International Journal of GEOMATE, Dec., 2016, Vol. 11, Issue 28, pp.2862-2865
Special Issue on Science, Engineering and Environment, ISSN: 2186-2990, Japan

EFFECT OF THE BIOEXTRACT TO CONTROL THE CURVULARIA LEAF SPOT AND CANKER OF LEMON

Vithaya Tavenooth

Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi, Thailand

ABSTRACT: Effects of the bio-extracts on controlling the Curvularia leaf spot pathogen (*Curvularia. sp*) and canker pathogen (*Xanthomonas campestris citri*) in lemon were assessed under laboratorial procedures at the Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi [RMUTT], Thailand, during June to July 2015. The bio-extracts were prepared through fermentation of sugar mixed with one part of water and one part of ground leaves and stems from 4 different species of plants, galangal, chili, basil and alata. Three categories of ingredients, a bio-based agar, four formulae [1-4] of the bio-extracts and distilled water were used to formulate testing media for this study. The growth rate and colony sizes of the pathogenic organisms were determined at 1, 3, 5, 7 and 14 days using a completely randomized design (CRD) experiment with four replications and five treatments. The results showed that *Curvularia sp.* growth was inhibited by all bio-extract formulae at the same degree from 3 days of exposure limiting all colony sizes to be smaller than 1.4 cm. On the other hand, formulae 1 and 2 bio-extracts showed better inhibition to the growth of the citrus canker bacteria (*Xanthomonas campestris citri*) over the rest of treatments. These results suggested that bio-extracts from some selected plants are capable to control plant diseases.

Keywords : Bio-extract, Curvulatia.sp, Xanthomonas campestris citri, Galangal, Chili, Basil, Alata

1. INTRODUCTION

Many small farms in the Northeast of Thailand have used some bio-extracts (BE) produced from plant or animal residues to reduce or replace chemical fertilizers and pesticides for crop production. The bio-extract could be applied either singly or combined with other organic based substances. The chemical pesticides are widely used for cultivation and disease control in production of most plants including lemon. These pesticides do not only raise production cost but in long term they also deteriorate soil qualities. In addition, these toxic chemicals pollute environment, spreading into farmer houses and are present in some agricultural products [1,6]. Currently, many imported plant growth promoters or fertilizers are found to be bio–extracts. Some of them are produced by fermentation, as this procedure enhances output qualities. Bio-extract fertilizer becomes one of the alternatives which minimizes chemical treatments. On the other hand, biological insect repellent serves for pest control and the effective microorganisms fight against plant diseases [1,2]. As crop quality is the primary issue, moving toward a biological procedure is important.

This procedure is supported by some reports on preventive properties of bio-organic substances in controlling plant diseases suggesting a potential use instead of chemicals. Thus, some biological controlling procedures on corn leaf-spot fungi (*Curvularia sp.*) and citrus-canker bacteria (*Xanthomonas campestris citri*) were tested using bio-extracts.

The aim of this study was to determine capability of 4 selected bio-extracts in inhibiting the growth of *Curvularia sp.* and *Xanthomonas campestris citri* at a laboratorial scale .

2. MATERIALS AND METHODS

The experiment was conducted at Rajamangala University of Technology Thunyaburi, Pathumthani, Thailand. A completely randomized design (CRD) experiment with 5 treatments and 4 replications was applied for this experiment. The treatments were 4 different combinations (formulae) of the bio-extract: 1) galangal, chili, basil and alata, 2) galangal, chili, and basil, 3) galangal and chili, 4) galangal, chili, basil and alata leavening P.D. 2. Distilled water was used as the 5th treatment. The responses to the treatments were indicated by the growth of the fungal mycelium or colony sizes of the bacteria on PDA media.

2.1 Bio-extract preparation

Stems and leaves of galangal (*Alpinianigra. L. Burtt*), chili *(Capsicum frutescens .L)*, basil (*Ocimu sanctum Linn*) and alata (*Senna alata L. Roxb.)* were chopped or ground into small pieces. The material from each plant was mixed with molasses at a ratio of 3:3. Some 20 liters of clean water was added to

each 3 kg of mixture. The mixture was allowed to ferment in a closed 20-liter bucket (anaerobic conditions) for 3 months at The Department of Plant Science in 2015. At maturation, the bio-extracted products were poured into plastic bottles, wrapped in tin foil and kept at 4°C as a stock solution for further experiment. Four testing formulae (1-4) were prepared from the stock using different doses of the extracts (table 1).

