ROLE OF DIFFERENT FUNGI FOR DEVELOPMENT OF DECLINE SYMPTOMS IN CITRUS, MANGO AND GUAVA

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ABSTRACT: *The major fungi that include Ceratocystis sp., Lasiodiplodia theobromae, Nattrassia mangiferae and Fusarium sp. have been consistently reported to cause tree decline in fruit, forest and ornamental trees. A study was conducted with hypothesis that symptoms development is similar for all of these fungi in citrus, mango and guava trees. An experiment to evaluate symptoms was conducted on 2 years old green house potted trees. Inoculation was done by the flap at stem and root injury methods. In the flap method a two 5mm plugs of freshly growing cultures were placed in T shape cut in the bark and wrapped with para film. In root injury method plants roots were injured with the help of a knife infested with the fungi and then covered with soil and followed by a soil drench with a spore suspension made from 5 plugs of 5mm from fresh cultures of fungi mixed in 200 ml sterilized distilled water. The trees were inoculated with individual fungi as well as in combination. The inoculated trees were assessed for symptoms of dieback, gummosis, bark splitting, canker and mortality. The results showed that the fungus C. fimbriata alone caused mortality in all three tree species. Other fungi alone produce symptoms like gummosis, bark splitting and rotting but not mortality. The root injury inoculation method was effective in symptoms development than the flap method. The study concludes that all fungi behave similarly in decline of citrus, mango and guava and hence similar management strategies may be adopted in the orchards of these fruit trees.*

Key words: Fungi, citrus, mango, guava, pathogenicity

INTRODUCTION

There are four major fungi, which have been consistently reported to cause the tree decline in fruit, forest and other ornamental trees. These fungi include *Ceratocystis* sp., *Lasiodiplodia theobromae*, *Nattrassia mangiferae* and *Fusarium* sp. The symptoms produced by the inoculation of these fungi alone or in combination produce decline like dieback, gummosis, bark splitting, canker formation and mortality of the tree [1, 2].

Different methods of pathogenicity have been used to confirm fungal association with decline [3,4,5]. Pathogenicity has been conducted using green house potted plants, branches of the mature trees as well as excised shoots inoculation. Mostly the flap method of inoculation has been used and it appeared to be successful [6].

The pathogenicity using different rating scales, while others just described the symptoms produced. However, the pathogenicity was confirmed after re-isolation of pathogens from artificially inoculated symptomatic plants.

During this study the aim was to know the role of four selected fungi was done to cause decline symptoms in citrus, mango and guava.

MATERIALS AND METHODS

The pathogenicity test was conducted on two years potted plants of citrus, mango and guava placed in the green house of Horticultural Research Institute, NARC, and Islamabad. The pathogenicity was done by using flap method and root injury method. The pathogenicity was determined looking at symptoms like bark splitting/cracking, gummosis, canker development and death of the plant.

Flap Method

In the flap method a 'T' shape cut was given in the bark of plants and two plugs of 5 mm from freshly growing cultures were placed by opening T-shape cut and then wrapped with the help of para film. As a control of this method plugs from Potato Dextrose Agar PDA without cultures were inserted (Fig 1).

Fig.1. Flap method inoculation

ROOT INJURY METHOD

In this method the plants roots were injured with the help of infested knife and then covered with soil. Spore suspension (5 plugs of 5mm from fresh cultures of fungi were mixed in 200 ml sterilized distilled water) was applied as a soil drench (Fig 2).

Fig.2. Root injury inoculation

The treatments in both methods are as follows:

T1= *Ceratocystis* sp.
T2= *Nattrassia* sp.
T3= *Botryodiplodia theobromae*
T4= *Fusarium* sp.
T5= *Nattrassia* + *Ceratocystis*

T6= *Nattrassia* + *B. theobromae*
T7= *Nattrassia* + *Fusarium*
T8= *Nattrassia* + *Ceratocystis* + *B. Theobromae*
T9= *Nattrassia* + *Fusarium* + *B. Theobromae*
T10= *Nattrassia* + *Ceratocystis* + *B. Theobromae* + *Fusarium*
T11= 5 mm plug from PDA (Control), media suspension for injury method
Each of the above treatment was tested in triplicate and a range of symptoms was observed on citrus, mango and guava seedlings.

RESULTS AND DISCUSSION

Inoculation on Citrus

The inoculation results of different fungi by flap method on 1 to 2 years potted citrus plants have been shown in the Table 1.

The results indicate that when *Ceratocystis* sp. alone (T1) was inoculated to the plants through flap method, symptoms like leaf drooping, stem canker and stem gummosis were observed and eventually the plants died. In case of root injury only leaf drooping was observed and the plants died. *Nattrassia* sp. inoculation alone (T2) showed bark splitting, gummosis and stem canker appeared but the plants survived. On inoculation of *Botryodiplodia theobromae* alone, root. Root injury method resulted in bark splitting and gummosis, there was no mortality of plants. On inoculation of *B. theobromae* alone (T3), stem canker and gummosis and in root injury only gummosis was shown without mortality of the plants in both methods. *Fusarium* sp. alone (T4) resulted in the only symptom i.e. leaf drooping and plants survived using both flap and injury method of inoculation. Fungi were also inoculated in different combinations. The combination of *Nattrassia* sp. + *Ceratocystis* sp.

