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### INTERNAL SPREAD OF FUNGI INOCULATED INTO MANGO LOGS

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#### ABSTRACT

The Mango (*Mangifera indica* L.) member of family *Anacardiaceae*, has become a fruit symbol of Pakistan. Its nutritional value is also overwhelming its importance. Various fungi have been reported on mango trees affecting different plant parts. The main objective behind this experiment was to observe the internal spread of these fungi within the mango logs. Mango logs of thickness about three inches were freshly cut from mango trees variety Samar Bahisht Chaunsa undergoing major pruning. The ends of the logs were immediately painted to avoid the moisture loss and wrapped up in the newspapers. Two fungi selected for inoculation included *B. theobromae* and *N. mangiferae* that has been observed on twigs, dry branches as well as on infected bark. The fungal plugs of both fungi were placed into wholes individually as well as in combination in the wholes made at center in the logs, later wrapped by para film at the point of inoculation. The most disastrous situation was observed when the combination of both fungi was inoculated in the mango logs. It not only aggravated the movement wildly all around but broke everything apart like bark removal and splitting of tissues, causing severe decomposition of logs.

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#### INTRODUCTION

Mango (*Mangifera indica* L.), member of family *Anacardiaceae*, has become a fruit symbol of Pakistan. It is deeply embedded in various cultures and rituals since its introduction in South Asia around 4000 years ago (Hill, 1952) as well as in Pakistan. Not only the attractive shapes and tastes make Pakistani mango unique globally, its nutritional value is also overwhelming its importance. The mango fruit contains 10-20% sugars, vitamin A, C and traces of vitamin B, too (Sarkiyayi et al., 2013).

Mango in Pakistan is grown on an area of 170.3

thousand hectares with production of 1336.5 thousand tonnes. Mango is susceptible to many diseases and disorders. The most lethal one is mango sudden decline disease and the most aggravating one, the mango malformation disease (Fateh et al., 2006).

Various fungi have been reported on mango trees affecting different plant parts. However, the most common fungi reported include *Botryodiplodia theobromae*, *Nattrassia mangiferae*, *Fusarium* sp. and *Ceratocystis* sp. These fungi have been reported from twigs, roots, barks and xylem portions of mango tree

(Asif et al., 2011).

*B. theobromae* and *N. mangiferae* have been reported to cause twig blight and dieback symptoms in mango as well as other fruit trees. Sometimes reported from the tree trunk, *Fusarium* has been found on flowers and in roots and to some extent on the bark as well. The only fungus that has been commonly found in the vascular bundles causing permanent wilt is *Ceratocystis* sp. (Fateh et al., 2016). Various inoculation techniques have been used to confirm the pathogenicity of reported fungi including flap method, root injury method, live tree inoculation etc. These methods however, do not provide the insight influence of the fungi attacking mango trees either being opportunistic or making their ways into the tissues with their sole or combined penetrating powers. Freshly cut stamps had been used for the forest trees in past but it was very interesting to use such method for fruit tree like mango. The main objective of this experiment was to observe the internal spread of these fungi within the mango logs.

#### **MATERIALS AND METHODS**

**Preparation of mango logs for experiment:** Mango logs of thickness about three inches were freshly cut from mango trees variety Samar Bahisht Chaunsa that underwent major pruning recently. The ends of the logs were immediately painted to avoid the moisture loss and wrapped up in the newspapers.

In the next step holes about half inch deep were made in the wood logs for the inoculation of various fungi to see their internal spread. One whole was made in the center of the logs. All efforts were made to conserve the moisture in the detached logs.

**Preparation of the fungal culture:** Two fungi selected for inoculation included *B. theobromae* and *N. mangiferae* that have been observed on twigs, dry branches as well as on infected bark. The fresh cultures grown on potato dextrose agar medium of both fungi were taken (about 7 days old cultures). The 5 mm plugs were made with the help of sharp cork borer. The plugs were aseptically placed on sterilized filter paper in a laminar flow.

