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Effects of Plant Extracts and Commercial Bactericides on *Erwinia Chrysanthemi* (Rhizome Rot Bacteria) in Banana Plants

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ABSTRACT

Isolation of *Erwinia chrysanthemi* (rhizome rot bacteria) from infected rhizome of banana plants in Twentay Township, Yangon Region was conducted in Microbiology Laboratory, Department of Botany, University of Yangon. Antibacterial activity on *Erwinia chrysanthemi* from the nine selected plant species and the four chemical bactericides were evaluated by paper disc diffusion methods. The solvent extracts of the eight plant samples: *Capsicum annuum* L., *Curcuma longa* L., *Languas galangal* (L.) Stuntz, *Nicotiana tobacum* L., *Piper nigrum* L., *Synzygium aromaticum* (L.) Merr. Perry, *Zingiber barbatum* Wall. and *Zingiber officinale* Rose indicated bioactivity on *E. chrysanthemi*. However, the extracts of Piper betel L. did not show antibacterial activity on *E. chrysanthemi*. Chemical bactericides: Agri-mycin 17, Ariston, Bio-save and Blocker 4E showed the weak activity on E. chrysanthemi.

KEYWORDS: *Antibacterial activity; Erwinia chrysanthemi; chemical bactericides; Plant extracts*

INTRODUCTION

Banana wilt occurs on mature plants before the fruit has ripened. Mature plants leaves were wilt and die. The leaves hang around the stem like a skirt. Internally, there is a rot of the pseudostem, sometimes accompanied by a foul smell. In this case, newly planted suckers are affected, leading to rotting and a characteristic foul smell. In older plants, rotting occurs at the collar, the part between the base of the pseudostem and soil, and this can lead to plants falling over after snapping at the base.

Rots are seen in the rhizomes, and sometimes cavities occur with dark margins. Banana wilt and rhizome rot are major diseases caused by a bacteria belonging to a genus *Erwinia*. The genus *Erwinia*, is one of the phytopathogenic *Enterobacteriaceae* that can cause soft rot diseases in a wide range of economically important crops. These bacteria can survive in soils, from where they are transmitted to plants by water, miscellaneous insects, or cultural techniques. Bacterial head rot or rhizome rot of banana is caused by bacteria (Wardlaw, 1950). The same disease were found in several banana growing regions of Karnataka (Khan and Nagaraj, 1998). But Chattopadhay and Mukherjee (1986) from West Bengal implicated *Erwinia chrysanthemi* as the casual agent.

Capsicum annuum L. is a species of the plant genus *Capsicum* native to southern North America and northern South America (Minguez and Hornero, 1994 and Latham, 2009). The genus *Capsicum* of the Solanaceae family has high economic value due to its use as a food (Menichini *et al.,* 2009).

Curcuma is a genus of about 100 accepted species in the family Zingiberaceae that are native to Southeast Asia, Southern China, the Indian Subcontinent, New Guinea and Northern Australia. The rhizome of *Curcumalonga* L. is laxative, carminative, diuretic, stimulant, in the treatment of itches, boils, abscess, leucoderma, eye diseases, pains, bruises and affections of the liver and jaundice (Leong-Skornickova *et al.,* 2008 & Skornickova *et al.,* 2010).

The leaf extract of *Nicotiana tobacum* L. was a popular pest control in the beginning of the 20th century (Wennig and Robert, 2009). In Brazil, leaves of *Nicotiana tobacum* L. are heated and the juice is squeezed out for making an intoxicating snuff. The leaf juice is taken orally to induce vomiting and narcosis. In Guatemala, hot water extract of the dried leaf is applied externally for ring worms, fungal diseases of the skin, wounds, ulcers, bruises, sores, mouth lesions, stomatitis and mucosa (Groark, 2010).

Piper betel is blessed as evergreen and perennial plant. Today some hardened betel chewers occur in Thailand, Myanmar and Indonesia (Chaveerach, 2006). Betel leaf is traditionally known to be useful for the treatment of various diseases like bad breath, boils and abscesses, constipation, headache, itches, leucorrhoea, swelling of gum, rheumatism, cuts and injuries (Deshpande *et al.*, 1970; Chatterjee and Pakrashi, 1995).