Table 1 Doses of herbs used in each recipe

Formula	Amount of herbs, kg			
	Galanga *(Alpinia nigra)*	Chili *(Capsicsicumfrutescens L.)*	Basil *(*Ocimu sanctum*)*	Alata *(Senna alata)*
1	3	3	3	3
2	3	3	3	-
3	3	3	-	-
4	3	3	3	-

Each plant extract was filtered through muslin cloth and this 100% plant extract solution was prepared. The extracts were poured in the flasks plugged with cotton and heated at 100°C for 10 minutes to avoid contamination [3]. The food poison technique was applied [4] by ratio PDA concentrations: 10 ml bio-extract: 1 ml prepared PDA (ratio:10:1**)** in test tubes and stored in refrigerator at 4°C.

The agar dilution assay was carried out according to **[5]** with a slight modification. Thirty nine grams of potato dextrose agar (PDA) powder was boiled until the agar completely dissolved in 1 L of distilled water. The solution was then sterilized using an autoclave at 121°C for 15 min. Some 10 ml of the sterilized PDA and 1 ml of bio-extract were mixed and plated on the sterilized petridish (8.5 cm in diameter).

Curvularia leaf spot pathogen was isolated using the dextrose agar medium and identified in a sterilized petridish. The pathogen was monograph and cultured using a standard procedure on the potato dextrose agar medium. Its mycelia discs of 7.0 mm in diameter were placed in the center of petri dishes containing media of different testing formulae. The growth rate of the fungi and colony sizes of the bacteria were determined at 1, 3, 5, 7 and 14 days.

2.2 Statistical analysis

The experimental data were statistically analyzed using SPSS statistical software version 17.0.

A one way analysis of variance at the significant level of 95 % was applied. The significance of differences between treatment means were analyzed using students 't' test procedure. The differences were shown by *, ** and *** indicating the significant level at P≤0.05, p≤0.01, and p≤0.001, respectively.

3. RESULTS

The data recorded during the course of investigation has been subjected to three-way classification. The conclusion was drawn on the basis of analysis of variance. The calculated value of F was compared with table value of F at 5% levels of significance for an appropriate degree of freedom. The result showed at 7 days that the colony diameters of the fungi on the formula 1, 2, 3, 4 and 5 (control) were 1.30, 1.35, 1.38, 1.35 and 4.05 cm, respectively. At 14 days, they were 1.33, 1.43, 1.50, 1.45 and 5.98 cm, respectively. It was clearly recognized that the *Curvularia sp.* growth was affected by all formulae from 3 days exposure onwards. This finding suggested that all formulae were able to prohibit the growth of *Curvularia sp.* at the same levels as they showed no difference in colony diameters as shown in table 2.

The growth of *Xanthomonas campestris citri* bacteria on the control medium was higher than those of all bio-extract formulae. This bacteria tended to grow less on formula 1 or 2. This result suggested that these two formulae were capable to prohibit the canker pathogenic bacteria.

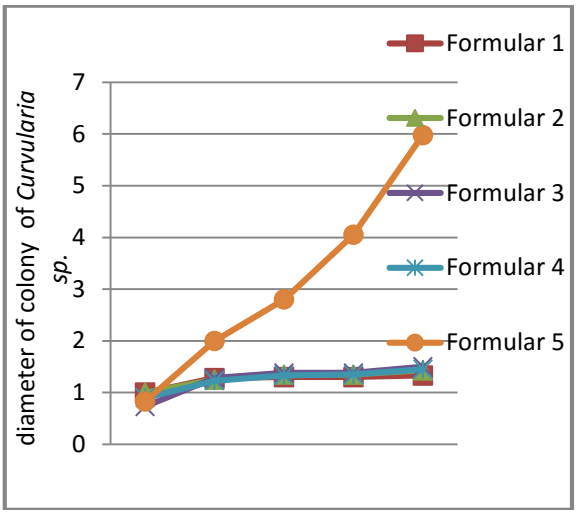

Fig. 1 The growth rate of *Curvularia sp.* on PDA mixed 5 formulae of bio-extracts, at 1, 3, 5, 7 and 14 days