**Inoculation of logs with fungal culture:** All the material needed was brought in the laminar flow. The fungal plugs of both fungi were placed into wholes individually as well as in combination in the wholes made at center in the logs, later wrapped by para film at the point of inoculation and further wrapped in the newspaper, sprinkled with sterilized water and placed in the incubator at  $25\pm 1$  °C.

For each treatment i.e. *B. theobromae*, *N. mangiferae*, their combination and control (only PDA culture plugs) five replicates were made.

**Assessment of the fungal spread:** The logs were placed in the incubator as long as 15 days and were cut longitudinally to observe the fungal spreads. The sections of the logs revealed the spread of individually and combined inoculated of the fungi. The movement was noted vertical, horizontal, irregular and diagonal. The distance was measured from the central point up to 2 and four centimeters for recording fungal movement. As the logs were contained to be fresh, symptoms and signs were also observed on each log.

#### **RESULTS AND DISCUSSION**

The individual inoculation of *B. theobromae* in each replication showed similar results. The movement from the central inoculation point on average is seen on both sides horizontally about 5mm. However, gummy spots and presence of other fungi growth was also observed at distance of 2 and 4 cm. Different color gums were observed in few replications (Table 1).

When *N. mangiferae* was inoculated alone, it showed somewhat similar results but the exception was that the fungal movement was a little more than *B. theobromae*. Moreover, no downward movement had been seen in the table 1, while it initiated with the inoculation of *N. mangiferae*. The bark cracks and decomposition with gums and growth of other fungi was also shown in this treatment (Table 2). The most disastrous situation was observed when the combination of both fungi was inoculated in the mango logs. It not only aggravated the movement wildly all around but broke everything apart like bark removal and splitting of tissues, causing severe decomposition of logs (Table 3).

The control treatment had obviously least effect on the mango logs. The lesions remained localized without any movements horizontal or the downward. Few gummy spots observed were point of interest but not so serious (Table 4). In the first case when *B. theobromae* was inoculated alone did not show much horizontal movement but no downward movements. This showed that the fungus might not have enough power to penetrate the hardwood of mango logs. However, being the superficial pathogen it influenced the bark only. This has been observed that *B. theobromae* is an endoparasite as well it does not cause much damage until it combines with other Botryosphaeriaceae members (Kannan et al., 2010; Pavlic et al., 2007).

Table 1. Observations for internal spread and symptoms caused by *B. theobromae* alone in mango logs.

<i>B. theobromae</i>	At point of Inoculation	2 cm from point of inoculation	4 cm from point of inoculation
R1	The blackening was observed at the point of inoculation with ~ 5mm horizontal spread on both sides. Shiny gum of dark color was also observed	Few gummy spots but no fungi were observed. The fungus could not reach at the distance of 2cm	Two fungal spores <i>Arthrobotrytis</i> sp and one gummy spot oozing from edges black streak visible in the longitudinal section.
R2	The central blackening as well as spread of 4 to 5 mm was observed horizontally on both sides	Gum under bark & Dark brown circular lesions were found on removing bark. However, no fungal movement from the center of inoculation reached this distance	Few light golden gum spots found. No fungal movement
R3	Bark cracked around center of inoculation. The blackening shifted to almost 5mm on left and 7.5 mm on the right side	Bark cracked but no fungal movement traced from the point of inoculation to this distance	Light brown gum spots and minor cracks on bark. No fungus in the section
R4	Dark brown decay of 5mm spread horizontally on both sides	Light brown spots on bark with minor bark detachment. No fungal movement up to this distance	Light brown gum spots on bark no fungal movement from the center
R5	Detachment of bark and dark brown lesions extending on both sides about 5.5 to 5.8 mm	Shiny gum spots and minor cracks on bark. No fungal movement	Same as in 2 cm

Table 2. Observations for internal spread and symptoms caused by *N. Mangifera* alone in mango logs.