Black pepper (*Piper nigrum* L.) is a flowering vine in the family Piperaceae. It is used as antiapoptotic, antibacterial, anti-colon toxin, antidepressant, antifungal, antidiarrhoeal, anti-inflammatory, antioxidative, antispermatogenic, antitumor, antithyroid, gastric ailments and insecticidal activity (Nisar *et al.,* 2012).

Cloves, *Syzygium aromaticum* (L.) Merr. & Perry, are the aromatic flower buds of a tree of the family Myrtaceae. Its native is Maluku Islands in Indonesia, and are commonly used as a spice. Cloves are used in the cuisine of Asian,

African, and the Near and Middle East countries, lending flavor to meats and curries (Dorenburg and Page, 2003).

Zingiber barbatum (meik tha lin) is a medicinal, therapeutic ginger found in Myanmar. It is used as the indigenous medicine. Ginger (*Zingiber officinale*) belongs to Zingiberaceae family. The part of the plant used is rhizome (Onyeagba *et al.*, 2004). Ginger inhibits Aspergillus, a fungus known for production of aflatoxin, a carcinogen (Nanir and Kadu, 1987).

Regarding to the above facts, the present research aimed to isolate *Erwinia chrysanthemi* (rhizome rot bacteria) from infected rhizomes of banana plants. The objectives of this research were to collect the selected plants samples, to extract various solvent extracts from parts used of the selected samples: *Capsicum annuum* L., *Curcuma longa* L., *Languas galangal* (L.) Stuntz, *Nicotiana tobacum* L., *Piper betel* L., *Piper nigrum* L., *Synzygium aromaticum* (L.) Merr. Perry, *Zingiber barbatum* Wall. and *Zingiber officinale* Roscoe, and to evaluate the antibacterial activity of selected plant species compared with four chemical bactericides: Agri-mycin 17, Ariston, Bio-save and Blocker 4E.

MATERIALS AND METHODS

A. Isolation of Erwinia chrysanthemi (rhizome rot bacteria)

The infected rhizomes of banana plants were collected from agricultural field in Twentay Township, Yangon Region. *Erwinia chrysanthemi* (rhizome rot bacteria) was isolated from the infected rhizome on yeast extract agar plates at Microbiology Laborotory, Department of Botany, University of Yangon (Phay, 1997).

B. Collection of the Plant Samples

The plant samples were collected from Twentay Township in Yangon Region to investigate their antibacterial activity on *Erwinia chrysanthemi*. These plant samples were shown in Table 1.

C. Preparation of the extracts from the selected plant samples

The collected plant species were thoroughly washed with water and then air dried at room temperature. When constant weight was obtained, the dried samples were pulverized by grinding machine and stored in air tight bottles for further use.

The dried powdered samples (6.0 g for each) of nine plant species were extracted with ethyl acetate, methanol and ethanol using percolation method. All these extracts were used for the determination of antibacterial activity on *Erwinia chrysanthemi*.

D. Chemical Pesticides

The four chemical bactericides such as Agri-mycin 17, Ariston, Bio-save and Blocker 4E that were used for antibacterial activity on *Erwinia chrysanthemi* .

E. Antibacterial Activity of the Selected Plants and Chemical Bactericides on Selected Plant Species

The antibacterial activity of various solvent extracts of the selected plants and chemical bactericides were tested on rhizome rot bacterium *E. chrysanthemi*. Antibacterial activity was conducted by using paper disc diffusion method (Davis and Stout, 1971) at the Microbiology Laboratory, Department of Botany, University of Yangon.

F. Test Agar Plates

Plant pathogenic bacterium, *Erwinia chrysanthemi* (50 µl) was added to 100 ml of assay medium (Sucrose Malt extract (SM) : sucrose 1.0 g, malt extract 0.3 g, NaCl 0.05 g, $CaCO_3$ 0.01 g, agar 1.8 g, distilled water 100 ml, pH 7) at 50˚C then poured into the plates.

