Antifungal Activity of Some Local Botanicals of Assam against *Pythium aphanidermatum* Inciting Storage Rot of Ginger

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**A B S T R A C T**

*Pythium aphanidermatum* causes post harvest rot of ginger which resulted in huge losses in quality as well as quantity of ginger. Concern over the use of agro-chemical to manage the pathogen raising the risk of food toxicity so plant extracts might be a potential alternatives to synthetic fungicides. In the study of antifungal activity of botanicals, ten botanicals (*Acorus calamus*, *Allamanda cathertica*, *Allium cepa*, *Allium sativum*, *Curcuma longa*, *Datura wrightii*, *Lasia spinosa*, *Laurus nobilis*, *Ocimum sanctum* and *piper betle*) were evaluated against *Pythium aphanidermatum* by poison food technique. Aqueous extracts (20%) of *A. sativum*, *A. cathertica* and *L. nobilis* significantly inhibited the growth of fungal pathogen. These three most effective botanicals were further tested at four different concentrations (5, 10, 15 and 20 per cent) and highest inhibition was exhibited by *A. sativum* (94.44%) at 20 per cent concentration followed by *A. sativum* (85.78%) at 15 per cent, *A. cathertica* (83.33%) at 20 per cent and *A. sativum* (76.44%) at 10 per cent concentration.

**Keywords** Post harvest rot, Ginger, Botanicals, *Allium sativum*, Inhibition

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**Introduction**

Ginger is the fourth most important spice in India with a production of 1047.19 thousand tons from an area of 160.48 thousand ha with productivity of 6.52 tons/ha (Annon, 2016-17). Globally, ginger is used in preparation of various foods for seasoning, flavoring and imparting aroma (Shukla and Singh, 2007). Medicinal uses of Ginger has been reported as anti-nausea, anti-clotting agent, antifungal, anti-inflammatory, antiseptic, antibacterial antiviral, antitussive, analgesic, circulatory stimulant, blood flow increasing agent and so on (Gunathilake and Rupasinghe, 2015). Assam produces 156.66 thousand tons of ginger with an average productivity of 8.88 t/ha (Annon, 2016-17). After harvesting,
ginger rhizomes are stored for seed and commercial purpose in different types of storage structures at least for a period of 6 -7 months (From Jan-Feb to Aug-Sep) for day to day fresh consumption under Assam condition. Ginger has been affected by many diseases in pre-harvest as well as post-harvest deterioration is the most important cause of loss of ginger during storage due to rotting, resulting into considerable loss to farmers. *Fusarium oxysporum* f. sp. *zingiberi*, *Pythium aphanidermatum* and *Pseudomonas solanacearum* have been reported as the major culprits for the storage rot of ginger in India (Dake and Edison, 1989; Dohroo, 1989; Sharma et al., 2017). Association of *Pythium pleroticum*, *P. aphanidermatum*, *P. equiseti* and *Fusarium solani* with ginger rot have also been reported (Rajan and Agnihotri, 1989). For effective management of storage rot of ginger, Carbendazim, Ridomil, Tospin M etc. are generally used (Grech and Swart, 1990; Sharma and Dohroo, 1991). The side-effects of synthetic fungicides and demand of fresh products without agrochemical treatment, particularly without post-harvest agrochemical treatment forcing us to find out alternative strategies for reducing losses due to post harvest decay that are perceived as safe by the public and pose negligible risk to human health as well as environment (Wisniewski and Wilson, 1994). So there is a need for alternative(s) of chemicals for management of plant diseases.

Eco-friendly plant extracts have shown to be great potential as an alternative to synthetic fungicides (Janisiewiez and Korsten, 2002; Zhang and Zheng, 2005). As alternative of chemicals, several works have been done on management of post harvest disease that include microbial antagonists and plant extracts as potential alternatives to synthetic fungicides (Chauhan and Joshi, 1990; Sarvamangla, 1993; Chaudhary, 2003; Bhardwaj et al., 2010). They are also best suited for use in organic food production in industrialized countries but can play a much greater role in developing countries as a new class of eco-friendly organic products for controlling diseases (Isman, 2006). Ram and Thakore (2009) evaluated efficacy of 19 plant extracts against *Fusarium solani* and *Pythium aphanidermatum* and they found that *A. sativum* and *Lantana camera* were most effective in vitro. In vitro test revealed that the extract of onion, garlic and agave were highly efficacious in limiting the mycelial growth of *Pythium aphanidermatum* (Dohroo et al., 2012).

