Antifungal potential of *Tinospora cordifolia* (Wild) Hook .f. & Thomson Leaf Extracts against Fruit Rot Pathogens of Papaya fruits.

Dr. Sawant S.G.

Department of Botany, K.K.M. College, Manwat, Parbhani, Maharashtra India.

**Abstract**: Plants extracts appear to be one of the most effective alternative methods of plant diseases control which are less harmful to human beings and environment.

In *vitro* antifungal activity of methanolic and ethanolic leaf extract of *T. cordifolia* against three postharvest fruit rot causing fungal pathogens viz. *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium digitatum* were tested by using radial growth technique. The result exhibited that both plant extracts had antifungal activity against the tested fungi. It is also proved that by using higher concentration of plant extract, inhibition level of mycelial growth get increased. Methanolic leaf extract of *T. cordifolia* shows better inhibition of *A. niger* than *Rhizopus stolonifera* & *Penicillium digitatum*.

**Medicinal Potential of Tinospora Cordifolia Against fruit rot pathogens.**

**Introduction**: Plants are known innumerable to contain biologically active compounds (Perumalasamy et.al 1999). According to Selvem and Thomas (2006) approximately 4000 plant species have been identified to have medicinal value and carry a few about 2030 species are under the cultivation. The curative properties of medicinal plant is due to the presence of various complex chemical substance of different composition which are found as secondary metabolite in one or more parts of the plants.

Nearly 2,50,000 (*Angios and Gymnos*) plant species are inhabitant of this planet, of these only about 6% have been screened for biological activity and 15% evaluated phytochemically (verpoorte,2000) Craker and Simon (2002) analysed the 64 medicinal and aromatic plants are temperate zone and reported their distribution. According to them 16.6% belongs to botany, 11.1% pharmacology 14.5% chemistry and 7% clinical studies while 0.5% in commerce.

Antifungal action of certain plant extract has got great potential as they can be handled easily and they have proved their fruitfulness in being true to their non-residual effect, systemic in their activity, easy biodegradable nature and stimulating host metabolism etc. Many herbal drugs are being produced and tested for systemic fungal infections and the need for new antifungals is ever increasing (Khan and Jain, 2000).
Plant extracts have long been speculated as vital factors to disease resistance and control against a wide range of fungi that infect crops (Mahadeven 1982; GerardEzhilanet al. 1994; Singh and Singh 1980; Kurucheveet al. 1997; Tiwari 1997). Oluma and Garaba (2004) have observed that crude extract of E. globulus and Ocimumgratissimum reduce radial growth of Phytophthiramphanidermatum by 44.5-100%.

Amadioha (2000) proved that O. gratissimum leaf extract was able to control spore germination and mycelial growth of R. oryzae. Narayan Rao et al. (1996), proved that using higher concentration of plant extracts raises inhibition level of the extracts.

Keeping this in view, the present study aim to evaluate the antifungal activities of the plant T. cordifolia (wild) hook. f. and Thomson which belongs to family menispermacae. It is a large deciduous, climbing herb found throughout India and also in Shrilanka, China and Bangladesh. It contains flavonoids, glycosides, saponins and some amount of phytosterols. These active constituents alone or in combinations are responsible for antioxidant activity.

Leaves of T. cordifolia are rich in protein and fairly rich in Ca and P. Different chemical constituents such as giloin, columbin, chasmanthin, palmatin, isocolumbin, tembetarine, cordioside, palmatin, tinosporin, tinosporic acid have been isolated from different parts of Tinosporacordifolia (5).

It is known to possess potential activities such as anti-spasmodic, anti-inflammatory, anti-allergic, antiperiodic, antiarthritic, antileptotic, antidiabetic, antioxidant, hepatoprotective, immunomodulatory and antimicrobial. Therefore this study focusses on the evaluation of antifungal properties of this plant extract against papaya fruit not pathogens.

**Material and Methods:**

1] **Sample Collection:**

A survey was conducted in the domestic market of Nanded and papaya samples which showed visible signs of fungal infection were collected from stock of fruits. Sixty such samples were collected from the 15 locations of Nanded in paper bags as described by Rasool et al. (5). And brought to our Research laboratory, Science collage, Nanded and categorized according to the visible symptoms of postharvest disease.

2] **Isolation of fungi:** Fungal pathogens present in diseased fruits were isolated on potato dextrose agar medium. Samples with visible disease symptoms were removed by a sterilized knife in such a manner as to contain the lesion edges too. These were then immersed in HgCl₂ for 2 minute for surface sterilization and washed thoroughly in sterile distilled water until all the HgCl₂ was washed away. The surface sterilized tissues were further cut into smaller pieces using a sterile blade and placed on PDA medium using disinfected forceps. The plates were incubated at 28°C for 5 days. The fungi were subcultured on PDA slants for further studies.

3] **Identification of Fruit rot fungal pathogens:**

Phenotypic identification was performed on standard growth conditions. Genus level identification was carried out based upon macro as well as microscopic characteristics. These included visible structures such as shape of conidia, colour of hyphae, septation,
pigmentation, fruiting bodies etc. by observing lactophenol cotton-blue stained sides, under the compound microscope at the magnification X10,X45,X100 (25) For this purpose a small quantity of the aerial mycelia with reprsentativespores was placed on drop of lactophenol cotton blue stain on a clean side.

A mounting needle was used to every spread the mycelia and the spores and subsequently a covership was gently placed with little pressure to eliminate air bubble. The slide was then observed under a binocular compound like microscope with X10 and X40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified as Onuorahet.al. (20).

