The potential use of plant extracts for controlling northern leaf blight on organic sweet corn production

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Abstract. Northern leaf blight caused by Helminthosporium turcicum is the most frequently occurring foliar diseases in sweet corn plants grown under humid environments. Finding an effective control measure for controlling the disease can be a challenging issue for organic growers. The present study was undertaken to investigate the potential use of plant products as the botanical fungicides for controlling northern leaf blight in sweet corn. The aqueous solutions of extracts mixture and its individual constituent extracts made from neem leaf, betel leaf, clove leaf, lemon grass leaf, and galangal rhizome were tested in petri dishes assay for their antifungal activity against H. turcicum. Lemongrass+galangal rhizome exhibited the most effective solution in suppressing the growth of H. turcicum (55%), as followed by galangal rhizome (50%), lemongrass+clove leaf (50%), betel leaf+galangal rhizome (50%), and cloveleaf+neem leaf (47.5%). Accordingly, these 5 solutions were selected and tested for their efficacy in controlling northern leaf blight on sweet corn plants under field condition. Lemongrass+galangal rhizome, galangal rhizome, lemongrass+clove leaf, betel leaf+galangal rhizome, and cloveleaf+neem leaf had similar effectiveness in reducing disease intensity ranged from 55% to 66% and leaf disease severity ranged from 44% to 52% as compared to the control. Betel leaf+galangal rhizome was relatively effective in reducing disease intensity (43.4%) but less effective in reducing leaf disease severity (24%). Application of the selected solution had reduced the ear length but improved the ear weight excluding betel leaf + galangal rhizome.

1. INTRODUCTION

Northern leaf blight (NLB) is known to be the most devastating foliar diseases in sweet corn. The disease is characterized by long elliptical, grayish-green or tan leaf lesions. As the disease develops, single lesions could collectively unify to form vast blighted areas [1]. The symptom usually begins on the lower leaves and then moves upward. Under favorable conditions, the lesions increase in size and number until very little living tissue remains [2].

Ear yield losses in sweet corn caused by NLB disease vary depending upon weather conditions, plant growth stage, number and position of leaves affected, and susceptibility or resistance of the plant varieties [3,4]. The loss is predominantly caused by the severe chlorotic and necrotic lesions in the leaf tissues that reduce the leaves capability in conducting photosynthesis to produce carbohydrate for grain filling [5]. Each 10% increase northern leaf blight infection can cause 2 to 8% of ear yield loss[4]. Similarly, old appearance and poor quality of ear resulted from NLB lesions on the husk will reduce the value of ear fresh market [6].
NLB is incited by the fungus *Helmintosporium turcicum* (syn. *Exserhisum turcicum*). In the tropic, the disease is endemic to sweet corn growing area of mid-altitude areas for having high humidity, low temperature and cloudy weather which is conducive to the growth of fungus [7]. The use of synthetic fungicides is commonly practiced as the control measure for the development of NLB [8,9,10]. For organic sweet corn growers, however, the avoidance of synthetic fungicides use for controlling the disease is a great challenge due to the rigorous restrictions in the crop production. Over the years, different alternative to synthetic fungicides has been tested with some promising levels of efficacy in controlling the diseases[11, 12, 13, 14]. The objective of this present study was to investigate the potential use of plant products as the botanical fungicides for controlling northern leaf blight in sweet corn.

2. MATERIALS AND METHOD

2.1. Laboratory experiment

2.1.1. Source of isolate *H. turcicum*

It was isolated from the leaf samples of sweet corn grown at NLB endemic area of Sukamarga, RejangLebong Regency, Bengkulu Province, Indonesia. The leaf samples were cut into 5 mm fragments and surface-sterilized in 0.5% sodium hypochlorite for 1 minute and washed with distilled water. The leaf fragments (5 mm) carrying chlorotic/ necrotic tissues were cultured on petri dishes containing potato dextrose agar (PDA) for 7 days incubation at room temperature. The resulting colonies showing typical features of *H. turcicum*[15, 16] were transferred on fresh media for further 7 days incubation to obtain a pure culture of the fungi.

2.1.2. Preparation of aqueous solutions of plant extracts

Prior to extraction, neem leaf (*Azadirachtaindica* A.Juss), betel leaf (*Piper betle* L.), clove leaf (*Syzygiumaromaticum* L.), lemongrass leaf (*Andropogannardus* L.), and galangal rhizome (*Alpinia galangal* L.) were washed with running tap water and placed between two towel papers to remove the excess water. As much as 0.5 kg of each plant material was cut into 5 mm pieces and finely ground with 0.5 L distilled water in a blender. The solution of plant extract was prepared for each material by filtrating the ground through a cotton cloth.

