties (i.e., 34 at national and 20 at state level) vulnerable to burl formation were further investigated for the burl shape, colour, surface texture, position of burl formation from ground level on the stem, the total number of burls per plant, the height of the first burl from ground and presence of gummosis (Figures 4, 5; Table 5, 6). Amongst them, two potential varieties that are of national interest *viz*., *Langra* (most preferred for its taste throughout the country) and *Rajapuri* (preferred for pickles) are selected for the detailed investigation in the present study. Though variety *Kesar* is famous from Gujarat, it is one of the resistant varieties and incidence of the burl formation was observed almost nil. Moreover, it is under cultivation at limited locations outside the state; therefore, not considered in the present study.

On the other hand, *Langra* and *Rajapuri* varieties are of national importance and one of the important choices of the consumers after top selected varieties. Moreover, the market rate is also reasonable for the common public from an economical point of view. Collection of samples will be also easy since both the varieties are frequently available and are under cultivation throughout the Gujarat state. Therefore, based on agro-climatic zones, four districts *viz*. Anand, Banaskantha (Dantiwada), Junagadh and Valsad (Pariya), were selected from Gujarat state (Figure 3). These four districts are known as potential areas for mango cultivation and production in the state. Mango trees growing naturally in the wild can be seen frequently in forest areas at many places. These trees were not considered in the survey due to inferior quality of fruits such as smaller size, more sour and low yield reflecting lower market value. However, they were found considerably resistant to burl incidence and found almost nil from this disease.

Table 5. Screening of different varieties/germplasms of *Mangifera indica* L. against burl disease throughout India

S. No	Germplasm	Total No. of individuals	Total No. of diseased plant	Burl incidence (%)	Fruit yield loss (%)
1	*Arka Aruna*	114	114	100	18.64
2	*Arka Punit*	175	107	61.14	6.18
3	*Alphanso × Baneshan*	32	8	25	0.83
4	*Alphanso × Sabja*	32	8	25	0.47
5	*Banganpalli*	152	74	48.65	8.25
6	*Badami Modal*	3	3	100	1.78
7	*Bathua*	747	410	54.88	9.84
8	*Banganpalli × Alphanso*	1	1	100	0.00
9	*Desi*	16	1	6.30	0.47
10	*Elite Ditla*	2	2	100	0.00
11	*Gopal bhog*	4	4	100	2.43
12	*Hybrid 10*	5	5	100	17.81
13	*Joshipura Junagadh*	2	2	100	0.00
14	*Khodi*	16	1	6.25	0.15
15	*Krishna bhog*	208	108	100	10.17
16	*Khaja Pasand*	4	4	100	3.91
17	*Kesar*	100	2**	2.00	0.00
18	*Langra*	7462	5268	70.59	20.22
19	Mahmood Vikarabad	98	98	100	21.56
20	*Mankurad*	1	1	100	0.00
21	*Mahuda Golkeri*	4	2	50.00	2.74
22	*Mahuvas*	6	6	100	7.77
23	*Malai*	5	5	100	5.48
24	*Neelphanso*	30	7	23.33	4.33

25	*Neelam*	14	4	28.57	1.14
26	*Olour*	100	20	20.00	2.30
27	*Prince*	12	6	50.00	2.80
28	*Rajapuri*	1248	276	22.11	5.37
29	*Seedling*	14	14	100	12.30
30	*Sindhu*	219	40	18.26	15.40
31	*Swarna Rekha*	5	4	80.00	7.27
32	*Sardar*	40	16	40.00	3.47
33	*Sukul*	621	398	64.07	14.30
34	*Tree 253*	8	8	100	4.00

** Only two plants of *Kesar* variety showed burls at Junagadh. (Stock of *Kesar* grafted on the scion of *Rajapuri* variety)

Table 6. List of different varieties/germplasm of *Mangifera indica* L. screened throughout India against burl disease, number of individuals/species, percentage of burl incidence and yield loss for the year 2017-2019.

Sr. No.	Germplasm	Total no. of individual	Burl infected individuals	Burl incidence (%)	Yield loss (%)
1	*Arka Aruna*	114	114	100	18.64
2	*Arka Punit*	175	107	61.14	6.18
3	*Alphanso × Baneshan*	32	8	25	0.83
4	*Alphanso × Sabja*	32	8	25	0.47
5	*Banganpalli*	152	74	48.65	8.25
6	*Bathua*	747	410	54.88	9.84
7	*Gopal Bhog*	4	4	100	2.43
8	*Hybrid 10*	5	5	100	17.81
9	*Khaja Pasand*	4	4	100	3.91
10	*Krishna Bhog*	208	108	100	10.17
11	*Langra*	7462	5268	70.59	20.22
12	*Mahmood Vikarabad*	98	98	100	21.56
13	*Mahuvas*	6	6	100	7.77

14	*Malai*	5	5	100	5.48
15	*Olour*	100	20	20.00	2.30
16	*Prince*	12	6	50.00	2.80
17	*Rajapuri*	1248	276	22.11	5.37
18	*Seedling*	14	14	100	12.30
19	*Sukul*	621	398	64.07	14.30
20	*Sindhu*	219	40	18.26	15.40

4.1 *Morphological Characterization of Burl*:

The burl shape was recorded globose in *Langra*, *Rajapuri*, *Sukul*, *Mahuvas*, *Olour, Seedling, Tree 253*, *Krishna Bhog, Hybrid 10*, *Alphanso* × *Baneshan*, *Alphanso* × *Sabja*, *Banganpalli*, *Gopal Bhog*, *Prince* and *Malai* while *Mahmood Vikarabad*, *Khaja Pasand*, and *Sindhu* have globose to elongated in shape (Figures 4, 5, 9, 10). Globose to semi-elongated shape was observed in *Elite Ditla*, *Joshipura Junagadh*, *Arka Aruna*, *Arka Punit*, *Neelphanso* and *Suvarna Rekha* (Figure 6).

The burl colour was also recorded for every variety under investigation, it was brownish-black in *Arka Aruna*, *Arka Punit*, *Mahuvas*, *Seedling*, *Tree 253*, *Rajapuri*, *Mahmood Vikarabad*, *Hybrid 10*, *Alphanso* × *Sabja* and *Banganpalli*, while blackish-brown in colour in *Khaja Pasand*, *Gopal Bhog*, *Prince*, *Malai*, *Sindhu*, *Seedling 68* and *Seedling 307*. Brownish-grey to brownish-black in colour was noted in *Langra* and *Bathua* whereas greyish-black colour was observed in *Sukul* and *Alphanso* × *Baneshan* variety (Figures 4, 5, 7, 9).

Surface features of the burls were also studied for different varieties. Germplasms like *Arka Aruna*, *Langra*, *Banganpalli* and *Seedling 307* had rough surface while it was medium rough in *Arka Punit*, *Alphanso* × *Baneshan*, *Alphanso* × *Sabja*, *Bathua*, *Gopal Bhog*, *Krishna Bhog*, *Olour*, *Tree 253*, *Rajapuri*, *Prince*, *Malai*, *Sindhu* and *Seedling 68* (Figures 6, 8). In some of the germplasm including *Sukul*, *Mahuvas*, *Seedling*, *Mahmood Vikarabad* and *Hybrid 10* burl surface was smooth while variety *Khaja Pasand* showed undulated surface. Secretion of gummosis was also noted from the burl which starts almost in the middle portion of the burl in *Langra* and *Rajapuri* variety but at many times it can be seen nearby burl occurrence (Figures 4B, 8I, 6, 7).

The surface was smooth in *Desi*, *Mahmood Vikarabad* and *Badami Modal* during the early stage of the burl development but subsequently, they became rough in fully grown trees. Burl formation in *Rajapuri*, *Mahmood Vikarabad*, *Badami Modal*, *Joshipura*

Junagadh, *Arka Aruna*, *Arka Punit* and *Mankurad* was noted on both sides (upper and lower) of the stems from the ground level, whereas burls were noted close to the ground level in *Langra*, *Elite Ditla* and *Neelphanso*. In the early stage of burl development, gummosis does not occur except in *Langra* variety, but as time passes and after the complete development of the burl, gummosis was seen in almost all germplasms (Figures 6, 8, 9, 11).

In Gujarat state, the burl shape was recorded globose in *Langra* and *Rajapuri* variety while globose to semi elongated shape was observed in *Mahmood Vikarabad*, *Elite Ditla*, *Joshipura Junagadh*, *Arka Aruna*, *Arka Punit*, *Neelphanso* and *Suvarna Rekha*. The colour of burl among all germplasm was grey to light brownish at an early stage of the burl development but after burl maturity, it became light brown to dark brown and later it became blackish in large-sized burls. The surface of the burls was rough in *Langra*, *Arka Aruna*, *Arka Punit*, *Joshipura Junagadh*, *Elite Ditla*, *Mankurad* and *Neelphanso* while it was medium to rough in *Rajapuri*, *Mahmood Vikarabad* and *Sindhu* variety (Figures 7, 8, 9, 11). During the survey in Gujarat, several burl infected trees were also found associated with termite attack (Figure 10). If compared within varieties, the burl size was larger in *Langra* (Figures 12A, B, 13) than in the *Rajapuri* (Figures 12A, D, 13). Formation of the first burl from the ground level was highest in *Rajapuri* while they generally appear close to the ground level in *Langra* variety (Figures 12A-D, 13).

4.2 *Disease Incidence and Burl Size*:

As mentioned earlier, out of 473 mango germplasms/varieties, about 34 varieties were affected with the incidence of burl disease, which is nearly 7.18 % throughout India. At the individual variety level, the highest incidence i.e., 100 % was observed in varieties *viz*. *Arka Aruna*, *Seedling*, *Elite Ditla*, *Mankurad*, *Joshipura Junagadh*, *Mahmood Vikarabad*, *Hybrid 10*, *Badami Modal*, *Banganpalli × Alphanso*, *Mahuvas*, *Tree 253*, *Krishna Bhog*, *Khaja Pasand*, *Gopal Bhog*, and *Malai* while it was minimal or lowest in *Desi* (6.30 %), *Khodi* (6.25 %) and *Kesar* (2.00 %) throughout the country (Table 5).

Similarly, in Gujarat, out of 167 germplasms/varieties nearly 20 varieties were susceptible and showed about 12% % disease incidence. Among these 20 varieties in Gujarat, 100% incidence was observed in varieties like *Arka Aruna*, *Badami Modal*, *Mankurad*, *Mahmood Vikarabad*, *Joshipura Junagadh*, *Elite Ditla*, *Banganpalli × Alphonso*, *Seedling*, followed by other varieties like *Langra* showed 81.81 %, in *Suvarna Rekha* it was 80 % and *Arka Punit* it was 77.77 % while the minimum incidence was noted in Kesar (2.00 %). If compared with the size of the burl, it was larger in *Langra* (36.00 cm^2), followed by *Desi* (32.00 cm^2), whereas it was minimal in *Mankurad* (10.0 cm^2). The maximum height of the first burl from ground level was observed in *Mahuda Golkeri* (210 cm) followed by *Desi* (180 cm) whereas it was minimum in *Arka Aruna* (23.33 cm) among the varieties investigated (Table 7).

Table 7. Burl size, its incidence and burl height from ground level in different varieties of *Mangifera indica* L. cultivated in different orchards in Gujarat state.

S. No.	Germplasm Name	Burl size (cm^2)	Burl incidence (%)	First burl height (cm)
1	*Arka Aruna*	17.00^{c}	100.00^{a}	23.33^{k}
2	*Arka Punit*	10.00^{cde}	77.77^{b}	78.75^{h}
3	*Badami modal*	3.00^{f}	100.00^{a}	150.33^{cd}
4	*Banganpalli x Alphanso*	13.00^{cd}	100.00^{a}	110.00^{e}
5	*Desi*	32.00^{ab}	6.30^{gh}	180.00^{b}
6	*Elite Ditla*	9.00^{cde}	100.00^{a}	90.00^{g}
7	*Joshipura Junagadh*	13.00^{cd}	100.00^{a}	65.00^{i}
8	*Khodi*	5.00^{de}	6.25^{gh}	72.50^{h}
9	*Langra*	36.00^{a}	81.81^{b}	31.23^{j}
10	*Mahmood Vikarabad*	17.00^{c}	100.00^{a}	88.33^{g}
11	*Mahuda Golkeri*	9.00^{cde}	50.00^{c}	210.00^{a}
12	*Mankurad*	1.00^{g}	100.00^{a}	150.00^{cd}
13	*Neelam*	15.00^{c}	28.57^{e}	150.00^{cd}
14	*Neelphanso*	3.00^{f}	23.33^{ef}	98.33^{f}
15	*Kesar*	3.00^{f}	2.00^{h}	38.00^{j}
16	*Rajapuri*	4.00^{e}	16.17^{fg}	102.23^{f}
17	*Sardar*	3.00^{f}	40.00^{d}	146.00^{d}
18	*Swarna Rekha*	4.00^{e}	80.00^{b}	157.00^{c}
19	*Sindhu*	2.00^{fg}	75.00^{b}	112.00^{e}
20	*Seedling*	26.00^{b}	100.00^{a}	90.00^{g}

Means with the same letters (a, b, c, d, superscript) in the column shows no significant variation at P = 0.05 level in Duncan's multiple range tests

Langra is very popular and widely cultivated throughout mango growing regions of India. Therefore, it is considered in the present investigation to study the incidence of burl disease, burl size and fruit yield loss. During the study, the highest incidence of burl formation in *Langra* was observed from Hyderabad (86 %) followed by Pariya region of Gujarat (81.81 %) and Uttarakhand (67.39 %), whereas the minimum incidence was recorded from Haryana (0.26%), whereas the New Delhi region was found free from burl incidence (i.e., 0.0 %). On the other hand, the size of the burl was observed maximum in mango orchards of Haryana (94.00 cm^2) followed by Uttarakhand (41.00 cm^2) and Gujarat (36.00 cm^2). It was minimum in individuals of *Langra* growing at Karnataka (14.00 cm^2) while no burls were observed in the mango orchards around New Delhi (Table 8).

Table 8. Screening of *Langra* variety of *Mangifera indica* L. for % burl incidence, burl size and yield loss growing at different locations of India

Sr. No.	Location	Incidence (%)	Burl size (cm^2) in diameter	Yield loss (%)
1	Karnataka	0.33	14	14.96
2	Bihar	53.1	29.00	20
3	Gujarat	81.81	36.00	25.5
4	Haryana	0.26	94.00	0
5	Hyderabad	86	18.00	14.82
6	Uttar Pradesh	90	15.00	11.31
7	New Delhi	0	0	0
8	Maharashtra	22.37	18.00	26.27
9	Uttarakhand	67.39	41.00	19

4.3 *Isolation and Identification of Causal Organism*:

Wood samples inoculated on PDA medium showed negative results and no fungal growth was observed. In contrast, overnight incubated surface-sterilized wood blocks in sterile distilled water showed changes in colour and it became turbid (Figure 14A, B). After 24 hours of incubation, 1 µl of water was used for inoculation for bacteria-specific media, which showed the colonies of bacteria. The obtained cultures were subsequently purified by serial transfer method and used to study colony characters and gram staining:

4.3.1 *Colony Characters, Gram Staining and On Plate Assay of Causal Organism*:

The obtained culture was stained with gram staining techniques using brightfield microscopy, and the stained slides showed the presence of rod-shaped bacteria that appeared pink coloured (Figures 14H, 16F). Based on these initial morphological characteristics of the isolated bacterial colonies were tentatively identified as *Agrobacterium* (Figure 14, 16).

Isolated bacteria were also tested for colony characterisation using on plate analysis by using specific media viz. NASA, Hofer's, MGY and Mac Conkey media (Figure 14C-G). One drop of the suspension was taken and spread on the above said bacterial media and incubated at 28-30 ºC temperature. After 24-48 hrs of incubation, bacterial colonies appeared on media plates and showed different colour on each medium; they are as follows:

4.3.2 *MGY Medium Plate*: *Agrobacterium* colonies were flat, spreading, fluidal, and white-cream colour after 24 -48 hrs. of incubation (Figure 14G).

4.3.3 *Hofer's Medium*: The bacterial colonies appear single with small size and white coloured (Figure 14E, 16D).

4.3.4 *NASA Medium*: *Agrobacterium* colonies turn into putative brick-red coloured after 24 -48 hrs. of incubation (Figure 14F, 16E).

4.3.5 *MacConkey* (*Broth*) *Assay*: Bacterial colonies turn yellow coloured because Bromocresol purple is a pH indicator in the medium; therefore, it turns yellow under acidic condition. Lactose fermenting organisms turn the medium yellow due to the acidity produced after lactose fermentation (Figure 14D).

4.3.6 *MacConkey (Agar) Plate Assay*: Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria. Lactose fermenting strains grow as red or pink coloured colonies (Figure 14D)**.**

4.3.7 *Molecular characterization*:

Subsequently, identification of the obtained bacterial culture was carried out by using molecular methods. The obtained sequences of 16S rRNA were compared with sequences available in the NCBI database using the Basic Local Alignment Search Tool in the NCBI GenBank database (www.ncbi.nlm.nih.gov) for confirmation of the identity of the bacterial species. Identification was done by 99 % base pair match of the sequence (Table 9) obtained to the closest available reference sequences (Leung, 1991). The sequence showed 100 % homology and the causal organism was identified as *Agrobacterium tumefaciens* Smith and Townend. These sequences were deposited in NCBI GenBank with Accession No. **MK835677**. Primers and obtained sequences from the bacterial isolates in the present study are as follows:

Primer 1 - 27F: 5'-AGAGTTTGATCCTGGCTCAG-3'

Primer 2 -1492R: 5'-GGTTACCTTGTTACGACTT-3'

***Contig Sequence* (16S): 1154 bp**

CCATACTCCAGGCGGATGTTTAATGCGTTAGCTGCGCCACCGAACAG
TATACTGCCCGACGGCTAACATTCATCGTTTACGGCGTGGACTACCAGGGTA
TCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTCAGCGTCAGTAATGGACC
AGTAAGCCGCCTTCGCCACTGGTGTTCCTCCGAATATCTACGAATTTCACCT
CTACACTCGGAATTCCACTTACCTCTTCCATACTCAAGATACCCAGTATCAA
AGGCAGTTCCGCAGTTGAGCTGCGGGATTTCACCCCTGACTTAAATATCCGC
CTACGTGCGCTTTACGCCCAGTAATTCCGAACAACGCTAGCCCCCTTCGTAT
TACCGCGGCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCGACTACCGTC
ATTATCTTCATCGGTGAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCAC
GCGGCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTG
CCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCCT
CTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAACTAG
CTAATCCAACGCGGGCTCATCCATCCCCGATAAATCTTTCCCCCGTAGGGCG
TATGCGGTATTAATTCCAGTTTCCCAGAGCTATTCCGCAGAGATGGGTAGAT
TCCCACGCGTTACTCACCCGTCTGCCACTCCCCTTGCGGGGCGTTCGACTTG
CATGTGTTAAGCCTGCCGCCAGCGTTCGTTCTGAGCCAGAACCAAAACTCTA
AAGGCCGGAAAAAAAGGTAGAAAAAGGGGGTTTCTTATTTTAAACTCGCCG
GTATACACCTCTCAACTTCTTATTGCACCTCCGAGGGTGGGGTTTTGAGACA
AAATATCTTCAAATAAAAGGGGGCGCGCCCCAAAAGAGAGGCGCGGGGGG
GGGTGGAACAAAAAGTTGGGGGGGGGTGGTTTTTGTTAGAGAATAACCGCA
CCTATAAAAGGACGGACGCCACATAGTTATTTAGACGCACCAGATATGTGT
GTTTCGCCCACTTCGCGGGGGGAGGAGGGGGGAGATTAAGAGCCCCGCACG
TGTGGAGGGAGGAAAACACAACCCCCCCCCCCGGCCCCCCC

Table 9. NCBI blast results showing maximum score, total score, query cover, E value and % Identity

Species Description	Max. score	Total score	Query cover	E-value	Identification (%)
Agrobacterium tumefaciens str. C58 linear chromosome, complete sequence	1493	2986	70%	0.0	99%
Agrobacterium tumefaciens str. C58 circular chromosome, complete sequence	1493	2986	70%	0.0	99%
Agrobacterium fabrum strain Z-22 16s ribosomal RNA gene, partial sequence	1491	1491	70%	0.0	99%
Agrobacterium fabrum strain C5816s ribosomal RNA, partial sequence	1491	1491	70%	0.0	99%
Uncultured compost bacterium clone 0B39 16S ribosomal RNA gene, partial sequence	1491	1491	69%	0.0	99%

4.3.8 *Phylogenetic Analysis of Obtained Sequences*:

The phylogenetic tree was constructed using a partial sequence of 16S rRNA using Maximum Likelihood (ML) analyses by RAxML software (Silvestro and Michalak, 2012). An ML analysis was run for 1000 bootstrap replicates under the GTR + I model to assess clade support. The obtained sequence from the pathogen was closely grouped with the sequence of *Agrobacterium tumefaciens* (KU955329) and *Agrobacterium radiobacter* (AJ389904) with a bootstrap value of 95 % compared to several members of the clade (Figure 15). Based on the dendrogram or phylogenetic tree, it was confirmed that the *Agrobacterium tumefaciens* is a causal organism responsible for burl disease.

