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RESEARCH ARTICLE

# STUDIES ON HISTOPATHOLOGY OF *TRIOZA JAMBOLANAE* ON INFECTED *SYZYGIUM JAMBOS* LEAVES FROM KOTA DISTRICT, RAJASTHAN

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**ABSTRACT**

The galls on the leaves of Syzygium jambos were found to be single or in clusters on the adaxial or sometimes on both the surfaces of the leaf. The galls were variable in size, as their length varied between 10-25 mm in length and 1-4 mm in breadth. They were irregular and tunnel like in shape. Young galls were greenish-yellow in colour and became brown at the time of maturity. The number of galls on a leaf varied from one to many. As 5-6 galls had been observed on a single leaf. The interior of the gall contained a circular or oval cavity which opened outside by a minute ostiole formed on the abaxial surface of leaf. The nutritive parenchyma cells are transformed into nutritive cells as the larva grazes and show increased lipid and protein concentrations as well as an increase in amylase activity which gets reflected by reduced concentrations of starch in the gall. As the gall reaches maturation, cell proliferation ceases, most of the gall tissues lignify, besides this the larva matures and finally adult is formed. Eventually the gall desiccates and adult comes out.



## INTRODUCTION

Plant galls are abnormal plant cells, tissues or organs formed as a result of stimulation by various parasites ranging from fungi and bacteria to insects and mites (Harris *et al*. 2003).

Generally three stages are involved in gall formation, which include initiation, growth and maturation. In this process cell proliferation, differentiation and hypertrophy occur which convert the inner-gall chamber lined with inner-gall tissue and the outer gall which is composed of cortical parenchyma. The growth stage consists mainly of cell expansion, leading to gall growth and inner-chamber growth. The larva grazes on the inner-gall tissue throughout development and as the larva increases in size, the cell layers of inner-gall tissue decrease.

Histopathological studies when correlated with physiological and biochemical ones could be helpful to determine the fundamental basis for plant tissue alterations that occur during insect parasitism. The histopathological changes in host are closely co-related with the galling life style of the insect species. The host gives response to the feeding or ovipositional stimulus of insect by morphogenetic changes.

Therefore, for the management of cecidozoan, it is of utmost importance to have basic knowledge about the histopathological changes induced by the psyllid (*Trioza jambolanae* Crawford) on *Syzygium jambos* (L.) Alston. Galls induced by *Trioza jambolanae* on the leaves of *Syzygium cumini* and *Syzygium jambos* are common in Kota district of the Rajasthan State. It causes great loss to leaves and fruits and seeds of the plant.

## METHODOLOGY

### *Effect on leaf*

### *Collection of Plant Sample*

For histopathological studies 15, 30 and 45 days old Galled and infected leaf samples were collected from naturally growing trees in the month of June and July in polythene bags. Galled leaf samples containing different ontogenetic stages of *Trioza jambolanae* were brought to the laboratory, washed carefully to remove dust, blotter dried and preserved in 4% formalin.

### *Tissue sectioning*

To study the difference between the anatomical structure of the healthy and *Trioza jambolanae* infected leaves, thin and fine sections of leaves were cut with the help of Microtome. Plant materials were dehydrated by passing in alcohol series and embedded in Paraffin wax (54°C). Wax embedded materials were sectioned at a thickness of 10-14 µm with microtome.

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***Staining***

De-waxed and processed sections were stained with 1 % safranin to study the internal structure of the galled and healthy plant leaf tissues.

***Effect on Seed germination***

To study the effect of galling insect on seed germination, both healthy and infected seeds were tested for germination ability and length of seedling was measured. 10 sets of petriplates were prepared, containing water soaked Watman No.1 filter paper. In first five sets healthy seed and in remaining diseased seeds were kept. 5-5 seed were held in each plate. After 7 and 15 days germination and length of seedling (plumule and redicle) was measured.

**Observations**

The galls on the leaves of *Syzygium jambos* were found to be single or in clusters on the adaxial or sometimes on both the surfaces of the leaf. The galls were variable in size, as their length varied between 10-25 mm in length and 1-4 mm in breadth. They were irregular and tunnel like in shape. Young galls were greenish-yellow in colour and became brown at the time of maturity. The number of galls on a leaf varied from one to many. As 5-6 galls had been observed on a single leaf. The interior of the gall contained a circular or oval cavity which opened outside by a minute ostiole formed on the abaxial surface of leaf. Anatomically following differences were seen in healthy and galled tissue.