2.1.3. Antifungal activity assay

The in vitro assay was carried out by placing a paper disk 1 cm in diameter that previously dipped in the solution of plant extract(s) and *H* turcicum colony 1 cm in diameter from pure culture on a petri dish (8 cm in diameter) containing PDA at 4 cm apart from each other (Figure 1). Fifteen solutions containing both single plant extract and the combination of two plant extracts were examined in this essay. The control was set up by placing the colony on the media alone. After 7 days incubation at room temperature, the antifungal activity on the medium was determined by the percentage of fungal growth inhibition as \([{(C-T)/C} \times 100\%]\), where C and T represent the radial fungal growth on control and treated media, respectively [17].
2.2. Field Experiment

2.2.1. Experimental site and design

The field experiment was conducted at the area where the H turcicum isolate was collected. The soil has previously been used for the organic production of sweet corn and other vegetables crops for two years. A randomized complete block design with three replications was used to allocate the treatments on the experimental plots consisted double row of sweet corn plants, each 400 cm in length and spaced 70 cm apart. The treatments were 5 solutions made of single or combination of two plant extracts showed high antifungal activity in the laboratory assay. These were galangal rhizome, lemongrass leaf + galangal rhizome, betel leaf + galangal rhizome, lemongrass leaf + clove leaf, and clove leaf + neem leaf.

2.2.2. Crop management and treatments application

Prior to planting, the soil was tilled and amended with 10 ton ha$^{-1}$ cow manure as basal fertilizer. Seeds of ‘Talenta’ sweet corn were sown at 2 to 3 cm deep with a plant-to-plant spacing of 20 cm to obtain 20 plants population in each row. As the additional fertilizer, a locally made liquid organic fertilizer as used by [18, 19] was sprayed to the plants 4 times during the growth period with two weeks interval. Similarly, the treatments of plant extract solutions were sprayed to the plants at 30, 44, 58, and 72 days after planting (DAP) with total volume of the application at 850 ml plant$^{-1}$. The solutions of plant extract were prepared using the same procedure as described in the laboratory experiment. Weed control was done manually at 21 and 45 DAP. No synthetic pesticides were applied during the experimentation. Harvest was carried out in the morning at 30 days following silking as the ears had fully developed and turned dark green, silks turned brown, and kernels were soft and plump with milky juice when squeezed.

2.2.3. Data collection and analysis

Data were collected for percentage of diseased plants, disease intensity, leaf disease severity, ear length, ear diameter, and ear weight. The percentage of diseased plants was measured as $P = (a/n) \times 100\%$, where $a =$ number of diseased plants in the plot population and $n =$ total number of the plant in the plot population. Disease intensity was measured as $DI = \frac{\sum (n \times v)}{(N \times Z)} \times 100\%$ [20], where $\sum (n \times v) =$ the sum of products when the number of diseased plants is multiplied by the corresponding grade of damaged leaves; $N =$ total number of plants observed, $Z =$ The scale’s highest grade.

The grade of damaged leaves was determined on the basis of symptomatic appearance on leaf surface and spread from lower to upper leaves as depicted on Figure 2, where $1 =$ no symptoms; $2 \leq 1\%$ of leaves were symptomatic; $3 = 1-10\%$ of leaves were symptomatic; $4 = 11-25\%$ of leaves were symptomatic; $5 \geq 50\%$ of the lower leaves were symptomatic, $< 25\%$ of center (the four leaves closest

![Figure 1. The arrangements of H turcicum colony (H) and paper disk dipped in plant extract solution (E) on the control plate (a) and treated plate (b).](image)
to the primary ear) and upper leaves were symptomatic; 6 = lower leaves were dead, > 50% of the center leaves, < 25% of upper leaves were symptomatic; and 7 = plant was dead [21]. Leaf disease severity was scaled into 1 to 4 grades on the basis of the lesion type of leaf sample at dough stage as shown on Figure 3, where 1 = stripe or narrow elliptical greenyellowish, 2 = narrow, long, elliptical gray lesion with greenyellowish, 3 = long, elliptical, gray lesion with green-yellowish border; and 4 = long, elliptical, gray or tan colored lesion[21]. Ear length, ear diameter, and ear weight were measured on the unhusked ear of 5 plant samples.

Figure 2. Scales of symptomatic appearances on leaf surface and spread from lower to upper leaves for determination of disease intensity (Reid and Zhu 2005).

Figure 3. Scale of lesion types on leaf at dough stage for determination of leaf disease severity (Reid and Zhu 2005).

The data were subjected to analysis of variance to show the significant differences among the solution of plant extract(s) on the observed traits. Means separation was carried out using least significant difference at level P < 0.05. The analysis was performed using SAS version 9 [22].

