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Application of biorational pesticides against *Ralstonia solanacearum* (Smith) on tobacco

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Abstract

The different biorational pesticides were evaluated against *R. solanacearum* (Smith) both *in vitro* and *in vivo* conditions. In the *in vitro* assay using spread plate method, fermented vegetables applied at the rate of 1ml/L of water showed a high degree of efficacy against the bacterial pathogen at one, two, and three days after incubation (DAI) with average inhibitory zones of 2.15 mm, 1.74 mm, and 1.44 mm, respectively. Under *in vivo* conditions, the inoculated tobacco plants manifested symptoms typical of bacterial wilt due to *R. solanacearum* (Smith). However, a comparable percent wilt incidence was noted on plants applied with fermented vegetables and other biorational pesticides. Moreover, the growth parameters of tobacco such as plant height, leaf length, fresh leaf weight, and the total number of harvested leaves per plant were not affected by the application of biorational pesticides. The results indicate that fermented vegetables are effective against *R. solanacearum* (Smith) under *in vitro* conditions, but did not show significant difference with the other treatments under *in vivo* conditions.

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Introduction

Tobacco is a crop primarily used in the production of cigarettes. With the overwhelming scientific evidence that smoking is harmful, other uses of tobacco were investigated. Instead of drying the leaves into cigarettes, researchers found promising new plant breeding techniques (NPBT) to turn tobacco leaves into efficient plant factories for medical, pharmaceutical, and cosmetic products. These plants will produce vaccines, antibodies, and other health-promoting substances including anti-aging or anti-inflammatory compounds (Goossens, 2018). Tobacco waste like dust, stalks, and discarded tobacco leaves are now promising components for various agricultural applications such as tobacco pesticide (Booker *et al.*, 2010) and composting material for fertilizer which makes the crop indispensable (Nguyen *et al.*, 2022).

Like any other crops, tobacco is susceptible to pest and diseases. Bacterial wilt is the most common and devastating soil-borne disease caused by *Ralstonia solanacearum* (smith). Currently, *R. solanacearum* is the most intensively studied phytopathogenic bacterium due to its lethal nature in tobacco and important solanaceous crops (Ahmed *et al.*, 2022). Bacterial wilt is a continuing threat to the tobacco industry worldwide causing great economic losses, thus searching for effective, sustainable, and environmentally sound treatment to mitigate the disease is of utmost importance. Integration of chemical and physical control strategies is an excellent option but each strategy has disadvantages in terms of environmental, human safety, and sustainability of control. The botanical antimicrobial substances from various plants, including secondary metabolites, have highly efficient sources of antimicrobial activity against agricultural plant pathogens. A great number of plant essential oils were tested for their antimicrobial activities, like methyl gallate, lansiumamide B and essential oils had inhibited the growth of *R. solanacearum* (Smith) (Gurjar *et al.*, 2012). At present, the application of biorational pesticides is now gaining more attention because these are eco-friendly pesticides that may offer an alternative option to manage bacterial wilt on tobacco.

Discerning the current situation of tobacco farmers with limited knowledge to mitigate the disease makes this research into realization. The study aims to evaluate the different biorational pesticides against *R. solanacearum* (Smith) under *in vitro* conditions*;* assess the effects of biorational pesticides on the incidence of tobacco bacterial wilt under *in vivo* conditions and evaluate the growth responses of tobacco applied with biorational pesticide.

Materials and methods

Treatments, duration and place of the study

The treatments used in the experiment were commercially available. The *in vitro* experiment was conducted at the Department of Plant Pathology, College of Agriculture, Central Mindanao University, University, Musuan, Bukidnon, while the *in vivo* experiment was conducted in Barangay Loguilo, Alubijid, Misamis Oriental, from February to June, 2020.

Experimental design and statistical analysis

Both *in vitro* and *in vivo* set-ups were laid out using Complete Randomized Design (CRD) with three replications per treatment. The Analysis of Variance (ANOVA) was carried out using Standard Tool for Agricultural Research (STAR). Treatment means were compared using Tukey's HSD to statistically determine the significant difference.

