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Screening of selected homeopathic medicines against *Colletotrichum musae* causing anthracnose of banana

Ashraful Alam[1*], Parag Mistry[2]
[1] Associate Professor, Department of Plant Pathology, Patuakhali Science and Technology University, Bangladesh
[2] Student, Department of Plant Pathology, Patuakhali Science and Technology University, Bangladesh

Abstract
The goal of this work is to provide a contribution on the effects of homeopathic drugs on fungi. Homeopathic treatments are prepared starting from a mother tincture of different substances, according to standardized protocol which consists in serial aqueous dilutions. Banana anthracnose caused by the pathogen *Colletotrichum musae* is one of the leading disease which cause enormous losses worldwide. Ten (10) different homeopathic medicines Sepia officinale, Belladonna, Sulphur, Kali iodatum, Thuja Occidentalis, Bryonia, Arsenicum Album, Calcaria sulp, Selenium, Phosphorus at a concentration of 3000 ppm with three potencies (Mother Tincture Q, 30X and 200X) of each medicine were selected and tested against growth of *Colletotrichum musae*. Interestingly, all the medicines inhibited the mycelial growth of the pathogen. Significantly the highest inhibition percentage of each potencies was found in Arsenicum Album Q (48.64%), Kali iodatum 30X (21.80%) and both Calcaria sulp and Phosphorus at 200X potency (19.92%) followed by other medicines. Out of all the homeopathic medicines with their three potencies Arsenicum album at Q potency was found to be most effective drug on mycelial growth inhibition of *C. musae*.

Keywords: banana, anthracnose, *Collectotrichum musae*, homeopathic medicines, potency

Introduction

Banana (*Musa* sp.) is one of the world's most important year round fruit which consumed globally and cultivated in humid and subtropical climes (Netshiheni, 2019). Bananas are high in potassium, low in fat, and a good source of vitamins and fiber. The crop is grown in more than 150 countries, producing 105 million tonnes of fruit per year worldwide where *India is the top most country of banana production in the world. As of FAOSTAT 2019, banana productioin of India was 30.5 million tonnes that accounts for 26.02% of the world's banana production. The top five countries (India, China, Indonesia, Brazil, and Ecuador) are account for 53.94% of its total production. In BBS 2020,* Bangladesh ranks eleventh position in terms of banana production and its, total estimated production was 817908 metric tons and cultivated area was 121777 acres in 2019-20. Anthracnose of banana is initially appear as brown spots, which become sunken lesions with orange or salmon-colored acervuli (Sun, 1988) [23]. It is the most important postharvest disease of banana that can result in 30-40% losses of marketable fruit **(**Bazie, 2014**).** It deteriorates the quality and nutritive value of the fruits and renders them unfit for marketing and consumption, thereby causing severe loss to farmers and traders. It is caused by *C. musae* which is the most important pathogen on wounded green and ripe banana fruits (Thangamani *et al*., 2011) [24].

The postharvest use of chemicals as fungicides is restricted in most countries (Serrano *et al.*, 2005) [21] as they are harmful for human health and reduces their commercial value. Consumer demand for agricultural commodities without pesticide residues is high (Serrano *et al.*, 2005; Cutler and Cutler, 1999) [21]. Thus, a new preservation technologies are needed, which have to be considered as human-safe and environmentally friendly (Duru *et al.*, 2003) [9]. So, the researchers are searching for alternatives to these agrochemicals. The homeopathic medicines may be the alternatives.

Homeopathic remedies are derived from substances that come from plants, minerals, or animals, such as red onion, arnica (mountain herb), crushed whole bees, white arsenic, poison ivy, belladonna (deadly nightshade), and stinging nettle.

However, currently the homeopathy is being used in various segments of agriculture, like: pest control and plant diseases (Bonato *et al.*, 2006; Kumar, 1980; Khanna and Chandra, 1976) increase of active principles or secondary metabolic in plants and influence on plant physiology (Bonato and Silva, 2003; Betti *et al*., 1997). For its contribution on the auto regulation of the soil plant-atmosphere system, the homeopathy can assist in the maintenance process of the plants health for its tonic effect and/or by inhibition effect directly on the pathological agents (Toledo *et al.*, 2011) [1]. Moreover, homeopathy is a low cost alternative to chemical fungicides, easy to use by farmers (Toledo *et al.*, 2011) [1].