4.4 *Pathogenicity Test*:

Pathogenicity of the bacterial isolate obtained from the burl was tested in two ways: i) Carrot disk essay for rapid testing and ii) inoculating the pathogen in young saplings of mango (*Mangifera indica* L.) and tomato (*Solanum lycopersicum* L.).

4.4.1 (i) *Carrot Disk Assay*:

Pure cultures of bacteria obtained from the burl were tested by carrot disk test to check the pathogenicity. Sterilised and aseptically transferred Carrot disc on Petri plates were overlaid with 100 ml of appropriate inoculum for one month period. After 15 days of incubation, initiation of callus like tissue was observed around the pericycle region i.e., on the inner side of the endodermis. A well-developed, white ring of meristematic tissues was distinctly visible after 20-25 days of inoculation (Figure 17A-F).

4.4.2 (ii) *Inoculation of The Pathogen in Mango and Tomato Saplings*:

A pathogenicity test was conducted on the three-month-old healthy plant of mango (variety *Langra*) and two months old tomato plants. A bacterial suspension containing approximately 10^8 CFU ml^{-1} inoculated in the stem by injecting the suspension in the stem or the paste of bacterial culture was applied on the wound created on the stem with a sterile razor or surgical blade and wounds were sealed with parafilm tape. The experiment was conducted from July to August and inoculated plants were grown in natural condition. After 2-3 months of inoculation, plants were examined, which showed small measurable outgrowth on both mango and tomato plants.

All the inoculated plants of mango (variety *Langra*) used for regeneration of burl showed 33 % incidence while tomato plants showed 20 % incidence of burl initiation. The pathogen was re-isolated from the inoculated saplings of mango as well as tomato and subjected to specific media (*i.e.*, Hofer's). Colonies appeared after 24 hrs and showed a similar result to those obtained from the burl wood. Control saplings were also injected with sterile distilled water or simply paste applied on wounds showed no symptoms and no pathogen could be isolated (Figure 18A-F).

4.5 *Fruit Yield Loss*:

The survey carried out at the national level showed the highest fruit yield loss in *Mahmood Vikarabad* (21.56 %) and *Langra* (20.22 %) followed by *Hybrid 10* (17.81 %), *Sukul* (14.30 %) *Sindhu* (15.40 %) and *Seedling* (12.30 %) whereas lowest fruit yield loss was recorded in *Olour* (2.30 %), *Alphanso* × *Baneshan* (0.83 %) and *Alphanso* × *Sabja* (0.47 %), respectively (Table 6). Due to the popularity and broadly cultivation, the yield loss in *Langra* variety was also recorded throughout India and the highest yield loss was recorded from Rahuri (26.27 %) and Gujarat (25.50 %) whereas New Delhi and Hisar region were found free from the burl disease resulting in no yield loss (Figure 19).

In Gujarat, both varieties i.e., *Langra* and *Rajapuri* were selected to investigate the fruit yield losses in four different zones of the state because both of them are popular and widely grown throughout the state. The maximum fruit yield loss was recorded in plants growing at Valsad in both the varieties *i.e.*, 24.58 % in *Langra* and 10.11 % in

Rajapuri whereas, the minimum fruit yield loss was observed at 17.25 % in *Langra* and 1.2 % in *Rajapuri* plants grown at Junagadh respectively. Followed by Valsad (Pariya campus), Anand campus stands second in case of high disease incidence and maximum yield loss in both the varieties (Table 10). As compared to *Rajapuri,* the variety *Langra* has more burl disease incidence and maximum fruit yield loss in trees growing in the Valsad (Pariya) region of south Gujarat.

Table 10. Fruit yield losses in *Langra* and *Rajapuri* variety *Mangifera indica* L. in four-zone of Gujarat state

S. No.	Location	Yield loss (%)	
		Langra	***Rajapuri***
1.	Anand	22.74	6.36
2.	Dantiwada	21.03	2.2
3.	Junagadh	17.25	1.2
4.	Pariya (Valsad)	24.58	10.11

4.6 *Correlation Between Disease Incidence, Burl Size and Fruit Yield Loss with Tree Age*: Correlation between age of the tree and formation of burl in *Langra* and *Rajapuri* varieties about the incidence of burl, their size and fruit yield loss showed an increase of incidence with the increase in the age of the trees. The highest disease incidence, burl size, fruit yield loss and several burls per plant were recorded in trees of both varieties having more than 40-years of age. The highest disease incidence (97.07 %), largest burl size (870.00 cm^2), maximum fruit yield loss (33.27 %) and the number of burls (1-20) per plant were recorded in more than 40-year-old plants, whereas the lower disease incidence (49.25 %), small burl size (30.0 cm^2), minimum fruit yield loss (10.1 %) and the number of burls (1-5) per plant were recorded in 10- to 20-year-old plants. (Table 11).

Table 11. Correlation between tree age, burl incidence, size, fruit yield loss and range of burls per individual tree in *Langra* variety of *Mangifera indica* L.

Tree age (year)	Disease Incidence (%)	Average burl size (cm^2)	Fruit yield loss (%)	Average range burl/plant
10-20	49.25^{c}	30.00^{d}	10.1^{d}	1-5
20-30	82.37^{b}	120.00^{c}	16.57^{c}	1-10
30-40	$93.92^{a, b}$	290.00^{b}	26.48^{b}	1-15
40-100	97.07^{a}	870.00^{a}	33.27^{a}	1-20

Similarly, in variety *Rajapuri*, the highest disease incidence, large burl size, maximum fruit yield loss and the number of burls per plant were recorded 32.36 %, 113.88 cm^2 10.15 % and 1-5 respectively in the age group above 40-year plants while the lowest incidence i.e., 6.25 %, burl size 8.1 cm^2, fruit yield loss 10.1 % and the number of burls per plant 1-2 were noted in young (10 to 20 years old) plants of *Rajapuri* (Table 12). The comparison of correlation studies with plant age is given in Figure 20.

Table 12. Correlation between tree age, burl incidence, size, fruit yield loss and range of burls per individual tree in *Rajapuri* variety of *Mangifera indica* L.

Tree age (year)	Disease Incidence (%)	Average Burl size (cm^2)	Fruit yield loss (%)	Average range burl/plant
10-20	6.25^{c}	8.1^{d}	1.20^{c}	1-2
20-30	12.50^{b}	19.68^{c}	2.67^{c}	1-3
30-40	15.91^{b}	32.04^{b}	5.85^{b}	1-4
Above 40	32.36^{a}	113.88^{a}	10.15^{a}	1-5

4.7 *Correlation Between Burl Incidence, Burl Size, Fruit Yield Loss with Climate*:

The climatic factors like temperature, relative humidity and rainfall (i.e., maximum and minimum) were evaluated in relation to the incidence of burl disease, size and fruit yield loss in a *Langra* variety and *Rajapuri* variety from four different parts of Gujarat. If compared within four regions of the state, the highest disease incidence (91.98 %), average burl size (870.00 cm^2), fruit yield loss (24.58 %) and the number of burls per plant (1-20) were recorded from Valsad (Pariya) region for the variety *Langra*. As compared to other parts of the state, the Valsad district receives more precipitation (mean annual rainfall 182.80 mm) and relative humidity (annual average 69.73 %) in this region, whereas the Anand district stands in the second position for rainfall and relative humidity (Table 13).

If correlated with climatic conditions, variety *Rajapuri* showed the highest disease incidence (26.31 %), average burl size (133.13 cm^2), fruit yield loss (10.11 %) and the number of burls per plant (3-5) from the Valsad region. However, the lowest incidence, smaller burl size, minimum fruit yield loss and a smaller number of burls per plant were positively correlated with relative humidity in the Junagadh region (Table 14). Similarly, variety *Langra* also showed the highest burl incidence (55.49 %), maximum burl size (40.00 cm^2) and fruit yield loss (17.25 %), a fewer number of burls per individual (1-5) in plants growing at Junagadh. Junagadh region of Saurashtra falls under the arid zones of India possessing the lowest relative humidity (60.59 %) and rainfall (Table 13). Variety *Rajapuri* also followed a similar trend for all the above-mentioned parameters including disease incidence, burl size, fruit yield loss and the number of burls per plant with lower relative humidity and rainfall from Junagadh (Table 14).

Table 13. Correlation between climate and burl incidence (%), burl size (mm), fruit yield loss (%), the average number of burls per plant, temperature (min and max), relative humidity (%) and rainfall (mm) in *Mangifera indica* cv. *Langra* in different growing locations of Gujarat state

Location	**Burl incidence (%)**	**Burl size (cm^2)**	**Fruit yield loss (%)**	**No. of burl/plant**	**Temperature (°C)**		**Relative humidity (%)**	**Average Rainfall (mm)**
					Max.	**Min.**		
Anand	88.36^{b}	290.00^{b}	22.74^{b}	1-15	34.07^{a}	19.61^{b}	65.59^{b}	100.94^{b}
Dantiwada	87.34^{c}	50.00^{c}	21.03^{c}	1-2	34.23^{a}	19.70^{b}	64.00^{b}	62.52^{d}
Junagadh	55.49^{d}	40.00^{d}	17.25^{d}	1-5	34.27^{a}	20.26^{a}	60.59^{c}	74.94^{c}
Valsad	91.98^{a}	870.00^{a}	24.58^{a}	1-20	33.37^{b}	18.74^{c}	69.73^{a}	182.80^{a}

Means with the same letters (a, b, c, d, superscript) in the column shows no significant variation at P = 0.05 level in Duncan's multiple range tests

Table 14. Correlation between climate and burl incidence (%), burl size (mm), fruit yield loss (%), the average number of burls/plants, temperature (min and max), relative humidity (%) and rainfall (mm) in *Mangifera indica* cv. *Rajapuri* in different growing locations of Gujarat state.

Location	Burl incidence (%)	Burl size (cm^2)	Fruits yield loss (%)	No. of burl/plant	Temperature (ºC)		Relative humidity (%)	Average Rainfall (mm)
					Max.	Min.		
Anand	24.83^{a}	25.50^{b}	6.36^{b}	2-4	34.23^{a}	19.70^{b}	64.00^{b}	62.52^{d}
Dantiwada	9.09^{b}	16.25^{c}	2.2^{c}	2-3	34.07^{a}	19.61^{b}	65.59^{b}	100.94^{b}
Junagadh	6.25^{b}	8.10^{c}	1.2^{c}	1-2	34.27^{a}	20.26^{a}	60.59^{c}	74.94^{c}
Valsad	26.31^{a}	133.13^{a}	10.11^{a}	3-5	33.37^{b}	18.74^{c}	69.73^{a}	182.80^{a}

Means with the same letters (a, b, c, d, superscript) in the column shows no significant variation at P = 0.05 level in Duncan's multiple range tests

4.8 *Biochemical Analysis of Fruits*:

As mentioned earlier, a detailed study on biochemical analysis of fruits collected from the healthy and burl affected trees were taken further in *Langra* and *Rajapuri* varieties. The main aim of this analysis was to measure chemical alterations in the quality of fruits, their nutritional value and stem. Fruits collected from burl affected and healthy (burl free) trees of both varieties were analysed for the content of Total Soluble Solids (TSS), sugars, acidity and ascorbic acid (Figure 21). Fruits of the burl affected plants of *Langra* variety showed maximum content of TSS (18.8 °brix), total soluble sugar (9.59 %), reducing sugar (2.11 %), non-reducing sugar (7.49 %) and ascorbic acid (129.39 %) while the acidity was found minimum (0.15 %).

A similar trend was also recorded in *the Rajapuri* variety, which showed the highest content of TSS (17.43 °brix), total soluble sugar (11.15 %), reducing sugar (4.38 %), non-reducing sugar (6.77 %) and ascorbic acid (12.28 %) in fruits of burl affected plants whereas the acidity was found minimum (0.14 %). The change in TSS, total sugar, reducing sugar, non-reducing sugar and ascorbic acid in fruit collected from affected and burl free plants are shown in Figure 22.

4.9 *Biochemical Analysis of Mango Stem Wood*:

The biochemical difference in the burl wood and wood of healthy stem of both varieties were analysed from three different portions of the stem: i) Proper burl tissue, ii) Transition portion between burl and normal-appearing stem and, iii) non-infected (normal/healthy) stem. Total eleven parameters such as total contents of moisture, ash, cellulose, fibre, lignin, total soluble sugar, reducing sugar, non-reducing sugar, total phenol, starch and ascorbic acid were examined and compared with normal and burl portions as mentioned above.

4.9.1 *Moisture Content in Wood*:

The moisture content of freshly collected wood from the burl and normal trees showed a significant difference in both varieties (Figure 23). Variety *Langra* showed the highest content of moisture in the burl portion of wood (6.28 % ± 1.402), followed by the transition portion (*i.e.*, the portion between burl and normal) of the stem (4.59 % ± 1.534), while it was minimal in the wood portion of the normal/healthy stem (3.61 % ± 1.171). A similar trend was also observed in *the Rajapuri* variety, which showed the highest (2.79 % ± 0.942) moisture content in the burl portion of wood, followed by the transition zone (2.67 % ± 0.715) and it was relatively less in normal stem (2.04 % ± 0.851). When compared within the variety, moisture content was found highest in *Langra* than *Rajapuri* and the declining order of moisture content (i.e., higher to lower) toward infected to healthy stems.

4.9.2 *Ash Content in Wood*:

Ash content showed considerable variations in the samples of burl wood, transition portion of wood between normal and burl, and normal stems of samples freshly collected from both varieties. In *Langra* the ash content was highest in the burl portion of the stem (3.52 % ± 0.926) and it was lowest in the transition portion (*i.e*., portion between normal and burl portion) of the stem (2.77 % ± 0.887), while in-between values were observed in the normal stems (2.97 % ± 0.691). In contrast, in the *Rajapuri* variety, the ash content was highest in normal/healthy stem (1.54 % ± 0.364), followed by the transition portion of the stem (1.33 % ± 0.227) and the lowest values (1.11 % ± 0.086) were observed in the wood of burl portion of the stem (Figure 24).

4.9.3 *The Cellulose Content in Wood*:

The cellulose content significantly increased in the wood of burl affected stems of both varieties (Figure 25). The percentage of cellulose content was highest in variety *Langra* in burl portions (21.71 % ± 2.87), followed by transition portions of the stem (17.62 % ± 2.72), whereas it was lowest in normal stems (14.48 % ± 1.58). A similar pattern of variations was also observed in the variety *Rajapuri*. The cellulose content was reported maximum in burl portions of the stem (30.70 % ± 1.905), followed by transition portions of the stem (28.66 % ± 2.121) and it was observed minimum in healthy stems (26.72 % ± 1.913).

4.9.4 *Lignin Content in Wood*:

Lignin is one of the important components of the secondary xylem (wood) and it not only plays important role in mechanical support but also plays a vital role in defence mechanism. Therefore, as per expectation, the percentage of lignin content was found highest in burl portions of the stem in both varieties of mango (Figure 26). The *Langra* variety has the highest per cent (24.32% ± 3.601) of lignin content in burl wood, followed by transition portions (20.81% ± 3.064) and whereas it was lowest in healthy/normal stems (18.40% ± 1.032). Similarly, *the Rajapuri* variety also showed a similar pattern of variations in lignin content (Figure 26). It was maximum in the wood of burl portions

(17.18% ± 2.281), followed by the transition portions (16.88% ± 1.657), and it was found minimum (15.13% ± 2.345) in healthy stems.

4.9.5 *The Xylem Fibre Content in Wood*:

The xylem fibres are the major constituent of the wood which contains cellulose and lignin. Examination of xylem fibre content showed that the percentage of fibres decreases drastically in the burl portion of wood in both varieties. Their percentage in *Langra* variety was minimal in burl portion (45.48 % ± 2.738), it was relatively more (48.68% ± 1.591) in transition portions of burl and normal looking stems while xylem of the healthy stem showed maximum (51.32 % ± 1.667) percentage (Figure 27). Likewise, in *the Rajapuri* variety, the percentage of xylem fibres was higher in the secondary xylem of healthy stems (59.10 % ± 2.44) and it was recorded lowest in the burl portions (48.67 %± 2.542) while it ranged in between (49.39% ± 2.513) the xylem of burl and healthy stems (Figure 27).

Maceration of normal, and burl infected portion of secondary xylem of both varieties showed that the length and width of the secondary xylem fibres were found maximum in the normal stem as compared to the affected portion of the secondary xylem. They were found highest in normal stems (1181µm ± 8.263 and 20 µm ± 0.873) and lowest in burl wood (665.53 µm ± 10.26 and 3.10 µm ± 0.475) of *Rajapuri* variety (Figure 28). Similarly, in variety *Langra*, the length and width were found highest in normal stem (1018.77 µm ± 34.61 and 3.48 µm ± 0.63) and lowest (67.87 µm ± 14.241) in the burl portions of the stem (Figure 28).

4.9.6 *Sugar Content in Wood*:

The total soluble sugar content in the xylem of both the germplasms also changed significantly in the stem portions of burl and healthy wood. The amount of total soluble sugar was found highest in burl wood of both varieties. The variety *Langra* showed the highest sugar content (38.45 % ± 1.891) in burl wood, relatively less in the transition portion of wood (36.22 % ± 2.112) and lowest (27.69 % ± 2.634) in healthy wood (Figure 29A). Likewise, in *the Rajapuri* variety; the amount of sugar was observed highest (48.02 % ± 3.025) in burl wood, followed by in transition portion (mean 30.36 % ± 2.327) and the lowest amount of sugar was recorded (15.85 % ± 1.543) in the wood of healthy stems (Figure 29A). Similarly, the concentration of the reducing sugar was also found highest in burl wood of variety *Langra* (18.56 % ± 1.741) and *Rajapuri* (26.46 % ± 2.244), whereas, it was found relatively less in the transition portion of the wood of *Langra* (17.25 % ±

1.872) and *Rajapuri* (13.19 % ± 1.464) and lowest in the healthy wood of *Langra* (12.04 % ± 1.63) and *Rajapuri* (5.51 % ± 1.09) varieties (Figure 29B).

The concentration of the non-reducing sugar was found maximum in the burl wood of both varieties as compared to the healthy wood. The burl wood of variety *Langra* has maximum content of non-reducing sugar (19.89 % ± 2.301), whereas the transition portion and normal xylem showed its minimum content (18.97 % ± 2.512 and 15.65 % ± 1.084), respectively (Figure 29C). Correspondingly, variety *Rajapuri* showed maximum content of non-reducing sugar in burl wood (21.56 % ± 2.44) and minimum in transition and a healthy portion of wood (17.17 % ± 1.202 and 10.34 % ± 1.061) respectively (Figure 29C).

4.9.7 *Starch Content in Wood*:

Starch is the most abundant source of sugar that contains high energy for plant growth and development. Its concentration is inversely related to sugar content. In the present study, no significant difference was recorded in the starch content of normal, burl wood and transition portion of wood in stems of normal and healthy trees. The highest starch content (1.19 % ± 0.716) was found in the healthy stem and lowest (0.70 % ± 0.021) in the burl wood of *Langra* (Figure 30). Analogous results are also recorded for *the Rajapuri* variety, which showed the highest content of starch in healthy wood (1.05 % ± 0.836) and lowest (0.74 % ± 0.0.167) in the burl wood (Figure 30).

4.9.8 *Ascorbic Acid*:

Analysis of ascorbic acid content in the stem wood showed that it was maximum in burl wood as compared to secondary xylem of the healthy trees of both varieties. The percentage of ascorbic acid content was noted highest (35.42 % ± 9.587) in burl wood gradually declined in the transition portion of the xylem (33.33 % ± 5.561) and it was recorded lowest in normal/healthy (25.00 % ± 4.745) wood of *Langra* variety (Figure 31). A similar trend was also noticed in the variety *Rajapuri.* The highest content of ascorbic acid was found in burl wood (41.67 % ± 3.312) followed by transition portion (33.33 % ± 3.354) and lowest in healthy (29.17 % ± 3.013) wood (Figure 31).

4.9.9 *Total Phenol*:

Phenol is the main components of the secondary metabolites that imparts resistance to the plant during pathogen attack. In the present study, total phenol content was analysed and its' presence was found highest in burl wood portion as compared to transition portion and healthy of wood of the stems of both the varieties of mango. The xylem of the burl portion of in variety *Langra* showed the highest phenol content (1.37 % ± 0.171) followed by transition portion of wood (1.25 % ± 0.152) and it was noticed lowest (1.02 % ± 0.144) in healthy stem (Figure 32). Likewise, burl wood of *Rajapuri* variety also showed higher content of total phenol (6.69 % ± 1.52), which declined in the transition portion of the wood (4.53 % ± 1.043) and found minimum in healthy 2.35 % ± 0.781) wood (Figure 32).

