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Studies on Transmission of Phytoplasma in Sesame

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was conducted to artificially transmit phytoplasma, the causal organism of sesame phyllody by mechanical sap inoculation and with insect vectors (*Orosius albicinctus* and *Hishimonus phycitis*). The experimental findings revealed that the phytoplasma could not be transmitted by sap inoculation. The mean transmission rate was found to be 54.67 per cent, with the highest rate of phyllody transmission (93.33%) observed in both treatments T8 and T9 with 3 insects/plant + 7 Days of Acquisition Feeding (DAF) + 5 Days of Inoculation Feeding (DIF) and 3 insects/plant + 7 DAF+ 7 DIF, respectively, while the lowest was observed in the treatment T1 with 3 insects/plant + 3 DAF+ 3 DIF (13.3%) phyllody transmission from infected to healthy sesame plants. All the treatments were found to be statistically significant over control, however, the treatments T_8 and T_9 with three insects/plant and with acquisition feeding period of 7 days and inoculation feeding of 5 and 7 days, respectively were found to be at par with each other statistically.

Keywords: Sesame; leafhoppers; sap inoculation; vector transmission.

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1. INTRODUCTION

Sesame (*Sesamum indicum* L.) commonly known as gingelly (nuvvulu in Telugu; *til* in Hindi, Punjabi, Bengali; tal in Gujarati; ellu in Tamil, Malayalam; rasi in Odia; beeja in Kannada) is one of the important oilseed crops grown worldwide. Sesame yield is relatively low due to non availability of high yielding and resistant varieties (biotic and abiotic stresses), its cultivation in marginal and sub-marginal lands with poor crop management, low harvest index, seed shattering and indeterminate growth habit [1]. Sesame phyllody is one of the major diseases caused by phytoplasma and is transmitted by leafhopper, *Orosius albicinctus* Distant [2]. The rate of transmission of phyllody disease from infected to healthy seedlings depends on the number of insect population, life stage, gender, flight behavior, quality of the host plant, movement between the plants, time spent on plants and mode of transmission of pathogen which ultimately influence the transmission efficiency of the disease [3]. It is also transmitted from infected to healthy plants *via* dodder (*Cuscuta* sp.), grafting and cuttings but cannot be transmitted through mechanical sap inoculation [4]. Hence, keeping in view the present investigation was carried out to conduct transmission studies of phytoplasma by sap inoculation and insect vector.

2. MATERIALS AND METHODS

2.1 Transmission Studies of Phytoplasma by Mechanical Sap Transmission

Phyllody infected leaf samples collected from the field were used for infectivity studies. Sesame leaves showing clear phyllody symptoms from the infected plants were colected, washed in running water thoroughly to remove dirt and were blot dried. The inoculum was extracted by macerating the infected leaves in the presence of pre-chilled 0.05M phosphate buffer (pH 7.0), (9 ml of potassium phosphate buffer per 1 g of leaf sample) in pre-chilled mortar and pestle. The resultant inoculum was squeezed through two folds of sterile muslin cloth, before using for mechanical transmission. Healthy, well established, actively growing three week old young sesame plants raised in insect proof cages in greenhouse, Insectary were used for mechanical transmission studies. Prior to inoculation, a pinch of celite was added to the filtrate. A sterile cotton pad immersed in the inoculum was gently rubbed over the leaves in single direction to cause mild injury. Immediately after inoculation, the leaves were washed with a jet of sterile distilled water to remove the excess inoculum and abrasive celite. The inoculated plants were labeled and kept in insect proof cages in glasshouse for symptom expression. Healthy seedlings of sesame plants were kept as control.

