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The Evaluation of Secondary Metabolites in *Saccharum officinarum* L. and *Mimosa invisa* Mart. as Natural Herbicides

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Abstract

The giant sensitive plant (*Mimosa invisa* Mart.) is a major weed found in the sugarcane (*Saccharum officinarum* L.) farming areas, which dominates and adversely affects the quantity and quality of the harvest. This weed poses a threat to both the sugarcane farmers and sugar companies because it causes about a 6 % to 9 % decline in the plant's biomass and a 0.09 % reduction in the crop yield. *M. invisa* has great competitiveness against the crop plant *S. officinarum* and also an increased population efficiency of 14.08 % in the first year (plant cane) to 38.55 % in the third year (3rd ratoon). Therefore, this research aims to determine the metabolic compounds in the rhizosphere of *M. invisa* and *S. officinarum* and assess their roles as allelochemicals. Research was conducted at the Laboratory of Agrotechnology, Faculty of Agriculture and Animal Science, University of Muhamadiyah Malang, and Central Laboratory of the Indonesian Legume and Tuber Crops Research Institute, Malang. The field research was carried out in the Research Garden Krebet Baru Sugar Factory, Malang, East Java, Indonesia. The descriptive qualitative research design was used and data were arranged randomly in groups of three treatments and three replications. The treatment details were planted in different plots of land each as, T_1: *Mimosa invisa* only, T_2: *Mimosa invi*sa mixed *Saccharum officinarum*, and T_3: *Saccharum officinarum* only. Furthermore, the analysis of metabolic compounds, using the Gass Cromatografy Mass Spectromotometry (GCMS), showed the presence of octadecanoic acid (an allelochemical compound) and methyl ester (2.06 %) in the rhizosphere of *M. invisa* and *S. officinarum*. These metabolite compounds are commonly used as herbicide activators.

Keywords: Allelochemical, Bio-herbicide, Eco-friendly technology, Giant sensitive plant, Sugarcane.

1. Introduction

According to the Central Statistics Agency (*Badan Pusat Statistik*) in 2019, the required consumption of sugar in Indonesia reached 5.1×10^6 t, while national sugar production is currently 2.36×10^6 t. In addition, there was a decline in national sugar production which was up to 2.22×10^6 t (Cindy, 2020). This decrease in sugar production was a result of the raw material, i.e. sugarcane (*Saccharum officinarum* L.), facing challenges such as competition with weeds which has, in turn, impacted the yield of sugarcane production. This weed poses a threat to both the sugarcane farmers and sugar companies because it causes about a 6 % to 9 % decline in the plant's biomass and a 0.09 % reduction in the crop yield. Additionally, the presence of this weed during the vegetative growth stage of the sugarcane crops results in a yield reduction of up to 40 %. Conversely, the weed competition at the growth stages of 3 wk, 6 wk, and 9 wk after planting the crop resulted in a decrease of 77.6 %, 50.6 %, and 4.7 % in the yield (Concenço *et al*., 2016; Faheem and Muhammad, 2015; Fanny *et al*., 2019; Gulshan and Bhagirath, 2020; Peng 2012; Sugar Research Australia, 2021).

In this stuation, the giant sensitive plant — *Mimosa invisa* Mart (also referred to in the literature as *Mimosa diplotrica*, C Wright) is a field crop weed that causes a decrease in the quality and quantity of crop production (Dania and Bamidele, 2006; Osariyekemwen, 2020; Prajal *et al*., 2020; Rao *et al*., 2018). It has been shown in previous research that *M. invisa* has great competitiveness against the crop plant *S. officinarum* and also an increased population efficiency of 14.08 % in the first year (plant cane) to 38.55 % in the third year (3rd ratoon) (Aekrathok, *et al.* 2021; Jayasree, 2006; Phil and Emilie, 2017; Zainol, 2017). Additionally, the mechanism of competition

