Weed extracts as potential biopesticides against Cabbage black rot in an upland of Southern Philippines

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Abstract. In vivo and in vitro experiments were conducted to evaluate different plant parts of Solanum biflorum Lour. in combination with Lantana camara Linn. as biopesticide against black rot of cabbage. There were nine treatments replicated three times. The least disease severity rating was consistently observed in T4 (fruits of S. biflorum + leaves of L. camara) which was comparable to T1 (Kocide) at 14, 21 and 28 Days After Treatment (DAT). Highly significant variation was observed among means on the number of harvested heads, weight of cabbage and adjusted yield per hectare. Furthermore, T1 (Kocide) and T4 (fruits of S. biflorum + L. camara leaves) had the most number of harvested heads comparable to T6 (roots of S. biflorum + leaves of L. camara). On the adjusted yield per hectare, T1 (Kocide) had the highest yield of 16,800 kg/ha which differed significantly from the other treatments. Among the extracts, T4 (fruits of S. biflorum + leaves of L. camara) had the highest mean of 9,600 kg/ha comparable to T6 (roots of S. biflorum) with 9,200 kg/ha. Based on the results, the fruits of S. biflorum and L. camara leaves proved effective in the control of black rot of cabbage.

1. Introduction
Cabbage (Brassica oleracea) is a leafy green or purple biennial plant, grown as vegetable due to its dense-leaved heads. Weight of cabbage heads generally range from 0.5 to 4 kilograms (1 to 9 lb). The Food and Agriculture Organization of the United Nations (FAO) reports that world production of cabbage and other brassicas for 2011 was almost 69 million metric tons (68 million long tons; 75 million short tons).

Almost half of these crops were grown in China, where Chinese cabbage is the most popular Brassica vegetables. Cabbage, however, is prone to several nutrient deficiencies, as well as multiple pests, bacterial and fungal diseases.

Black rot, caused by the bacterium Xanthomonas campestris pv. campestris, is considered the most serious disease of crucifer crops worldwide [1]. This disease is also known as blight, black stem, black vein, stem rot and stump rot. All crucifer crops are susceptible to black rot; radish and kale, however, are less easily infected. Plants that are not in the crucifer family are not susceptible. Yield can be affected in several ways: infected plants may die prematurely, heads may remain small and quality may be reduced because of symptoms on the marketable part of the plant. Removing symptomatic leaves increases production costs. Soft rot may develop after black rot, further reducing quality and storage life.

Black rot, caused by the bacterium Xanthomonas campestris pv. campestris (Xcc), is a significant disease of cabbage and other crucifer crops worldwide. The pathogen thrives in warm, wet weather, spreading from plant to plant by splashing water, wind-blown water droplets, and by workers or animals.
moving from infected fields to healthy fields. Xcc can spread rapidly during transplant production in greenhouses or seed beds, and could be spreading long before any symptoms are observed. The bacterium can infest seed, infecting young seedlings as they emerge [2].

The control of black rot is difficult and relies on the use of pathogen-free planting material and the elimination of other potential inoculum sources (infected crop debris and cruciferous weeds) [3]. Contaminated cabbage has been linked to cases of food-borne illness in humans.

Thus, this study was conducted to produce a biopesticide against black rot of cabbage. This study specifically aimed to evaluate the efficacy of different plant parts of Solanum biflorum Lour. in combination with Lantana camara Linn. as biopesticide against black rot of cabbage; and determine the effects of the different treatments on the yield performance of cabbage.

2. Methodology
The pathogenic bacterium was isolated from cabbage leaves infected with black rot using streak plate method. A part of the infected tissue was cut and suspended in sterile water and allowed to stand for a few minutes or until the bacterium oozed out. A loopful of the suspension was streaked on the surface of plated Nutrient Agar. The agar plate was incubated for 24 hours. Using a sterile wire loop, a single colony was streaked on an agar slant to produce pure culture.