2.2 Transmission Studies of Phytoplasma by Insect Vector

The phytoplamas was artificially transmitted with the help of two leafhopper species that were found in sesame field *i.e*., *O. albicinctus* and *Hishimonus phycitis*. An aspirator comprising a glass tube (10 cm length and 2 cm diameter) and a rubber tube of 15 cm length was used for the collection of leafhoppers. The leafhoppers were collected from sesame field by gently turning the leaves upwards and sucking with an aspirator. The leafhoppers were released on sesame plants maintained in insect proof cages for rearing. Infected sesame twigs showing phyllody symptoms were maintained in acquisition cages. The leafhoppers were collected from the rearing cages using an aspirator and were released into acquisition cages for acquaintance of phytoplasma inoculum. The hoppers were given different acquisition feeding periods (3, 5 and 7 days) as detailed in Table 1. Sesame seeds were sown in pots and seedlings were maintained in insect proof cages for inoculation studies. After giving prescribed acquisition feeding, the leafhoppers were removed carefully from the acquisition cages and transferred to inoculation cages as approved (3, 5 and 7 days) for transferring the inoculum from infected plants to healthy seedlings. The cages were kept in greenhouse for symptom expression. After prescribed period of inoculation feeding, the insects were killed using imidacloprid @ 0.3 ml l^{-1}. Data was recorded on rate of per cent phyllody transmission and number of days taken for development of phyllody symptoms.

3. RESULTS AND DISCUSSION

3.1 Mechanical Sap Transmission

Mechanical inoculation of sap collected from phyllody infected leaves into the young healthy sesame (variety Gowri) plants at three weeks stage induced no mechanical dissemination of phytoplasma. The plants that were sap inoclulated and maintained in insect proof cages did not produce any phyllody symptoms during the period of observation. The results clearly indicated that there was no mechanical transmission of phytoplasma into healthy plants (Table 2).

Table 1. Treatment details of insect transmission studies

Treatment No	Treatments
T_1	3 insects/plant + 3 DAF+ 3 DIF
T_2	3 insects/plant + 3 DAF+ 5 DIF
T_3	3 insects/plant + 3 DAF+ 7 DIF
T_4	3 insects/plant + 5 DAF+ 3 DIF
T_5	3 insects/plant + 5 DAF+ 5 DIF
T_6	3 insects/plant + 5 DAF+ 7 DIF
T_7	3 insects/plant + 7 DAF+ 3 DIF
T_8	3 insects/plant + 7 DAF+ 5 DIF
T_9	3 insects/plant + 7 DAF+ 7 DIF
T_{10}	No inoculation (Control)

DAF- Days of Acquisition feeding; DIF- Days of Inoculation feeding

3.2 Transmission with Leafhopper, *Orosius albicinctus*

The results of artificial transsion of sesame phyllody phytoplasma through leafhopper (*O. albicinctus*) are summarized in Table 3. The sesame phyllody disease caused by phytoplasma was successfully transmitted by leafhopper, *O. albicinctus.* The percentage of phyllody transmission by *O. albicinctus* ranged from 13.3% to 93.3% in the different treatments combination, while 0% was recorded in control. There were no findings of phyllody transmission and symptom expression in control pots that were maintained in insect proof cages.

Treatment T3 recorded the highest per cent (46.7%) phyllody transmission in the 3 Days of Acquisition Feeding (DAF) treatment combinations and was least in treatment T1 (13.3%). The per cent phyllody transmission increased as DAF increased. The per cent phyllody transmission was also observed to increase as the Days of Inoculation Feeding (DIF) increased. At 5DAF, the highest per cent phyllody transmission was observed in treatment T6 (3 insects/plant + 5 DAF+ 7 DIF) *i.e.*, 86.7% as compared to per cent phyllody transmission recorded in treatment T4 (3 insects/plant + 5 DAF+ 3 DIF) which had the least per cent phyllody transmission of 53.3%. At 7 DAF, the treatment T8 and T9 (7 DAF+5 DIF and 7 DAF +7 DIF) recorded the highest percent phyllody transmission (93.3%). From the transmission study, the mean transmission rate of phyllody transmission was 54.67 per cent. The number of days taken for first appearance of phyllody symptoms in all the treatment combinations (T1-T9) was in the range of 29-41 days. All the treatments were found to be statistically significant over the control. However, the treatments T_8 and T_9 which recorded the highest per cent phyllody transmission were statistically not different.