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between weeds and cultivated plants growing around them is through allelopathy, whereby biochemicals released by the weeds inhibit the growth of other plants around them (Abu-Romman *et al*., 2015; Chauhan, 2008; Dania and Bamidele, 2006; Gulshan and Bhagirath 2020). Conversely, allelopathy occurs when a chemical component produced by plants interacts with other plants growing around them. It is used by weeds to compete with other plants growing around them (*Aliyu et al*, 2018; Ejaz, 2003; Erida *et al*. 2019). Subsequently, the results obtained will reveal that *M. invisa* weed extract can inhibit the germination of seeds of other plants growing in its vicinity, giving it the potential of a bioherbicide (Albuquerque *et al*., 2011; Ejaz, 2003; Hussain *et al*., 2021; Permatasari *et al*., 2020; Sadiqullah *et al*., 2019). Therefore, this research aims to determine the metabolite compounds in the giant sensitive plant (*M. invisa*) weed rhizosphere and sugarcane plant (*S. officinarum*) together with their roles as allelochemicals.

2. Materials and Methods

2.1. Field Research

This research was conducted in the upland (non-irrigated) area of the Research Garden Farming Facilities (*Bina Sarana Tani*) Krebet Baru Sugar Factory, Bululawang, Malang, East Java, Indonesia (coordinant 120° 37' 30" to 70° 58' 10") in Latosol soil, pH 5.5. Additionally, the climate classification of Schmidt and Ferguson was included in type C (slightly wet) grading. The average monthly rainfall data for the last 10 yr also showed that it amounted to 1 898.2 mm, with rainfall of about 102.5 d.

The plant *S. officinarum* was planted in a double row system using the stem cutting BL (Bululawang) cultivar, with a center-to-center spacing of 100 cm to 110 cm and a width of 50 cm to 60 cm. The fertilizers NPK were used at the rates of 150 (N), 105 (P_2O_5), and 150 Kg (K_2O), respectively. The BL stem cutting was also planted in October during the beginning of the rainy season.

Consequently, the seeds of *M. invisa* were taken from the sugarcane cultivated fields and sown in polybags. These weeds were then maintained up to a height of approximately 10 cm. Furthermore, after the sugarcane seeds had sprouted, these weeds were planted with them according to the treatment.

The descriptive qualitative design was used for this research, and the experimental units were arranged randomly in groups consisting of three treatments and three replications. T_1 plot of land was planted with *M. invisa* only, T_2 with *M. invi*sa and *S. officinarum*, and T_3 with *S. officinarum* only. The data was analyzed using the standard deviation with the Equation (1) (Adinurani 2016 ; Iftikhar and Hayat, 2021)

$$s = \sqrt{\frac{n\sum_{i=n}^{n} x_i^2 - (\sum_{i=1}^{n} x_1)^2}{n(n-1)}} \quad (1)$$

Notes: S = standard deviation ; X_i = value of X to i ; n = sample size

2.2. Laboratory Test

This research was conducted in the Laboratory of Agrotechnology, Faculty of Agriculture and Animal Science, University of Muhamadiyah Malang, and the Central Laboratory of the Indonesian Legume and Tuber Crops Research Institute, Malang, East Java, Indonesia within February to May 2019. Furthermore, the metabolite compound was analyzed using a Gas Chromatography-Mass Spectrophotometry (GC-MS) type QP2010S Shimadzu (Japan), semi-polar column RXi-5MS, with Helium carrier gas flow rate of 0.5 mL min^{-1}, and pressure of 27.4 kPa. The initial temperature of the GC 120 ^oC oven was increased with a speed of 5 ^oC min^{-1} until it reached 320 ^oC min^{-1} and the sample volume of 1 uL to 2 uL (Ahmed and Annadurai, 2017). Also, the data obtained were analyzed with the NCBI (National Center for Biotechnology Information) database to obtain specific metabolites.

