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Report of B-Biotype Whitefly (*Bemisia tabaci* Gennadius) in Northern Karnataka

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Abstract B-biotype whitefly *Bemisia tabaci* (Gennadius) reported for first time in pumpkin fields of Northern parts of Karnataka by using symptomatic and biochemical diagnosis asays such as silverleaf disorder and filter paper dot blot techniques which can able to distinguish infestation of B-biotype whitefly in plants from Indian indigenous biotypes easily by symptomatic expression, slivery color leaf on upper surface of leaf. Where as in filter paper dot blot technique, homogenate samples from B-biotype whitefly on Whatman filter paper express deep blue color but bomogenate samples of indigenous biotype shows slight blue color and these techniques found quickly and reliably for diagnosis, detect and differentiation of biotype.

Keywords B-biotype, Filter paper dot blot technique, Pumpkin, Silver leaf assay, Whitefly.

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Introduction

Whiteflies (Hemiptera: Aleyrodidae) have been recorded from all the tropical and sub tropical areas of the world, as well as many temperate regions. Amingst the known whitefly species, *Bemisia tabaci* (Gennadius) is considered the most economically important attacking over 600 plant species. *B. tabaci* is both multivoltine and polyphagous and a vector of economically important plant viruses. The *B. tabaci* was first collected and described as *Aleyrodes tabaci* (Gennadius) from tobacco, *Nicotiana* spp. in Greece in 1889. It was subsequently renamed as *B. tabaci*. The total life cycle of the whitefly from egg to adult stage varied from 13 to 72 days depending upon seasonal conditions.

In India, *B. tabaci* was first recorded in 1905. The center of origin of *B. tabaci* was suggested to be the Indian subcontinent because of the presence of large number of natural enemies against it [1—3]. It is defined 10 biotypes on the basis of esterase banding

Table 1. Estimation of the number of B-biotype female adults required for the induction of silverleaf symptoms on the pumpkin variety Pumpkin hybrid, MAHY-1 (Mahyco)[1]. [1]Each group of insects inoculated each plant for 48 h, [2]Symptom severity was recorded three weeks after inoculation using a 0–5 point scale, where, 0=no symptoms and 5=severest symptoms.

No. of B biotype adults used per plant	No. of plants with symptoms/ inoculated	Silverleaf incidence(%)	Silverleaf severity[2]
0	0/25	0	0
1	14.25	56	1.8
2	22/25	88	2.4
5	25/25	100	2.9
10	25/25	100	3.2
20	25/25	100	3.8
30	25/25	100	4.1

patterns viz., A, B, D, E, G, H, J, K, L, and M. The B biotype was first recorded in the Kolar district of Southern Karnataka, during summer season (March–June) of 1999 but no report of B biotype from Northern Karnataka.

The present study having the basic idea is that formation of silverleaf disorder on upper surface of infested leaf by B-biotype whitefly which is because of high esterase activity in B-biotype whitefly compared to indigenous. This can be used as a symptomatological diagnosis tool for detecting B-biotype whitefly. It is observed that leaf silvering was a physiological disorder of marrows, *Cucurbita pepo*, exacerbated by drought silvered leaves and spaces between the upper epidermis and the mesophyll cells and within the mesophyll. These unique symptom is used to detect the B-biotype. It has obtained 100% silver leaf symptoms incidence with 48 h feeding by twenty adult sweet potato whitefly (SPWF) (B biotype).

Filter paper dot blot method was based on the high esterase activity in B-biotype whitefly compared to indigenous. This technique developed by Shankarappa et al. [4] for identification of B-biotype whitefly samples, which had an advantage over the dot blot method as it requires very minimal quantities of reagents and materials and could be performed within short time at field level. Which is a simple and effective biological assay was developed using pumpkin cv Big for identification and presence of B-biotype whitefly. The present study deals with detection of B-biotype whitefly by using simple and effective biological assay such as silver leaf disorder technique and filter paper dot blot test to record B-biotype whitefly first time in Northern parts of Karnataka.