Whole plant of Solanum biflorum and Lantana camara leaves were collected and brought to the Natural Science Research Center (NSRC) Laboratory for preparation. Roots, leaves and fruits of S. biflorum were segregated and disinfected separately in 10% chlorine solution for 3 minutes and blotted dry in a piece of sterile cheesecloth. Disinfected plant parts were then homogenized separately using sterile mortar and pestle. Sterile water was added to plant parts at 1:1 ratio. Sap was squeezed out using sterile muslin cloth and then stored in a clean beaker through refrigeration until needed. The leaves of L. camara were also homogenized separately in sterile mortar and pestle. Pure sap was separated using sterile muslin cloth. A 1:1 ratio of the stock solution of S. biflorum plant extracts and L. camara leaves were prepared as treatments.

The experiment was laid out in Randomized Complete Block Design (RCBD) with nine treatments replicated three times. The experimental treatments include: T1 - Kocide (Positive Control), T2 - Distilled Water (Negative Control), T3 - Solanum biflorum roots + Lantana camara leaves, T4 - Solanum biflorum leaves + Lantana camara leaves, T5 - Solanum biflorum fruits + Lantana camara leaves, T6 - Roots of S. biflorum, T7 - Leaves of S. biflorum, T8 - Fruits of S. biflorum and T9 - Leaves of L. camara.

The poison technique was used in the assay of the different crude extracts against Xanthomonas campestris pv. campestris. A 1:100,000 bacterial suspension was prepared. One (1) ml of the bacterial suspension, 1 ml of the treatment and 10 ml of Nutrient Agar (NA) were dispensed in sterile Petri dishes. To thoroughly mix, the plates were agitated in a circular manner until the mixture was homogeneous and allowed to stand to solidify. The plates were sealed with parafilm to avoid the evaporation of the volatile compounds. Plates were incubated at room temperature for 48 hours. Bacterial colonies were counted and recorded.

The experimental site (in vivo) was at Victory, Lantapan, Bukidnon. The total land area of 350 sq meters was prepared by plowing and harrowing twice to remove deeply-rooted weeds and to loosen the soil. Seeds were sown in a seed box with sterile soil. Two-week-old seedlings were planted separately in a soil mounted in rolled banana leaves “lukong” and placed in a partially-shaded area for shelter during heavy rain. Seedlings were exposed to the field condition for a week before transplanting by watering and gradual exposure to sunlight.

Thirteen-day-old seedlings were transplanted in a plot at a distance of 25 cm between rows and 25 cm between hills. Transplanting was done late in the afternoon in order to prevent high mortality resulting from seedling dehydration. Vermicompost was applied based on the recommended rate after the result of soil analysis. Soil samples were analyzed at the Soil and Plant Analysis Laboratory (SPAL). Replanting was done to replace the missing hills.

Pure culture of the test organism was inoculated to cabbage plants 24 hours before application of the organic pesticides. Inoculation was done early in the morning. One week after inoculation, treatment
application was done and at weekly basis thereafter. Disease assessment was done prior to treatment application. Blight was assessed using a rating scale of $0 – 4$ ($0 = \text{no symptoms}, 1 = \text{trace amount}, 3 = 1.5$ and $4 = >2.0 \text{cm}^2$ of diseased leaf tissue [4].

Growth parameters such as number of heads, weight of cabbage and yield per hectare were gathered. The yield per hectare was computed using the formula:

$$
\text{Yield per ha.} = \frac{\text{Yield per plot (kg)}}{1 \text{ ton}} \times \frac{10,000 \text{ sq.m}}{1,000 \text{ kg}} \times \frac{1 \text{ ha}}{1 \text{ sq.m}}
$$

3. Results and Discussion

3.1. Number of colony forming units (CFU) of Xanthomonas campestris pv. campestris as affected by the S. biflorum and L. camara extracts at 48 hours after treatment

Colony Forming Units (CFU) of Xanthomonas campestris pv. campestris as influenced by the different combinations of S. biflorum and L. camara are presented in Table 1 and Figure 1. Highly significant variation among treatment means was observed.

In vitro assay proved that the different combinations of plant parts affected the CFU of Xanthomonas campestris pv. campestris. Treatment 2 (roots of S. biflorum + leaves of L. camara) and T9 (fruits of S. biflorum) had lesser CFU with $10.6 \times 10^8$ and $11 \times 10^8$, respectively, which are comparable to the positive control ($T_1$- Kocide) with $7.3 \times 10^8$ CFU. The highest mean CFU was observed in T9 (leaves of L. camara alone) with $24.5 \times 10^9$.