Table 1. Pathogenicity of fungi associated with citrus decline by using flap and Root injury method inoculation

Treatments	Method	Decline symptoms in citrus				
		Leaf drooping	**Bark splitting**	**Stem canker**	**Stem gummosis**	**Mortality**
T_1	Flap	+	-	+	+	+
	Root injury	+	-	-	-	+
T_2	Flap	-	+	+	+	-
	Root injury	-	+	-	+	-
T_3	Flap	-	-	+	+	-
	Root injury	-	-	-	+	-
T_4	Flap	+	-	-	-	-
	Root injury	+	-	-	-	-
T_5	Flap	+	+	+	+	+
	Root injury	+	+	-	+	+
T_6	Flap	-	-	+	+	-
	Root injury	-	+	-	+	-
T_7	Flap	+	+	-	+	-
	Root injury	+	+	-	+	-
T_8	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_9	Flap	+	+	+	+	-
	Root injury	+	+	-	+	-
T_{10}	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_{11}	Flap	-	-	-	+	-
	Root injury	-	-	-	+	-

+ = Symptoms present ; - = Absent

Table 2. Pathogenicity of fungi associated with mango decline by using Flap and Root injury method of inoculation.

Treatments	Methods	Decline symptoms in mango				
		Leaf drooping	**Bark splitting**	**Stem canker**	**Stem gummosis**	**Mortality**
T_1	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_2	Flap	-	+	-	+	-
	Root injury	-	-	-	+	-
T_3	Flap	-	+	+	+	-
	Root injury	-	+	-	+	-
T_4	Flap	+	-	+	+	-
	Root injury	+	+	+	+	+
T_5	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_6	Flap	-	+	+	+	-
	Root injury	-	+	+	+	-
T_7	Flap	+	+	+	+	-
	Root injury	+	+	-	+	-
T_8	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_9	Flap	+	+	+	+	-
	Root injury	+	+	+	+	-
T_{10}	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_{11}	Flap	-	-	-	+	-
	Root injury	-	-	-	+	-

+ = Symptoms present ; - = Absent

Table 3. Pathogenicity of fungi associated with guava decline by using Flap and Root injury inoculation method

Treatments	**Methods**	**Decline symptoms in guava**				
		Leaf drooping	**Bark splitting**	**Stem canker**	**Stem gummosis**	**Mortality**
T_1	Flap	+	-	+	+	+
	Root injury	+	-	+	+	+
T_2	Flap	-	+	-	+	-
	Root injury	-	+	-	+	-
T_3	Flap	-	+	+	+	-
	Root injury	-	+	-	+	-
T_4	Flap	+	-	+	+	-
	Root injury	+	-	-	+	-
T_5	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_6	Flap	-	+	+	+	-
	Root injury	-	+	+	+	-
T_7	Flap	+	+	-	-	-
	Root injury	+	+	-	+	-
T_8	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_9	Flap	+	+	+	+	-
	Root injury	+	+	+	+	-
T_{10}	Flap	+	+	+	+	+
	Root injury	+	+	+	+	+
T_{11}	Flap	-	-	-	-	-
	Root injury	+	-	-	+	-

+ = Symptoms present; - = Absent

(T5) produced complete set of decline symptoms i.e. leaf drooping, bark splitting, stem canker, gummosis in flap method, however, in root injury stem canker was skipped, eventually plants mortality occurred in both cases. The combination of *Nattrassia* sp + *B. theobromae* (T6) through flap method showed similar symptoms as *B. theobromae* alone (T3) while, root injury showed bark splitting and gummosis. Plants survived in all cases. The combination of *Nattrassia* sp +*Fusarium* sp (T7) produced similar symptoms i.e. leaf drooping, bark splitting and gummosis but no plant mortality took place. The inoculation combination of *Nattrassia* sp + *Ceratocystis* sp + *B. theobromae* (T8) also showed similar symptoms for flap and root injury method causing all decline symptoms and eventual plant mortality. T9 (*Nattrassia* sp + *Fusarium* sp + *B. theobromae*) produced all decline symptoms except the mortality in flap method , skipping stem canker in case of root injury. Inoculation combination of *Nattrassia* sp + *Ceratocystis* sp + *B. theobromae* + *Fusarium* sp (T10) also produced all symptoms and consequently plants died in both flap and root injury method. The control plants without inoculation of any fungi only produced minor gummosis (Table 1). Most common fungi *L. theobromae* is associated with dieback and is steadily isolated from various tissues (twigs, bark, vascular tissue and fruits) of affected plants [7,8,9]. The fungi *L. theobromae* has been reported to cause gummosis of Jatropha podagrica in China [10] and root rot and collar rot disease on J. curcas in India [11].

Inoculation on Mango

The inoculation results of different fungi by flap and root injury method through soil drenching the fungal spore suspension on mango plants have been shown in the Table 2.