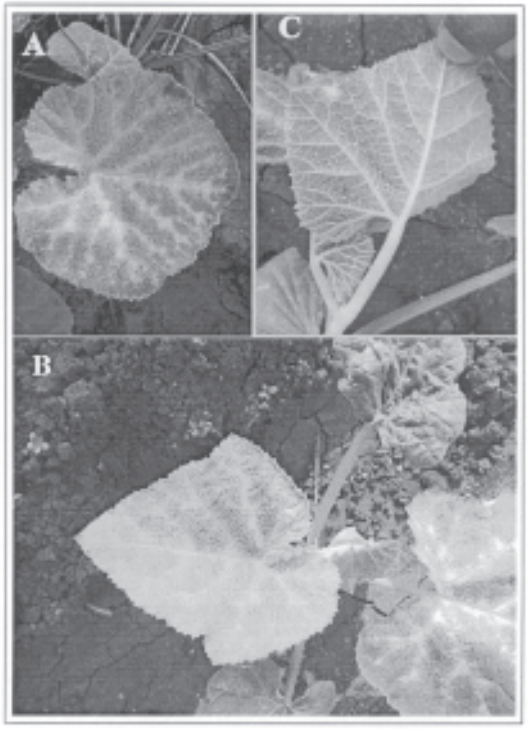

Fig. 1. Pumpkin plants showing silver leaf under field condition (A, B) and B-biotype whiteflies colony in pumpkin plant showing silver leaf (C).

Materials and Methods

The present investigation was carried out at Department of Plant Pathology, K. R. C. College of Horticulture, Arabhavi, Gokak (Taluk), Belagavi (District), Karnataka during the year of 2014-15.

Collection of B-biotype whitefly sample

The B-biotype whitefly sample was collected from

Fig. 2. Whitefly *Bemisia tabaci* adults maintained on cotton plants in insect proof net house.

different experimental plot of K. R. C. College of Horticulture, Arabhavi, Gokak (Taluk), Belagavi (District), Karnataka by observing the leaf silvering, a physiological disorder in pumpkin *Cucurbita moschata*, exacerted by drought silvered leaves and spaces between the upper epidermis and the mesophyll cells and within the mesophyll which is shown in Figure 1.

Maintenance of whitefly cultures

Cultivation of cotton and pumpkin plants

The experiment was proceeded with the raising of cotton seedlings (*Gossypium hirsutum*). The seedlings were raised in 4 × 6 cm polythene bags containing soil and compost mixture in 2:1 proportion and maintained under both insect proof wooden cages

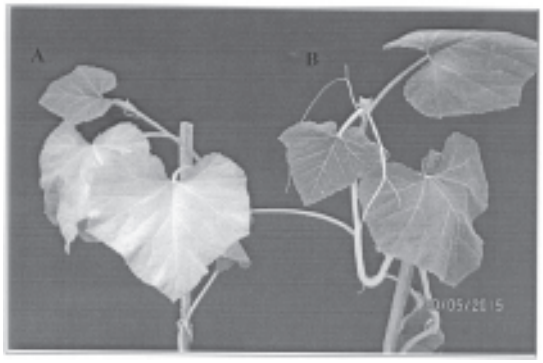

Fig. 3. The symptoms induced in pumpkin plants after feeding of female B-biotype whitefly. A. Silver leaf pumpkin and B. Pumpkin plants after feeding indigenous whitefly served as control.

and insect proof net house for whitefly culture maintenance on cotton (Fig. 2). Pumpkin hybrid MAHY-1 (Mahyco) seeds were used to raise seedlings in 4 × 6 cm polythene bags containing soil and compost mixture in 2:1 proportion and maintained under insect proof net house. pumpkin plants were maintained in insect proof net house and 1 to 2 leaf stage seedlings were used for B-biotype whitefly inoculation.

Establishment and maintenance of 'B' biotype whitefly culture

The pure culture of the 'B' biotype whitefly (*Bemisia tabaci*) was used for the current investigation was originally collected from pumpkin (*Cucurbita moschata*) plant showing silver leaf symptoms at experimental plot of K.R.C College of Horticulture, Arabhavi (Fig. 1). The collected whiteflies were maintained on cotton (*Gossypius hirsutum*) (Fig. 2) and kept in insect proof wooden cages (45 × 45 × 30 cm) and covered with muslin cloth on all the sides except the front side. The whitefly culture was maintained on healthy cotton grown in polythene bags (4 × 6 cm) under insect proof cages. These cages were provided with temperature of 28 to 30°C in an insect proof net house by covering transparent roof sheet.