4.10 Anatomical Examination:

Wood anatomy of healthy (normal) stem and burl was also investigated for both varieties using histological methods. When compared with healthy and affected wood tissue, drastic alterations were not only observed in the composition of wood of healthy and burl affected trees but also showed alterations in the structure of the individual xylem elements in both species. Structure and composition of healthy (burl free stems) wood of both varieties i.e., *Langra* and *Rajapuri* remained similar while the pattern of deformation in the xylem structure of burl and transition portion between normal looking and burl wood shared a similar pattern of xylem elements orientation and deformation. Therefore, a description of both varieties is provided commonly unless and the specific difference is pointed out variety wise.

4.10.1 *Structure of (Healthy) Normal Secondary Xylem*:

In transverse view, the secondary xylem of healthy (normal, *i.e.*, burl free) stem was diffused porous with indistinct growth rings (Figure 33A). It was composed of vessels, tracheids, axial and ray parenchyma cells (Figure 33A-E). Vessels mostly solitary but radial multiples of 2-3 vessels were also observed frequently (Figure 33B). They were mostly circular but tangentially they may be oval to elliptic in outline with the presence of a simple perforation plate arranged transverse to slightly oblique on their terminal ends. In tangential view, vessel elements showed alternate bordered pits that were oval to circular in outline, measuring from 7-9 µm in diameter. Vessel elements were 96-280 (185 ± 5.987) µm in length and 64-165 (112 ± 2.891) µm in tangential diameter. Axial parenchyma cells were vasicentric to an aliform-vasicentric forming a wide sheath of parenchyma cells around vessels (Figure 33A, B). Xylem fibres non-septate, tapered at the ends and wide at the middle. They were measured from 989-1372 (1181 ± 8.263) µm in length and 18-23 (20 ± 0.873) µm in width. Xylem rays were uni-biseriate and heterocellular whereas tri-seriate rays were observed infrequently. In tangential view, terminal ray cells mostly procumbent or vertically upright while in between cells were arranged in 2-3 rows, irregular in size and maybe square to polygonal in shape (Figure 33C-E). They were measured from 178-329 (248 + 3.632) µm in height and 29-59 (36 $\pm$ 2.88) µm in width, whereas diameter of ray cells was measured from 10-30 (24 $\pm$ 0.98) µm.

4.10.2 *Structure of Burl and Transition Wood*:

As mentioned earlier, there are no variety-specific alterations in the secondary xylem formed in the burl wood and wood portion between burl and normal (henceforth referred to as transition wood for both the varieties.

Unlike normal secondary xylem, extreme structural alterations were noticed in the burl wood. The composition of the secondary xylem of the burl showed an increase in isodiametric cells looking like lignified callus cells while axial parenchyma and fibres were reduced significantly. All the cell types of secondary xylem such as fibres, vessel axial and ray parenchyma cells (Figures 34A-D, 35A-C) were observed. However, as seen in transverse sections all the cells lost their morphological characteristic and became deformed. They were asymmetrical in shape and exhibited substantial discrepancy in their size as compared to those of normal/healthy stems and branches (Figures 34A-D, 35A-B, 36A-D). Length of the secondary xylem fibres decreased significantly while the percentage of isodiametric parenchyma cells looking like ray cells were augmented greatly (Figure 37A-D).

The structure of the secondary xylem varied at different positions of burl developmental stages. In transverse view, a 10 cm diameter burl of both the varieties showed loss of normal arrangement of axial and radial elements in the transition part of the burl (Figure 35C). As shown in Figure 35C, the axial elements were oriented in both radial and transverse direction within the same transverse section of the burl xylem. The secondary xylem of this part showed a considerable increase in ray cells diameter (Figure 37A-D) in tangential sections, measuring about 33-70 (46 $\pm$ 1.73) µm as compared to those of ray cells (13-30 [19 $\pm$ 0.98] µm) of healthy xylem (Figure 33C, D). The presence of rhomboidal crystals in the ray cells of both normal and burl xylem is a common character and they were observed frequently in both varieties (Figure 36b).

Though they observed frequently, they were relatively more in burl wood. The secondary xylem of this portion was characterized by the presence of deformed and irregular shape and sized cells with short fibres and large ray cells (Figure 36A-D). In contrast, cells appearing like tracheids were shorter than the vessel elements and showed the presence of elongated, oval to oblong pits with reduced borders (Figure 36A, C).

Similar types of pits were also observed on the ray cells (Figure 37A). The secondary xylem elements *viz*. vessel elements and ray cells of this portion were occluded with tyloses (Figures 36D, 37B-D). Tyloses in these cell types were smaller in size if compared with those of normal vessel elements (Figure 37B). They were larger than starch grains and found adjacent to the pits on the cell walls. These tyloses were measured from 22 to 59 (33 $\pm$ 3.238) μm in diameter. They were also observed in the relatively wider vessels (Figure 37B) located in the transition portion of the burl xylem (Figure 37E, F).

On the other hand, the core part (i.e., inner portion) of the burl showed complete deformation of the xylem elements with a greater number of isodiametric cells looking like lignified callus cells or wound tissue (Figure 35F, G). In this portion, the frequency of large diameter vessels decreased significantly while narrow diameter vessels appearing like tracheids and parenchyma cells with bordered pits were more common (similar to those shown in Figures 36A, C, 37A). Similar to the transition portion, this region of wood also showed the formation of tyloses in narrow vessels and ray cells. The core portions of the burls were composed of fibres and vessels that were arranged as spirals and circles (Figures 34A, D, 35A) while parenchyma cells were more or less isodiametric (Figures 2B, C, 3A), and they were intermixed with fibres (Figures 34A, 35B) or randomly oriented in serpentine fashion (Figure 35A, D).

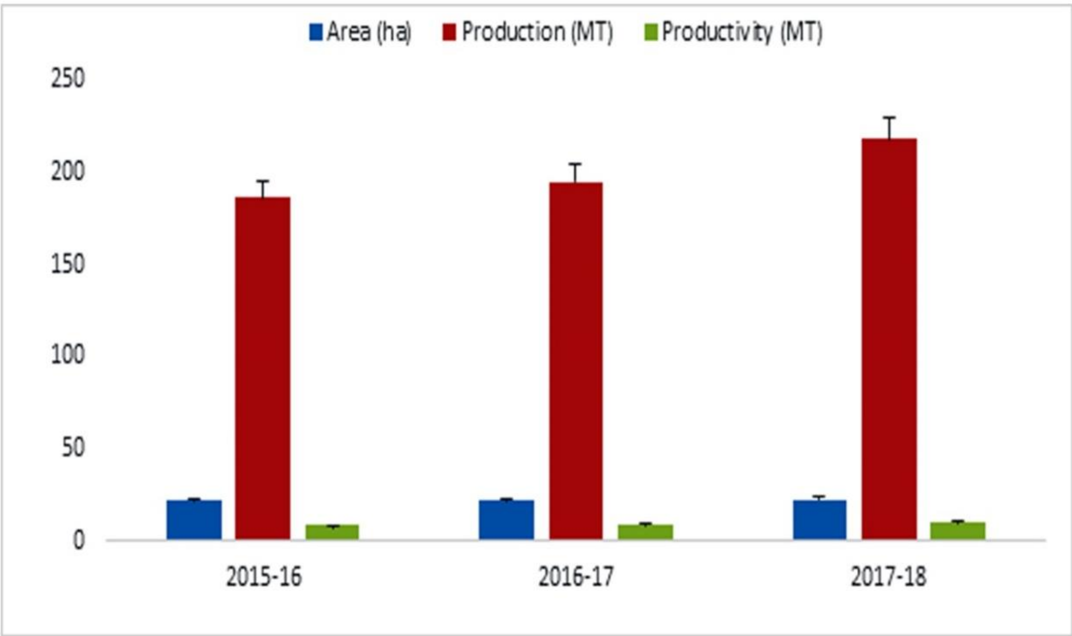

Figure 1. Area, production and productivity of Mango in India.

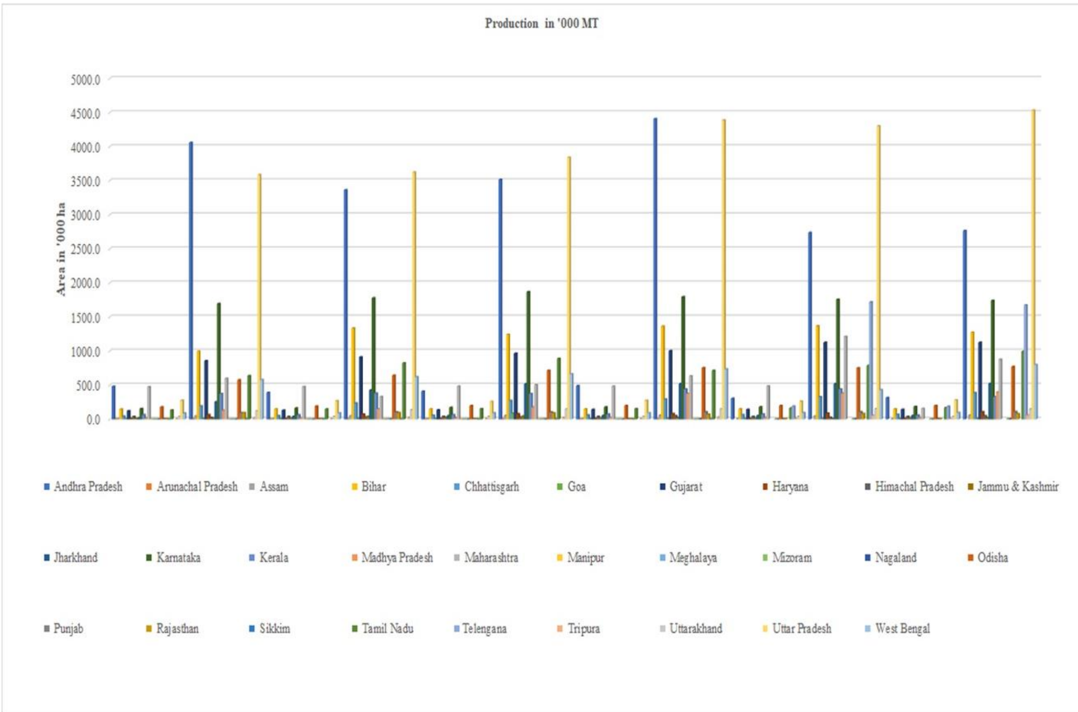

Figure 2. Area and production of mango in different state of India.

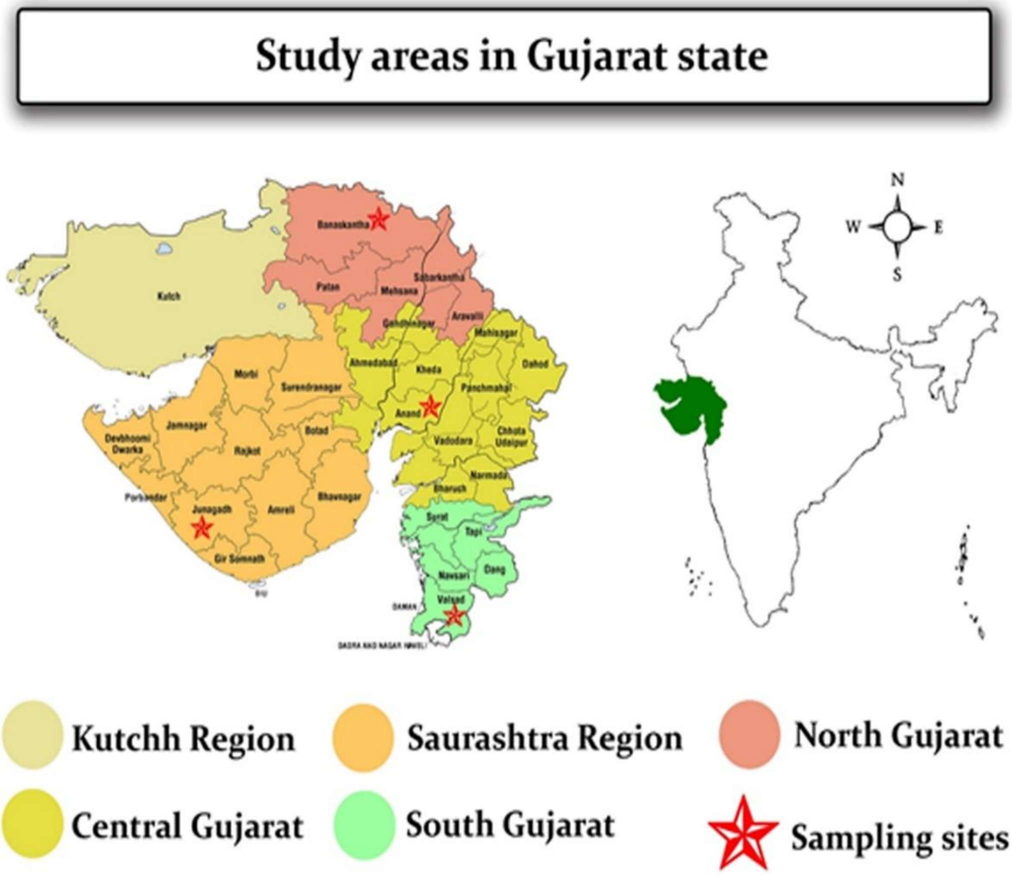

Figure 3. Study area of Gujarat state selected for burl study.

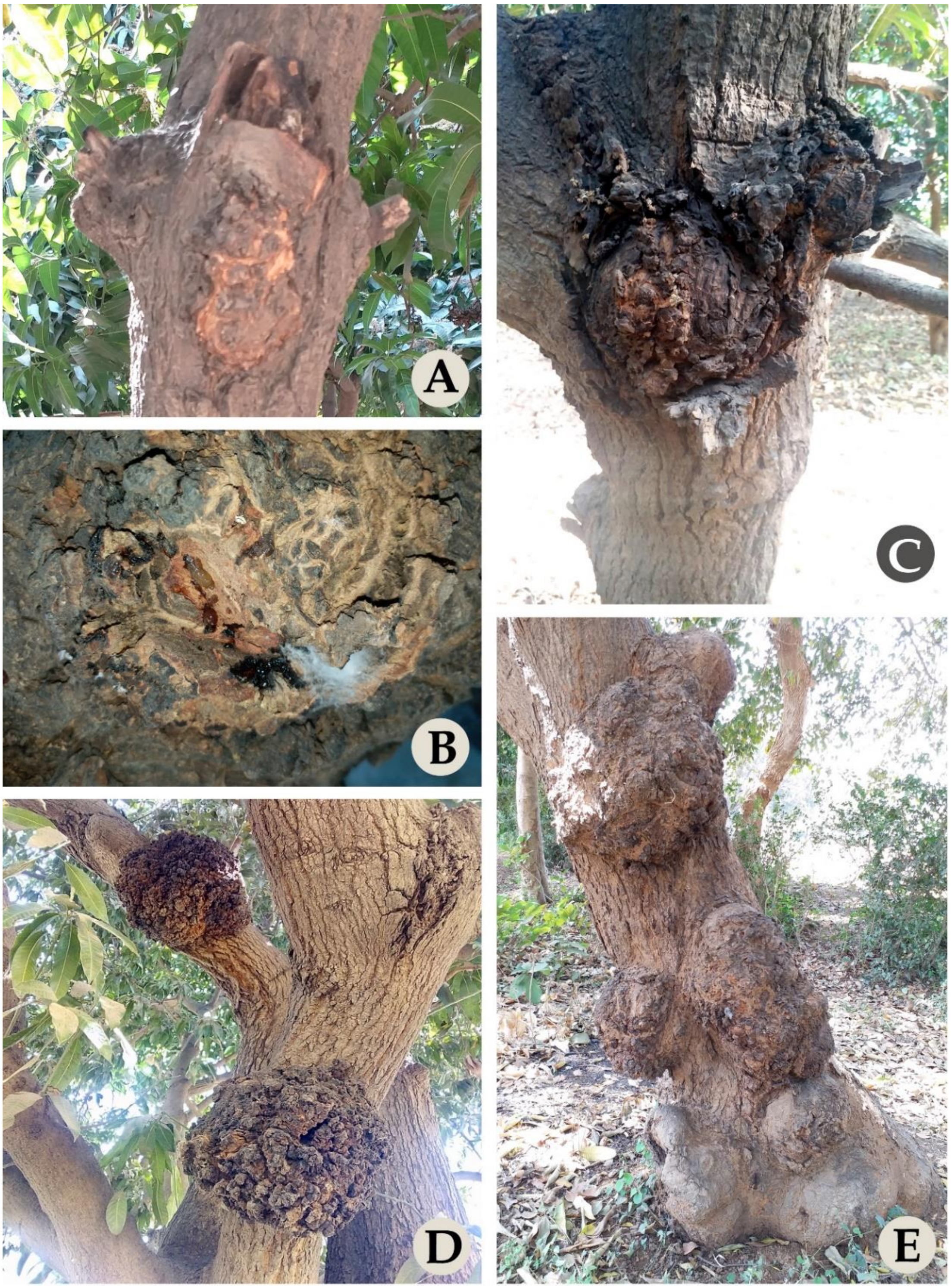

Figure 4. Variation in burl morphology (A) Small size burl, (B) Gummosis in burl, (C) Size and no of burl increasing, (D, E) Burls with different size, shape and colour.

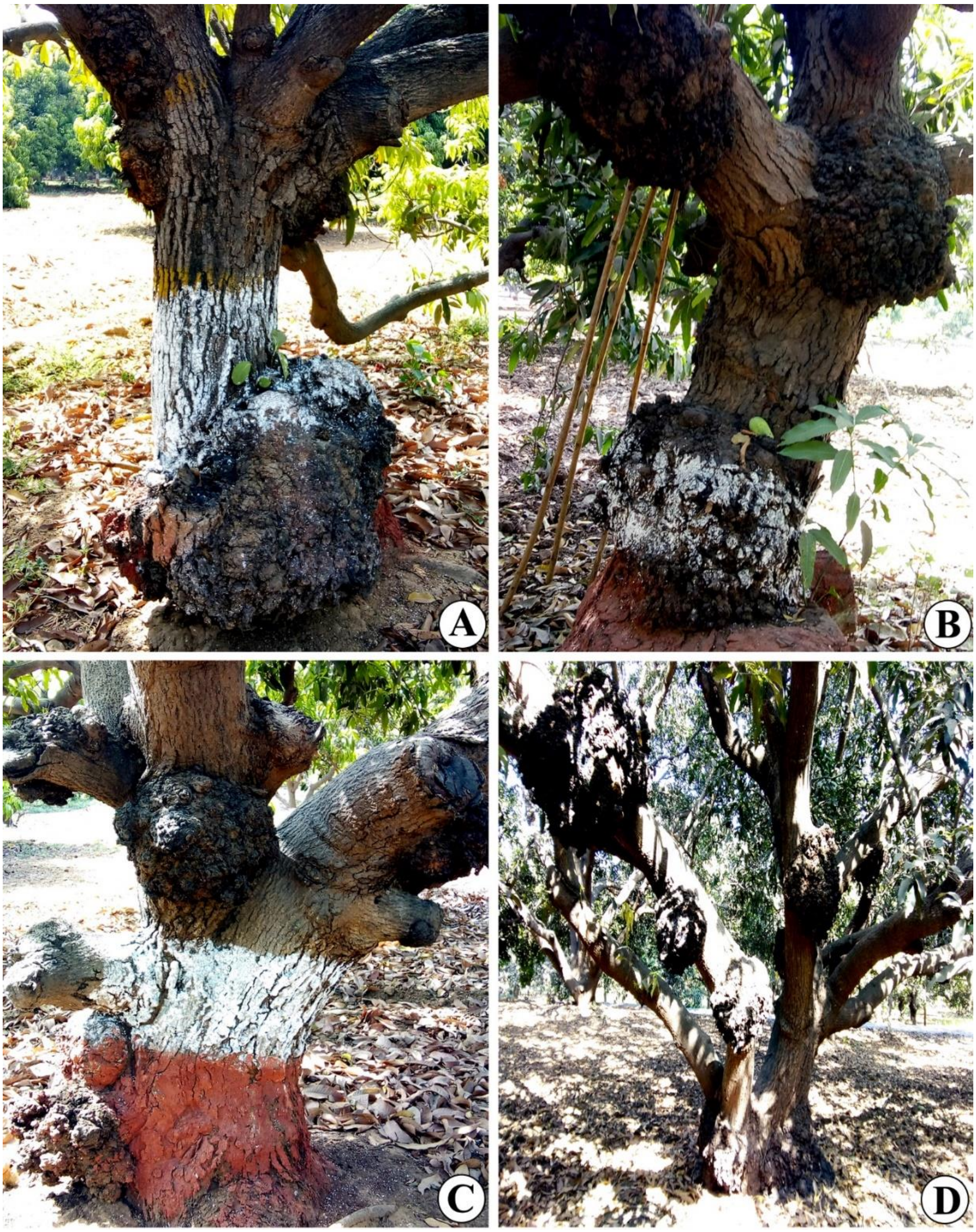

Figure 5. Large size of burl with globose shape in *Langra* variety (A) Single burl on main trunk with big size, (B, C) Globose shape burl on main and primary branches, (D) Large size of burls

