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Guava Wilts a Multidimensional Malady: Role of Guava Stem Borer and Concomitant Fungi in Effecting the Pathogenic Process in India

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Abstract

Study involving surveys undertaken in guava cultivation belt of Uttar Pradesh comprising of Allahabad-Varanasi, Unnao-Kanpur in Eastern and Central region respectively as well as Badaun and Farrukhabad in Western region of the state indicate that the guava orchards in the former two regions are mainly afflicted by stem borer, *Bactocera rufomaculata* and bark eating caterpillar, *Indarbela quandrinotata* which seem to pave the way to infection of *Fusarium oxysporum* f. sp. *psidii*. Stem borer affected guava plants consistently yielded the colonies of *Fusarium oxysporum f sp psidii* from live worm, wood scrapings from the borer infested hole and soil/roots of guava orchards in Badaun and Farrukabad exhibited the fungal induced typical wilt. Analysis of the fungal distribution on dried/healthy branches from different set of plants comprising of healthy, 30%, 90% and 100% wilt revealed the prevalence of *Fusarium oxysporum* f. sp. *psidii*, in the lower/middle portions whereas *Botrydiplodia theobromae* and *Pestalotiopsis psidii* in peripheral branches. A positive correlation was noticed between of *Fusarium oxysporum* f. sp. *psidii*, in plants exhibiting 30% wilt as compared to negative correlation between *B. theobromae* and wilt (30%). However, in the plants displaying 100% wilt, role of *B.theobromae* and *Pestalotiopsis psidii* was more pronounced as compared to *F. oxysporum* f. sp. *psidii*. Our study sheds the light on varying degree of contribution made by the insects and fungi in effecting the wilt disease witnessed in guava and flagged the crucial issue that fungal centric approach has miserably led the failure of durable and reproducible management schedule(s) developed so far in different guava cultivation pocket(s) of the country.

Keywords: Guava wilt; Stem borer; *Bactocera rufomaculata*; *Fusarium oxysporum* f. sp. *Psidii*; *Pestalotiopsis psidii*; Partial least square; Principal component

Introduction

Guava wilt since its emergence on the patho-system horizon has been one of the most perplexing problems on account the of the lack of authentic identity of the causal factor(s) which could be validated and withstand all factors linked to prevailing ecosystem across the guava growing regions. A number of pathogenic microbes including fungi, bacteria *viz*., *Botrydiplodia theobromae, Clitocybe tabescens Cephalosporium sp,Cylidrocarpon sp, Macrophomina phaseoli, Fusarium oxysporum f psidii, Myxosporium psidii, Fusarium moniliforme F.solani, Rhizoctonia bataticola, Septofusidium sp, Penicillium vermoensenii, Pseudomonas sp, Erwinia psidii*, etc. have been considered responsible for the malady-the wilt across the globe [1-6]. Role of plant nematode; *Helicotylenchus dihystera* too has come to the fore in effecting the wilt either as a sole factor or co-factor [7]. Recently the identification of *Meloidogyne enterolobii* especially in the guava nurseries, their role in its further spread in guava cultivation belt of Madhya Pradesh, Rajasthan, Uttar Pradesh and Tamilnadu along with its association with *Fusarium oxysporum* f. sp. *psidii* has added another worrisome dimension to the woes of thriving guava nursery industry and guava orchardists as well [8-11]. The puzzle shrouding the identity of the causal factor across the board and inconsistency pertaining to the valid identity of the pathogen(s) has largely impacted the development of durable and consistent management strategy across the spectrum of agro ecologies having commercial cultivation of guava. Till date a durable management strategy for tackling the problem of guava wilt is still a far cry. In view of these surveys were undertaken with reference to the varying degree of association/occurrence of biota including bark eating caterpillar, stem borer and fungi *viz*., *Fusarium oxysporum* f. sp. *psidii*, *Botrydiplodia theobromae* associated with the wilt affected guava orchards. The study was aimed to quantify and analyse the factors responsible for the onset of wilt, its further persistence and the extent of their involvement in the wilt at different stages in different guava growing regions of U.P. [11]

Materials and Methods

Random surveys were undertaken in 7, 3, 4 and 5 locations in commercial guava growing belts of Allahabad-Varanasi, Unnao-Kanpur Badaun and Farrukhabad of Eastern, Central and Western regions of U.P. respectively. Soil/root samples were collected and visual observations were recorded on the wilting status and borer infestation in the plants. Status of fungal induced wilt and borer were based on the total number of plants included in programme and the number of plants exhibiting wilt/borer infestation. It was expressed in terms of percentage. Three to five plants were selected at each site.

Three guava plants each were selected from four categories based on plant health status *viz*., healthy, 30%, 90% and 100% wilted. Three branches each from central and peripheral region of the tree canopy were selected in healthy and wilted plants exhibiting 30%, 90% and 100%, marked and divided in four sections of 5 cm each and categorized as upper, upper middle lower middle, lower for the study. Pieces measuring 1-2 mm were cut from each marked category (healthy, 30%, 90% and 100% wilt) of each branch portion (Upper, upper middle, lower middle and lower). Bits were washed thoroughly in 0.01% mercuric chloride and five such bits were placed in 90 mm petri dishes having 15 ml of sterilized PDA with three replications. Observations on fugal colonies were made after 10 and 15days of inoculations. Isolations of nematodes and fungi were made as per standard methods [12,13].