3.3 Transmission with Leafhopper, *Hishimonus phycitis*

The transmission of sesame phyllody disease by insect vector, *Hishimonus phycitis* is presented in (Table 4). No single plant developed typical phyllody symptoms after introduction of insects during the period of observation. The results from the transmission studies with leafhopper, *H. phycitis* showed that the leafhopper could not transmit phytoplasmsa from infected sesame plants to healthy seedlings. Hence, *H. phycitis* is not an insect vector in the transmission of sesame phyllody disease caused by phytoplasma.

The findings from the transmission studies of phytoplasma by mechanical sap transmission in this study support earlier report by Akhtar et al. [5] when they reported that sap transmission of chickpea phyllody was not achieved by mechanically transmissible. Similarly, earlier research findings by Gogoi et al. [4], Karra [6] and Yadav et al. [7] using mechanical sap inoculations did not induce any typical phyllody symptoms on healthy sesame seedlings. However, Akhtar et al. [8] reported that the leafhopper, *O. albicinctus* transmitted 60 percent phyllody disease from phyllody infected sesame plants to healthy plants by showing symptoms of floral virescence and proliferation with acquisition and inoculation feeding of seven days, respectively. The finding in this study that phytoplasma is transmitted by leafhoppers is supported by earlier research finding by several authors [9,4,10,11].

The findings that the per cent phyllody transmission increased as DAF and DIF increased are in accordance with earlier research findings [12,4,5,13,11]. Jayashree et al. [12] noticed increase in percent disease transmission of pumpkin yellow vein mosaic virus and decrease in number of days required for symptom expression with an increase in acquisition feeding period and inoculation feeding period. Gogoi et al. [4] reported increase in sesame phyllody disease transmission with increase in acquisition feeding (AFP) 3 days

Table 2. Transmission of sesame phyllody disease by mechanical sap inoculation

Test plant	No. of plants taken	No. of plants inoculated	No. of plants showing phyllody symptoms	Days taken for appearance of phyllody symptoms	Per cent phyllody transmission
Sesame	150	150	0	0	0.00

Table 3. Transmission of sesame phyllody disease by insect vector, *Orosius albicinctus*

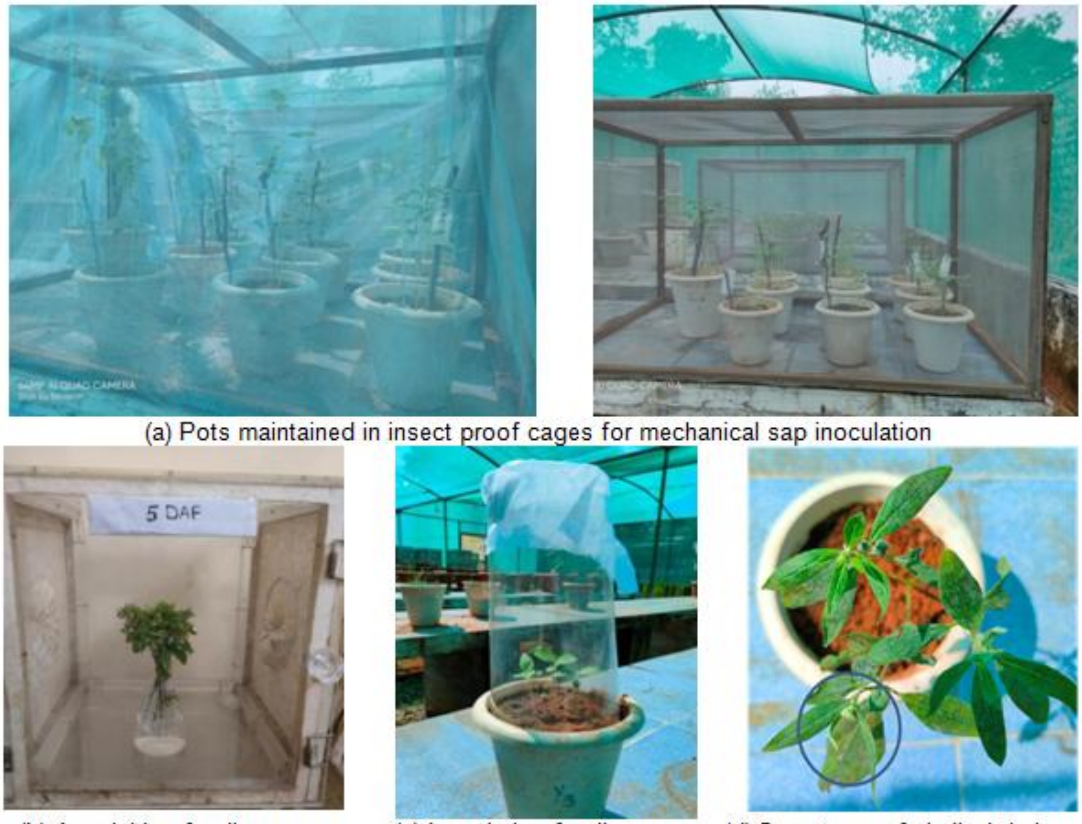
Treatment No	Treatments	Days required for appearance of phyllody symptoms	Per cent phyllody transmission (%)
T_1	3 insects/plant + 3 DAF+ 3 DIF	38-41	13.3^h
T_2	3 insects/plant + 3 DAF+ 5 DIF	33-36	26.7^g
T_3	3 insects/plant + 3 DAF+ 7 DIF	33-34	46.7^f
T_4	3 insects/plant + 5 DAF+ 3 DIF	32-38	53.3^e
T_5	3 insects/plant + 5 DAF+ 5 DIF	32-36	60.0^d
T_6	3 insects/plant + 5 DAF+ 7 DIF	29-33	86.7^b
T_7	3 insects/plant + 7 DAF+ 3 DIF	31-35	73.3^c
T_8	3 insects/plant + 7 DAF+ 5 DIF	31-34	93.3^a
T_9	3 insects/plant + 7 DAF+ 7 DIF	29-31	93.3^a
T_{10}	No inoculation (Control)	0	0.00^i
Mean			**54.67**
F-test			**Sig.**
SEm(±)			**1.37**
CD (P=0.05)			**4.05**
CV (%)			**4.38**