***Anatomy of the Healthy Leaf***

*Syzygium jambos* is a dicotyledonous plant bearing dorsiventral leaves. The cross section of healthy leaf showed adaxial and abaxial epidermis consisted of small rectangular cells. Stomata were present only on abaxial surface. The mesophyll tissue consisted of two components. First, two layers of compactly arranged palisade cells below the upper epidermis, and second 3-4 layers of spongy parenchyma below the lower epidermis. The cells of spongy parenchyma were large, rounded and thin walled with small intercellular spaces. A single layer of palisade like cells was also present below the abaxial epidermis. Tannin occurred only in some of epidermal and spongy parenchyma cells. The vascular bundles were embedded in the parenchyma tissue. Glands containing aromatic oil were observed on both adaxial and abaxial surfaces. (Plate-1)

***Gall Histology***

*Trioza jambolanae* caused tunnel galls on the young leaves of *Syzygium* and it modified the internal structure of the leaf. The adaxial epidermis of the young gall was in the continuation with the adaxial epidermis of unaffected part. While the abaxial epidermis was ruptured at some places due to oviposition by the female. Gall mesophyll was not differentiated into palisade and spongy parenchyma as the healthy mesophyll but represented by simple ruptured parenchyma. The main bulk of gall tissue was composed of thin walled, closely packed parenchyma cells. In young galls cells the outer (adaxial) epidermis contained chloroplast. However in mature galls, it was completely absent. In mature gall most of the parenchyma cells were filled with tannin. A mechanical zone (3-4 layered) was observed below the adaxial epidermis which is composed of large sized, thick walled sclereids. Several vascular bundles were seen in scattered pattern on either side of gall cavity (Plate-1).

The gall cavity was round to oval in cross section and contained single cecidozoan. The cells of the inner layer, lining the gall cavity were rectangular in shape with dense cytoplasm. This layer was in continuation with the abaxial epidermis of the leaf. There was no characteristic nutritive zone around the gall cavity. Below adaxial and abaxial glands containing aromatic oil were found (Plate-1). Stomata were not observed on gall epidermis.

***Gall formation***

Gall formation was initiated by the attack of an insect. The cecidozoa generally attack on very young leaves surrounding the growing tips of the plant. New attacks also continued on the young leaves; as a result large number of gall appeared at various stages of their development. The attack was generally confined to the abaxial surface of the leaf. The adult female of *Trioza* punctured abaxial surface of the young leaf for egg laying and remained attached there for a continuous period of 4 to 6 days. Due to deposition of the eggs the cecidogenetic stimulus spreaded and resulted in mitotic activity.

The microscopic examination of the affected area of the blade showed a change in the normal histological pattern. In the beginning, cell proliferation of the abaxial epidermis and the adjoining mesophyll cells do not greatly occur below the cecidozoa but it is vigorous around it. This resulted in the formation of a specific zone of vigorously proliferated cells. The mesophyll cells of the adaxial side of the infected area were greatly hypertrophoid and were in a state of proliferation. Later on hyperplasia and hypertrophy spreaded in all the parenchymatous cells of the infected area of the blade, which included both adaxial and abaxial surfaces. Simultaneously both inner and outer epidermal cells keep pace with the process of division. As a result the affected part of the blade arched itself out of the level of the blade. The bulging was on the adaxial side and corresponding invagination on the abaxial side. In this way typical pouch like structure was formed, in which the larval stages were arrested. Concurrently the rapidly dividing abaxial mesophyll projected downwards leaving a passage which developed into a small opening (ostiole) of the gall.

**Table- 1**Shoot-Root Length of both Healthy and Infected seedlings (Results are mean of 5 replicates)

| S. No. | % seed germination ± S.E. | | Length of plumule (cm) ± S.E. | Length of radical (cm) ± S.E. |
|---|---|---|---|---|
| 1. | Healthy | 100 (±1.9832) | 4.3(±0.7845) | 2.1(±0.3263) |
| 2. | Infected | 64(±0.4563) | 2.5(±0.4536) | 1.6(±0.5622) |

In mature galls the mesophyll cells adjacent to the adaxial epidermis became sclerenchymatous to form mechanical zone.