Isolation and purification of R. Solanacearum (Smith)

Infected tobacco plants showing internal and external symptoms were obtained in the municipality of Alubijid, Misamis Oriental. Infected stalks of tobacco plants were surfaced sterilized by immersion in 10% sodium hypochlorite for 2 minutes then rinsed in sterile distilled water three times. The disinfected stalks were cut into 5-10 cm and placed in a test tube containing sterile distilled water to allow bacterial oozing. A loopful of suspension was streaked on tetrazolium chloride agar (TZCA) plates and incubated for 24 to 48 hours. Colonies that were large, elevated, fluidal, and entirely white or with pale red center in TZCA plate culture were further sub-cultured using nutrient agar (NA) slants (Agrios, 1997).

In vitro assay of biorational pesticide against R. Solanacearum (Smith)

The assay was performed by dipping sterile paper disks for 5-10 minutes on the prepared treatment solutions. Four paper disks were placed equidistantly on NA plates seeded uniformly with standardized suspension of *R. solanacearum* (Smith) using spread plate method. The culture plates were incubated for 24 to 28 hours at room condition and were observed daily for the presence of zone of inhibition until three days of incubation using this formula.

Zone of Inhibition = (D1 + D2 - PD)/2

Where:

D1 = Diameter of the widest inhibitory zone

D2 = Diameter of the narrowest inhibitory zone

PD = Paper disk diameter

In vivo assay of biorational pesticide against R. Solanacearum (Smith)

Field soil from tobacco growing area was collected and pasteurized as planting medium while healthy tobacco seedlings (Baltek/Native variety) were source locally for the pot experiment. The pasteurized soil was transferred in flour bags measuring 20" × 30" and with six holes at the bottom for drainage. Tobacco seedlings in trays were transplanted in empty flour bags at 60 days after sowing. This was done late in the afternoon. The biorational pesticides were drenched at four weeks after transplanting following the recommended rate. Subsequent applications were done at two weeks intervals until the termination of the study. Inoculation was performed by drenching the base of tobacco seedlings with 200 ml of standardized bacterial suspension. Sample plants were monitored for the incidence of bacterial wilt from the time of inoculation of *R. solanacearum* (Smith) up to the termination of the study. This was computed by counting of infected plants divided by the total number of plants assessed multiplied by 100.

% Disease Incidence = {(Total No. of Infected Plants)/(Total No. of Plants Assessed)}×100

Results

Table 1 confers, that wilting incidence is evident at one week after inoculation (WAI). T_3 (Fermented vegetables at 1ml/L) with 3.71% infection, T_2 (Control-Inoculated) and T_4 (Lactic acid at 6ml/L), both with 1.85% infection. There was no infection observed in T_5 (Tea tree extract at 5ml/L) and T_6 (*Bacillus subtilis* at 1.5ml/L). Subsequently, T_2 (Control-Inoculated) increased to 11.11% but this did not differ significantly to the percent infection on tobacco applied with Fermented vegetables (T_3), Lactic acid (T_4), Tea tree extract (T_5) and *Bacillus subtilis* (T_6) with 3.71, 7.41, 1.85 and 3.71, respectively at 2 WAI. Furthermore, the wilt incidence continued to increase in treatments applied with biorational pesticides at 3 and 4 WAI, but these values were statistically similar to the percent infection on *R. solanacearum*-inoculated plants without biorational pesticide application (T_2).

Table 1. Percent wilt incidence *R. Solanacearum* (Smith) on tobacco in response to different biorational pesticides

Treatment	Weeks after inoculation (WAI)			
	1	2	3	4
T_1-Control (Uninoculated)	0.0	0.0b	0.0^{b}	0.0^{b}
T_2- Control (Inoculated)	1.85	11.11^{a}	12.97^{a}	12.97^{a}
T_3- Fermented vegetables (1ml/L)	3.71	3.71^{ab}	3.71^{ab}	7.41^{ab}
T_4- Lactic acid (6ml/L)	1.85	7.41^{a}	7.41^{ab}	12.97^{a}
T_5- Tea tree extract (5ml/L)	0.0	1.85^{a}	3.71^{ab}	3.71^{a}
T_6- Bacillus subtilis (1.5ml/L)	0.0	3.71^{ab}	3.71^{ab}	3.71^{a}
Ftest	ns	*	*	*
CV%	25.70	26.92	26.61	26.92