Although the different parts of L. camara were proven for ethnopharmacological potency, as reported by [5], the leaves of L. camara has an antimicrobial component that is effective against some pathogens and microorganisms. The result of the study also show that leaf extract was more effective when combined with the roots of S. biflorum extract.

Table 1. Number of colony forming unit (CFU) of Xanthomonas campestris pv. campestris as influenced by the different treatment combinations of Solanum biflorum and Lantana camara at 48 Hours After Treatment (HAT)

| TREATMENTS | CFU in 1:1,000,000 |
|------------|------------------|
| $T_1$ - Kocide (Positive Control) | $7.333,333,333^1$ |
| $T_2$ - Water (Negative Control) | $20,333,333,333$ $^{abc}$ |
| $T_3$ - Roots of S. biflorum + Leaves of L. camara | $10,666,666,667^fghi$ |
| $T_4$ - Leaves of S. biflorum + Leaves of L. camara | $14,000,000,000^fghi$ |
| $T_5$ - Fruits of S. biflorum + Leaves of L. camara | $18,333,333,333^{abdef}$ |
| $T_6$ - Roots of S. biflorum | $21,666,666,667^ab$ |
| $T_7$ - Leaves of S. biflorum | $19,000,000,000^{bcde}$ |
| $T_8$ - Fruits of S. biflorum | $11,000,000,000^{ghi}$ |
| $T_9$ - Leaves of L. camara | $24,666,666,667^a$ |
| F-test | ** |
|--------|----|
| CV (%) | 23.68 |

Means followed by the same letter in a column are not significantly different at 5% level of probability

** = highly significant

| TREATMENTS | R1 | R2 | R3 |
|------------|----|----|----|
| T1         | ![Image](image1) | ![Image](image2) | ![Image](image3) |
| T2         | ![Image](image4) | ![Image](image5) | ![Image](image6) |
| T3         | ![Image](image7) | ![Image](image8) | ![Image](image9) |
| T4         | ![Image](image10) | ![Image](image11) | ![Image](image12) |
| T5         | ![Image](image13) | ![Image](image14) | ![Image](image15) |
| T6         | ![Image](image16) | ![Image](image17) | ![Image](image18) |
Figure 1. In-vitro assay of the weed extracts against Xanthomonas campestris pv. campestris

3.2. Mean percent severity of bacterial blight (Xanthomonas campestris pv. campestris) as affected by S. biflorum and L. camara extracts at 7, 14, 21 and 28 days after treatment

Table 2 shows the mean percent severity of bacterial blight (Xanthomonas campestris pv. campestris) as affected by weed extracts at 7, 14, 21 and 28 days after treatment. Figure 2 shows cabbage plants assessed for black rot infection at 7 to 28 days after treatment.

Based on the result, plants applied with T₈ (roots of S. biflorum) had the least severity rating of 1.17 while T₂ (Negative Control) had the highest severity rating of 2.12. However, non-significant variation among treatment means was observed.

At 14 DAT, highly significant variation among treatment means was noted. The least severity rating was observed in T₅ (fruits of S. biflorum + leaves of Lantana camara) which was comparable to T₁ (Kocide - Positive Control), T₃ (roots of S. biflorum + leaves of L. camara) and T₈ (fruits of S. biflorum) with an equal mean of 1.58. The highest mean was recorded in T₂ (Water-Negative Control).