***Effect on seed germination***

Generally *Trioza jambolanae* attacks on the leaf of *Syzygium* tree, but sometimes insect invaded into seeds also. The pulp of the fruit damaged due to this infection and it reduces the seed germination capacity also. After 7 days all the healthy seeds showed 100% germination and average length of the shoot and root was calculated to be 4.3 cm and 2.1 cm respectively. However in the case of infected seeds, out of total 25 seeds (5 replicates-5 seeds per plate) only 16 seeds found to be germinated; i.e. only 64% germination was observed and length of shoot was 2.5 cm while length of root was 1.6 cm (Table-1, Plate-1).

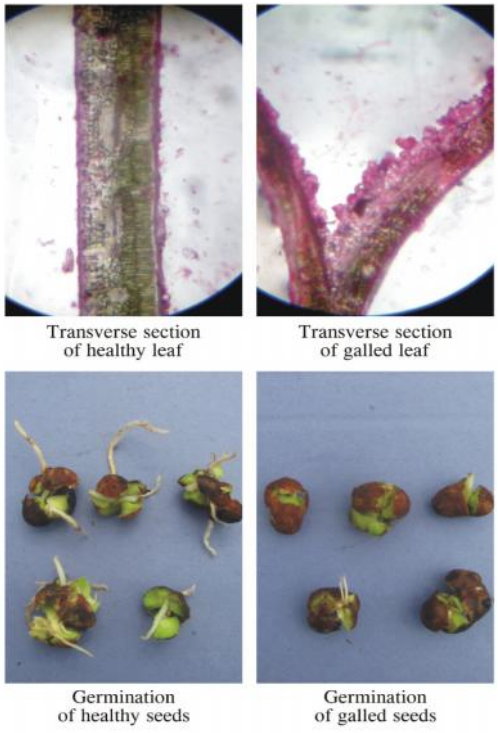


**Plate-1** Effect on Tissues and Seed Germination due to insect infestation

## RESULT AND DISCUSSION

**Histopathology of Infected plant**

Gall-inducing insects have profound effects on their hosts. These insects live within the plant tissues and induce tumor-like growths that provide them with food, shelter, and protection from natural enemies (Raman *et al*. 2005). Psyllids induce gall formation by sucking sap from the underside of a leaf, triggering pit formation around the nymph. It is concluded that in closed leaf galls, the nymph instar resides opposite to the site of entry, at the bottom of the pit, protected from environmental factors, particularly desiccation, drying out.

During the developmental process from Nymphal instar-I to Nymphal instar-V the feeding site shifts from gall parenchyma to vascular phloem. Parenchyma cells lining the nymphal chamber shrinks due to snapping of water transport mechanism.

*Trioza* adults are small winged, coloration is either pale green or brown with ochraceous markings. Forewings are hyaline with pronounced veination. Adults have been observed feeding on *Syzygium* leaves, presumably imbibing phloem.

Sometimes *Trioza jambolanae* also invades into the seeds of mature fruits. In this condition it losses the germination capacity and size of seedlings. As compare to healthy only 64% germination occurs in infected seeds. Size of plumule in healthy is 4.3 cm and in infected is 2.5 cm while size of radical is 2.1 cm in healthy and 1.6 cm in galled. It indicated that the value of seed and fruit also losses due to the infection.

Gall-forming insects are also known to manipulate their host plants and induce changes in source-sink relationships in a way that is beneficial to larval development. Since insects derive their nutrition from gall tissue, the gall becomes a sink for different nutrients and energy that is vital for the insect's growth (Raman 2003; Raman and Abrahamson. 1995).

## CONCLUSION

From the host parasite interaction study it can be concluded that during penetration and pathogenecity remarkable morphological and anatomical changes brought about by the pathogen for successful establishment and development on the host plant, resulting in a circular or oval cavity which opened outside by a minute ostiole on the abaxial surface of leaf. Due to deposition of eggs the cecidogenetic stimulus spreaded and resulted in mitotic activity. Anatomically abaxial epidermis was ruptured at some places due to oviposition by the female and mesophyll converted into ruptured parenchyma. A mechanical zone was also observed below the adaxial epidermis which is composed of large sized, thick walled sclereids. Sometimes insect also invaded into seeds which resulted in the reduction of germination capacity.

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