**Materials and Methods**

For evaluation of botanicals, different plant parts like i.e. fresh leaves of *Acorus calamus*, *Allamanda cathartica*, *Datura wrightii*, *Lasia spinosa*, *Laurus nobilis*, *Ocimum sanctum* and *piper betle*, bulbs of *Allium cepa* and *Allium sativum*, rhizome of *Curcuma longa* were collected from various localities of Assam for the preparation of aqueous plant extracts. The method reported by Shekhawat and Prasad (1971) was followed for botanical extracts preparation with certain modifications. Collected botanicals were washed thoroughly in sterile distilled water and crushed in grinder by adding equal amount (100ml) of sterilized distilled water (1:1 W/V). After grinding, the extract was filtered through muslin cloth and finally the extracts were centrifuged at 10,000 rpm for 20 minutes in centrifuge (Remi C 24) at room temperature. The supernatant was taken as standard plant extract solution (100%). The selected botanicals were first screened for their antifungal activity against *Pythium aphanidermatum* at 20 per cent concentration by ‘poisoned food technique’ (Nene and Thapliyal, 2000). For this, PDA medium was prepared in 250 ml Erlenmeyer flasks and sterilized. Twenty (20) ml of 100 per cent aqueous plant extracts of each botanicals were
aseptically added to 80 ml molten PDA (Potato Dextrose Agar) in flasks respectively so as to get the final concentration of 20% of the extracts in the medium. PDA without any extract served as control. The media was poured in 9 cm Petri plates at the rate of 20 ml per plate. The fungal culture disc using a cork borer (5mm diameter) from the tip, obtained from a 7 days old culture were taken and inoculated in the centre of Petri plates aseptically after solidification of the medium and incubated at 28±1°C. Three most promising botanicals were further tested against Pythium aphanidermatum, in four different concentrations viz., 5, 10, 15 and 20 per cent, respectively.

The diameter of the colony is measured when the mycelium fully covered the Petri plates of control plate and the percent inhibition of the mycelial growth was calculated by the formula of Vincent (1927).

\[ I = \left( \frac{C - T}{C} \right) \times 100 \]

Where, \( I \) = Inhibition of mycelial growth (%)

\( C \) = Growth in control (mm)

\( T \) = Growth in treatment (mm)

Five replications were maintained for each concentration of the treatments in a completely randomized design. The observed data was analyzed by OPSTAT package of programs (Sheorlan, 2006) after angular transformation.

**Results and Discussion**

The results (Table 1) indicated that some botanicals significantly reduced the mycelial growth of the pathogen while other showed less inhibition over control (90 mm). Among the all ten botanicals tested A. sativum recorded highest inhibition (94.44%) followed by A. cathertica (83.33%), L. nobilis (68.66%) and A. calamus (57.55%). Per cent mycelial growth inhibition recorded in C. longa (54.22%) followed by L. spinosa (35.55%) and D. wrightii (35.11%). The per cent inhibition in A. cepa, O. sanctum and P. betle were recorded as (28.44%), (19.77%) and (12.66%) respectively.

Perusal of the data (Table 2 and Fig. 1) revealed that irrespective of different concentrations, all the botanicals showed significantly higher inhibitory effect on the mycelial growth of P. aphanidermatum as compared to control and highest inhibition was recorded in A. sativum followed by A. cathertica and L. nobilis respectively. Among the three botanicals tested at four different concentrations (5%, 10%, 15%, and 20%), the maximum mycelial inhibition was recorded in A. sativum (94.44%) at 20 per cent concentration which was found to be significantly superior over rest of treatments. This was followed by A. sativum (85.78%) at 15 per cent, A. cathertica (83.33%) at 20 per cent and A. sativum (76.44%) at 10 per cent. The effect of A. cathertica (67.56%) at 10 per cent was statistically at par with L. nobilis (68.67%) at 20 per cent, similarly the effect of A. sativum (68.67%) was at par with L. nobilis (68.67%) at 20 per cent. The least inhibition of mycelial growth of P. aphanidermatum was observed in L. nibilis (46.67%) at 5 per cent. Irrespective of concentrations of plant extracts, A. sativum recorded maximum mean growth mycelial inhibition (81.33%) followed by A. cathertica (71.72%) and minimum mean mycelial growth inhibition was recorded in L. nobilis (58.72%).