Table 2 Average colony diameter of *Curvularia sp.* on PDA mixed with 5 formulae of bio-extracts at 1, 3, 5, 7 and 14 days

Date / Formula	1	3	5	7	14
1	1.00	1.28	1.3	1.30	1.33
2	1.00	1.25	1.35	1.35	1.43
3	0.73	1.28	1.38	1.38	1.50
4	0.90	1.23	1.33	1.35	1.45
5	0.83	2.00	2.80	4.05	5.98

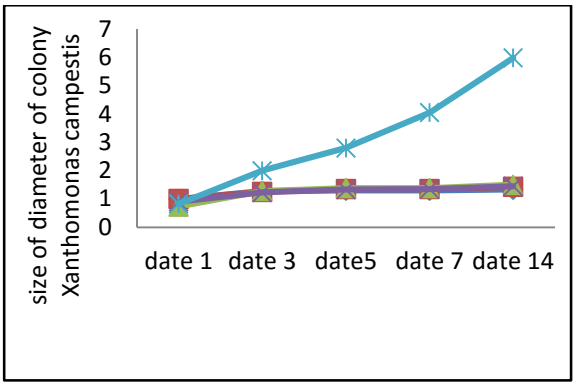

Fig. 2 The growth rate of *Xanthomonas campestris citri* on PDA mixed with 5 formulae of bio-extracts, at 1, 3, 5, 7 and 14 days

Table 3 Average colony diameter of *Xanthomonas campestris citri* on PDA mixed with 5 formulae of bio-extracts, at 1, 3, 5, 7 and 14 days

Formula	Diameter of colony				
	1	3	5	7	14
1	0.70b	0.70c	0.70d	0.70c	0.70d
2	0.70b	0.78c	0.78d	0.78c	0.85cd
3	0.70b	0.98b	1.03c	1.10b	1.10bc
4	0.70b	1.13b	1.20b	1.20b	1.28b
5	0.78a	1.48a	1.53a	1.60a	2.65a

4. DISCUSSION

All formulae of the bio-extracts were effective in controlling *Curvularia sp.* growth from 3 days. They demonstrated by having colony diameters smaller than 1.4 cm with no significant difference. On the other hand, the growth of citrus canker pathogen (*Xanthomonas campestris citri*) was slowed down by the formulae 1 and 2 of bio-extracts in comparison with the other two bio-extracts mixtures including the control. This indicated that bio-extracted from medicinal plants tend to have ability to control plant diseases. In addition, the bio-extracts indicate the potential of selected plant species as a source of natural fungicidal material or bactericide material. Antifungal and anti-bacterial activity was confirmed by all of the selected plant species. The results of this research coincided with the previous report [6]. The results revealed that different extracted formulae did not vary in their efficacy in inhibiting the mycelia growth and bacterial growth of the tested pathogens.

5. CONCLUSIONS

Bio-extracted from medicinal plants tend to have ability to control plant diseases. In addition, the bio-extracts indicate the potential of selected plant species as a source of natural fungicidal material or bactericide material. Antifungal and anti-bacterial activity was confirmed by all of the selected plant species. The results of this research coincided with the previous report [6]. The results revealed that different extracted formulae did not vary in their efficacy in inhibiting the mycelia growth and bacterial growth of the tested pathogens.

6. ACKNOWNLEDGEMENT

The author is thankful to Faculty of Agricultural Technology, Rajamangala University of Technology Thanyaburi, Thailand for research funding, providing necessary facilities and help.

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International Journal of GEOMATE, Dec., 2016, Vol. 11, Issue 28, pp. 2862-2865.

MS No. 1288 received on July 27, 2015 and reviewed under GEOMATE publication policies.

Corresponding Author: Vithaya Tavenooth