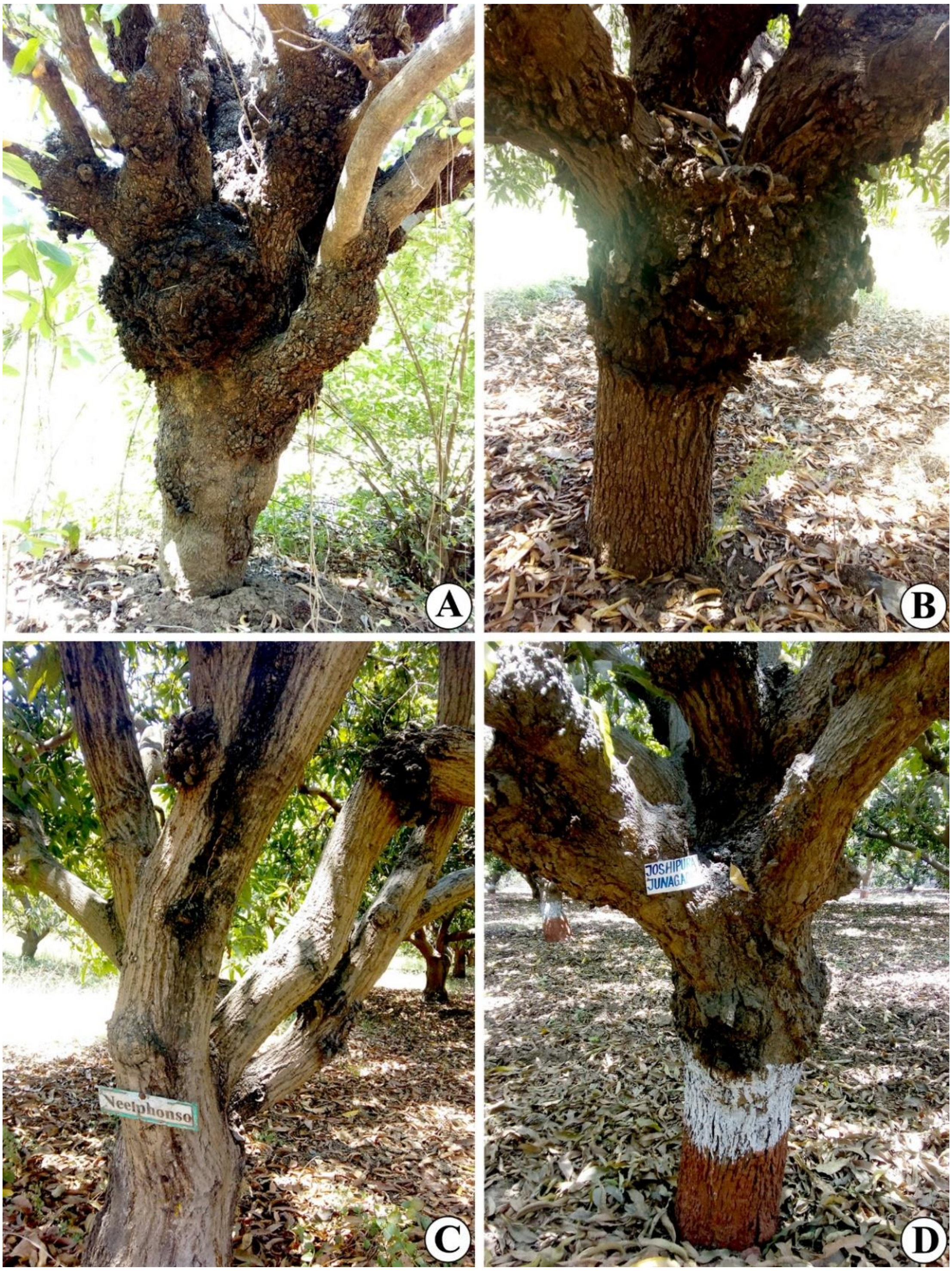

Figure 6. Morphology of burl in different mango varieties (A) Burl in *Bathua* germplasm entirely cover stem and lower branches, (B, C, D) burl showing rough surface and scaly bark with different shape, colour and size.

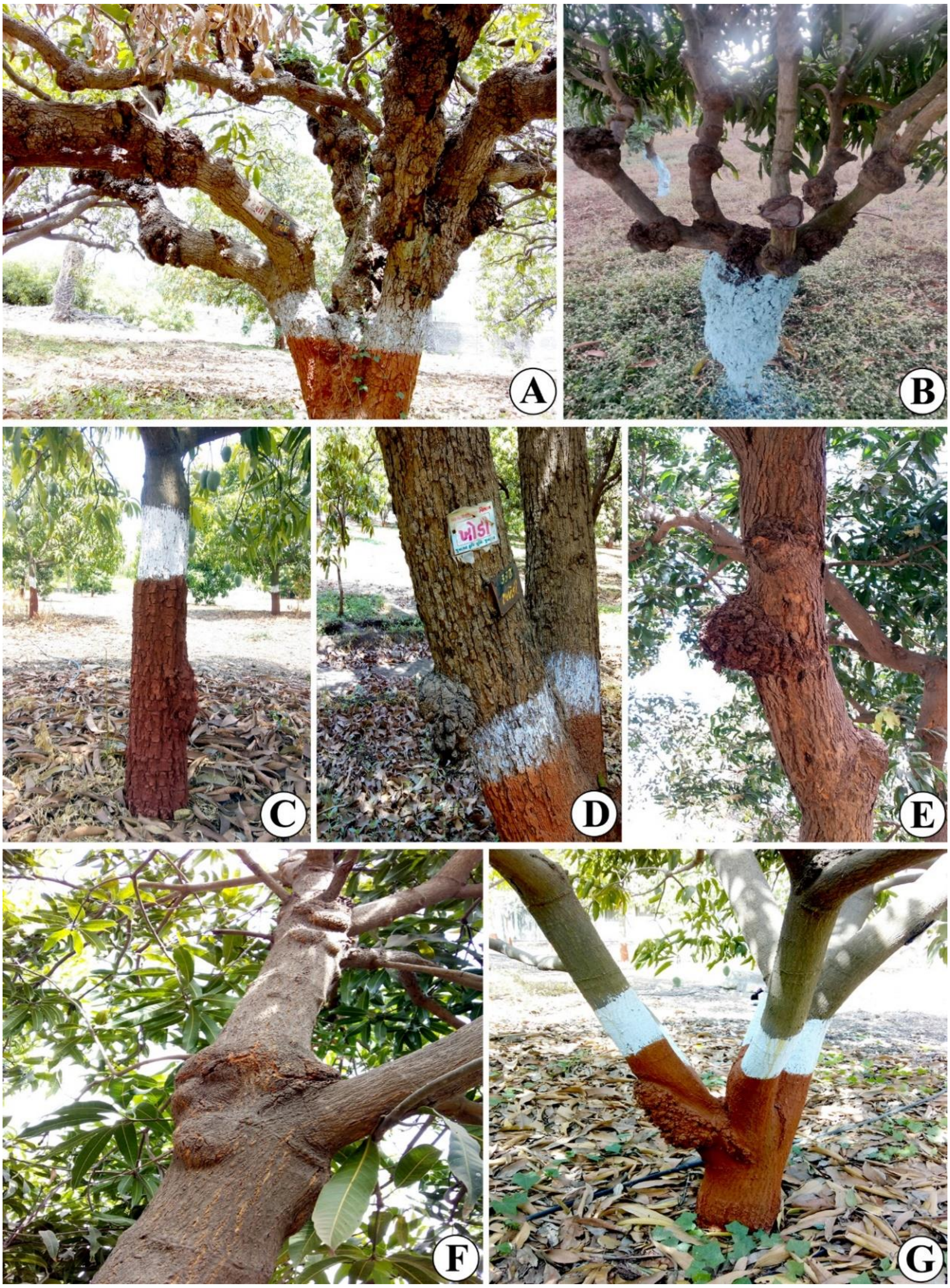

Figure 7. Variation in burl; (A) Entire plant covered with small burls, (B) Burl shape, colour and size in *Mahmood Vikarabad*, (C) Young burl on *Rajapuri* × *Kesar*, (D, E) Single burl in grey and brown to dark brown colour, (F) Burl in globular shape with smooth surface (G) Small elongated burl on *Langra*

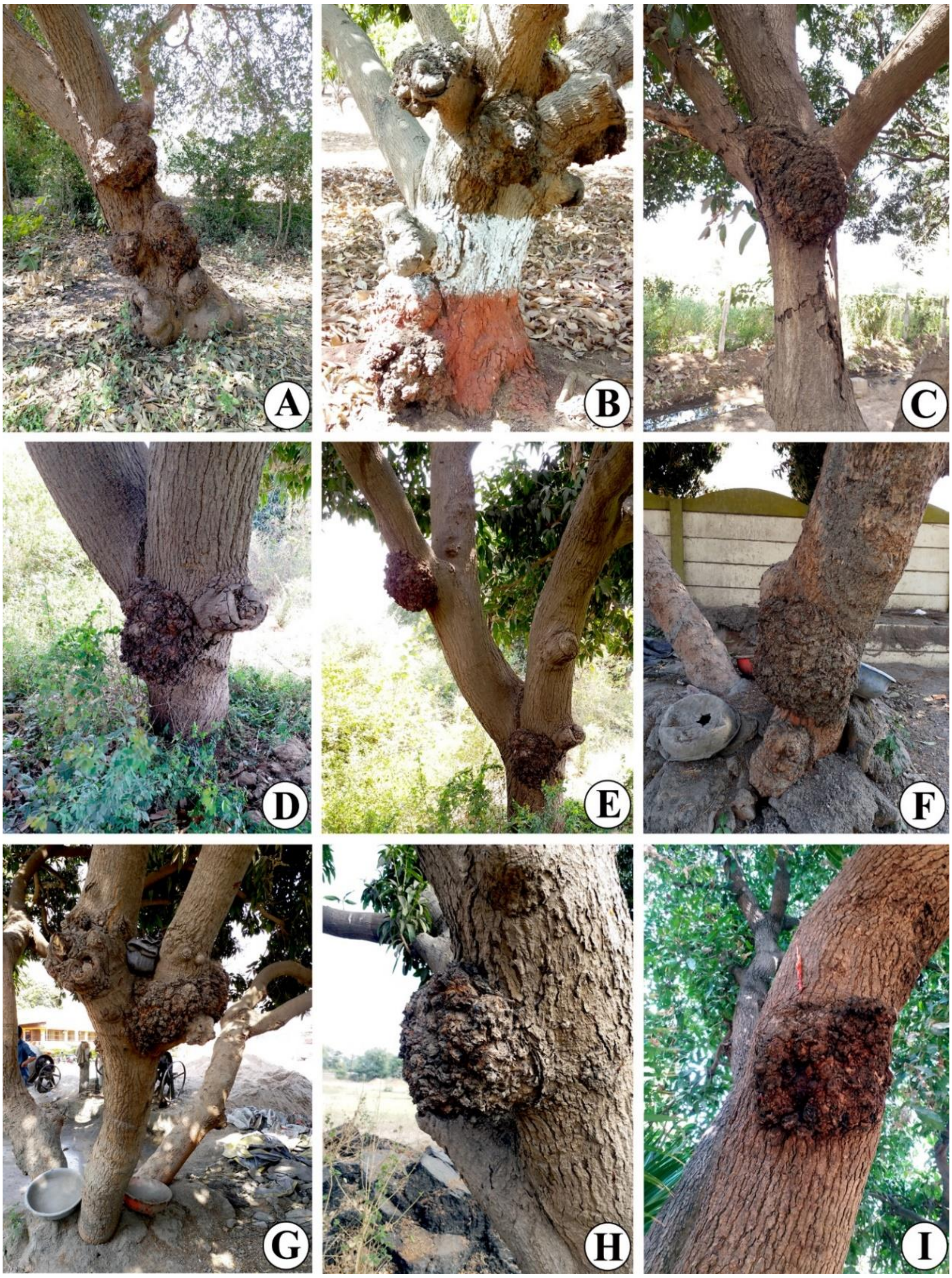

Figure 8. Burl morphology in various germplasm; (A) *Rajapuri*, (B) *Langra*, (C) *Olour*, (D) *Krishan Bhog*, (E) *Arka Punit*, (F) *Seedling*, (G) *Seedling 307*, (H) *Hybrid*, (I) *Rajapuri*

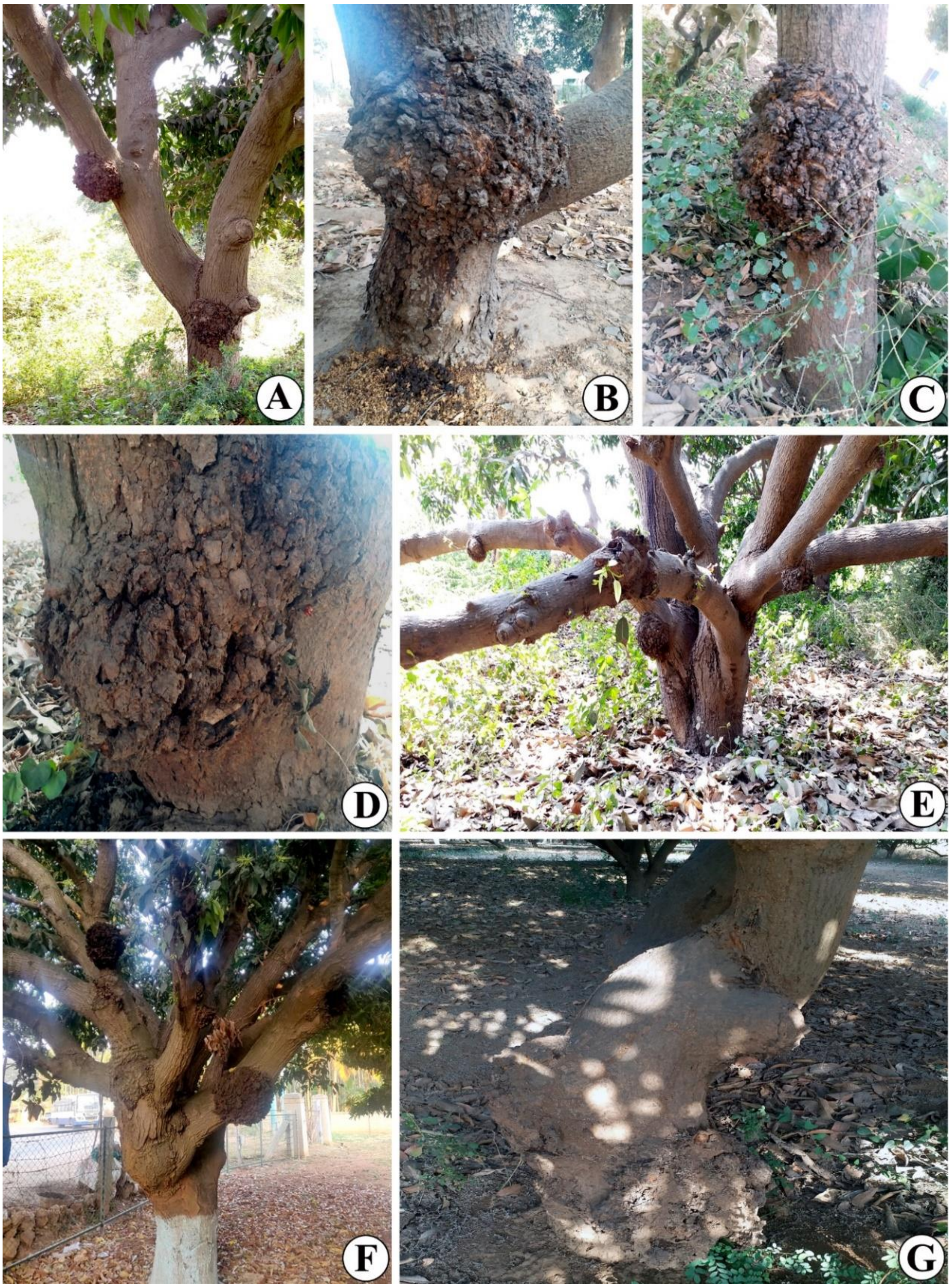

Figure 9. Burl on main trunk and primary branches showing various size, shape and colour; (A) *Krishan Bhog*, (B) *Langra*, (C) *Sindhu*, (D) *Arka Punit*, (E) *Rajapuri* (G) *Alphanso* × *Sabja*

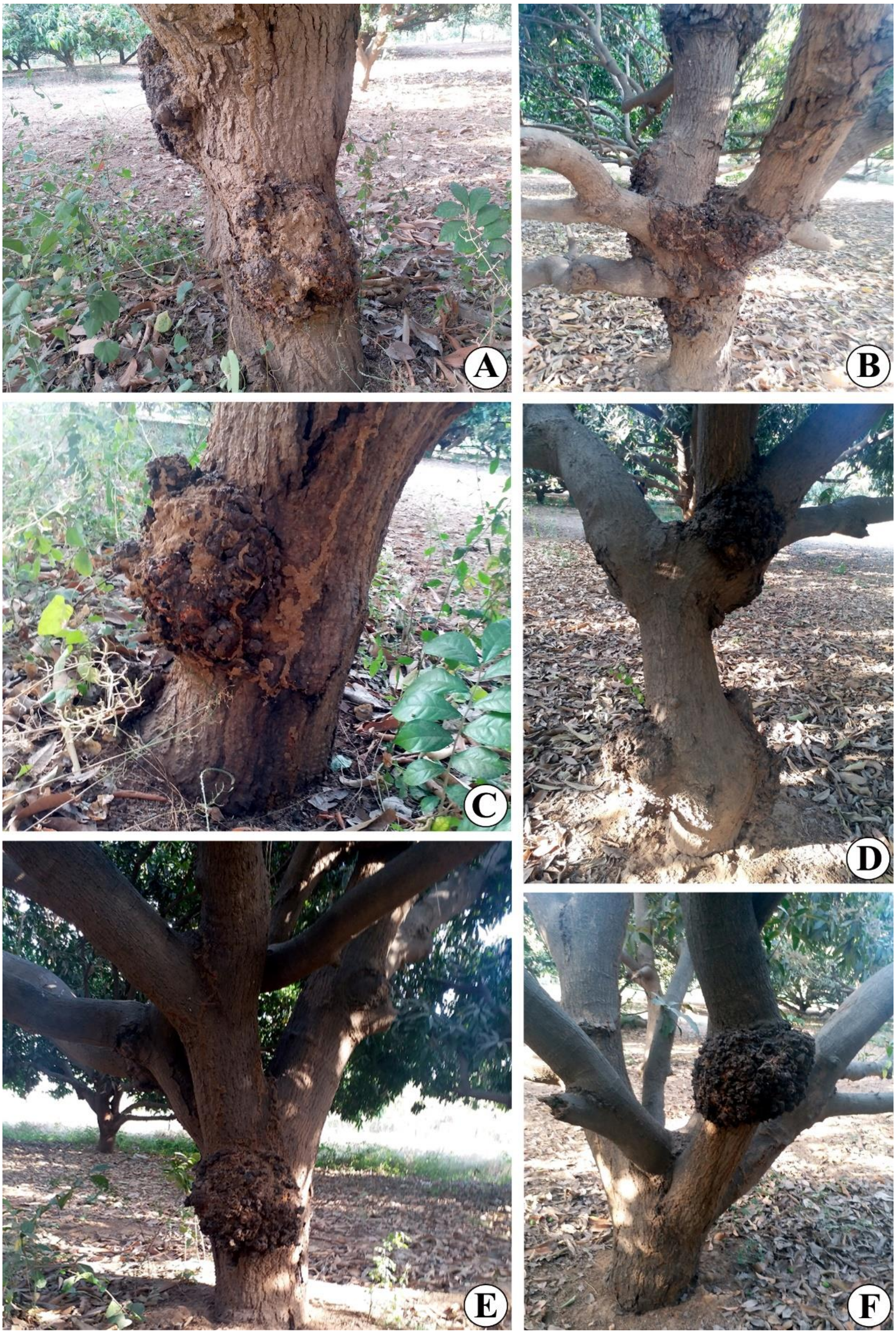

Figure 10. Termite or insects attack on mango germplasm in Gujarat.