Amongst the tested botanicals, bulb extracts of A. sativum recorded highest inhibition on mycelial growth of the pathogens followed by A. cathertica and L. nobilis respectively. The results of the present study are in agreement
with those reported by several workers (Vijaya et al., 2007; Ram and Thakore, 2009; Jadhav et al., 2013; Chaudhary et al., 2017) who reported *A. sativum* highly effective against many fungal pathogens.

**Table 1** Efficacy of different botanicals (20%) on mycelial growth of *Pythium aphanidermatum*

| Treatments          | Mycelial growth* (mm) | Mycelial growth inhibition over control (%) |
|---------------------|-----------------------|--------------------------------------------|
| T1: *Acorus calamus* | 38.20                 | 57.55 (49.32)**                           |
| T2: *Allamanda cathertica* | 15.00          | 83.33 (65.88)                              |
| T3: *Allium cepa*    | 64.40                 | 28.44 (32.21)                              |
| T4: *Allium sativum* | 5.00                  | 94.44 (76.33)                              |
| T5: *Curcuma longa*  | 41.20                 | 54.22 (47.40)                              |
| T6: *Datura wrightii*| 58.40                 | 35.11 (36.31)                              |
| T7: *Lasia spinosa*  | 58.00                 | 35.55 (36.58)                              |
| T8: *Laurus nobilis* | 28.20                 | 68.66 (55.94)                              |
| T9: *Ocimum sanctum* | 72.20                 | 19.77 (26.38)                              |
| T10: *Piper betel*   | 78.60                 | 12.66 (20.82)                              |
| T11: CoC (0.3%)      | 0.00                  | 100.00 (89.55)                             |
| T12: Control         | 90.00                 | 00.00 (3.69)                               |

SEd (±) 0.60

CD (p = 0.05) 1.22

*Mean of five replication

**Data in parentheses represents angular transformation**

**Table 2** Efficacy of botanicals (5, 10, 15 and 20%) on mycelial growth of *Pythium aphanidermatum*

| SI. No. | Treatments          | Mycelial growth inhibition over control (%) | Mean (mm) |
|---------|---------------------|--------------------------------------------|-----------|
|         |                     | 5   | 10   | 15   | 20   |         |
| 1       | *Allamanda cathertica* | 61.33 (51.55)* | 67.56 (55.28) | 74.67 (59.78) | 83.33 (65.90) | 71.72 (58.13) |
| 2       | *Allium sativum*     | 68.67 (55.96) | 76.44 (60.98) | 85.78 (67.85) | 94.44 (76.36) | 81.33 (65.29) |
| 3       | *Laurus nobilis*     | 46.67 (42.96) | 56.44 (48.70) | 63.33 (52.73) | 68.67 (55.94) | 58.72 (50.08) |
|         | CoC (0.3%)           | 100.00 | 100.00 | 100.00 | 100.00 |         |
|         | Control              | --   | --   | --   | --   |         |

SEd (±) 0.23

CD (p=0.05) 0.46

CV (%) 1.26

* Data in parentheses represents angular transformation
Fig.1 Efficacy of botanicals (5, 10, 15 and 20%) on mycelial growth of *P. aphanidermatum* (after 8 days of inoculation)

*Allamanda cathertica*

5% 10% 15% 20%

*Allium sativum*

5% 10% 15% 20%

*Laurus nobilis*

5% 10% 15% 20%

CoC (0.3%) Control
Plant based pesticides i.e. botanicals being relatively economical, safe and non-hazardous show antifungal activity against many fungal pathogens (Vijaya et al., 2007; Ram and Thakore, 2009; Jadhav et al., 2013; Chaudhary et al., 2017). On the other hand, Assam as well as other North East states of India is well known for the rich biodiversity.

Hence, use of rich potential of the botanicals available in the state may be a comparative advantage and an alternative for the management of plant disease diseases. Eco-friendly management approach like use of botanicals more particularly A. sativum, A. catherica and L. nobilis may be used as an integral part of integrated disease management and it also has prospect as an alternative to reliance only on fungicide.

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