2.3. Roots Extract of Saccharum officinarum and Mimosa invisa

The roots of *S. officinarum* and *M. invisa* weeds were extracted using the reference-based method (Gusthinnadura, *et al*. 2017; Kokosa, *et al*., 2019; Ram, *et al*. 2019). Conversely, the roots of both plants were taken 15 cm to 20 cm from the base of the rootstock in a circle as deep as 25 cm to 30 cm, to ensure they remained intact without damage. After collection, these roots were washed with water and extracted as follows; 10 g of the roots sample were crushed by Bamix, and 1:2.5 mL of absolute methanol solvent was added. The Rotator shaker gemmy-VRN-360 was then used as an extractor for 2 h. Afterward, 5 mL supernatant was added at an absolute methanol ratio of 1:1, after which the extract underwent centrifugation using the Centrifuge Digital DLABDM 0412 at a speed of 4 000 rpm for 15 min (1 rpm = 1/60 Hz).

Furthermore, 7.5 mL each of supernatant and cold methanol was added in the ratio 1:1. This also underwent centrifugation using Centrifuge Digital DLABDM 0412 at a speed of 4 000 rpm for 15 min. Lastly, the pellets were taken and 1 mL absolute methanol was added in preparation for GC-MS testing which was done at a retention time of 39.46 min.

3. Results and Discussion

3.1. Results

The results of the analysis of four rhizosphere samples from *M. invisa* and *S. officinarum* plants showed the presence of many types of allelopathic compounds, also known as allelochemical compounds. These different compounds are identified as shown in the following, Figure 1, Figure 2, and Figure 3.

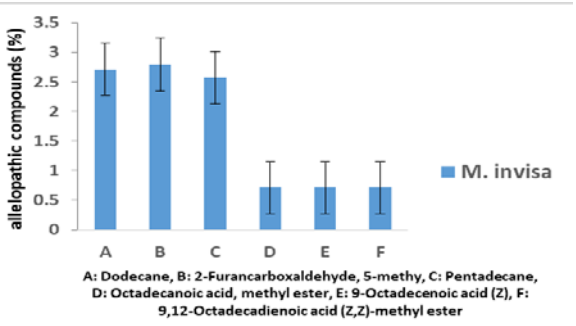

Figure 1. Allelochemical compounds from rhizosphere samples of *M. invisa*

From Figure 1 above, the results showed that the *M. invisa* root extracts from the rhizosphere when grown alone contained allelochemical compounds such as Dodecane (6.83 %), 2-Furan carboxaldehyde, 5-methyl (7.28 %), and Pentadecane (6.10 %).

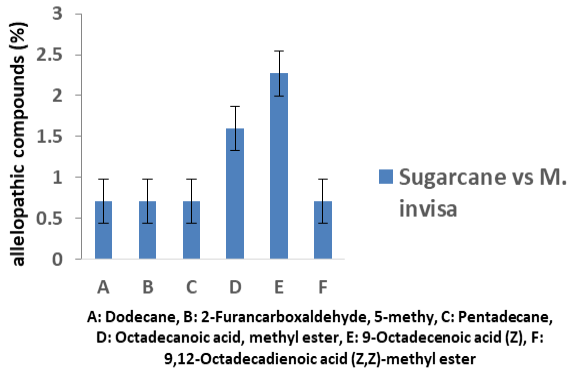

Figure 2. Allelochemical compounds from rhizosphere samples of *S.officinarum* mixed with *M. invisa*.

Figure 2 above showed both *S. officinarum* and *M. invisa* in the rhizosphere when grown side by side contained Octadecanoic acid, methyl ester (2.06 %), and 9,12-Octadecadienoic acid (Z, Z)-methyl ester (3.86 %).

