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The Allelopathic effect of *Mangifera indica* Leaves on Mustard Seeds

Luningning M. Mendoza[1*], Danila S. Paragas[2]

[1]Occidental Mindoro State College, Sablayan, Occidental Mindoro, Philippines; [2]Chemistry Department, Central Luzon State university, Munoz, Nueva Ecija, Philippines.

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This study was conducted to evaluate the allelopathic activity of *Mangifera indica* leaves against seed germination of mustard seed. Specifically, it aimed to: extract the components of *M.indica* leaves using ethanol; determine the presence of tannins using Ferric chloride test; and assess the allelopathic property of *M.indica* leaves using mustard seed as seed bioassay. *M. indica* leaves were air dried, ground into powder and extracted using ethanol. The crude extract was concentrated using water bath. The crude extract was diluted with distilled water to make 10 mg/mL stock solution. Other concentrations 2, 4, 6 and 8 mg/mL were also prepared using the stock solution. Assessment of the allelopathic effect of *M. Indica* ethanol extract on mustard seeds was done. Results showed that the crude extract inhibited the germination and growth of mustard seeds. Tannin was isolated from the crude extract by preparative thin layer chromatography (TLC). The tannin was scraped from the TLC plate and was dissolved in hexane. The dried tannin was then diluted to different concentrations and tested on the germination and growth of mustard seed. Results show that the semi-purified tannin did not inhibit the germination and growth of mustard seed.

Keywords: allelopathy, mustard seed, *Mangifera indica*, germination

Introduction

Allelopathy is the suppression of the growth of neighboring plants by chemicals released from a plant or plant parts. These chemicals are the secondary metabolites from plants that interact with the environment and possess allelopathic activities (Wardle *etal.* 2011). Allelopathy is believed to be involved in many natural and manipulated ecosystems and it plays an important role in the evolution of plant communities, exotic plants invasion and replant failure (Ridenour *et al.* 2001). Many plant species are able to produce and release bioactive compounds which are secondary metabolites into the environment and are capable of suppressing the growth of other plants. Such chemicals include tannins, phenolic acids, lignins, alkaloids, flavonoids,

[*]**CoresspondingAuthor:**Luningning M. Mendoza; **E-mail:** luningningmmendoza@gmail.com

coumarins and terpenoids and are present in all plant tissues including leaves, stems, roots, rhizomes, flowers, fruits and seeds, and even in pollen grains (Ahmad *et al* . 2011) .

Decrease in crop yields due to weeds result from their multiferous ways of interfering with crop growth and crop culture. Weeds compete with crops for one or more plant growth factors such as mineral nutrients, water, solar energy and space and they hinder crop cultivation operations. During the 20th century, pronounced attention was paid to the use of herbicide to suppress the growth of weeds which compete with crops in absorbing the soil's nutrients (Heap, 2014). However, the use of herbicides during the past years has raised doubts about the safety from their continuous use because they are toxic and endocrine disruptors in human cell line (Gasnier *et al.* 2009; Macías *et al*. 2007). Allelopathy is a natural and environment friendly technique which may prove to be a tool for weed management and thereby increase crop yields. The allelopathic activities of plants make them candidates as natural herbicides (Gasnier *et al.* 2009). Extracts from flowers, leaves, stems and roots could be used to control the growth of weeds (Mesnage *et al.* 2013). The main principle in allelopathy arises from the fact that plants produce thousands of chemicals and many of these chemicals are released by leaching, exudation, or decomposition processes. Subsequently, some of these compounds which are known as allelochemicals alter the growth or physiological functions of receiving species. The most commonly found allelochemicals arecinnamic acid (Mesnage *et al.* 2013; Chon *et al*. 2003) and benzoic acids (Ye *et al.* 2006), flavonoids, and various terpenes (Einhellig *et al*. 2004), these compounds are known to be phytotoxic (Singh *et al*. 2003).

Mangifera indica leaf litters are being used in composting-vermicomposting (Gajalakshmi *et al*. 2005; Prakash and Karmegam 2010; Nayeem-Shah *et al.* 2015), biodegradation (Isaac and Nair 2005; Musvoto *et al.* 2000), and allelopathic studies against fungi, germination of wheat seed (Shafique *et al.* 2007), and germination and growth of *Zea mays* (Yan *et al*. 2006), growth and propagative capacity of nutsedge (Harborne, 1998). This study attempted to find out if the leaves of *M. indica* have the capacity to control or suppress the growth of weeds using mustard seeds.

Materials and methods

Materials, Chemicals, and Reagents

The ethanol used as extracting solvent and the analytical reagents such as ferric chloride, gallic acid, toluene, acetone and ethyl acetate were available in

Analytical Chemistry Laboratory, Department of Chemistry, Central Luzon State University.

Sample Collection and Preparation

Leaves of *Mangifera ind*ica collected from SitioTuburan, Poblacion, Sablayan, Occidental Mindoro were cleaned using tap water, then air dried for about one week. The leaves were then chopped into small pieces, ground, and pulverized using the blender.