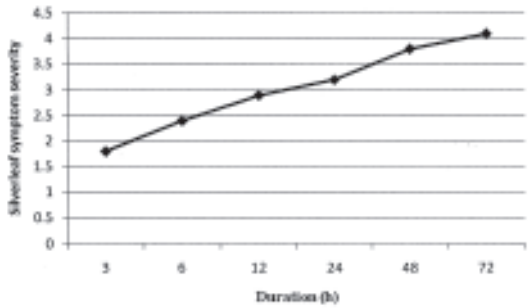

Fig. 4. The effect of the feeding period of B biotype on the induction of silverleaf symptoms meassured by using a 0–5 scale.

Identification of 'B' biotype whitefly

Silver leaf disorder technique for confirmation of 'B' biotype whitefly

Silver leaf disorder technique for confirmation of 'B' biotype whitefly in pumpkin (*Cucurbita moschata* L. (Duch) ex 1 am) hybrid MAHY-1 (Mahyco) seedlings followed based on protocol of Shankarappa et al. [4].

Raising of healthy seedlings

Healthy pumpkin (*Cucurbita moschata* L. (Duch) ex 1 am) hybrid MAHY-1 (Mahyco) seedlings are taken for silver leaf disorder technique study. Seedlings were grown from seeds in 4 × 6 cm polythene bags filled with soil and compost mixture in 2:1 proportion, which were kept in an insect proof cage. Seedlings were used for experiment is at first true leaf geowth stage.

Preparation of cages and feeding of pumpkin seedlings with 'B' biotype whitefly

Plastic tubes of 8 cm in height and 7 cm in diameter were taken and the bottom portion was removed with the help of soldering rod. Muslin cloth was fixed to the removed portion to avoid the accumulation of excess moisture inside the cage. A small hole (0.5 cm) was made on the middle portion of the tube to release the whiteflies. 'U' shaped cut was given on the top portion of the tube. The growing leaf from the tip of the plant was selected and inseted into the plastic tube, through the hole (1.0 × 1.0 cm) made at the mouth of the tube, plugged with cotton and tube was enclosed with cap. Then the whiteflies were released through the small hole then covered the hole with cotton plug. The tube was tied to the bamboo stick with rubber band.

Testing of pumpkin seedlings for silver leaf symptom

The experiment was under taken to determine whether *B. tabaci* is capable of inducing silvering in pumpkin (*Cucurbita moschata* L. (Duch) ex 1 am). A group of 30 adult 'B' type whiteflies were collected from colony and are allowed to feed on ten seedlings of pumpkin hybrid MAHY-1. The seedlings were exposed to whitefly for 48 h and plants were periodically assessed for silver leaf symptom.

Testing to determine the number of B biotype whitefly required to induce silver leaf symptom

In order to determine the number of B-biotype nymphs required to induce silver leaf symptom, groups of 1, 2, 3, 5, 20 and 30 B-biotype females were exposed to each of the 25 pumpkin seedlings (hybrid MAHY-1) using leaf cages. Adults were allowed to feed for 48 h, after which they were removed. The exposed seedlings were miantained in insect-proof cages for the development of silver leaf symptoms. Plants exposed with indigenous whitefly served as control. Latter silver leaf symptom severity was recorded on a scale ranging from 0–5 scale.