Samples from soil, root and wood scrapings were collected from the plants infested with guava stem borer (*Bactocera rufomaculata*). Three plants having borer infestation were marked and composite soil/root samples were taken from three plants each. Similarly the stem wood scrapings (1.0 g) from the upper as well as lower portion of the hole caused by the borer were collected in triplicate from three set of holes from each of the three plants marked for the purpose. Isolation from the soil, roots and wood scrapings were made as per standard methods and observations made after 10 and 15 days of incubation.

The portion of branch (Lower, Lower Middle, Upper Middle, Upper) affected by wilting, frequencies of fungi; Botrydiplodia theobromae (Bot), Pestiliopsis psidii (Pet), Fusarium oxysporum psidii (Fus) and extent of wilting of plant (SW) subject to multivariate analysis; principal component analysis and partial least square factor analysis through software SAS 9.3.

Purpose of analysis was to understand the role of fungi and explain the role played by each fungus during the wilting process in multivariate set up. Principal component analysis was applied for generation of loadings for status of wilting of plant (SW) along with fungi *Fusarium oxysporum* f. sp. *psidii* (Fus), *B.theobromae* (Bot), *Pestalotiopsis psidii* (Pet) on component (PC1&2) to quantify the significant role (positive or negative), during process of wilting of plant. The partial least square analysis was applied for understanding the crucial role in terms of proportion of variation explained by fungi in muti-collinearity and wilting of plant environment. The factors (1 &2) were applied for quantification of the proportion of variation explained in individual fungi.

Results and Discussion

Data presented in **Figure 1** revealed that the orchards located in Allahabad-Varanasi and Kanpur-Unnao belt were found to have 85.7% and 66.66% infestation of stem borer. Conversely the plants exhibiting typical wilt symptoms were 14.2% and 33.33% only. Conversely guava orchards located in commercial cultivation belt of Badaun and Farrukhabad districts of Western exhibited fungus induced wilt (100%). Our observation indicate that the orchards exhibiting wilt in the Eastern and Central part of U.P., basically have a major problem of insect infestation *viz*., bark eating caterpillar, *Inderbella quandrinotata* and stem borer, *Bactocera rufomaculata* which at later stages of infestation paves the way to fungal infection especially *F.oxysporum* f. sp. *psidii*, which besides soil and root samples was also consistently isolated from live worm as well wood scrapings from the upper portion of the hole **(Figure 2)**.

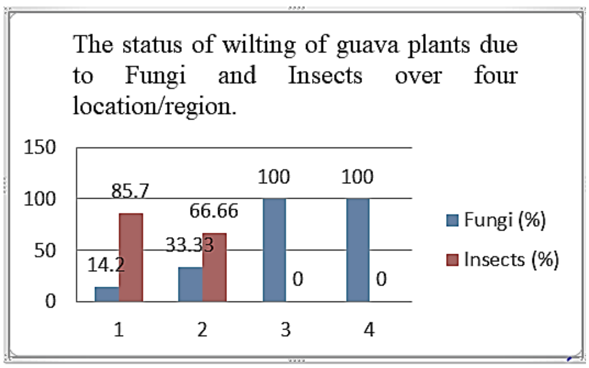

Figure 1: Wilting of guava plants affected by Insects and fungi in different regions.

Data from borer infested plants revealed that *Fusarium oxysporum* f. sp. *psidii* was the predominant fungi for the 19,3,4,4 colonies of the fungus were isolated from soli root, live worm and wood scrapings from the upper portion of hole respectively. Scrapings from lower region of the hole did not yield any fungal colony. A mutualistic interaction has been observed between bark beetles, mites and fungi [14].

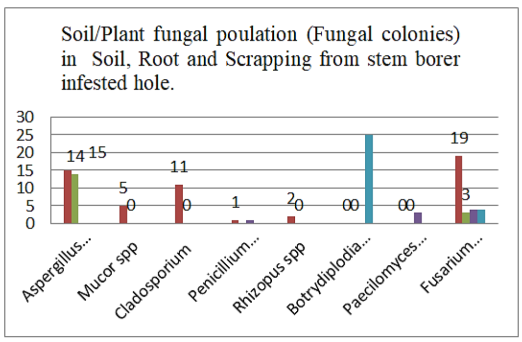

Figure 2: The distribution of fungal colonies in soil root and scrapping.

Data in respect of infection caused by three fungi *viz*., F.oxysporum f. sp. psidii, *Botrydiplodia theobromae* and *Pestalotiopsis psidii* on the four designated portion of branches (Upper, Upper middle, Lower middle and Lower and plant health status *viz*., healthy, 30% wilt, 90% wilt, 100% wilt) subjected to the principal component analysis revealed that more than 58% variation in infection in the portion of branches occurred due to significant contributions made by all the three fungi as the status of wilting had less loading coefficient 0.32, *B.theobromae* would have played negative role with highest loading 0.96.