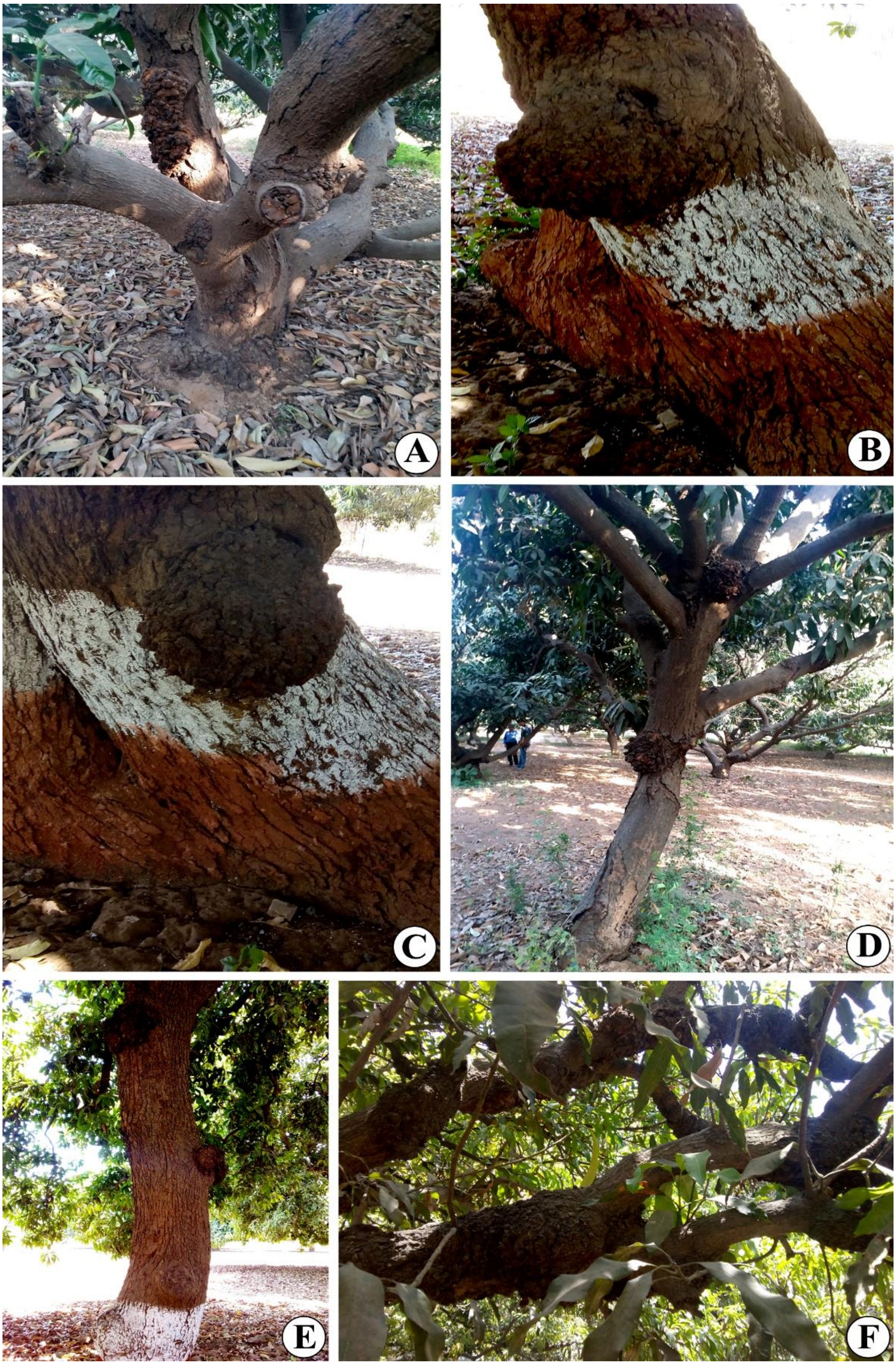

Figure 11. Burl colour and surface in Mango germplasm; (A) *Langra*, (B, C, E) *Rajapuri*, (D) *Arka Punit*, (F) *Joshipura Junagadh*

Figure 12. Comparison of burl size and shape in *Langra* (A, B) and *Rajapuri* germplasm (C, D).

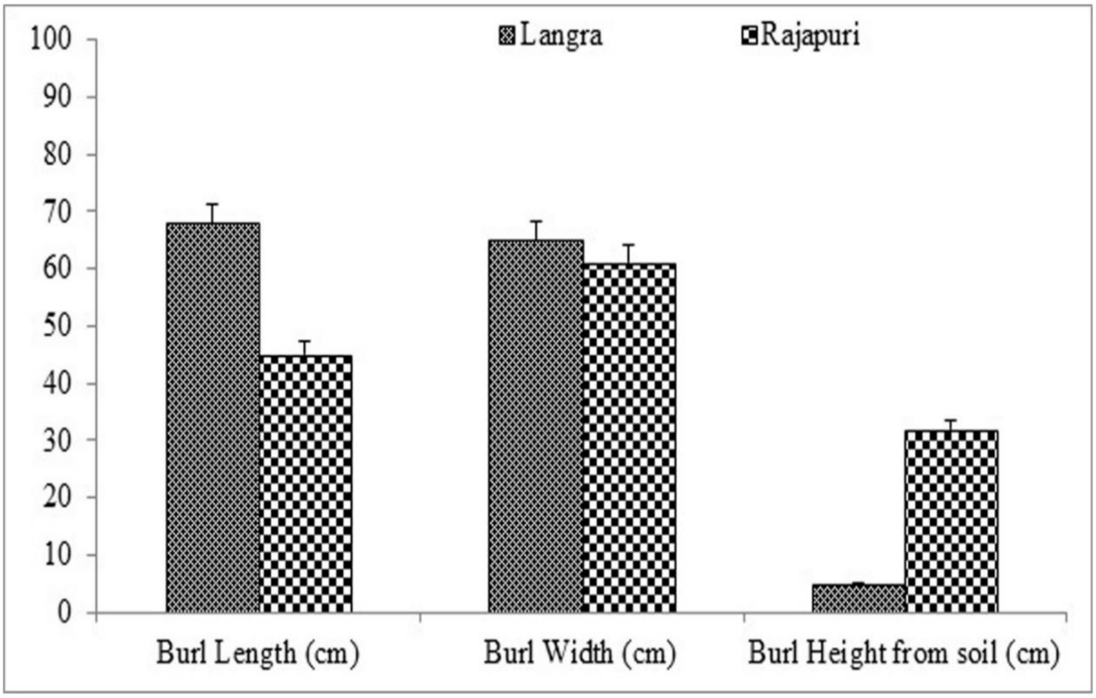

Figure 13. Comparative study of burl morphology in *Langra* and *Rajapuri* varieties.

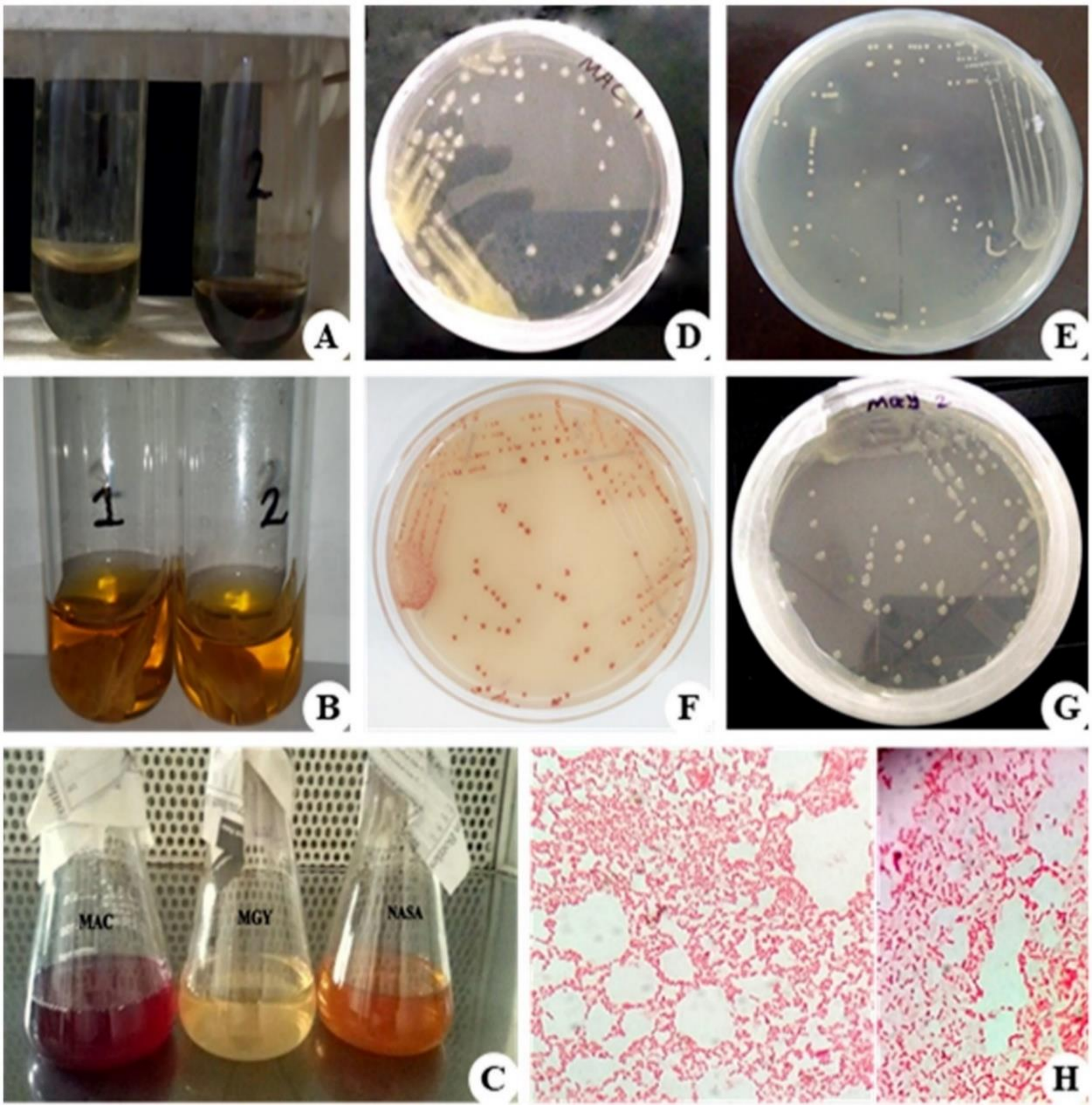

Figure 14. Bacteria isolation and their morphology on different media, Bacterial suspension (A, B), different media (C), colony on *MacConkey* media (D), (E) Hoffer's media, (F) NASA media (G) MGY media (H) Gram staining

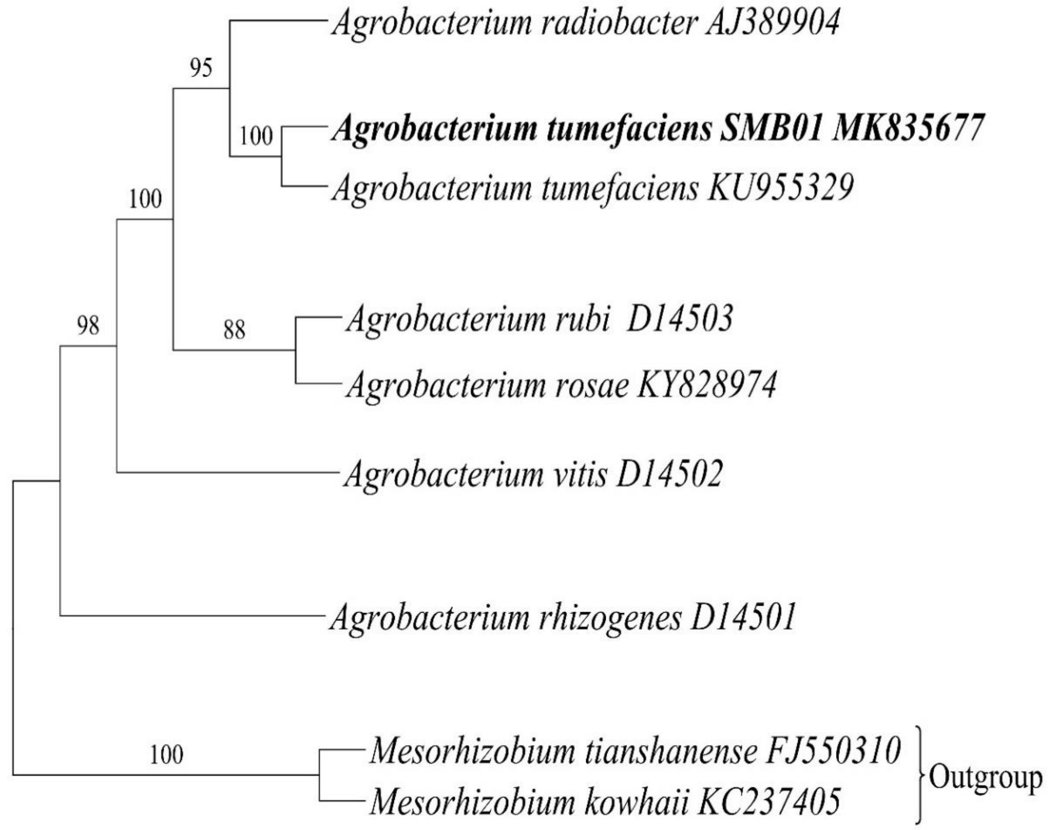

Figure 15. Phylogenetic analysis of *Agrobacterium* isolated from mango burl with ex-type retrieved from NCBI GenBank

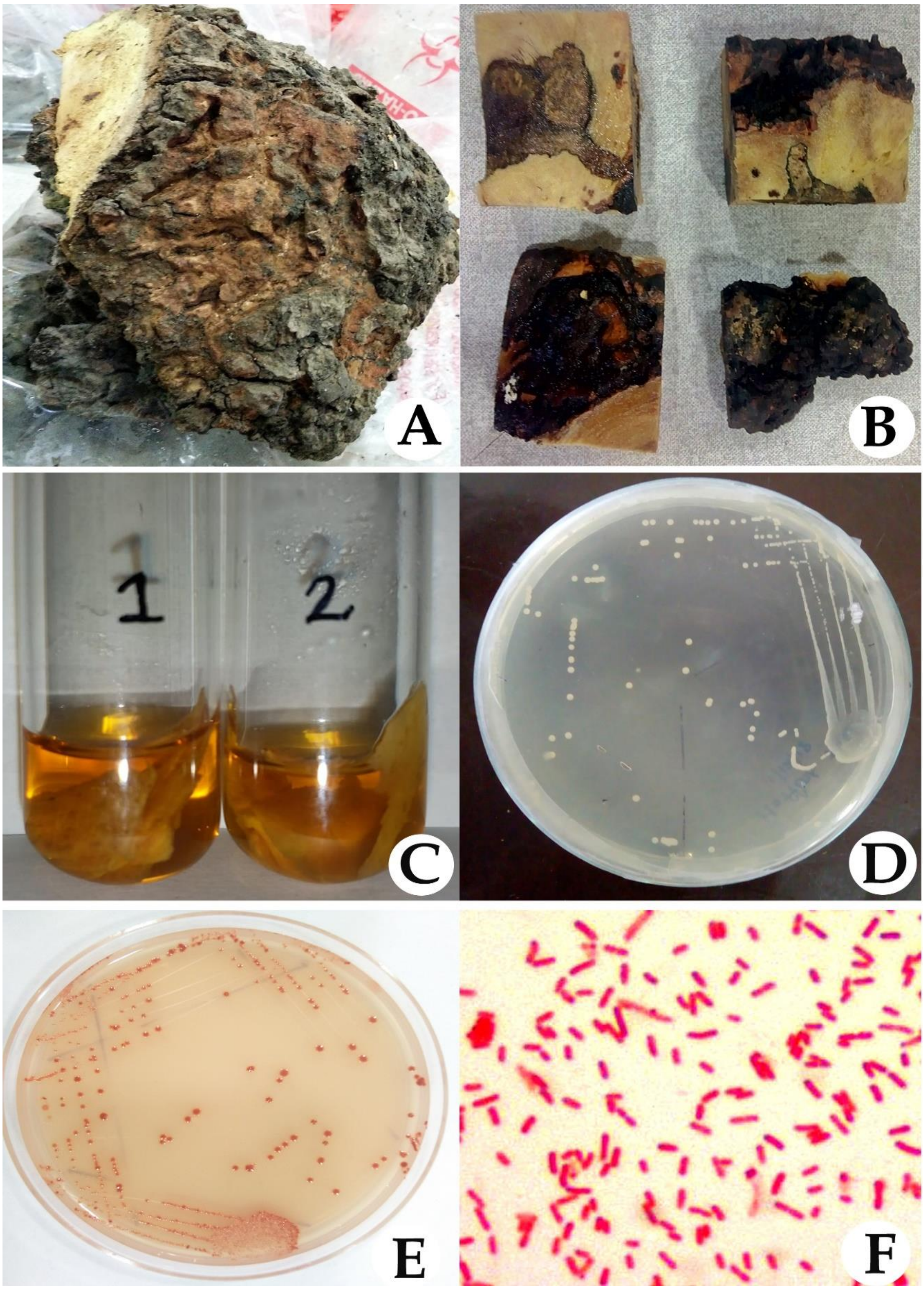

Figure 16. Pathogen isolation from wood blocks and suspension in distilled water (A, B, C), Bacterial growth on Hofers and NASA medium (D, E) and morphological identification by gram staining (F).

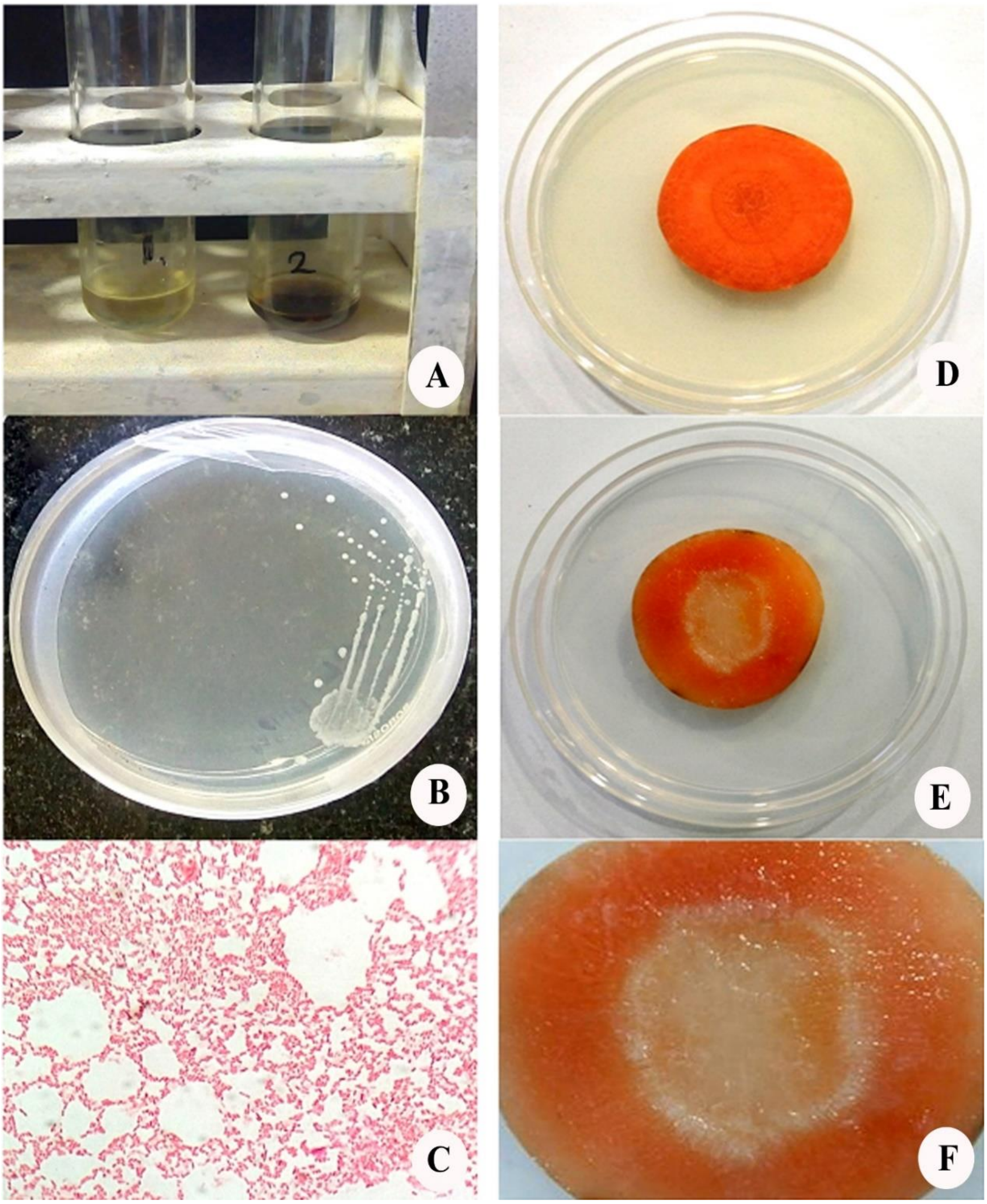

Figure 17. Pathogenicity test on carrot disc assay, (A) Suspension preparation, (B) Bacterial colony isolation on Hofers media, (C) Gram staining, (D) Inoculation of bacterial colony on Carrot disc, (E, F) Callus formation or initiation of tumour cells