The use of homeopathic medicines to control various pathogens was highly effective and safe for fruit and environment. Inhibitory effect of homoeopathic drugs such as Lycopodium, Thuja, Arsenicum, Zincum etc. against *Alternaria alternata, Fusarium moniliforme, Gloeosporium psidii, Colletotrichum gloeospoioides* and *Pestalotia sp.* and certain fruit rot pathogens have been reported (Baviskar and Suryawanshi, 2015; Khanna and Chandra, 1992 and 1989; Wilson *et. al*.,1991; Chandra *et. al*., 1981). But in our country, the research on homeopathic medicine for controlling plant pathogens is very scanty. Considering the above facts, the goals of the present study was:

- To find out a suitable homeopathic medicine against *C. musae* for controlling anthracnose of banana.

Materials and Method

Banana (*Musa* sp.) having anthracnose symptoms were collected from Pirtola bazaar, Dumki, Patuakhali. Selected 10 homeopathic medicines were collected from different homeopathic dispensaries of Patuakhali city, Bangladesh**.**

Isolation and Identification of fungi

Banana's showing symptoms of anthracnose with sunken lesion were collected. The areas showing sunken lesion were cut into 5×5 mm by using a flame sterilized surgical scissors. Pieces of surface cut were put onto PDA containing petri plates and incubated at 25°C. After 5 days of incubation the fungal hyphal tips were transferred by sterilized 2.5 mm mycellial block cutter to another PDA containing petri plates for pure cultures. The pure cultures of the fungal pathogen were confirmed under compound microscope with 100X magnification. The oval shaped, hyaline and both end round conidia were observed which is identitical *C. musae* (Barnett and Barry, 1972) [2] (Figure 1). Finally the fungal cultures were stored at 4°C in refrigerator.

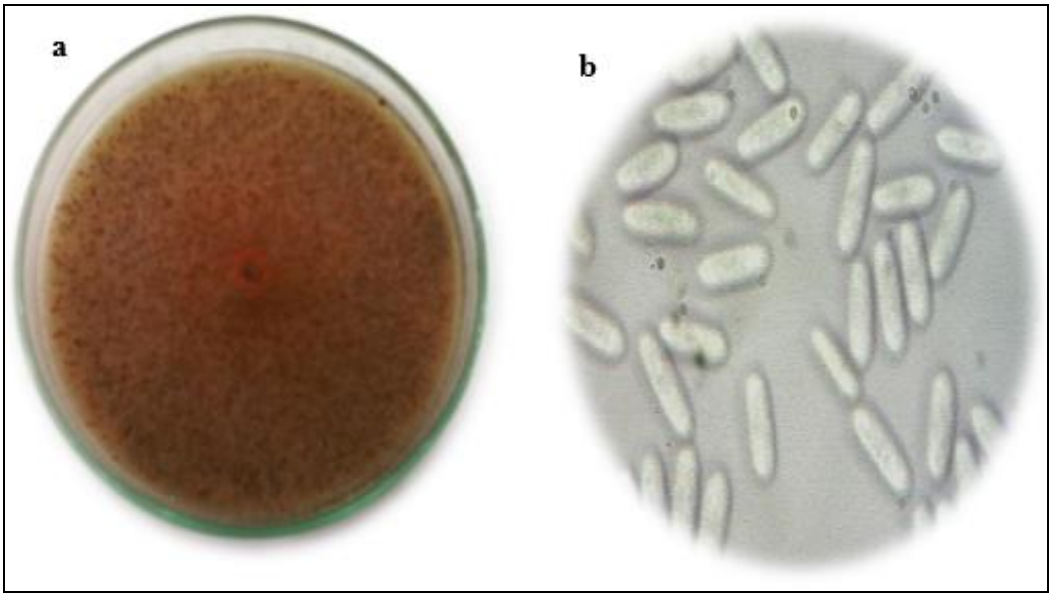

Fig 1: Five days old pure culture of *C. musae* on PDA containing sterilized petriplates a) numerous orange color conidial masses on the upper surface of the plates and b) Conidia of *C. musae* under compound microscope with 100X magnification.