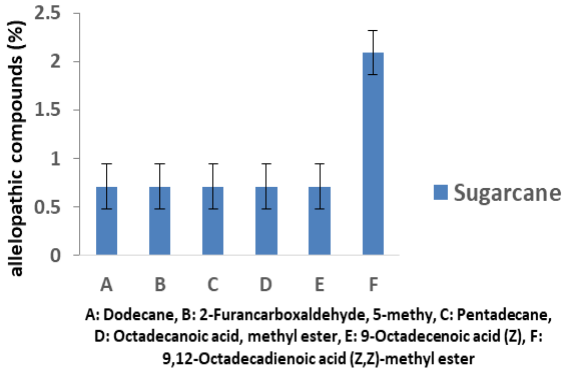

Figure 3. Allelochemical compounds from rhizosphere samples of *S. officinarum*

The Figure 3 above shows that the results of the identification of *S. officinarum* root extract from the rhizosphere when grown alone contained allelochemical compounds such as 9-Octadecenoic acid (Z) 4.64 %.

The results of the GC-MS chromatogram of dodecane compounds are presented in Figure 4.

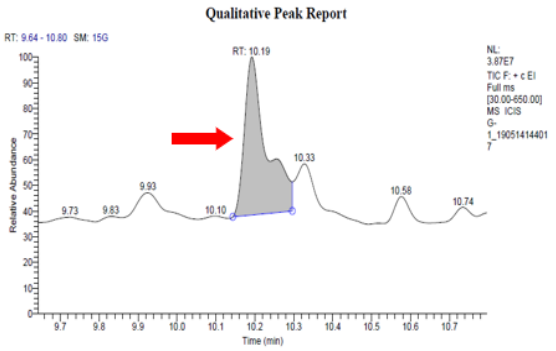

Figure 4. The GC-MS chromatogram of dodecane compounds.

Figure 4 above showed that the rhizosphere of the weeds contained allelopathic compounds such as dodecane (6.83 %). Additionally, dodecane is a metabolite compound in plants that has the molecular formula $C_{12}H_{26}$ with a molecular weight of 170.33 g mol^{-1}. This compound is classified as essential oil which has the chemical name dihexil. Furthermore, dihexil is used in micro-extraction solvents for the formation of the herbicide, Triazine, which is an active herbicide used to control both grassy and broadleaf weeds. (Kokosa, 2019).

The results of the GC-MS chromatogram for the compound 2-furan carboxaldehyde, 5-methyl are presented in Figure 5.

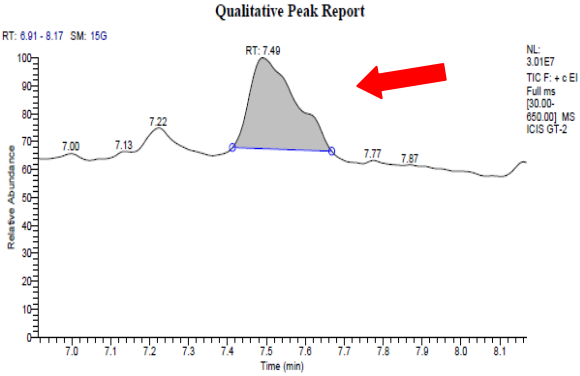

Figure 5. The GC-MS chromatogram for the compound 2-furan carboxaldehyde, 5 methyl.

Figure 5 above shows the results of the GC-MS chromatogram of 2-furan carboxaldehyde, 5-methyl compounds. Conversely, the compound 2-furan carboxaldehyde, 5-methyl, with the molecular formula, $C_6H_6O_2$ are found in weed root extracts and belong to a class of furfural compounds that have allelopathic potential in plants. In addition, it is also one of the main allelochemical compounds in the methanol root extract from the marigold plant (Genus: *Tegetes* L.) (Chotsaeng, 2018).

The results of the GC-MS chromatogram of pentadecane compounds are presented in Figure 6.