It was observed that *Ceratocystis* sp. alone (T1) produced complete symptoms of decline using flap and injury method which led to plants mortality. *Nattrassia* sp. alone (T2) showed bark splitting and gummosis in flap while, only gummosis in root injury but the plants survived. Inoculation of *B. theobromae* alone (T3), showed bark splitting, canker and gummosis in both pathogenicity methods. *Fusarium* sp. alone (T4) resulted in leaf drooping canker and gummosis in case of flap

method and plants survived. However, in case of root injury T4 plants died with complete set of decline symptoms resulting in mortality. The combination of *Ceratocystis* sp + *Nattrassia* sp. (T5) produced all symptoms by two methods and mortality occurred. The combination of *Nattrassia* sp + *B. theobromae* (T6) showed bark splitting, canker and gummosis in flap as well as root injury inoculation. The combination of *Nattrassia* sp +*Fusarium* sp (T7) in case of flap incoculaion produced all decline symptoms but no mortality. In root injury all symptoms except canker were shown and plants survived. The inoculation combination of *Nattrassia* sp + *Ceratocystis* sp + *B. theobromae* (T8) similar symptoms as T5 and eventual plant mortality observed. T9 (*Nattrassia* sp + *Fusarium* sp + *B. theobromae*) through both methods all decline symptoms produced but no mortality recorded. Inoculation combination of *Nattrassia* sp + *Ceratocystis* sp + *B. theobromae* + *Fusarium* sp (T10) also produced all symptoms and consequently plants died. The control plants without inoculation of any fungi only produced gummosis (Table 2).

The results of the present studies are in close confirmation of [12] who recorded similar symptoms of dieback and found that after artificial inoculations with *Lasiodiplodia theobromae, Phoma sp*., and two *Fusarium sp*., only the *L. theobromae* caused the disease. Similarly [13] reported *L. theobromae* associated with mango trees severely affected with gummosis. Moreover [14]. *L. theobromae, C. gloeosporioides, R. solani, Pestalotia mangiferae, Phoma sp., S. rolfsii* and *F. solani* as pathogenic. He also reported that a mixed infection was common and L. theobromae was the primary cause of the disease, and necrosis caused by other pathogens may facilitate invasion by *L. theobromae.*

Inoculation on Guava

The inoculation results of different fungi by flap and root injury method on 1 to 2 years potted guava plants have been shown in the Table 3.

The results indicate that inoculation of *Ceratocystis* sp. alone (T1) was inoculated to through flap and root injury produced all decline symptoms except bark splitting but resulted in mortality. *Nattrassia* sp. alone (T2), produced bark splitting and gummosis without mortality by both methods. The inoculation of *Botryodiplodia theobromae* alone (T3), bark splitting, stem canker and gummosis was observed by flap method; bark splitting and gummosis were observed by root injury inocuation. In both cases plants remained alive. *Fusarium* sp. alone (T4) resulted in leaf drooping, stem canker and gummosis while by root injury leaf drooping and gummosis appeared. None of the plants died in all cases. The combination of *Nattrassia* sp. + *Ceratocystis* sp. (T5) produced complete set of decline symptoms as in citrus and mango through both methods resulting in mortality. The combination of *Nattrassia* sp + *B. theobromae* (T6) showed similar symptoms through both methods, skipping leaf drooping; without plant mortality . The combination of *Nattrassia* sp +*Fusarium* sp. (T7) through flap method produced leaf drooping and bark splitting, while in root injury method it resulted leaf dropping, bark splitting and gummosis without mortality of plants. *Nattrassia* sp. + *Ceratocystis* sp. + *B. theobromae* (T8) produced similar symptoms as T5 and eventually plant died using both inoculation method. T9 (*Nattrassia* sp + *Fusarium* sp. + *B. theobromae*) produced all decline symptoms except the mortality in both methods. Inoculation combination of *Nattrassia* sp + *Ceratocystis* sp. + *B. theobromae* + *Fusarium* sp. (T10) also produced all symptoms and consequently plants died. The control plants without inoculation of any fungi only produced gummosis (Table 3).

The results in totality show that *Ceratocystis* sp. is the most fatal fungus which results in the mortality of plants whether alone or in the combination of other fungi. Although the *Ceratocystis* isolate used in this study was taken from mango and inoculated on both guava and citrus as this has not been isolated from these fruits. The study indicate to avoid the invasion of Ceratocystis sp. in citrus and guava as well.

The other fungus which can result in the mortality of the plants is *Fusarium* sp. in combination with other fungi but through root injury method only. *Nattrassia* sp. and *B. theobromae* only produce symptoms like bark splitting,

gummosis and canker development but generally plants survive. However, they can aggravate the disease in combination with *Ceratocystis* sp. and *Fusarium* sp.

CONCLUSIONS

These studies are quite complex and with the repetition can show different results based on the concentration of inoculum,, initial health of the plants, climate change and other affecting factors but provide a good picture of strengths and weaknesses of different fungi in causing decline symptoms in the fruit trees. Based on these studies it will be easy for devising management practices for the decline disease.

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