G. Paper Disc Diffusion Assay

After solidification, paper discs impregnated with the extracts and chemical pesticides (5.0µg/ml per disc) were applied on the test plates and these plates were incubated for 24 - 48 hrs at 30°C. After 24 - 48 hrs, clear zones (inhibitory zones) surrounding the test discs were measured. These zones indicated the presence of the bioactive compounds which inhibited the growth of pathogenic bacteria. Paper disc size is 6.0 mm (Davis and Stout, 1971).

TABLE1. Collected plant samples

No.	Scientific name	Part used
1	*Capsicum annuum* L.	Fruits
2	*Curcuma longa* L.	Rhizome
3	*Languas galangal* (L.) Stuntz	Rhizome
4	*Nicotiana tobacum* L.	Leaves
5	*Piper betel* L.	Leaves
6	*Piper nigrum* L.	Seeds
7	*Synzygium aromaticum* (L.) Merr. & Perry	Flowers
8	*Zingiber barbatum* Wall.	Rhizome
9	*Zingiber officinale* Roscoe	Rhizome

RESULTS AND DISCUSSION

A. Antibacterial Activity of the Selected Plants and Chemical Pesticides

Screening of antibacterial activity of various solvent extracts of the selected plants and chemical bactericides was carried out on *Erwinia chrysanthemi*. The results of inhibitory zones that appeared on the samples were given in Tables 2, 3 and Fig. 1 - 10.

In this experiment, the ethyl acetate, methanol and ethanol extracts of *Languas galangal* (L.) Stuntz. and *Synzygium aromaticum* (L.) Merr. & Perry exhibited high activity on *E. chrysanthemi* while these three extracts of *Capsicum annuum* L., *Curcuma longa* L., *Nicotiana tobacum* L., *Piper nigrum* L. *Zingiber barbatum* Wall. And *Zingiber officinale* Roscoe. indicated weak activity on *E. chrysanthemi*. However, all extracts of *Piper betel* L. did not show antibacterial activity on *E. chrysanthemi* as seen in Table 2 and Fig. 1 - 9. Chemical bactericides: Agri-mycin 17, Ariston, Bio-save and Blocker 4E showed weak activity on *E. chrysanthemi* as shown in Table 3 and Fig. 10.

Table2. Antibacterial activity of various solvent extracts of the selected plants

No.	Sample	EA ext.	MeOH ext.	EtOH ext.
1	*Capsicum annuum* L.	*	*	*
2	*Curcuma longa* L.	*	*	*
3	*Languas galangal* (L.) Stuntz.	**	**	**
4	*Nicotiana tobacum* L.	*	*	*
5	*Piper betel* L.	-	*	-
6	*Piper nigrum* L.	*	*	*
7	*Synzygium aromaticum* (L.) Merr. & Perry	**	**	**
8	*Zingiber barbatum* Wall.	*	*	*
9	*Zingiber officinale* Roscoe	*	*	*

** = high activity
* = Weak activity
- = No activity

TABLE3. Antibacterial activity of chemical pesticides on *Erwinia chrysanthemi*

No.	Chemical pesticides	Inhibitory zone	Activity
1	Agri-mycin 17	*	*
2	Ariston	*	*
3	Bio-save	*	*
4	Blocker 4E	*	*

* weak activity = 10-14 mm,
** high activity = 15-18 mm
- = no activity,

EA ext.= ethyl acetate extract, MeOH ext.= methanol extract, EtOH ext. = ethanol extract

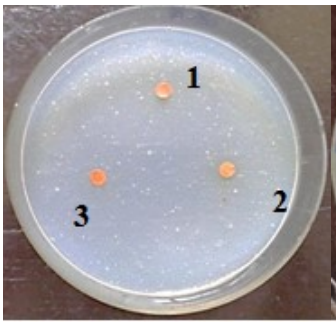

Fig.1. Inhibitory zone of *Capsicum annuum* L.