Pathogenecity test

Pathogenecity test was done using healthy banana. Healthy bananas were surface sterilized with 70% ethanol for 30 seconds.

Then the excess ethanol was removed by using sterilized cotton. The samples were injured softly by flame sterilized pointed needles in some places. Conidial suspension was prepared from the pure culture of *C. musae.* Then the samples were dipped into the conidial suspension, after that it was taken into separate polybags. Then the banana's were incubated at 25±1°C and checked regularly for developing characteristic symptoms.

Selection of homeopathic medicines

Ten homeopathic medicines each of three potencies were selected based on antifungal activities against different pathogen in many experiments (Khare and Atri, 1994; Chaurasia, 1997). Potency= Potency is the strength of a homeopathic remedy. Q (Mother tincture) = A mother tincture is the extract of the original drug substance; the extract being made with alcohol or water in certain proportion. 30X= A potency of 30X means 1: 10000 dilution of the original substance. 200X= A potency of 200X means 1: 10^{60} dilution of the original substance. The homeopathic medicines are listed in the Table 1.

Table 1: List of homeopathic medicines used in this study

Name of homeopathic medicines	Potencies	Concentration	References
Sepia officinale, Belladonna, Sulphur, Kali iodatum, Thuja Occidentalis, Bryonia, Arsenicum Album, Calcaria sulp, Selenium, Phosphorus	Q, 30X and 200X	3000 ppm	Baviskar and Suryawanshi, 2015; Khanna and Chandra, 1992 Wilson *et. al*.,1991; Chandra *et. al*., 1981

Preparation of concentration of medicines and Mixing with PDA medium

For each potency of each homeo medicine, 3000 ppm concentration was prepared. 60 µL medicine was added separately in each test tube containing 20 ml PDA medium (1 ppm = 1 µL/L). Then it was mixed by shaking manually. The mixture was poured in 80 mm sterilized Petri-dishes and kept it for solidification. Each plate was considered as a replication. For control treatment no medicine was added. All the activities were undertaken under aseptic condition. Three replications were used for each potency.

Evaluation of homeopathic medicines against *C. musae* Inoculation and incubation

The evaluation of homeopathic medicines were followed Poison Food Technique (Baviskar and Suryawanshi, 2015) [3]. After mixing of medicines the plates were inoculated at 2.5 mm mycelial disc of five days old pure culture of *C.*

musae at the centre. The plates were incubated at 28±1°C. After seven days of incubation, the mycellial growth rate (mm/day) data were collected.

Measurement of radial growth and calculation of percent inhibition

After the growth of mycelium rim of plates in control plate the two cross-wise radial growth of each plate was recorded. The average of two diameters measured and computed. Percentage inhibition of growth was calculated by using the following formula (Alam *et al.*, 2017).

$$\text{Percent of inhibition} = \frac{X - Y}{X} \times 100$$

Where,

X= Average growth of *Colletotrichum musae* in control petridishes

Y= Average growth of *Colletotrichum musae* in each medicine treated petridishes

Experimental design and data analysis

The experiment was laid out in Completely Randomized Design (CRD) with three replications. The data were analyzed statistically by using Minitab software version 17 and means were compared with Tukey's method at 95% confidence level.

Results and Discussion

Effect of Q potency (Mother Tincture)

All the selected ten homeopathic medicines at Q potency were found significant inhibition at 5% level of significance where Arsenicum album > Sepia officinale and Selenium > Thuja Occidentalis > Phosphorus, Calcaria sulp and Sulphur > Kali iodatum and Bryonia > Belladonna followed by 48.64% > 19.50% > 12.79% > 10.48, 9.43 and 8.60 > 7.13 and 6.50 > 2.94 (Figure 2). Significantly the highest mycelia growth inhibition percentage (48.64%) was found in Arsenicum album which was statistically different with other medicines. Both Sepia officinale and Selenium showed same inhibition percentage (19.50%) which was lower than Arsenicum album. The lowest inhibition percentage (2.94%) of mycelia growth was found in case of the medicine Belladonna was statistically different with all other medicines.