Preparation of the Plant Extract

About 100 grams of the ground and pulverized sample was soaked in container containing approximately 200 mL ethanol (80%). The bottle was covered and set aside for two days at room temperature. Then, the mixture was filtered using a Whatmann filter paper no. 1. The filtrate was concentrated using water bath. The concentrated crude extract was stored in a tightly covered amber bottle to avoid deterioration of the active components and was kept in a refrigerator until analysis.

Test for tannins

Test with ferric chloride solution

One milliliter of extract was taken in a test tube and 5 drops of 1% $FeCl_3$solution was added. Formation of black precipitate indicates the presence of tannins.

Seed Bioassayof the Crude Extract

In order to determine the allelophatic property of *M. indica* leaves, the concentrated crude extract was air dried. The air dried sample was measured and a stock solution of 10 mg/mL was prepared. Concentrations of 2, 4, 6, 8 mg/mL were prepared from the stock solution.

Test for seed germination (Crude Extract)

Mustard seeds were placed in ELISA plate and were treated with 0.5 mL of each concentration of the crude extract. Distilled water was used as control. Four seeds were used in each treatment and two replicates were made. The percent germination was recorded after ten days.

Thin Layer Chromatography

A 50-mL beaker with a watch glass as cover was used as a developing chamber. The beaker was lined with filter paper around the sides to prevent "edge effect." The chamber was filled with the appropriate solvent system and equilibrated for 5 minutes.

The crude extracts were spotted on the thin layer plate using capillary tube. The spotted plates were placed inside the equilibrium chamber with the solvent system composed of 3:1:4 toluene: acetone: ethyl acetate. The chamber was covered and the solvent was allowed to travel until the fronts just reached about 1cm before the edge of the coating. Then the plates were removed from the chamber and allowed to dry.

Seed Bioassay (Semi- purified Tannin)

The tannin was scraped from the TLC plate and was dissolved in hexane. It was then dried using water bath. The dried tannin was then diluted to different concentrations such as 0.1, 0.25, 0.5 and 0.75 mg/mL.

Test for seed germination (Semi- purified Tannin)

Mustard seeds were planted in ELISA plate and were treated with 0.5 mL of each concentration of the tannin. Distilled water was used as control. Fout seeds were used in each treatment and two replicates were made. The seeds were observed for ten days.

Results

Extraction of the Bioactive Component

The leaf ethanolic extract of *M.ind*ica was filtered and evaporated in a water bath until it became syrupy. The concentrated extract gave a viscous dark green color.

Test for Tannin in the Crude Extract of Mangifera indica

The crude extract of *Mangifera indica* formed green to black solution upon treatment with ferric chloride. According to Harborne 1998, a classic way to detect simple extract is to add a solution of 1% $FeCl_3$ in water. The Fe^{+3}ions

form complexes with the tannins forming a green or black color on the extract after addition of $FeCl_3$.

Seed Bioassayof the Crude Extract

To evaluate the allelopathic property of the plant, mustard seed was used for the seed bioassay. The mustard seeds were treated with 2, 4, 6, and 8 mg/mL of the crude extract. Distilled water was used as the control, four mustard seeds were used in each treatment. Three replicates were made and the results were shown in Table 1.

Table 1. Evaluation of the allelopathic property of *Mangifera indica* crude extract using mustard seeds.

Concentration (mg/mL)	**Description of the effect After 10 days**
0	Seeds germinated with 4-5 mm radicles and 10 mm hypocotyl with root hairs
2	Seed coats were broken with 2 mm radicles
4	Seed coats were broken with 1.5 mm radicles
6	Seed coats were broken with 1.2 mm radicle
8	Seed coats were broken with 1.1 mm radicles
10	Seeds did not germinate. No opening of the seed coat was observed

After ten days, the seeds in the control group sprouted and formed root hairs (Fig. 3A). From the initial length of mustard seed which is 1mm it became 15 mm which means that there was 14-mm difference indicating seedling growth (Table 1 and Fig. 1A). In the concentration of 2 mg/mL, a short radicle can be seen with the aid of a microscope (Fig.1B). The length of the seed became approximately 2 mm. For 4 mg/mL concentration, radicle can also be seen using the microscope (Fig.1C) but shorter than in the treatment with 2 mg/mL concentration, only about 0.5 difference from the initial length of the mustard seed.