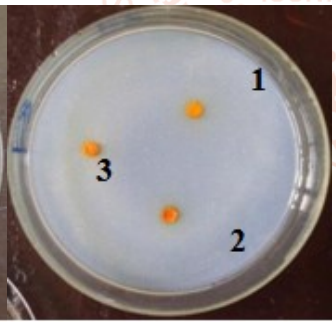

Fig.2. Inhibitory zone of *Curcuma longa* L.

Fig.3. Inhibitory zone of *Languas galangal* L.

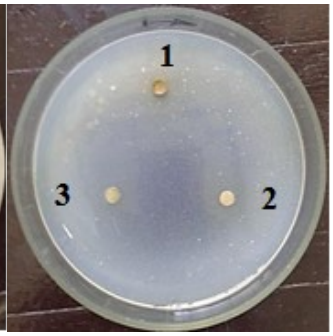

Fig.4. Inhibitory zone of *Nicotiana tobacum* L.

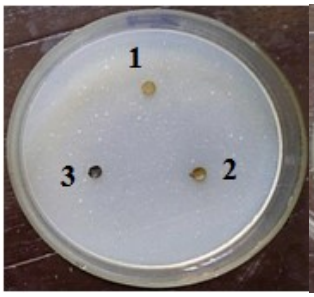

Fig.5. Inhibitory zone of *Piper betel* L.

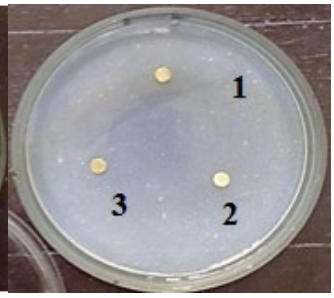

Fig.6. Inhibitory zone of *Piper nigrum* L.

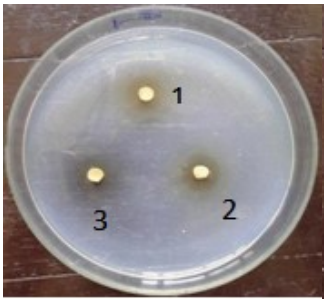

Fig. 7. Inhibitory zone of *Synzygium aromaticum* L.

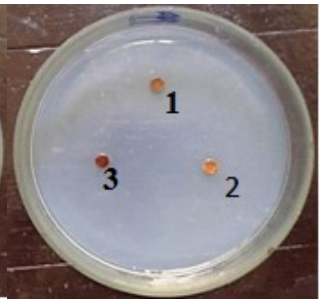

Fig. 8. Inhibitory zone of *Zingiber barbatum* Wall.

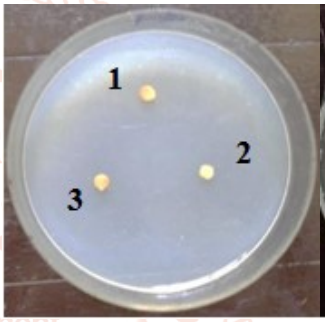

Fig.9. Inhibitory zone of *Zingiber officinale* Roscoe

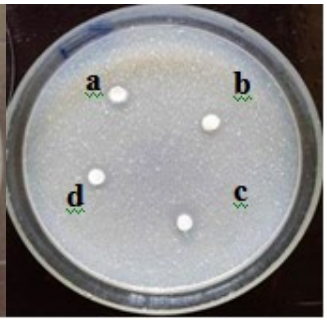

Fig.10. Inhibitory zone of chemical bactericides

1 = Ethyl acetate extract, **2** = Methanol extract, **3** = Ethanol extract

a = Agri-mycin, **b** = Ariston, **c** = Bio-save, **d** = Blocker 4E

In the course of antibacterial activity on *Erwinia chrysanthemi* of the nine selected plant species and the four chemical pesticides show that, all extracts of the eight plant samples: *Capsicum annuum* L., *Curcuma longa* L., *Languas galangal* (L.) Stuntz, *Nicotiana tobacum* L., *Piper nigrum* L, *Synzygium aromaticum* (L.) Merr. & Perry, *Zingiber barbatum* Wall. and *Zingiber officinale* Roscoe indicated bioactivity on *E. chrysanthemi*. However, the extracts of *Piper betel* L. did not show antibacterial activity on *E. chrysanthemi*. The four chemical bactericides: Agri-mycin 17, Ariston, Bio-save and Blocker 4E showed weak activity on *E. chrysanthemi* in this research.