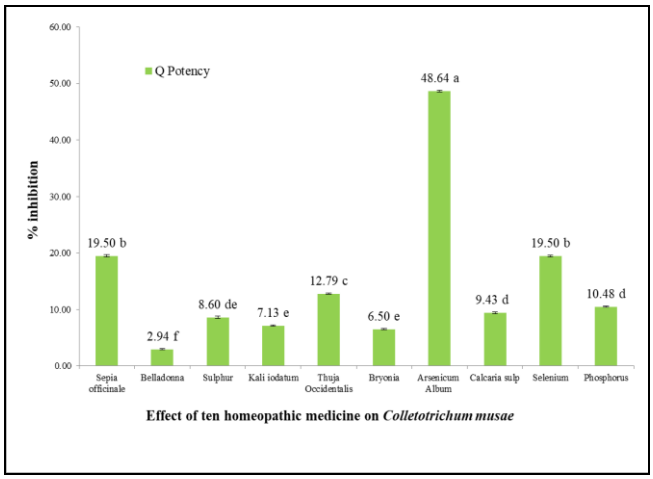

Fig 2: Effect of ten homeopathic medicines at Q potency against *C. musae*

Effect of 30X potency

Same medicines at 30X potency were found significant at 5% level of significance. The highest inhibition percentage (21.80%) of mycelia growth was found in case of Kali iodatum which was statistically similar with Selenium and Phosphorus but statistically different with rest of the medicines (Table 2). The second highest inhibition percentage (15.93%) of mycelia growth was found in case of Thuja occidentalis. The lowest inhibition percentage (8.39 %) of mycelia growth was found in case of Sepia officinale.

Table 2: Inhibition percentage of *C. musae* by different homeopathic medicines at 30X potency

Name of homeopathic medicine	Inhibition Percentage at 30X Potency
Sepia officinale	8.39 d
Belladonna	11.11 c
Sulphur	12.15 c
Kali iodatum	21.80 a
Thuja Occidentalis	15.93 b
Bryonia	12.99 c
Arsenicum Album	21.38 a
Calcaria sulp	11.74 c
Selenium	19.28 a
Phosphorus	19.91 a
CV (%)	6.45
Level of significance	0.05

Effect of 200X potency

The graph shows that mycelial growth of *C. musae* was considerably influenced by following ten homeopathic medicines with 200X potency at 5% level of significance (Figure 3). Calcaria sulp and Phosphorus induced maximum growth inhibition (20.13%) of which was statistically similar with Kali iodatum. Selenium (14.68%) showed the second highest inhibition percentage of mycelial growth. The lowest inhibition percentage (5.45%) was found in case of Sulphur was statistically similar with Bryonia but statistically different with all other medicines.

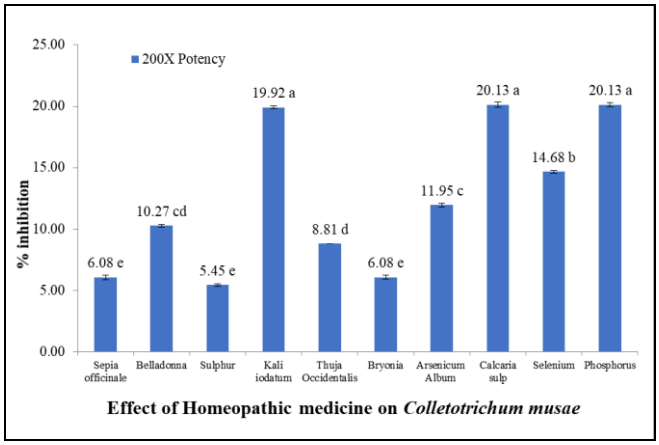

Fig 3: Effect of homeopathic medicine at 200X potency against *C. musae*

Conclusion

Homeopathic medicines are cheaper and have no side effects so effort was made to study the effect against *C. musae* of anthracnose disease of banana. Among the medicines were observed Arsenicum album Q was most effective and showed 48.64% mycelial growth inhibition.

This was followed by Sepia officinale and Selenium > Thuja Occidentalis > Phosphorus, Calcaria sulp and Sulphur > Kali iodatum and Bryonia > Belladonna.To this end, future studies would also be advisable to perform investigations into the potentisation process itself and to adopt standardized potentisation techniques.

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