Conversely *P. psidii* would interact positively vis a vis the wilt as compared other concomitant fungi recorded from the infected branches with loading as 0.91.

However, *F. oxysporum* f. sp. *psidii*, with a low loading (0.66) too would be contributing positively with reference to its role in effecting the pathogenic process. At 23.62% variation in the infection observed in the branches, *B.theobromae* and *P. psidii* would contribute significantly due to significant loading (0.93). The role of *F. oxysporum* f. sp. *psidii* at this particular stage seems to be non-significant.

However, with 17.61% variation the contribution of *F. oxysporum* f. sp. *psidii*, (0.71) and *P. psidii* (-0.38) became significant again. Our study indicates and highlights the role/ contribution of the fungal pathogen/weak parasites/ opportunistic fungi at various stages of the wilt development process.

However, when the role of individual fungi occurring in the system is taken into consideration with respect to wilting of plant as a whole, partial least square analysis generated factors for understanding the role of *B.theobromae* (1.01) seems to be major one followed by *P. psidii* (1.28) and *F. oxysporum* f. sp. *psidii* (0.62).

On the other hand considering the role of individual fungi in wilting process, more than 95% variation could be explained by single factor (F1) in case of *B.theobromae* whereas the two factors (F1, F2) would explain the role of *F. oxysporum* f. sp. *psidii* and *P. psidii*.

Principal component and partial least square factor analysis also has flagged the core issue and confirmed the contention that though *F. oxysporum* f. sp. *psidii*, plays a pioneering role in initiation of wilt but following its establishment in the pathosystem, the other co inhabitants *viz*., *B.theobromae* and *P. psidii* amongst fungi and *Bactocera rufomaculata*, *Inderbella quandrinotata* amongst insects too starts contributing in the process.

Statistical analysis of the data collated on prevalence and frequency of the three fungi indicate that at 30% wilt, *F. oxysporum* f. sp. *psidii* seems to play the predominant role while at higher percentage of wilting *B.theobromae* and *P. psidii* seem to contribute significantly **(Figures 3 and 4).**

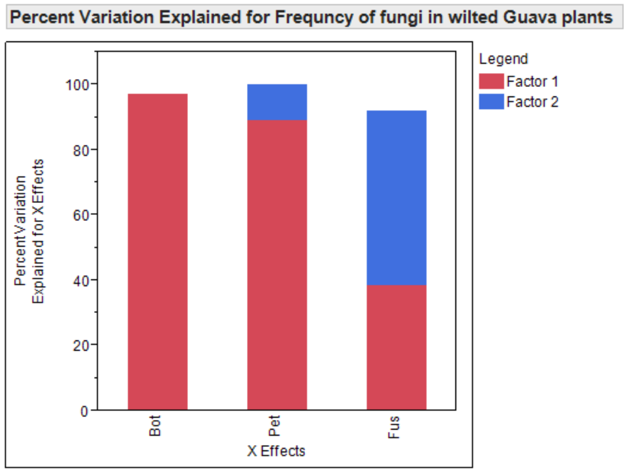

Figure 3: The variation in frequency distribution of fungi explained through factors (1 and 2).

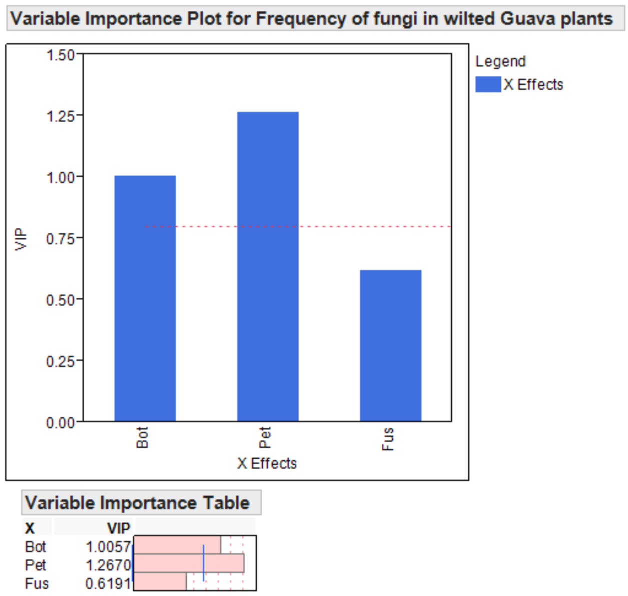

Figure 4: The importance of fungi as significant contributor in wilting of guava plants.

Conclusion

The problem of guava wilt has largely been attempted with focused thrust on the fungi. However, our studies have brought to fore that the problem needs to be probed with multidimensional approach, keeping the complexity of ecosystem in view. The failure in tackling the problem and the development of workable management also corroborates our view point. We propose that a combo-schedule comprising of soil treatment for *F.oxysporum* f. sp. *psidii* plugging the holes for *Bactocera rufomaculata, Inderbella quandrinotata* and spray coupled with pruning for *B.theobromae* and *P. psidii* would in all probability be an effective and durable solution to the problem.

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