Means in acolumn followed by a common letter are not significantly different at 0.05 level using Tukeys HSD test. (Tukey, p > 0.05). *= Significant, ns= not significant

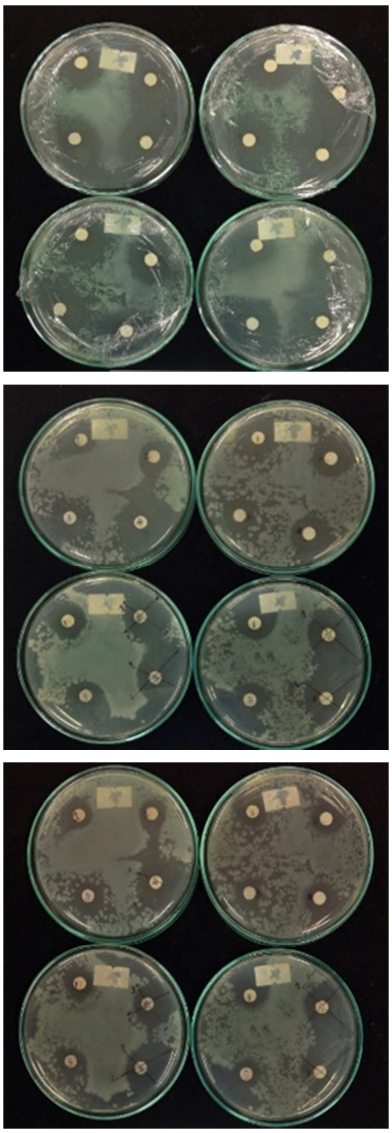

Fig. 1. Inhibitory zones on the growth of *R. solanacearum* (Smith) on nutrient agar (NA) applied with fermented vegetables at one (A), two (B), and three (C) days after incubation

Fig. 1 revealed that among the four biorational pesticides, only fermented vegetables (T_3) exhibited an inhibitory effect on the *R. solanacearum* (smith) that was monitored one, two and three days after incubation (DAI). The widest zone of inhibition was observed at day one after incubation with the mean value of 2.15 mm followed by the decreasing mean values of 1.75 mm and 1.44 mm at two and three DAI, respectively. Further, the results showed that the effectiveness of fermented vegetables at 1ml/L (T_3) under *in vitro* conditions decreased with time of incubation.

Discussion

The efficacy of fermented vegetables against the pathogen is attributed to its unique composition. The liquid-based product contains enzymes that degrade cell walls of fungi causing lysis and death. It has been found effective against *Colletotrichum, Verticillium, Fusarium, Pythium, Phytophthora, Rhizoctonia, Botrytis, Sclerotinia, Alternaria, Puccinia, Uromyces* and other pathogenic agents (Baltic BioIndustry, 2021). Although reports indicated that the product had antifungal effects but probably there are other components from the fermented vegetables that has the potential in combating bacterial pathogens like *R. solanacearum* (Smith) as observed in the *in vitro* experiment. According to Khameneh *et al.* (2019), plant-derived compounds have displayed more potential applications in combating bacterial infections in plants and animals. These phytochemicals may include alkaloids, sulfur-containing compounds, terpenoids and polyphenols.

Conclusion

Based on the study, the following conclusions are drawn. The application of fermented vegetables at 1ml/L inhibits the growth of *Ralstonia solanacearum* (Smith) but its longevity of effectiveness decreases with time of incubation under *in vitro* conditions. Biorational pesticides namely, Lactic acid, *Bacillus subtilis*, Tea tree extract and Fermented vegetables applied through drench method, showed comparable results in controlling bacterial wilt on tobacco under *in vivo* conditions and does not enhance the growth parameters of tobacco particularly the height, leaf length and width, fresh leaf weight and total number of leaves per plant.

Recommendations

The application of fermented vegetables at 1ml/L showed promising results in the *in vitro* bioassay but comparable with the treatments in the field experiment against *R. solanacearum* (Smith) causing wilt on tobacco.

However, it is imperative to conduct an experiment under field conditions using fermented vegetables at different rates to determine the appropriate dosage and frequency of application of this product that would potentially control *R. solanacearum* (Smith)

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