<i>N. Mangifera</i>	At point of Inoculation	2 cm from point of inoculation	4 cm from point of Inoculation
R1	Light brown lesions in the center but extending up to 1cm on both sides horizontally and almost 3mm in downward direction. Detachment of bark was observed	Bark detachment The fungus could not reach at this point	Orange color <i>Acremoniella</i> type unknown fungi found on bark but no fungal movement from point of inoculation
R2	Light brown lesions on both sides as well as downward from 3 to 5 mm, little cracks on bark	Bar cracks and golden gum spots	Few golden gum spots
R3	Bark detachment with gummy material under it. Horizontal movement on both sides about 1cm	No fungal movement, light gum spots	Light brown gum spots and minor cracks on bark. No fungus in the section
R4	Fungus was much more aggravated extending up to 1.5 cm on sides and 1 cm downward	Detachment of bark as well as minor splitting of tissues under the bark	Bark detached and light brown gum observed on surface.
R5	The fungus spread on both sides up to 5mm and downward 3mm	Minor bark cracks and tiny golden gum spots. No fungal movement.	Light golden gum spots on bark

Table 3. Observations for internal spread and symptoms caused by combination of fungi in mango logs.

<i>B. theobromae</i> & <i>N. Mangifera</i>	At point of Inoculation	2 cm from point of inoculation	4 cm from point of Inoculation
R1	Cracked bar with golden gum. Central lesion extending up to 2.5 cm on left, 3.9 cm right and 1 cm downward	Fungal movement crossed this point horizontally with dark brown lesions in the section	<i>Aspergillus</i> growth on cracked bark surface mixed with gums and splitting of tissue underneath bark
R2	Internal spread 4.5 cm on both sides and 3.2 cm downward The entire bark separated	Fungal lesions passed from this point. Downward movement up to 3.5 cm	Fungal lesions crossed the downward spread almost 4 cm
R3	Bark detachment with dark brown gum and lesions extending up to 2.5 cm on all sides	Fungal lesions of dark brown color crossed this point	Bark decomposed along with surface under it. <i>Aspergillus</i> growth found with gum mixture.
R4	Horizontal movement up to 2 cm on both sides the bark rotten with dark brown gum, downward movement up to 1 cm	Detachment of bark as well as lesions reaching this area. Downward movement up to 1.2 cm	Bark cracks with gums but lesions not reaching this point
R5	The fungus spread on both sides up to 4.5 cm and downward 3cm, all bark detached	Almost similar situation as at the central inoculation	Same as 2 cm

Table 4. Observations for internal spread and symptoms caused by control in mango logs.

PDA Culture only	At point of Inoculation	2 cm from point of inoculation	4 cm from point of Inoculation
R1	Few bark cracks and light brown lesions within the whole	No effects	No effects
R2	Light brown lesions extending about 1.5 mm on both sides	Few bark cracks and shiny light gum	Light gum spots
R3	Light brown lesions at the central point without extension	Light gummy spots	No effects
R4	Bark cracks with gum lesions in the centre with no movements	Light gum shining	Few golden gum spots
R5	Lesions at inoculation point without movements	No effects	No effects

*B. theobromae* has been reported from mango, shisham, guava and loquat in Pakistan causing somewhat similar symptoms in the bark and underneath (Kazmi et al., 2007; Masood et al., 2010).

*Natrassia mangiferae* moving all around showed its behavior to affect tissue more severely than *B. theobromae*. Due to this character it is also involved even in postharvest diseases as well. Similar to *B. theobromae* it becomes more aggressive when combined with other fungi of this family (Pérez et al., 2009). In the hardwood, *N. mangiferae* movement upward and downward

coincide with the research of Sutton and Dyko, 1989, state that *N. mangiferae* attacks the cambium and spreads downward, causing, wilt dieback and decline depending on tree species involved.

The most serious situation is caused by the combination of both *B. theobromae* and *C. mangiferae* (Crous et al., 2006). This lethal combination has been reported to cause death of many forest, fruit and ornamental trees (Alizadeh et al., 2000).

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