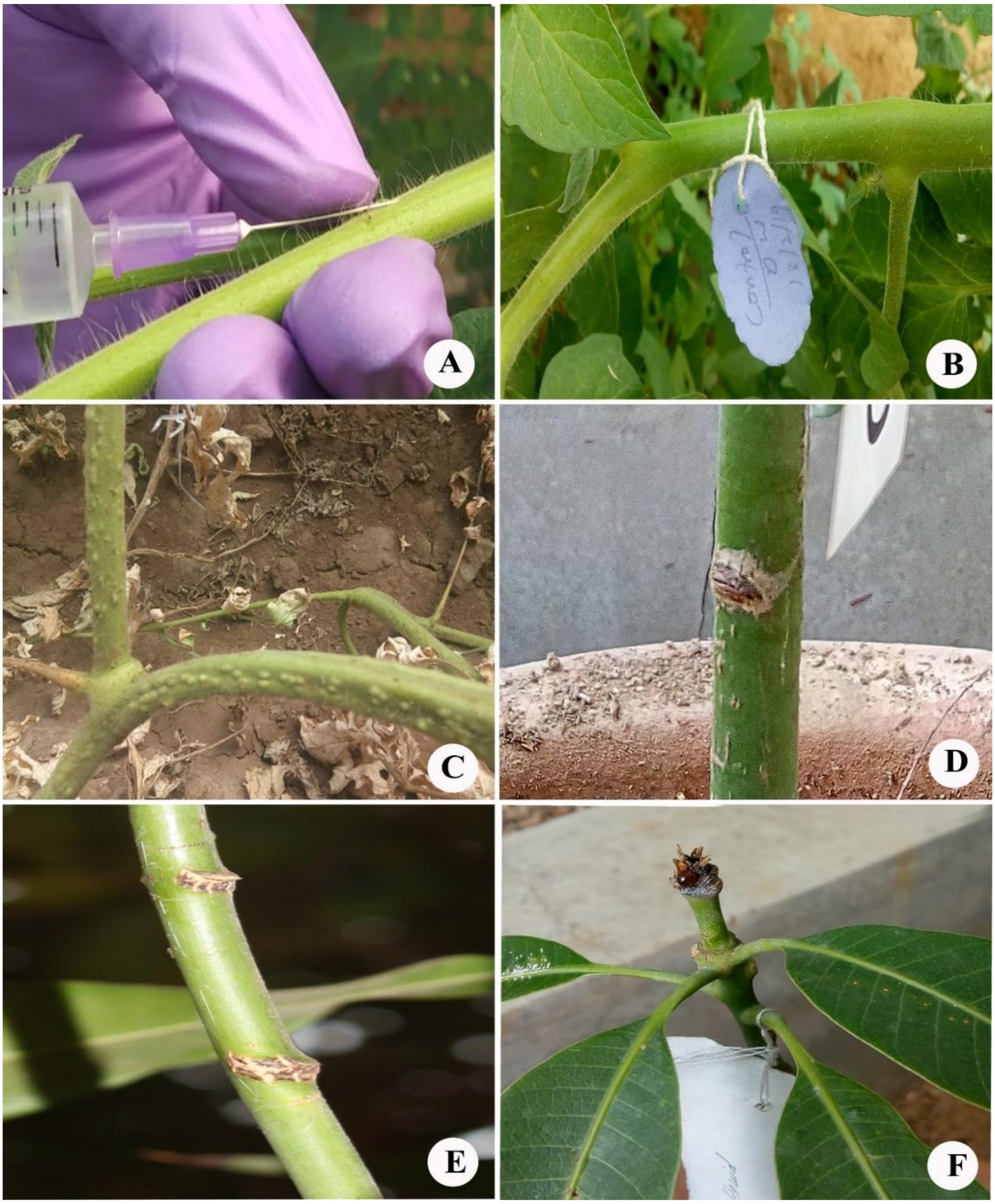

Figure 18. Pathogenicity test on tomato and mango plant; (A) Inoculation of *Agrobacterium* in tomato plant, (B) Control plant inoculated with distilled water, (C) Small galls initiation after inoculation of 2 months, (D, E) Burl initiation on mango plant, (F) Gummosis formation in young inoculated mango plant

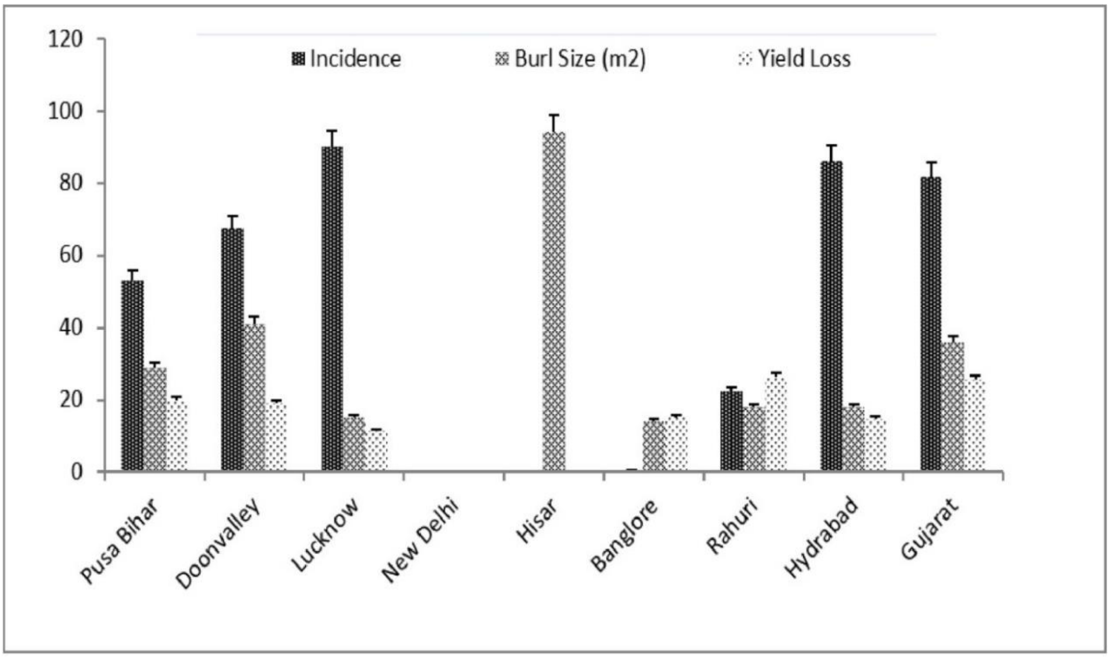

Figure 19. Incidence, burl size and yield loss in *Langra* cultivar throughout India

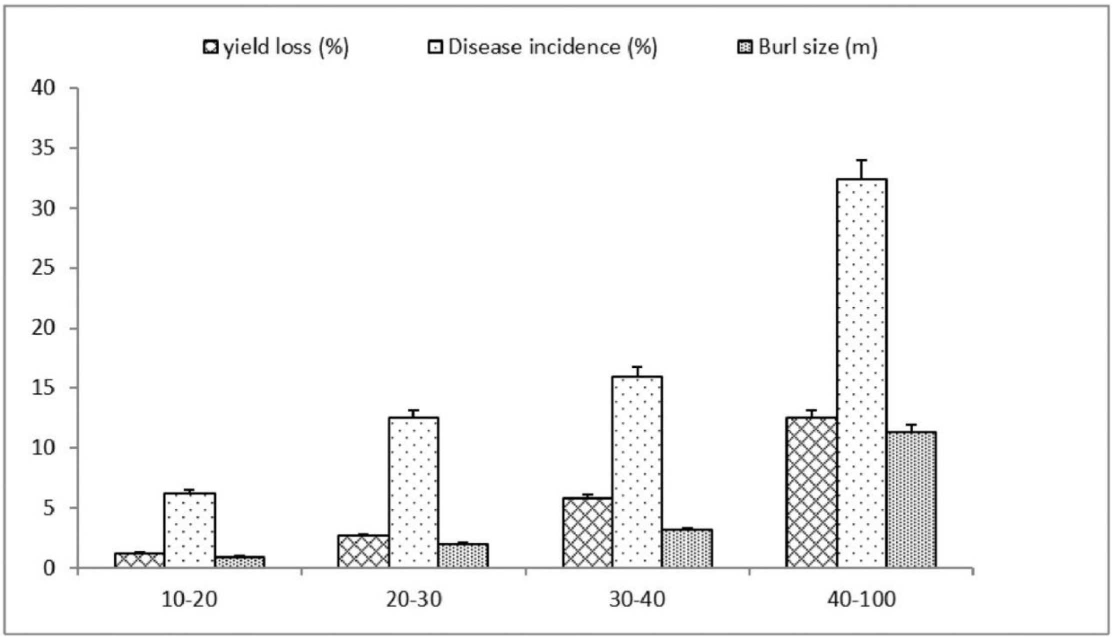

Figure 20. Correlation of burl disease with different age group from 10 to 100 year

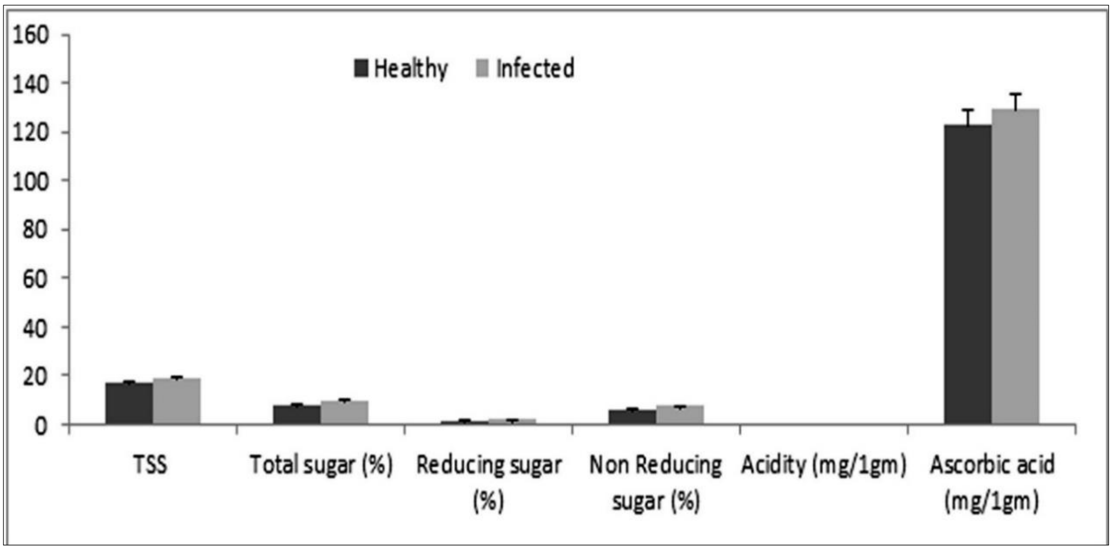

Figure 21. Biochemical analysis of fruits in burl infected and non-infected trees of *Mangifera indica* cv. *Langra*

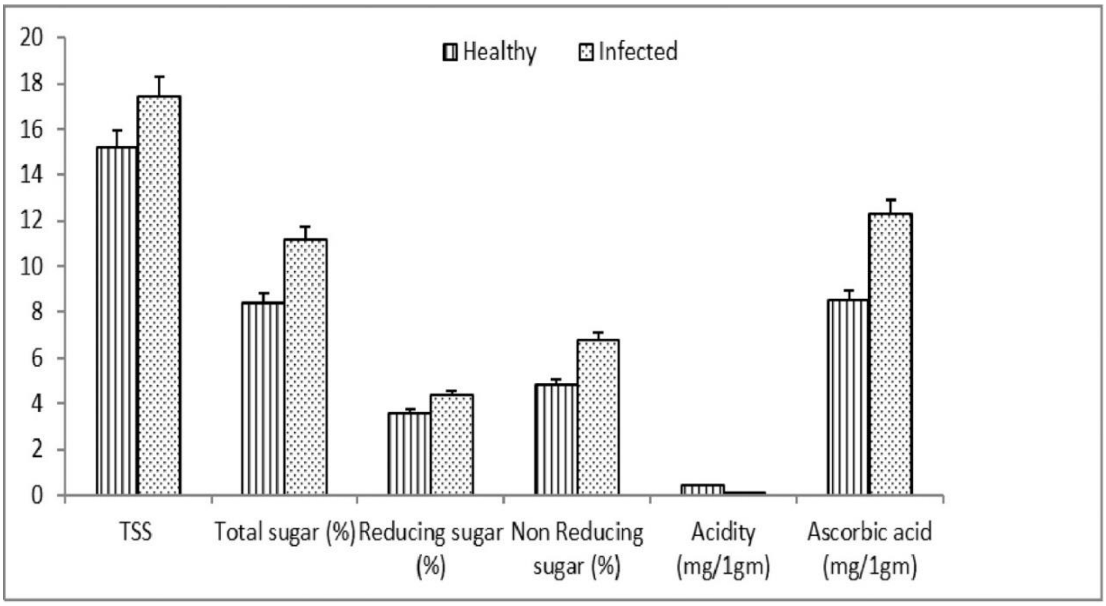

Figure 22. Biochemical analysis of fruits in burl infected and non-infected trees of *Mangifera indica* cv. *Rajapuri*.

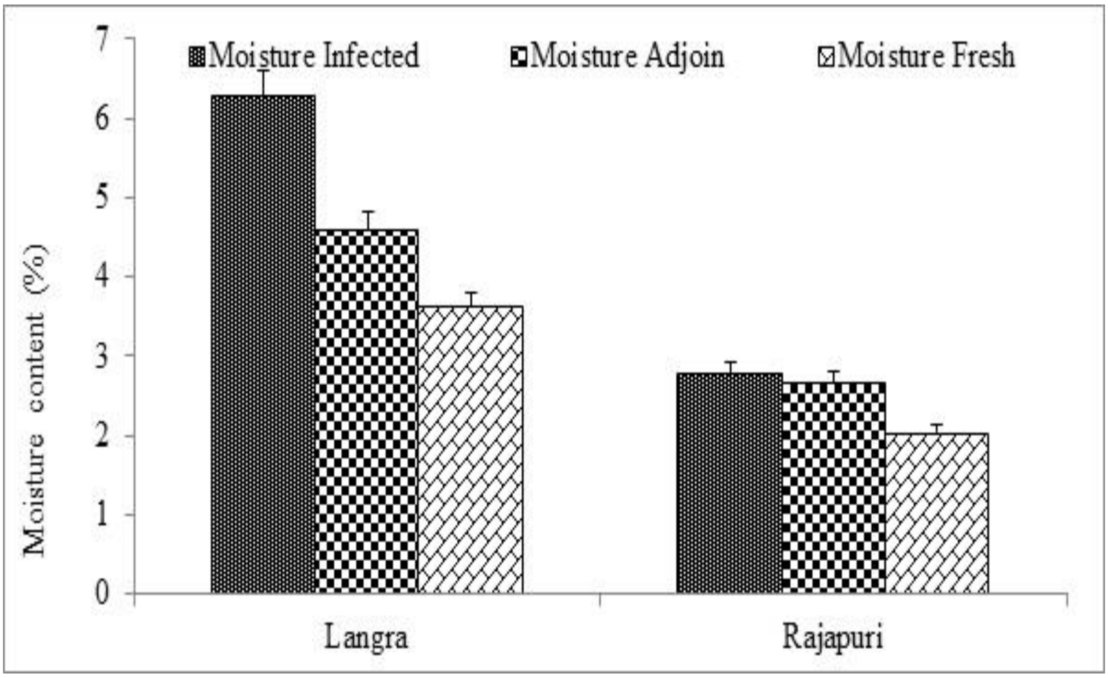

Figure 23 Comparative changes in Moisture content (%) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

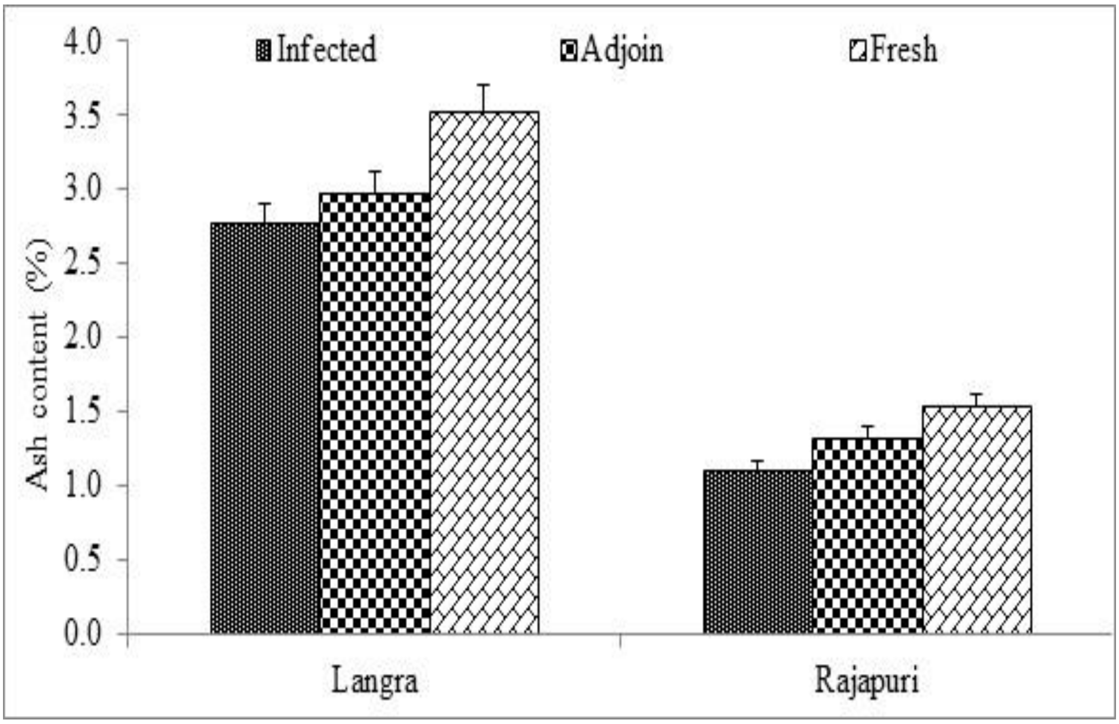

Figure 24. Comparative changes in Ash content (%) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

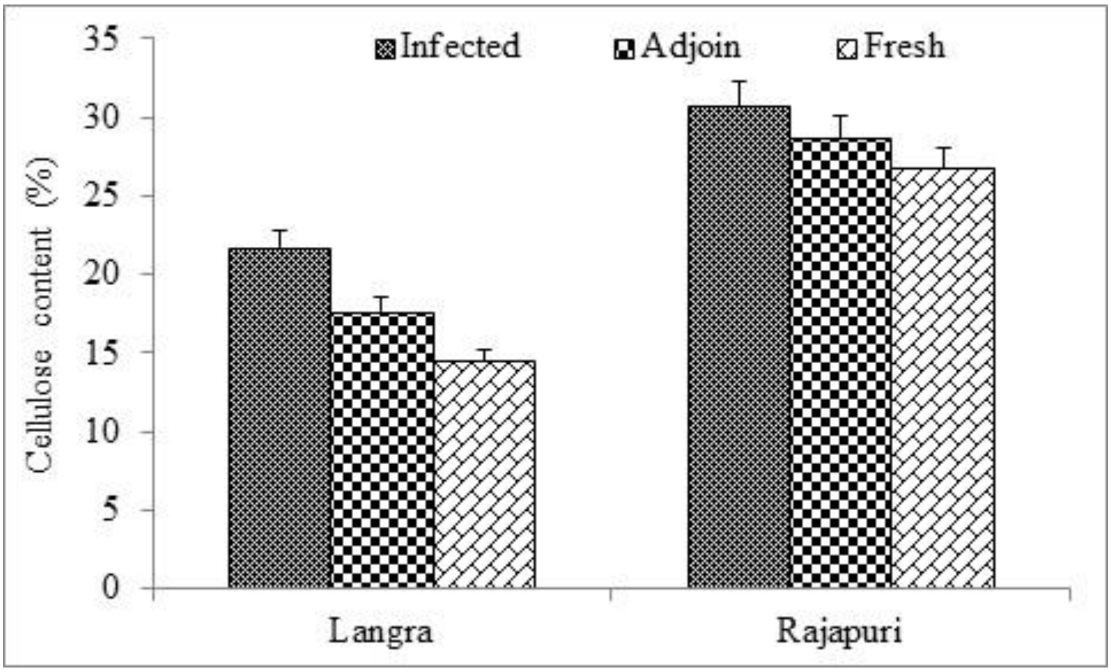

Figure 25. Comparative changes in Cellulose content (%) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