DAF – Days of Acquisition Feeding; DIF – Days of Inoculation Feeding; Sig – Significant at 5 per cent level of significance

Table 4. Transmission of sesame phyllody disease by insect vector, *Hishimonus phycitis*

Treatment No	Treatments	Days required for appearance of phyllody symptoms	Per cent phyllody transmission (%)
T_1	3 insects/plant + 3 DAF+ 3 DIF	0	0.00
T_2	3 insects/plant + 3 DAF+ 5 DIF	0	0.00
T_3	3 insects/plant + 3 DAF+ 7 DIF	0	0.00
T_4	3 insects/plant + 5 DAF+ 3 DIF	0	0.00
T_5	3 insects/plant + 5 DAF+ 5 DIF	0	0.00
T_6	3 insects/plant + 5 DAF+ 7 DIF	0	0.00
T_7	3 insects/plant + 7 DAF+ 3 DIF	0	0.00
T_8	3 insects/plant + 7 DAF+ 5 DIF	0	0.00
T_9	3 insects/plant + 7 DAF+ 7 DIF	0	0.00
T_{10}	No inoculation (Control)	0	0.00
Mean			**0.00**
F-test			-
SEm(±)			-
CD (P=0.05)			-
CV (%)			-

DAF – Days of Acquisition Feeding; DIF – Days of Inoculation Feeding; Sig – Significant at 5 per cent level of significance



(a) Pots maintained in insect proof cages for mechanical sap inoculation

(b) Acquisition feeding cage

(c) Inoculation feeding cage

(d) Symptoms of phyllody in insect transmitted pots

Plate 1. Phytoplasma transmission studies in green house

(49.38%) to 5 days (64.57%). Akhtar et al. [5] recorded success in the transmission of chickpea phyllody from infected to healthy chickpea plants by insect vector, *O. orientalis* with transmission rate of 70 per cent. However, the results from this study are in contradiction with finding by Jutimala et al. [14] who reported that *H. phycitis* was found to transmit sesame phyllody phytoplasma with a transmission rate of 83.33 per cent to healthy sesame plants. The presence of phytoplasma in *H. phycitis* was confirmed by nested PCR assays and concluded that *H. phycitis* can be considered as a new vector of phyllody from North East region of India in addition with *O. albicinctus.*