Moreover, the ethyl acetate, methanol and ethanol extracts of fruits from *Capsicum annuum* L. showed activity on bacterial pathogen *E. chrysanthemi*. Soetarno (1997) reported that ethanol extracts of the fruits of Capsicum showed antimicrobial activities against Gram (+) and Gram (-) bacteria, and fungi. Santos *et al.,* (2012) also stated the inhibitory effects of the ethyl acetate extract (1.25µg/ml) from *Capsicum annuum* L. fruit. Therefore, the present finding is in agreement with the statement of Soetarno *et al.,* (1997) and Santos *et al.,* (2012).

The ethyl acetate, methanol and ethanol extracts of *Curcuma longa* L. rhizome indicated antibacterial activity on *E. chrysanthemi*. This finding is in agreement with the results of Araújo & Leon 2001, who reported that the ethanol (50%) extract of *Curcuma longa* L. rhizome was effective as insect repellent against houseflies. It had the effect on growth of pathogenic bacteria and possessed antibacterial and antifungal activity on some test organisms.

In this study, all polar solvent extracts of *Languas galangal* (L.) indicated high activity on *E. chrysanthemi* that is similar to the results by Riyanto (2009) who investigated that the extracts of *Languas galangal* L. rhizome showed antibacterial activity on some species.

The extracts of dry leaves from *Nicotiana tobacum* L. exhibited bioactivity on *E. chrysanthemi*. This finding had an agreement with the statements by Akbar (2012) and Jehan and Mohammad Shafi, (2012). Akbar (2012) stated that the leaves extracts of *N. tobacum* L. had effects on bacterial growth. Jehan and Mohammad Shafi (2012) reported that the different polar solvent extracts of *N. tabacum* L. revealed the different ranges of antibacterial activities at different concentrations.

The polar solvent extracts of *Syzygium aromaticum* (L.) Merr. & Perry flower showed high antibacterial activity on *E. chrysanthemi* in this experiment. Deans *et al.,* (2006) reported that the essential oil from clove *S. aromaticum* (L.) Merr. & Perry exhibited significant antimicrobial activity against a collection of 25 different genera of test bacteria including animal and plant pathogenic bacteria.

The rhizome extracts of *Zingiber barbatum* Wall. and *Zingiber officinale* Roscoe indicated the antibacterial activity on *E. chrysanthemi* in this study. These results were similar to Suhad *et al.*, (2012) and White (2007). Suhad *et al.,* (2012) stated that the aqueous extract of ginger, *Zingiber officinale,* roots showed clear antibacterial activity against pathogenic bacteria; Gram-negative and Gram-positive bacteria. White (2007) also reported that Ginger has strong antibacterial activity and to some extent of antifungal properties.

CONCLUSIONS

In conclusion, the ethyl acetate, methanol and ethanol extracts of *Languas galangal* (L.) Stuntz and *Synzygium aromaticum* (L.) Merr. & Perry possessed higher antibacterial activity on *E. chrysanthemi* than that of chemical bactericides in this research work. Therefore, the rhizome extracts of *Languas galangal* (L.) Stuntz and the flower extracts of *Synzygium aromaticum* (L.) Merr. & Perry should be used to inhibit the rhizome rotting disease, *E. chrysanthemi* in banana plants instead of chemical bactericides that can pose many side effects to living things and their environments. Application of chemical bactericides created the soil fertility depletion and also ecosystem pollution. For banana cultivars, the dried powders of *Languas galangal* (L.) Stuntz rhizome and *Synzygium aromaticum* (L.) Merr. & Perry flower should be applied to inhibit the rhizome rot bacterial disease caused by *E. chrysanthemi* in banana cultivation. Bio bactericides which once obtained in the laboratory can be multiplied by sub-culturing and therefore it can be used for long term in the actual field. The consequence is production cost reduction in banana cultivation and thus it can be said to be economic feasible for the gardeners.

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