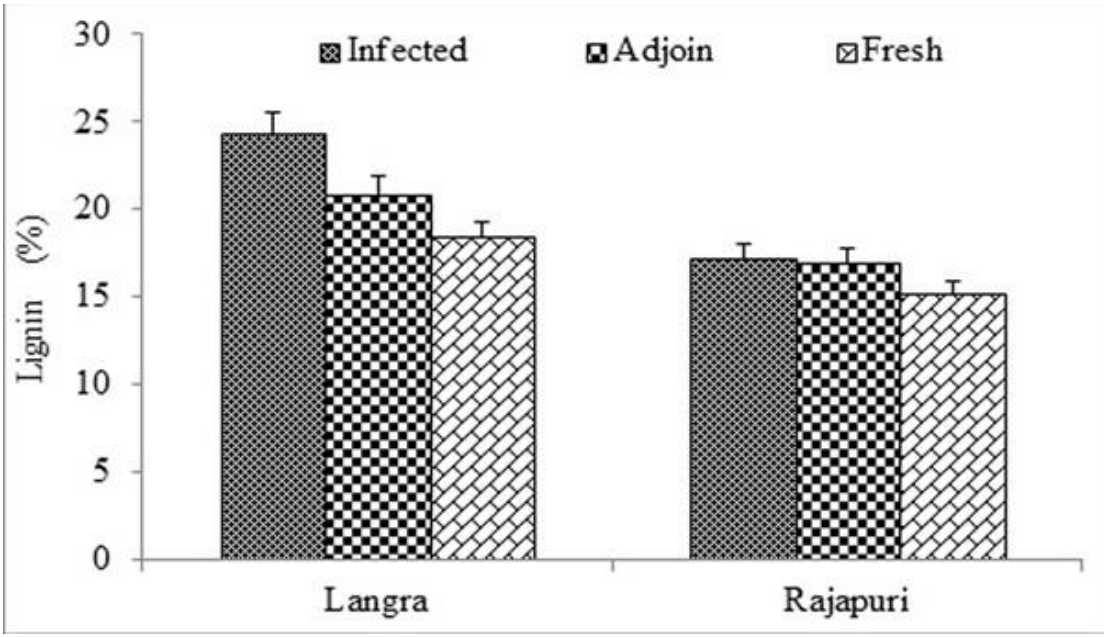

Figure 26. Comparative changes in Lignin content (%) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

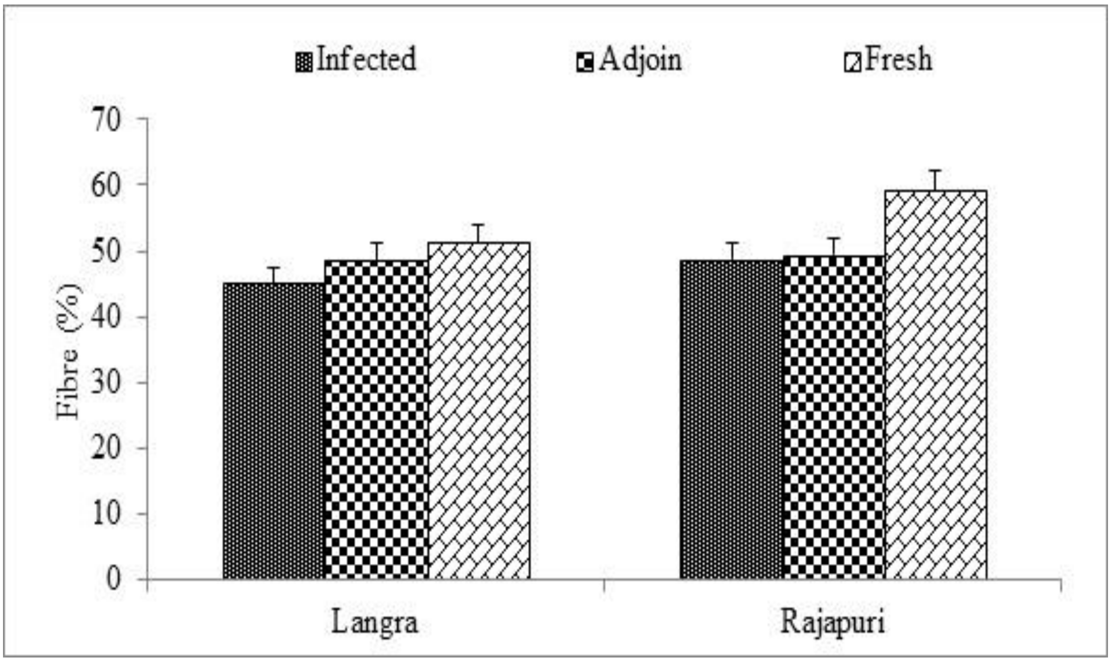

Figure 27. Comparative changes in fibre content (%) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

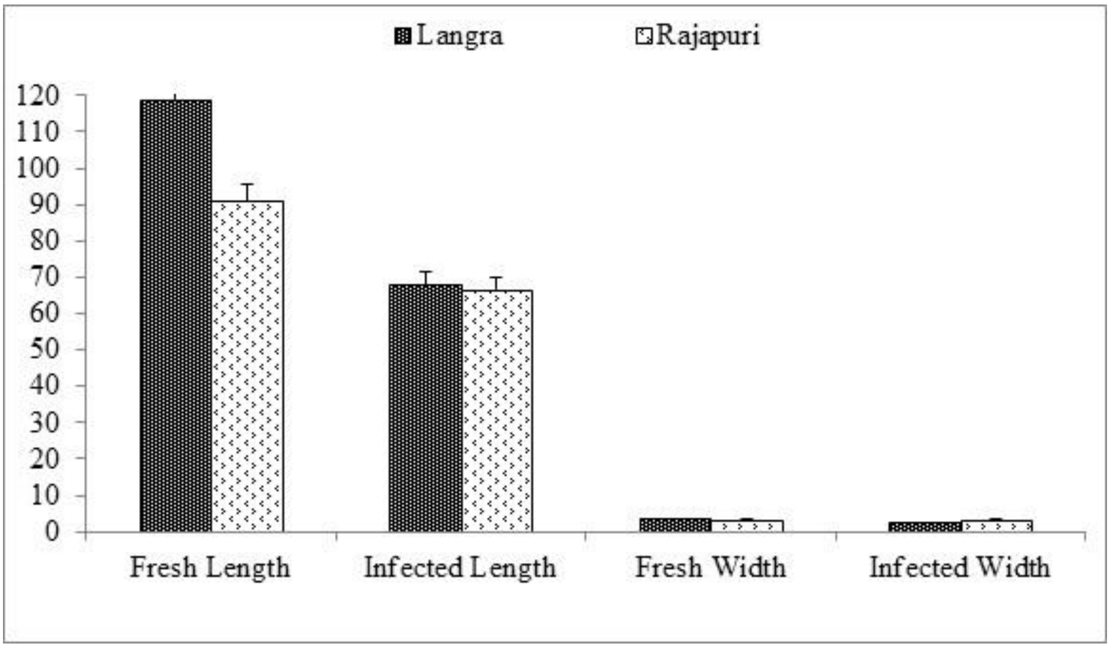

Figure 28. Changes in fibre length and width of fresh and infected stem of *Langra* and *Rajapuri* variety.

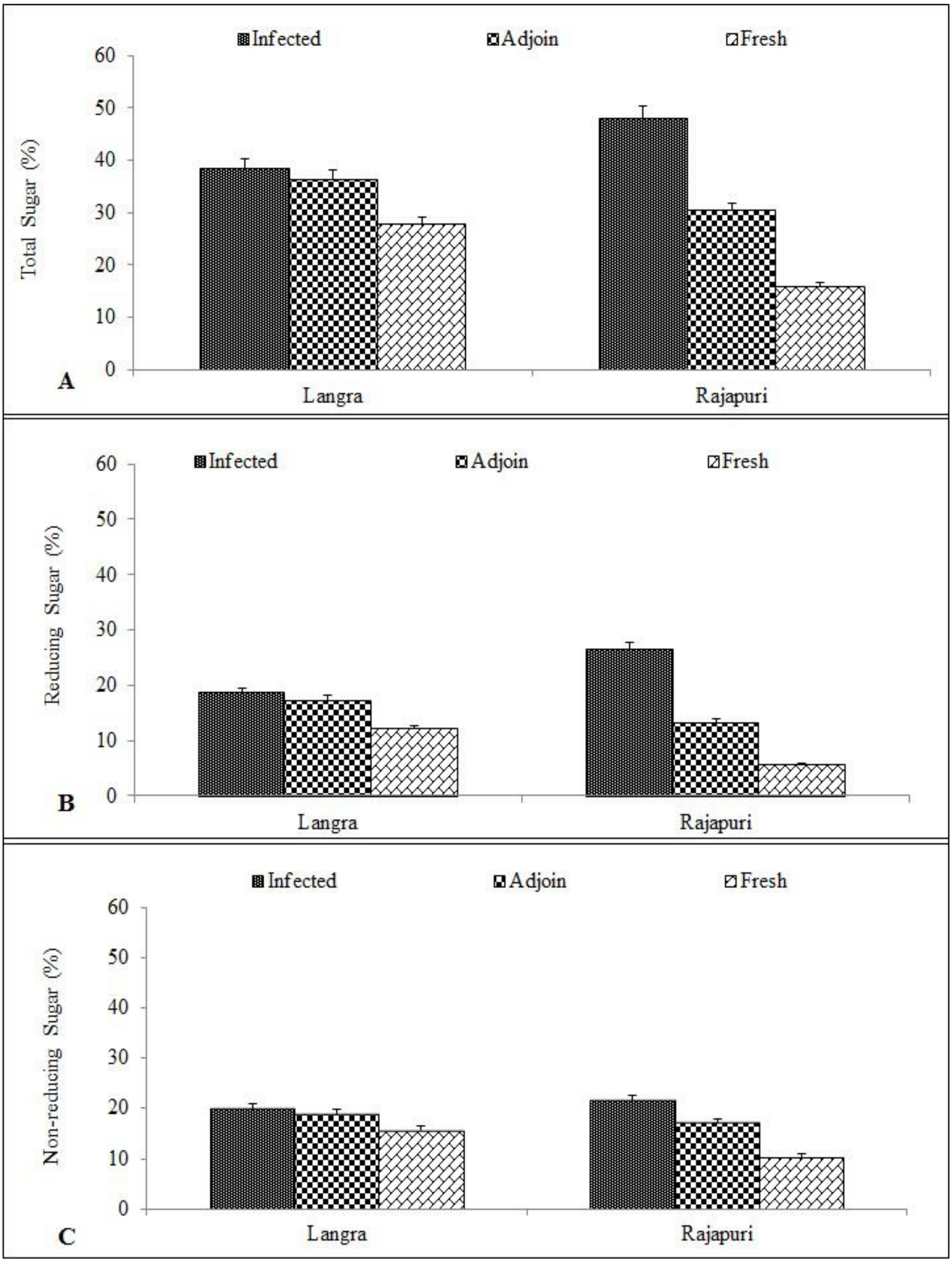

Figure 29. Comparative changes in per cent of Total soluble Sugar (A), Reducing sugar (B) and Non-reducing sugar (C) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

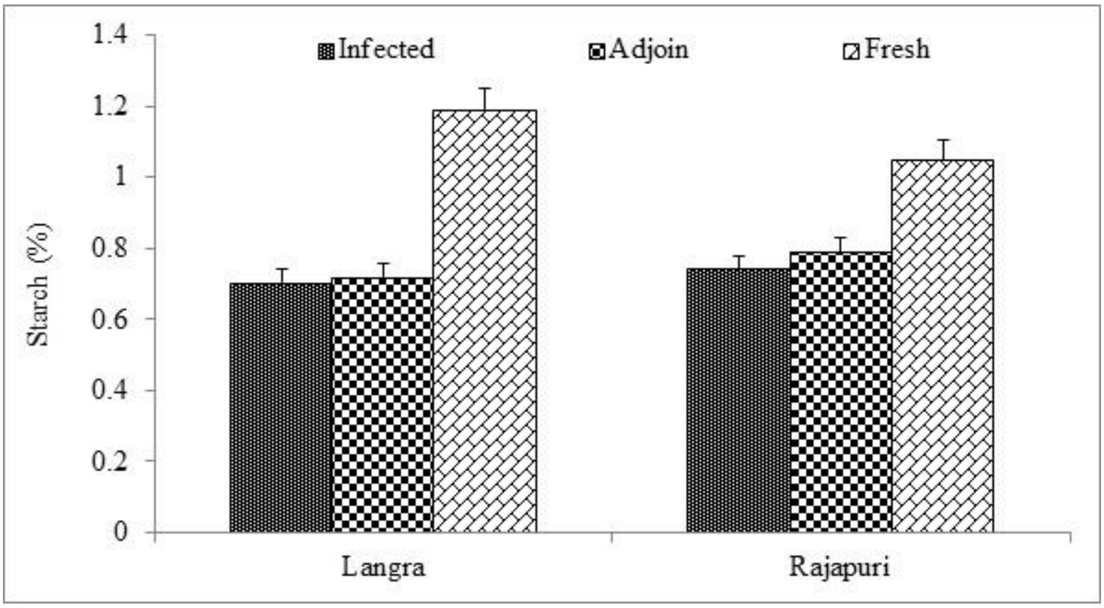

Figure 30. Comparative changes in Starch (%) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

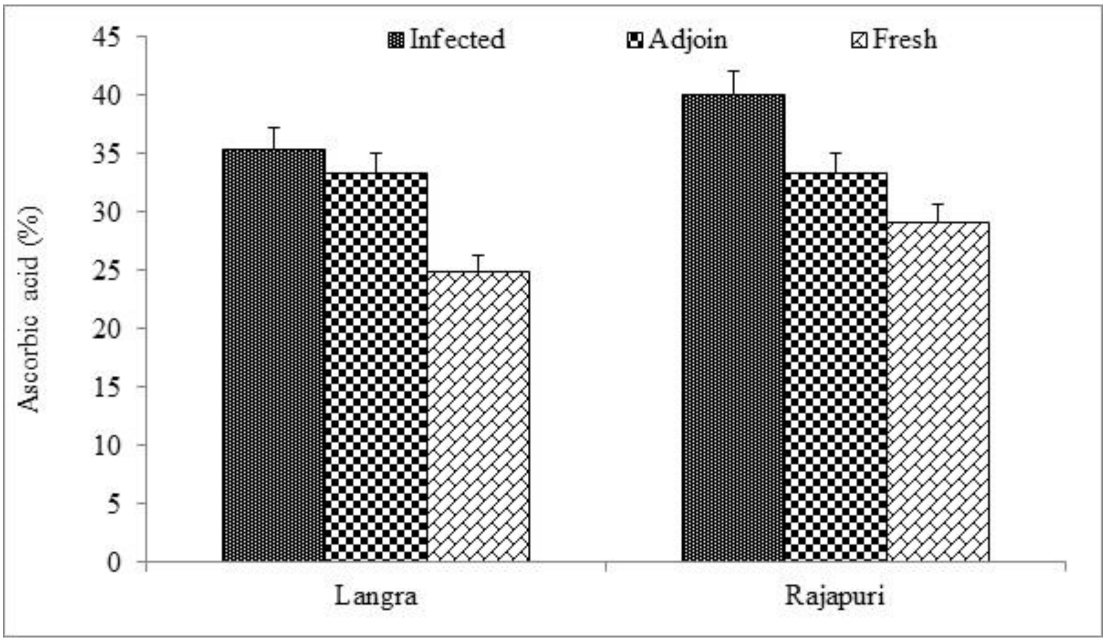

Figure 31. Comparative changes in Ascorbic acid (%) in fresh, adjoin and infected stem of *Langra* and *Rajapuri* variety.

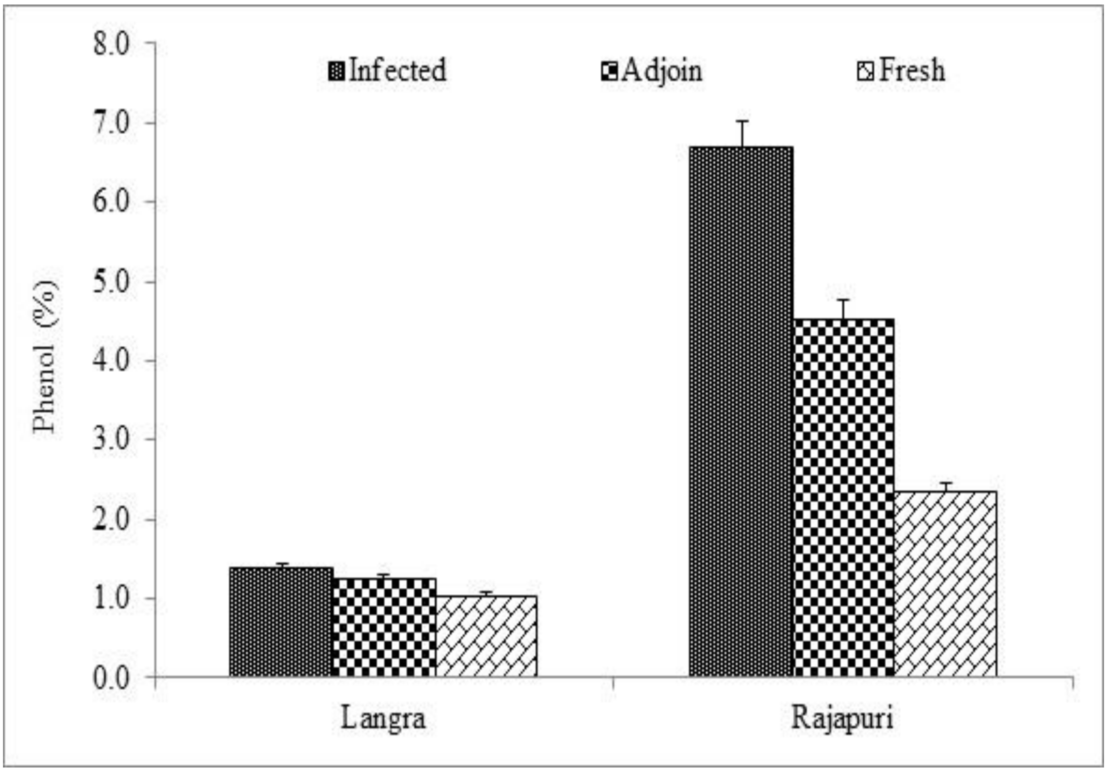

Figure 32. Comparative changes in Phenol (%) in fresh adjoin and infected stem of *Langra* and *Rajapuri* variety.

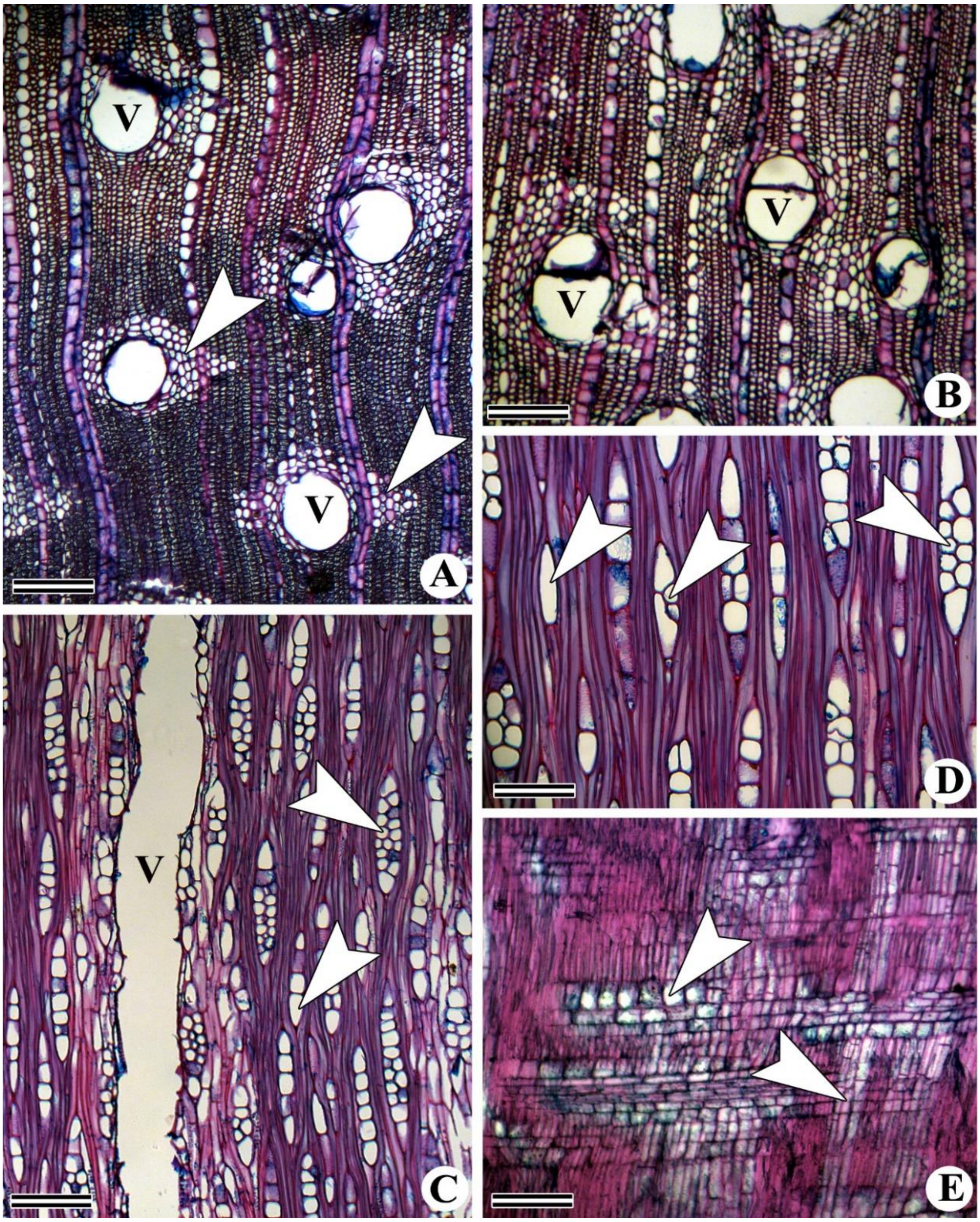

Figure 33. Transverse (A, B), Tangential (C, D) and radial (E) view of secondary xylem of healthy (i.e., free from burl) wood of *Mangifera indica* collected from *Langra* and *Rajapuri* variety.