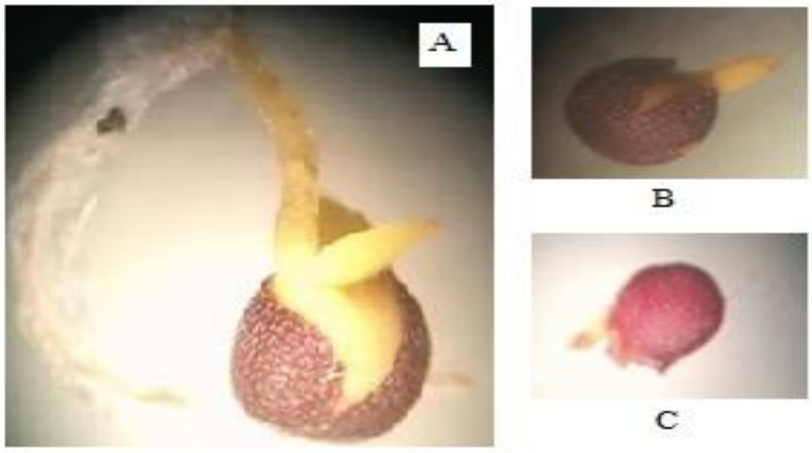

Figure 1. Microscopic view of **(A)** control mustard seed **(B)** treated with 2 mg/mL crude extract and **(C)** treated with 4 mg/mL crude extract after 10 days.

In terms of 6 mg/mL concentration, about 0.2 mm difference from the initial was seen (Fig. 2A). In the 8 mg/ mL concentration, very short radicle was observed which was not visible to the naked eye. It was approximately 0.1 difference in length compared to its initial length (Fig. 2B). Finally, the highest concentration that is 10 mg/mL, the mustard seed was totally inhibited from germination. As noticed in the microscope, the cover of the seed was almost intact (Fig.2C).

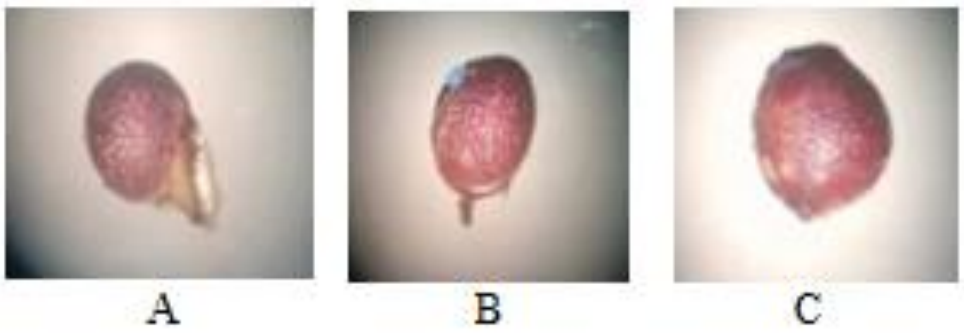

Figure 4. Microscopic view of the mustard seed treated with **(A**) 6 mg/mL crude extract, **(B)** 8 mg/mL crude extract and **(C)** 10 mg/mL crude extract after 10 days.

Seed Bioassay of the Semi-purified Tannins

To assess the allelopathic effect of the tannins from *Mangifera indica*, mustard seed was also used as seed bioassay. The mustard seeds were treated with smaller concentrations: 0.1mg/mL, 0.25 mg/mL, 0.5mg/mL, and 0.75mg/mL of the semi-purified tannins. Distilled water was used as the control, four mustard seeds were used in each treatment. As shown in Table 2, the semi-purified tannin did not inhibit the germination and growth of mustard seed. The longest radicle was observed with the seeds treated with 0.1 g/mL concentration and was longer than the control group.

Table 2. Assessment of the allelopathic property of *Mangifera indica* semi purified-tannins using mustard seeds.

Concentration(mg/mL)	Initial Length (mm)	Final length (mm)	Difference
0	1.0	6.3	5.3
0.10	1.0	12.4	11.4
0.25	1.0	2.2	1.2
0.50	1.0	2.6	1.6
0.75	1.0	2.8	1.8

Discussion

According to Macias *et al*. (2008), allelochemicals such as phenolic acids and associated compounds are most common growth inhibitor of plants. Tannins belong to phenolic secondary metabolites. The presence of tannin in *Mangifera indica* leaves has the ability to inhibit seed germination of mustard seeds. All the test concentration of the crude extract inhibited the germination and growth of mustard seeds. This implies that even the lowest concentration of 2 mg/mL crude extract from *M.indica* leaves can inhibit the germination of mustard seed. This result is supported by Muscolo *et al*. (2008) who explained that phenols, like tannins may cause inhibitory effect in germination of seeds. This is because tannins are gibberellins antagonist (Corcoran *et al.* 1972). Measured effect of tannin from the crude extract was obtained by getting the final and initial lengths of the roots and shots after 10 days. When semi-purified tannin was applied to mustard, all mustard seeds germinated at different concentrations. A concentration of 0.1 mg/mL has activated the germination of mustard seed. There are also reports that tannins can also stimulate seedling growth and germination (Muthukumar *et al.* 1985).

In conclusion, the components of *Mangifera indica* can be extracted using ethanol. The crude tannins present in the leaves of *Mangifera indica* can inhibit the growth of the mustard seeds. The semi-purified tannin did not inhibit the germination and growth of mustard seed.

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