3. RESULTS AND DISCUSSION

3.1. Antifungal activity assay
The overall performances of the aqueous solutions made from different plant extracts in suppressing the growth of *H. turcicum* mycelia are depicted in Table 1 and Figure 1. Although a total inhibition of the fungal growth was not observed in the assay, most of the extracts exhibited some degrees of
inhibition, notably Lemongrass+galangal rhizome (55%), galangal rhizome (50%),
lemongrass+clove leaf (50%), betel leaf+galangal rhizome (50%), and cloveleaf+neem leaf
(47.5%). The efficacy of these plant materials in controlling the fungal growth has been
reported many workers [23, 24, 25, 26, 27].

![Figure 4](image)

**Figure 4.** The in vitro assay showing the antifungal activity of the 15 aqueous solutions of plant extracts on the growth of *H. turcicum* mycelia

| No. | Plant extract                     | Fungal growth inhibition (%) |
|-----|-----------------------------------|------------------------------|
| 1   | Lemongrass leaf                   | 12.5                         |
| 2   | Betel leaf                        | 0.0                          |
| 3   | Clove leaf                        | 35.0                         |
| 4   | Neem leaf                         | 37.5                         |
| 5   | Galangal rhizome                  | 50.0                         |
| 6   | Lemongrass leaf + betel leaf      | 42.5                         |
3.2. Disease incidence and plant yield performance under field conditions
Most of the studies regarding the use of plant extracts in controlling plant pathogens were conducted under laboratory environments and their results are hardly tested under field conditions. In this study, it was revealed that foliar application of 5 aqueous solutions of plant extracts had significant effects on disease intensity, leaf disease severity, ear length, and ear weight (Table 2).

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Table 2. Mean squares of disease incidence and plant yield performance as treated with aqueous solution of plant extracts

| Source of variation | df | Diseased plant | Disease intensity | Mean Square |
|---------------------|----|----------------|-------------------|-------------|
| Block               | 2  | 52.59ns        | 114.44ns          |             |
| Plant extract       | 5  | 28.92 ns       | 632.87**          |             |
| Error               | 10 | 26.93          | 39.09             |             |

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Values with * or ** are significant at P=0.05 or 0.01; value with ns are not significant at P=0.05

A closer inspection of the mean values presented in Table 3, it can be noted that the treatments had reduced the disease intensity and leaf disease severity caused by NLB as compared to the control. Lemongrass + galangal rhizome, galangal rhizome, lemongrass + clove leaf, betel leaf + galangal rhizome, and clove leaf + neem leaf exhibited similar effectiveness in reducing disease intensity ranged from 55% to 66% and leaf disease severity ranged from 44% to 52% as compared to the control. Betel leaf + galangal rhizome was relatively effective in reducing disease intensity (43.4%) but less effective in reducing leaf disease severity (24%). In all cases, the application of plant extracts had shortened the ear length by 3.4 to 3.9 cm (12 to 13%) as compared to the control, but ear weight was increased by 3 to 62 g (1 to 18%). The efficacy of these plant extracts could possibly be enhanced by adding a natural surfactant, such as aqueous rind extract of Sapindusrarak, in the solution as to minimize the effect of rain or excessive moisture [28].
### Table 3. Mean values of disease incidence and plant yield performance as treated with 5 aqueous solutions of plant extracts

| Plant extract                  | Diseased plant (%) | Disease intensity (%) | Leaf disease severity (Score) | Ear length (cm) | Ear diameter (cm) | Ear weight (g) |
|-------------------------------|--------------------|-----------------------|-------------------------------|-----------------|-------------------|----------------|
| Control                       | 100 a              | 60.6 a                | 2.5 a                         | 28.60 a         | 5.56 a            | 346 c          |
| Galangal rhizome              | (100%)             | (100%)                |                               |                 |                   |                |
| Lemongrass leaf+galangal rhizome | 92.3 a       | 20.6 c                | 1.3 c                         | 24.80 b         | 6.00 a            | 396 ab         |
| Lemongrass leaf+clove leaf    | (92%)              | (34%)                 |                               |                 |                   |                |
| Betel leaf+galangal rhizome   | 100 a              | 34.3 b                | 1.9 b                         | 24.73 b         | 5.53 a            | 349 bc         |
| Clove leaf+neem leaf          | (100%)             | (45%)                 |                               |                 |                   |                |

The differences among values marked with the same letter are not significant on LSD test at P=0.05. Values in brackets are the percentage form of relative change from control.

### 4. CONCLUSION

Aqueous extract solutions of galangal rhizome, lemongrass leaf+galangal rhizome, betel leaf+galangal rhizome, lemongrass leaf+clove leaf, and clove leaf+neem leaf have shown their potential use for suppressing _H. turcicum_ growth and controlling NLB to enable sweet corn plants of maintaining their yield. Further studies are required to develop suitable formulations of the extract to gain the improved efficacy and handling.

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