Scale bars: A, E = 200 µm; B = 100 µm; C = 100µm; D = 50 µm.

Figure 33. Transverse (A, B), Tangential (C, D) and radial (E) view of secondary xylem of healthy (i.e., free from burl) wood of *Mangifera indica* collected from *Langra* and *Rajapuri* variety.

A: Structure of secondary xylem from healthy (i.e., free from burl) tree. Note the outline and arrangement of vessels (V) and lozenge-aliform axial parenchyma cells (arrowhead) in *the langra* variety.

B: Secondary xylem of the healthy (i.e., burl free) tree of *Rajapuri* variety showing the distribution of vessels and axial parenchyma. Note the species-specific variation in the arrangement of axial parenchyma around the vessel elements.

C: Arrangement of axial elements of secondary xylem and structure of rays in secondary xylem of healthy trees. Arrowheads indicate variations in the shape and size of ray cells in the variety *Langra*.

D: Relatively enlarged view of secondary xylem in tangential longitudinal view of the secondary xylem in variety *Rajapuri* showing variations in the ray size.

E: Radial view of healthy secondary xylem of variety *Langra*. Arrowhead indicates ray cells.

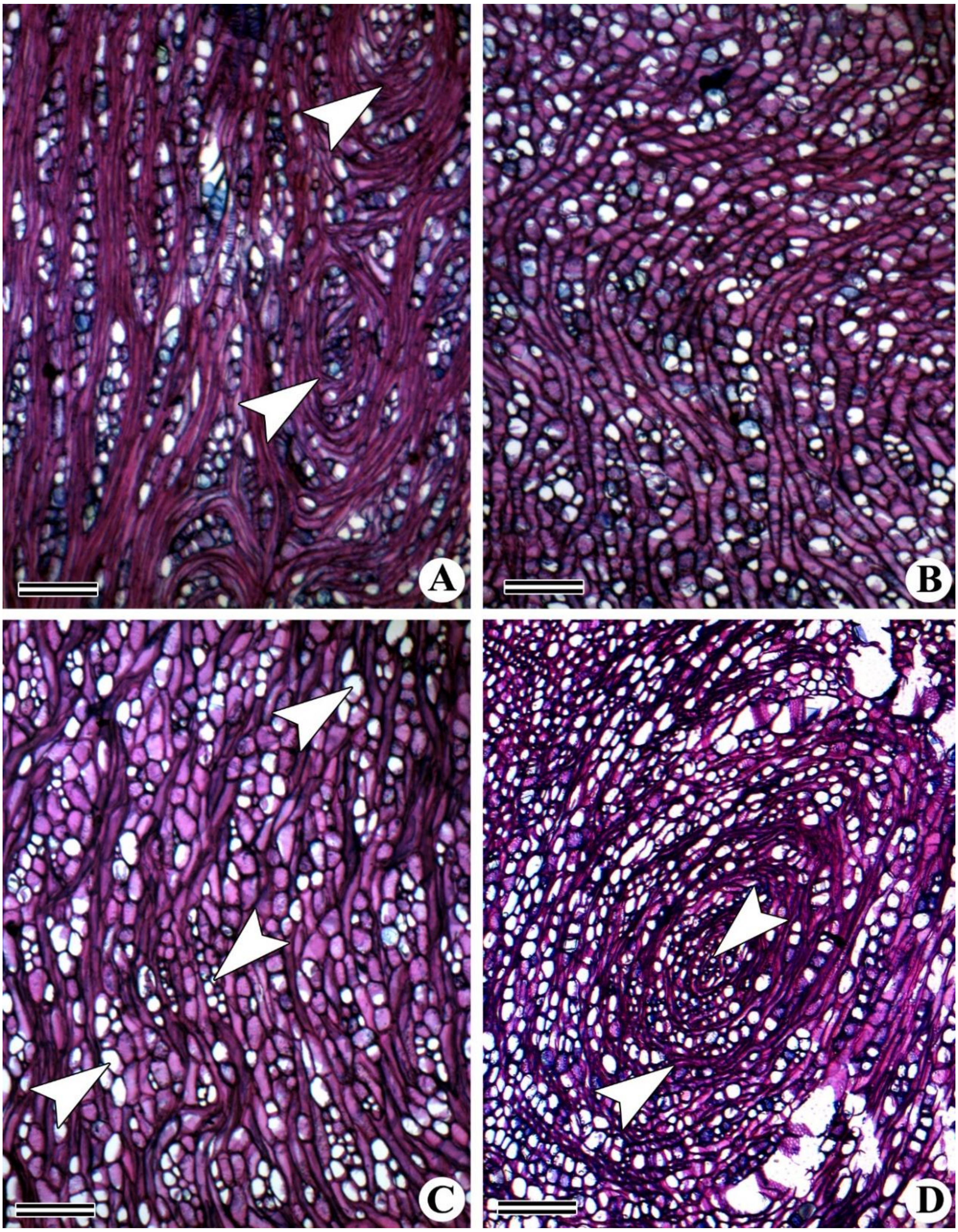

Figure 34. Tangential longitudinal (A-D) view of burl wood of *Mangifera indica* variety *Langra* and *Rajapuri* showing alterations in the axial arrangement of the xylem derivatives.

Scale bars: A-D = 200 µm.

Figure 34. Tangential longitudinal (A-D) view of burl wood of *Mangifera indica* variety *Langra* and *Rajapuri* showing alterations in the axial arrangement of the xylem derivatives.

A: Increase in the number of ray parenchyma, absence of wide diameter vessels while some of the fibres showing circular arrangement (arrowheads).

B: Deformed xylem from the burl region showing complete loss of axial elements like fibres and vessels in the xylem. Note the increased level of axial and ray parenchyma cells.

C: Structure of burl xylem showing an increased level of ray cells intermixed with axial parenchyma while vessels and xylem fibres are absent. Note the size of the ray cells (arrowhead).

D. Deformed xylem from the burl region showing complete loss of axial polarity of the xylem derivatives while some of the fibres oriented circularly (arrowheads).

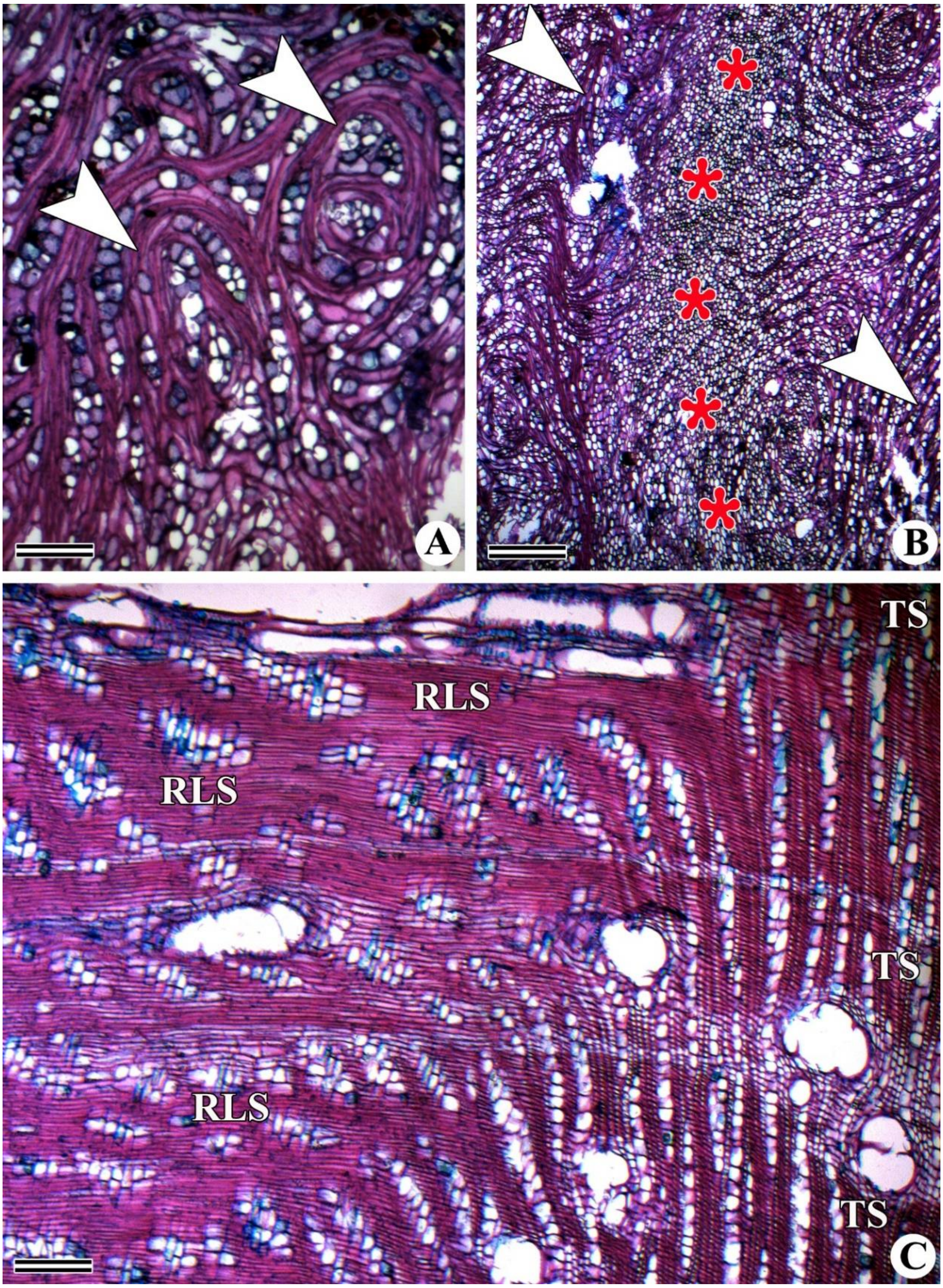

Figure 35. Tangential longitudinal (A, B) and transverse (C) view of secondary xylem of burl wood of *Mangifera indica* variety *Langra* and *Rajapuri*.
Scale bar: A = 200 µm, B = 500 µm; C = 50 µm.

Figure 35. Tangential longitudinal (A, B) and transverse (C) view of secondary xylem of burl wood of *Mangifera indica* variety *Langra* and *Rajapuri.*

A: Deformed xylem from the burl region showing irregular and circular orientation of fibres (arrowheads).

B: Transition portion of the xylem of the healthy and point of burl initiation showing the arrangement of xylem derivatives. Extreme left and right-side portion of the image (arrowheads) and central portion showing part of the xylem towards healthy and burl wood, respectively. Xylem portion marked with an asterisk shows the junction zone.

C: Transverse view of xylem connecting the burl showing the orientation of xylem derivatives. Note their orientation in different plains like transverse (TS) and radial (RLS) view in the same sections. Note the size of the ray cells (arrowheads). V = vessel.

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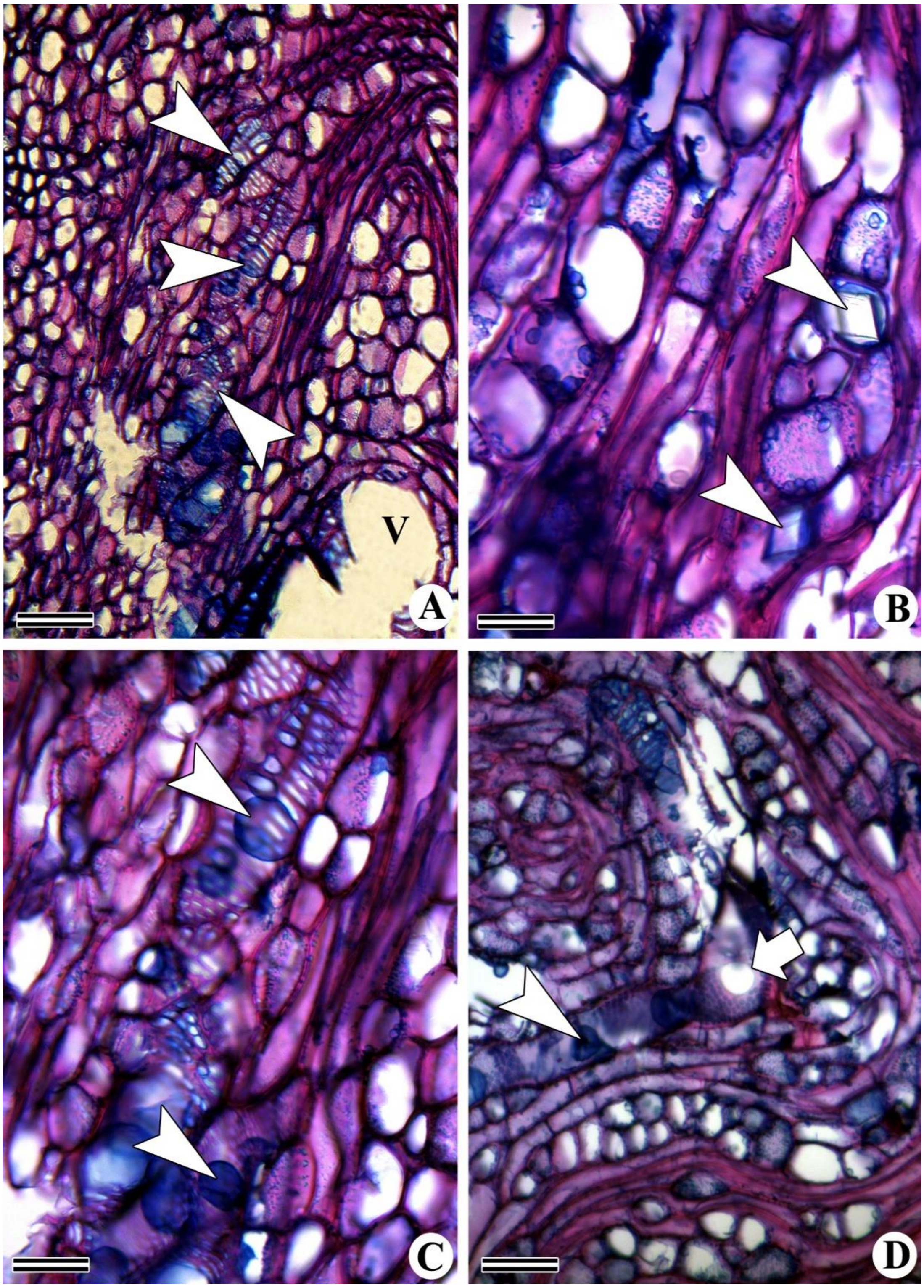

Figure 36. Tangential longitudinal (A-D) view of the deformed secondary xylem of burl portion of *Mangifera indica* variety *Langra* and *Rajapuri*.

Scale bar: A – D = 100µm.

Figure 36. Tangential longitudinal (A-D) view of the deformed secondary xylem of burl portion of *Mangifera indica* variety *Langra* and *Rajapuri.*

A: Composition of burl xylem. Note the wide vessel (V) while arrowheads indicate tracheid like narrow cells with tyloses. F = Fibres, R = ray cell.

B: Xylem portion of the tumour showing rhomboidal crystal in ray cell (arrowheads).

C: Enlarged view of Figure 36A showing tracheid like/narrow vessels occluded with tyloses (arrowheads). Note the pit size of these elements.

D: Narrow vessel element (arrow). One of the narrow vessels occluded with tyloses (arrowhead) and structure of other xylem derivatives in the *Rajapuri* variety.

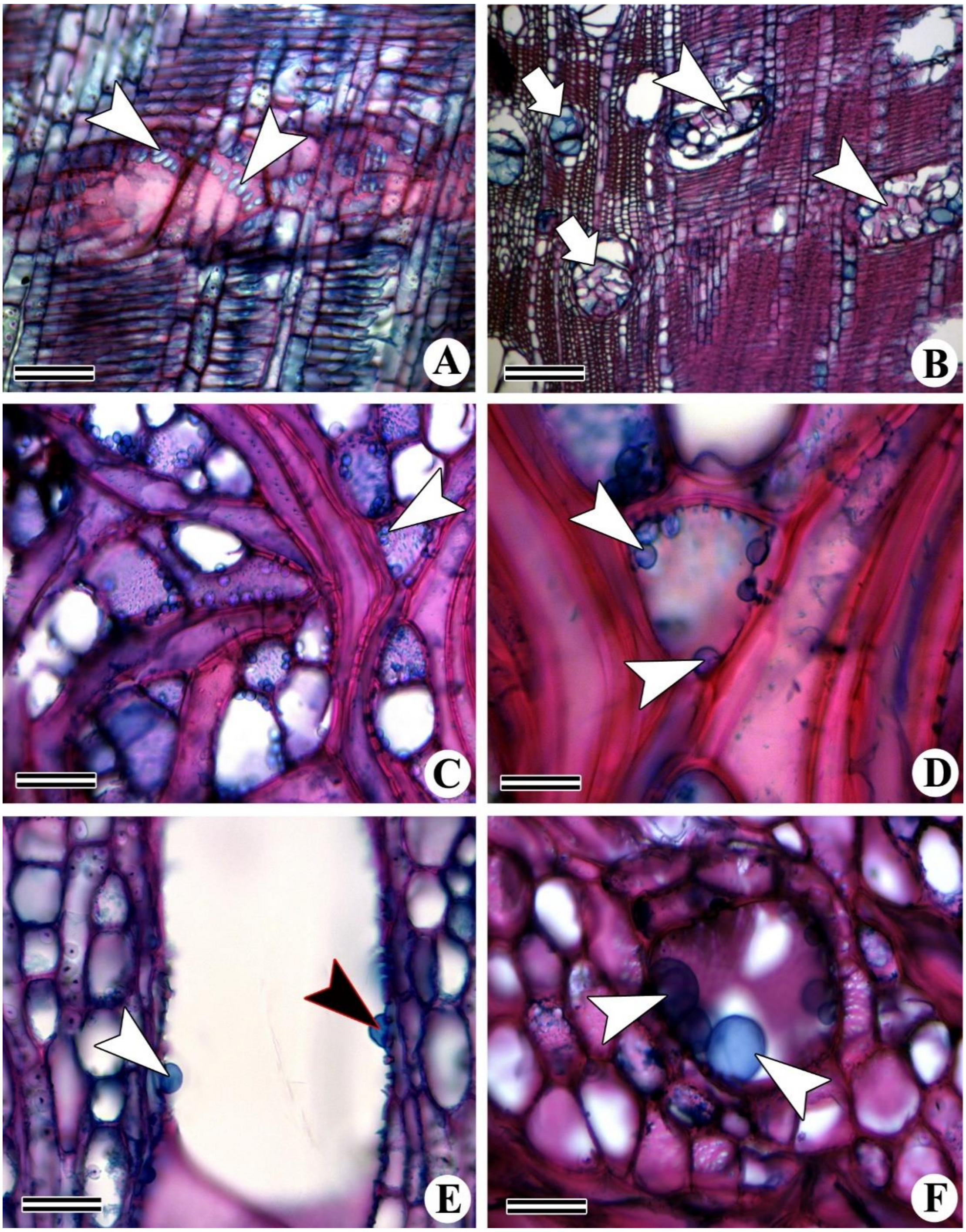

Figure 37. Transverse (A, B) and the tangential view of the deformed secondary xylem of the burl portion of *Mangifera indica* variety *Langra* and *Rajapuri*.

Scale bar: A = 100µm; B =100 µm; C = 50µm; D = 20 µm;

Figure 37. Transverse (A, B) and the tangential view of the deformed secondary xylem of the burl portion of *Mangifera indica* variety *Langra* and *Rajapuri.*

A: Transition portion of the secondary xylem of the burl showing the irregular orientation of axial parenchyma. Note the large-sized simple pits on their lateral walls (arrowhead).

B: Transition portion of the burl xylem showing vessels occluded with tyloses. Note the polarity of the vessel elements (arrowheads). Arrow showing axial arrangement.

C: Burl portion of the burl xylem showing short fibres and ray cells. Arrowhead indicates tyloses in the ray cells

D: Enlarged view of secondary xylem portion of Figure 37C showing tyloses in one of the ray cells (arrowheads).

E: One of the vessels occluded with tyloses (arrowheads) and structure of other xylem derivatives in the *Rajapuri* variety

F: One of the deformed, large parenchyma cells occluded with tyloses (arrowhead).