Table-1

**Characteristics of isolated fungal pathogens from diseased Papaya fruit.**

<table>
<thead>
<tr>
<th>Fungal pathogens</th>
<th>Name of disease</th>
<th>Characteristics of disease</th>
<th>Shape and size of conidia</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>Blackrot</td>
<td>Initially white colonies but later on become black from centre as conidia appears.</td>
<td>Globose shaped conidia with 4-5 um in diameter</td>
</tr>
<tr>
<td><em>R. stolonifer</em></td>
<td>Softrot</td>
<td>Colonies shows white cottony appearance</td>
<td>Ovate/polygonal angular 100-250 um</td>
</tr>
<tr>
<td><em>P. digitatum</em></td>
<td>Green mold</td>
<td>Initially colonies are white which become green as conidia are produced</td>
<td>Oval shaped conidia with 6-15 um long in size.</td>
</tr>
</tbody>
</table>

Table-2

**Frequency of occurrence of fungi in various fruits.**

<table>
<thead>
<tr>
<th>Name of Fungal pathogen</th>
<th>No. of samples</th>
<th>Frequency of Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. niger</em></td>
<td>17/20</td>
<td>85</td>
</tr>
<tr>
<td><em>R. stolonifer</em></td>
<td>28/30</td>
<td>93.33</td>
</tr>
<tr>
<td><em>P. digitatum</em></td>
<td>04/10</td>
<td>40</td>
</tr>
</tbody>
</table>

Preparation of Plant extract:-

The plant material was collected from wild population in rainy season. 10gms of *T. cordifolia* leaves were weighed and washed first in running water and then surface sterilized in 0.1% HgCl₂ solution for 2-3 minutes. Then it was ground with 50% methanol same procedure is repeated and the same amount of plant material was ground with 50% ethanol.

Both of the extract was passed through two layers of muscin cloth and centrifuged at 3000 rpm. for 5 minutes. The supernatant was made up to 20 ml using 50% ethanol and 50%
methanol. Further the extract was diluted to 25,50,75 and 100%. The test fungi were isolated by pure culture method from the stock and preserved in slants under freeze storage until further use. The in vitro antifungal activity of methanol and ethanol extracts of leaves were assessed on the basis of radial growth rate of fungi. About 15 ml of sterilised PDA medium with 5 ml of the extract of different concentration were poured into each sterilized petriplates under aseptic conditions. After the solidification of the medium the test fungi were inoculated aseptically plated were incubated at 30°C in incubator.

Three replicates were maintained simultaneously suitable control (without plant extract) were also maintained throughout the study. Fungal growth was observed from the third day onwards and the radial growth of fungi were measured on the 5th day after inoculation.

**Observation and Result:-**

**Effect of different conc. of methanol leaf extracts from T.cordifolia on three postharvest pathogens of papaya fruit.**

<table>
<thead>
<tr>
<th>Fruit rot pathogens</th>
<th>Diameter of fungal growth (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>A. niger</td>
<td>06</td>
</tr>
<tr>
<td>R. stolonifer</td>
<td>10</td>
</tr>
<tr>
<td>P. digitatum</td>
<td>08</td>
</tr>
</tbody>
</table>

**Effect of different conc.of ethanol leaf extract from T.cordifolia on three postharvest fruit not pathogens of papaya fruit.**

<table>
<thead>
<tr>
<th>Fruit rot Pathogens</th>
<th>Diameter of fungal growth (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>A. niger</td>
<td>07</td>
</tr>
<tr>
<td>R. stolonifer</td>
<td>12</td>
</tr>
<tr>
<td>P. digitatum</td>
<td>10</td>
</tr>
</tbody>
</table>

**Discussion:-**

The present study showed that a number of fungi are associated with postharvest rot disease of papaya fruits. These fungi includes Aspergillus niger, Fusarium solani, Penicillium digitatum, Rhizopus stolonifer etc. There have been previously reported as fruit rot pathogens of papaya fruits (Pathak et.al, 1976; Alvarez and Nishijima, 1987). It was observed that the presence of wounds on the fruits for disease occur as intact fruits showed no infection. This observation is similar to that of Nishijima et.al (1990) showing that Rhizopus requires a break in cuticle for successful infection to occur.
Similarly unripe fruits were less infected by this isolated fungus this is inline with the findings of Elmoussoul et al. (2001) who reported that the green papaya fruits contain enzyme *papain*, which could possibly inhibit *fusarium sp, A.niger, F.solani and B.theobroma* while *Penicillium* acted as a secondary invader on lesions caused by other fungi.

This observation agrees with the reports of Flentje (1965) and Wilhelm (1967) showing that such organisms when tested against the host in the absence of the primary pathogen would have no effect. Investigation into the antifungal properties of *T.cordifolia* leaf extracts on the growth of fungal isolates shows that these crude extracts possess some inhibitory components which causes significant reduction in mycelial growth of the tested fungal pathogen. This agrees with the results of S.M. Nagarajan et al. (2006) reported similar results of this plant extract against certain pythopathogenic fungi.

Investigation therefore suggested to purify and characterize the active components of these plant extracts. Efforts should also be made to screen the flowers, stem and roots of these plants for possible antifungal activity.

**Acknowledgement:**

The author wishes to thank the Department of Botany, Research Laboratory, Science College, Nanded for it’s immense co-operation and support.

**References:**