The same trend was observed at 21 and 28 DAT. T₅ (fruits of S. biflorum + leaves of L. camara) consistently had the least severity rating comparable to T₁ (Kocide - Positive Control) with an equal mean of 1.68 and T₈ (fruits of S. biflorum) and T₆ (roots of S. biflorum) with 1.86 and 1.96, respectively.

| TREATMENTS                             | DAYS AFTER TREATMENT (DAT) |
|----------------------------------------|-----------------------------|
|                                        | 7   | 14  | 21  | 28  |
| T₁ - Kocide (Positive Check)           | 1.22| 1.58| 1.66| 1.68a |
| T₂ - Water (Negative control)          | 2.12| 3.96| 4.00| 4.00g |
| T₃ - Roots of S. biflorum + Leaves of L. camara | 1.38| 1.58| 2.06| 2.92de |
| T₄ - Leaves of S. biflorum + Leaves of L. camara | 1.83| 1.96| 2.96| 3.12def |
| T₅ - Fruits of S. biflorum + Leaves of L. camara | 1.25| 1.52| 1.58a| 1.68a |
| Treatment                  | Mean 1 | Mean 2 | Mean 3 | Mean 4 |
|----------------------------|--------|--------|--------|--------|
| T6 - Roots of *S. biflorum* | 1.17   | 1.62   | 1.85   | 1.96   |
| T7 - Leaves of *S. biflorum* | 1.92   | 2.22   | 2.58   | 2.78   |
| T8 - Fruits of *S. biflorum* | 1.42   | 1.58   | 1.82   | 1.86   |
| T9 - Leaves of *L. camara*   | 1.92   | 2.46   | 2.96   | 3.12   |

F-test | ns | ** | ** | ** |
C.V. (%) | 19.00 | 21.18 | 17.25 | 16.35 |

Means followed by the same letter in a column are not significantly different at 5% level of probability (DMRT)

** = highly significant
ns = not significant

T1
(Kocide – Positive Control)

T2
(Water - Negative Control)

T3
(Roots of *S. biflorum* + Leaves of *L. camara*)

T4
(Leaves of *S. biflorum* + Leaves of *L. camara*)
T5
(Fruits of *S. biflorum* + leaves of *L. camara*)

T6
(Roots of *S. biflorum*)

T7
(Leaves of *S. biflorum*)

T8
(Fruits of *S. biflorum*)

T9
(Leaves of *L. camara*)

Figure 2. Cabbage plants showing black rot symptoms at: (A) 7, (B) 14 and (C) 21 days after treatment

In the study of [6], plant extracts presented antibacterial activity against clinically relevant pathogens (gram positive and gram negative). *L. camara* leaves extract was active against *P. vulgaris* and *V. cholerae* (MIC 128 μg/mL for both strains); against *P. vulgaris* and *P. aeruginosa* (MIC 8 μg/mL) and two strains of *E. coli* (MIC 16 μg/mL for the multi-resistant strain). Previous studies using extracts from *Lantana* species showed that they were able to inhibit the growth of gram-positive bacteria strains.

Table 3 shows the number of harvested heads, weight of cabbage and adjusted yield per hectare as affected by the different treatments. Highly significant variation was observed among treatment means.

Result of the study shows that T1 (Positive Control - Kocide) and T4 (fruits of *S. biflorum* + *L. camara* leaves) had the greatest number of harvested heads which was comparable to T5 (roots of *S. biflorum* + leaves of *L. camara*) with an equal mean of 24.00. Treatment 6 (roots of *S. biflorum*) and T8 (leaves of *L. camara*) had 22.50 and 20.50 heads, respectively.

The mean weight of harvested heads was also presented in Table 3. The heaviest was recorded in T1
(Positive Control - Kocide) with an average of 8.4 kgs which differed significantly from the other treatments, followed by T₅ (fruits of *S. biflorum* + *L. camara* leaves) with 4.8 kgs. This result can be attributed to the less number of infected leaves on these treatments compared to treatments with severe infection.

**Table 3. Number of cabbage heads, weight and adjusted yield per hectare of cabbage as affected by the different treatments**