Leafhopper species belonging to cicadellidae regarded as the major insect vectors transmitting sesamum phyllody of which, *O. orientalis* is the major insect vector transmitting the phyllody disease in India, Iran, Turkey and other Asian countries [15, 8, 16]; *O. albicinctus* in India [4] and Iran [15]. However recent reports have implicated another species (*Hishimonas*) as vector in the transmission of sesamum phyllody from infected to healthy plants [17,14].

In all our field experiments at S.V. Agricultural College, Tirupati; *O. albicinctus* was found to be most abundant in addition to other species such as *H. phycitis* which were less abundant. The aimed to demonstrate the ability of both the species of leafhopper *i.e*., *O. albicinctus* and *H. phycitis* to acquire and transmit the disease from infected to healthy sesame plants. The results revealed that *O. albicinctus* was the main vector in transmitting the disease from infected plant to healthy plants and did produce the phyllody symptoms. However, studies with *H. phycitis* did not produced any conclusive results on appearance of symptoms even after 40 days of inoculation, where the insect was given a maximum period of 7 days of acquisition and 7 days of inoculation feeding period. This probably could be due to the occurrence and abundance of different leafhopper species across various geographical locations, along with differences in genetic variation among the population of same species at different geographical locations, which could affect the acquisition and transmission of the phyllody by the insect vector. Among the leafhopper species that were found to be vectors of sesamum phyllody, *Hishimonus* sp. was found to be more abundant in Northern part of India [17] and was able to transmit the disease to healthy plants, whereas, *Orosius* spp. was found to be more prevalent in Southern India [18,19]. Additionally, the genetic differentiations among the *H. phycitis* populations, at different geographical location could also affect the vector capability in transmitting the disease [20]. Because of the variation in in the different geographical areas, the abiotic factors also vary and accordingly can influence the acquisition and transmission of phyllody by the insect vector and the expression of symptom [21,22]. The abiotic factors present at Tirupati with the vector *Hishimonus* may be different from the experimental conditions IARI, New Delhi where Nabi et al. [17] carried out their study, which might have contributed to non-appearance of phyllody symptoms in the present investigations. Also mere acquisition of phytoplasm in the insect vector doesn’t mean that the insect can transmit the disease successfully from an infested plant to a healthy plant and the success of the transmission by the vector also depends on the quantity or titer load of the pathogen during the acquisition period [23].

It has also been demonstrated that the latent period *i.e*. the period between acquisition of phytoplasma (AAP) and the ability of the insect vector to transmit the phytoplasma into a healthy plant ranges from 7 to 80 days, which is also temperature dependent [24,25,26]. In our experiments we have given an maximum of 7 days as inoculation period for the phyllody infected insect vector (after a maximum of 7 days of acquisition period on phyllody infected plant) which could also be one of the reasons for absence of appearance of any phyllody symptoms when *Hishimonus* was used as vector in the present transmission studies. However the studies on insect vector transmission with species of leafhoppers other than *O. albicinctus* is still in the infancy stage and further studies on identification of all the available leafhopper species at different agro climatic zones have to be undertaken by taxonomic keys as well as molecular identification and the acquisition, inoculation, latent period of phyllody in the insect vector and the presence of phyllody and the titer load in the insect vector as well as presence and expression of symptoms in the healthy plants have to confirmed with insect vector transmission studies as well as molecular confirmation with the help of specific primers.

4. CONCLUSION

The transmission studies conducted with two leafhopper species, *O. albicinctus* and *H. phycitis* confirmed that leafhopper, *O. albicinctus* was the potential insect vector responsible for dissemination of phytoplasma, the causal organism of sesame phyllody from infected plants to healthy sesame seedlings. The findings revealed that leafhoppers play a major role in transmission of phytoplasma under field conditions. The sap inoculated plants did not produce any significant phyllody symptoms during the period of observation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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