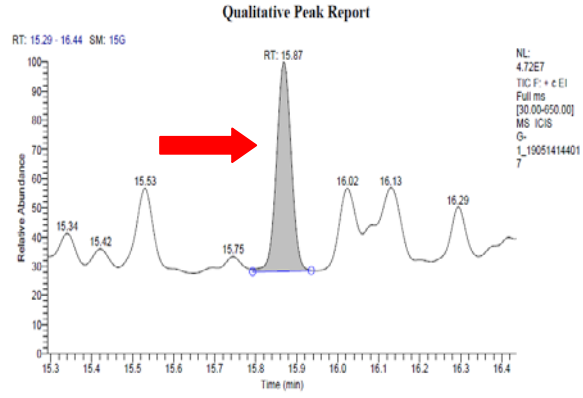

Figure 6. The GC-MS chromatogram of pentadecane compounds

The results in the figure above showed that pentadecane metabolites with the molecular formula $C_{15}H_{32}$ are a group of essential oils whose presence in plants functions as metabolites. Conversely, the pentadecane compounds released by plants have the potential to cause negative effects on the growth of surrounding plants. Furthermore, several essential oil components screened for allelopathic activity showed growth inhibition in *Lactuca sativa* L. and *Lactuca perenne* L. (Jones, 2012).

The results of the GC-MS chromatogram for the compound 9-Octadecenoic acid (Z, Z) are presented in Figure 7.

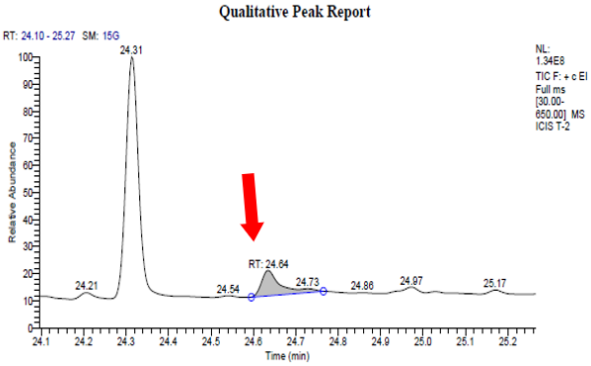

Figure7. The GC-MS chromatogram for the compound 9-Octadecenoic acid (Z, Z)

The results in Figure 7 above showed that the *S. officinarum* and *M. invisa* rhizosphere contained 9,12-octadecadienoic acid (Z, Z), methyl ester (3.86 %) compounds. Additionally, the *S. officinarum* root rhizosphere extraction contains the allelochemical compound 9,12-octadecadienoic acid (Z, Z) which has the molecular formula $C_{19}H_{34}O_2$ and is known to inhibit plant's growth. Karimi *et al*., (2011) stated that the ethyl acetate extract of walnut tree roots containing n-hexadecanoic acid, 9, 12-octadecadienoic acid (Z, Z), and 8-octadecenoic acid was able to suppress the germination and growth of cabbage (*Brassicaoleracea* L. var. capitata) seedlings (Chauhan, 2008).

4. Discussion

The results of the GC-MS analysis showed that the extracts of *S. officinarum* and *M. invisa* in the environment of different rhizosphere ecosystems contained different secondary metabolite compounds. This is presumbly due to the different conditions of the plant rhizosphere ecosystem making the compound contents within also different. Therefore, the research results showed that the diversity of secondary metabolite compounds was influenced by the environment in which the plants grew, specifically the soil nutrient content and the type of material to be identified (Asadi *et al.*, 2019; Sirikantaramas, 2008).

Furthermore, plants were shown to have growth organs that contained metabolite compounds. Conversely, the process of synthesis of plant secondary metabolite compounds is influenced by several factors including genetics, environmental stress, and physical factors (Aliyu *et al*., 2018; Andrew *et al*., 2014; Darmanti, 2018). Additionally, the difference in the content of these compounds is due to the different metabolite synthesis patterns between *S. officinarum* and *M. invisa*. The results of the identification of metabolite compounds in the four samples contained the same pattern of interaction of metabolites between these two plants for several compounds.