| Treatments                                      | Number of Heads | Weight (kg) | Adjusted Yield (kg/ha) |
|------------------------------------------------|----------------|-------------|------------------------|
| T₁ – Kocide (Positive Control)                 | 24.00<sup>a</sup> | 8.40<sup>a</sup> | 16,800<sup>a</sup> |
| T₂ – Water (Negative control)                 | 8.50<sup>ef</sup> | 1.60<sup>fg</sup> | 3,200<sup>de</sup> |
| T₃ - Roots of *S. biflorum* + Leaves of *L. camara* | 21.50<sup>abc</sup> | 4.20<sup>bcd</sup> | 8,400<sup>c</sup> |
| T₄ - Leaves of *S. biflorum* + Leaves of *L. camara* | 13.00<sup>e</sup> | 2.40<sup>f</sup> | 4,800<sup>d</sup> |
| T₅ - Fruits of *S. biflorum* + Leaves of *L. camara* | 24.00<sup>a</sup> | 4.80<sup>b</sup> | 9,600<sup>b</sup> |
| T₆ - Roots of *S. biflorum*                     | 22.50<sup>ab</sup> | 4.60<sup>bc</sup> | 9,200<sup>bc</sup> |
| T₇ - Leaves of *S. biflorum*                    | 8.50<sup>ef</sup> | 1.60<sup>fg</sup> | 3,200<sup>de</sup> |
| T₈ - Fruits of *S. biflorum*                    | 20.50<sup>abcd</sup> | 4.20<sup>bde</sup> | 8,000<sup>cd</sup> |
| T₉ - Leaves of *L. camara*                     | 8.50<sup>ef</sup> | 1.60<sup>fg</sup> | 3,200<sup>de</sup> |
| F-test                                         | **             | **          | **                     |
| C.V. (%)                                       | 11.38          | 18.36       | 13.18                  |

Means followed by the same letter in a column are not significantly different at 5% level of probability

** = highly significant

On the adjusted yield per hectare, T₁ (Positive Control - Kocide) had the highest yield of 16,800 kg/ha which differed significantly from the other treatments. However, among the extracts, T₅ (fruits of *S. biflorum* + leaves of *L. camara* had the highest mean of 9,600 kg/ha which was comparable to T₆ (roots of *S. biflorum*) with 9,200 kg/ha.

4. **Summary, conclusion and recommendation**

The study was conducted with the following objectives: to evaluate the efficacy of different plant parts of *Solanum biflorum* Lour. in combination with *Lantana camara* Linn. as biopesticide against black rot of cabbage; and to determine the effects of the different treatments on the yield performance of cabbage.

The *in vivo* study was conducted at Victory, Lantapan, Bukidnon while the *in vitro* experiment was conducted at Natural Science Research Center (NSRC), Central Mindanao University, Musuan, Maramag, Bukidnon. The study was laid out in Randomized Complete Block Design with nine treatments and replicated three times.

Statistical analysis showed no significant variation among treatment means on the percent severity of bacterial blight (*Xanthomonas campestris* pv. *campestris*) at 7 days after treatment. At 14 DAT, however, highly significant variation among treatment means was observed. The least severity rating was observed in T₄ (fruits of *S. biflorum* + leaves of *L. camara*) which was comparable to T₁ (Positive
Control - Kocide). At 21 and 28 DAT, T4 (fruits of S. biflorum + leaves of L. camara) consistently had the least severity rating comparable to T1 (Positive Control - Kocide).

Highly significant variation was also observed among means on the number of harvested heads, weight of cabbage and adjusted yield per hectare as affected by the different treatments. Result of the study showed that T1 (Positive Control - Kocide) and T4 (fruits of S. biflorum + L. camara leaves) had the greatest number of harvested heads which was comparable to T4 (roots of S. biflorum + leaves of L. camara).

The heaviest heads were observed in T1 (Positive Control - Kocide) with an average of 8.4 kgs which differed significantly from the other treatments, followed by T4 (fruits of S. biflorum + L. camara leaves) with 4.8 kgs. These results can be attributed to the less number of infected leaves on the treatments compared to those with severe infection.

On the adjusted yield per hectare, T1 (Positive Control - Kocide) had the highest yield of 16,800 kg/ha which differed significantly from the other treatments. Among the extracts, T4 (fruits of S. biflorum + leaves of L. camara) had the highest mean of 9,600 kg/ha which was comparable to T6 (roots of S. biflorum) with 9,200 kg/ha.

Based on the results of the study, combining the fruits of S. biflorum and L. camara leaves proved effective in the control of black rot of cabbage. It is recommended that the active principle in the plants be identified. Furthermore, the efficacy of the extracts be tested in other cabbage-growing areas in the Philippines.

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