Esterase based detection technique

Filter paper dot blot test

A single female adult B-biotype was homogenized in a microfuge tube in 15 ml of protein extraction buffer, as described by Byrne and Devonshire. [5]. The sample was applied onto Whatman filter paper (125

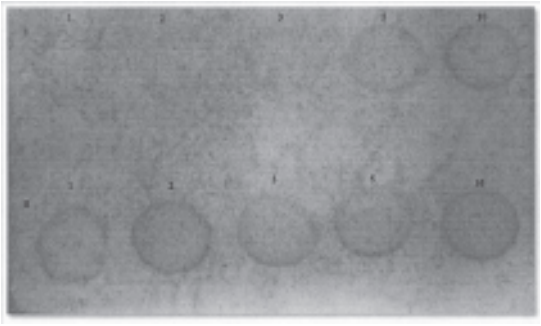

Fig. 5. Development of deep blue color on filter paper blotted with homogenate from B-biotype whitefly samples (B). Slight blue color with homogenate of indigenous biotype (A).

mm) using a micropipette, air dried for a few minutes and incubated in 15 ml of substrate solution (napthyl acetate and fast blue RP) for 30 min at 30°C. The membrane was destained using the destaining solution with occasional shaking, air dried and observed for variation in color intensity between the indigenous (faint blue) and B biotype (intense blue) samples.

Results and Discussion

Silver leaf disorder technique

Reaction of pumpkin seedlings to 'B' biotype whitefly feeding

Pumpkin hybrid, MAHY-1 (Mahyco) tested for their ability to induce silver leaf symptoms upto 'B' biotype whitefly feeding. All the ten plants tested produced 100% incidence. The hybrid showed prominent vein clearing was the first visible symptom followed by silvering of leaves and expressed symptoms, typical to those of pumpkin [4]. All pumpkin seedlings developed symptoms in about 12—20 days (Fig. 3).

A minimum of 6 h feeding on pumpkin by B-biotype adults was essential to induce silver leaf symptoms. Exposure of more than 12 h resulted in 100% silverleaf incidence, often with severe symptoms. Silverleaf symptom severity increased with an increase in the exposure period. A single femele B-biotype whitefly induced silver leafing on 56% plants, while five or more females caused 100% incidence. The most severe symptoms (rating 4.1) were observed when 30 females fed on each plant (Table 1 and Fig. 4). Similar type of results are obtained in study conducted by Shankarappa et al. [4].

Esterase based detection technique

Filter paper dot blot test

Dot blot rechnique is useful for instant detection of B-biotype whiteflies on the basis of color development by esterase activity. This technique involved applying whitefly extraction onto Whatman filter paper, staining with napthyl esterase substrates and recorded the differences in color intensity between the biotypes. The presence of B-biotype was confirmed by the development of deep brown color on filter paper blotted with homogenate from B-biotype whitefly samples while faint brown color with homogenate of indigenous biotype. This method was found suitable for esterase analysis of both individual and groups of adults (Fig. 5). Shankarappa et al. [4] observed similar type result in pumpkin, gherkin and squash genotypes.

The B-biotype was first recorded in the Kolar district of Southren Karnataka state of South India, during the summer growing season (March–June) of 1999. A symptomatological study, Silver leaf disorder technique was conducted by Shankarappa et al. [4] in pumpkin, gherkin and squash genotypes and observed the prominent vein clearing was the first visible symptom followed by silvering of leaves and expressed symptoms which is supporting the present research work. Similar type study also conducted in squash, zucchini plants by De Barro et al. [6] Seruwagi et al. [7] and found that these techniques are having simple, cost-effective detection protocols which were used in this experiment, alternative to those techniques which require substantial capital investment and skilled personnel such as detection using RAPD polymorphisms and mtCOI sequences [8]. Silverleaf assay has been used to detect B-biotype worldwide as

it is the most invasive of the biotypes; and, therefore, the likelihood of the individual being B is very high [4, 7, 9].

However, in South India pumpkin, are more popular and grown extensively throughout the year. For these reasons, the ability of the B-biotype to induce silverleafing on pumpkin genotypes was absorbed. The pumpkin hybrids, MAHY-1 (Mahyco) were shown highly susceptible and expressed silver leaf symptoms. Esterase profiles showed that, the indigenous and B-biotype adults were different, which allowed easy discrimination of the two biotypes. Moreover, esterase levels were considerably greater in B-biotype compared to the indigenous adults [4]. By knowing these unique properties such as simple, cost effective and quick. This silverleaf disorder technique and filter paper dot blot method was used to diagnosis, detect and differentiate B biotype whitefly from indigenous biotype (A).

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