Consequently, the rhizosphere of *M. invisa* contained a dodecane allelopathic compound (6.83 %), which is a plant metabolite compound that has a molecular formula of $C_{12}H_{26}$ and a molecular weight of 170.33gmol^{-1} (Ram *et al*., 2019; Karimi *et al*., 2011). This dodecane compound is classified as essential oil which has the chemical name dihexil and is used in micro-extraction solvents for the formation of Triazine herbicide (Kokosa, *et al*., 2019). Similarly, this triazine is an active ingredient of herbicides used to control broadleaf and grassy weeds. Additionally, compound 2-Furan carboxaldehyde, 5-methyl with the molecular formula $C_6H_6O_2$, found in weed root extracts, is a class of furfural compounds that has allelopathic potentials in plants (Ram *et al.*, 2019). Moreso, pentadecane metabolite compounds, with the molecular formula $C_{15}H_{32}$, is a group of essential oils in plants functioning as metabolites with the potential to cause negative effects on the growth of plants surrounding them. Conversely, it was also shown that some components of essential oils which were screened for allelopathic activity showed growth retardation (Enyiukwu and Ononuju, 2016; Karimi *et al*., 2011; Lucas *et al*., 2021).

Furthermore, allelochemicals do not affect the activity of cells that synthesize and store them because plants have a mechanism of resistance to self-produced toxic compounds (Aliyu *et al*., 2018; Asyraf and Micheal, 2011; Chauhan, 2008). This resistance mechanism occurs through a biosynthetic process outside the cell such as in the secretory cell wall, storing it in vacuoles. Other than that, the allelochemical is transported from the cytoplasm to the vacuole by vesicles, where they experience enzymatic detoxification. Conversely, there is a mutation in the gene encoding the protein at the target sites of allelochemicals leading to a non–toxic accumulation of these chemicals in the vacuole (Erida *et al*., 2019; Marvillo *et al*., 2011; Sirikantaramas *et al*., 2008). Further, the allelochemical compounds released by roots into the soil can cause growth disorders in plants depending on the concentration of the allelochemicals. Additionally, these chemicals, when released into the environment, result in interactions between its biotic and abiotic factors such as physical-chemical processes, absorption by plants, and microbial breakdown (Albuquerque *et al*., 2011; Darmanti, 2018).

Consequently, chemicals that are secreted from plants that affect other plants are called allelochemicals (Riajeng, 2019; Suresh *et al*., 2017). Moreover, these chemicals are secondary metabolites produced by plants that can inhibit or stimulate the growth of plants in the vicinity. Therefore, most of these chemicals are classified as plant secondary metabolites which are produced from primary metabolites. A wide variety of these chemicals has been identified including phenolic acids, coumarins, terpenoids, flavonoids, alkaloids, glycosides, and gluconates (Karimi *et al*., 2017). Additionally, potential allelochemicals are found in almost all plant tissues, including the leaves, flowers, fruits, seeds, and root tissues. Also for decades, these chemicals were mostly used in agriculture, forestry, ornamental plants, and gardening. The results showed that they contain several naturally occurring chemical substances such as alcohol, organic acids, aliphatic and aromatic components.

Moreover, the effect of competition in plant communities can occur indirectly on individual plants, and this is usually due to allelochemical interactions. Therefore, based on the results of this research and discussion, it is concluded that the allelochemicals secreted

by donor plants will be released into the environment through root exudation by the process of diffusion, evaporation from leaves, leaching, and biomass decomposition (Euis and Ratag, 2017; Mohammad *et al*., 2021; Sirikantaramas *et al*., 2008). Similarly, the effect of these chemicals on seed germination, seedling growth gives it its allelopathic property. Therefore, allelopathy is a chemical component produced by plants to interact with other plants that grow around them (Erida *et al*., 2019; Lucas *et al*., 2021). It is used by weeds to compete with other plants that grow around it. The results of this research showed that weed extract can inhibit the germination of seeds of other plants growing in its vicinity, giving it the potential to be developed as a bioherbicide.

5. Conclusion

It can be concluded, based on the results of this research, that the *S. officinarum* rhizosphere contains an octadecanoic acid allelochemical compound, and methyl ester (2.06 %). Additionally, weed roots and *S. officinarum* each contained metabolite compounds which are usually used as herbicide activators.

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