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Research Article

Bioactive compound from mangoes leaves extract as potential soil bioherbicide to control amaranth weed (*Amaranthus spinosus* Linn.)

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Abstract: Bioherbicide is important approach for sustainable farming practices. One of plant that has potentially as bioherbicide, which is environmentally safe, is mango. Mango leaf extract is useful as bioherbicide because it produces allelochemical compounds, which could inhibit the weed growth. This research was designed to study the effect of several mangoes species leaves extract to control dominant weed (amaranth). Split plot design was implemented using mango species (S) as the main plot; S1 (*Mangifera odorata* Griff.), S2 (*Mangifera foetida* Lour) and S3 (*Mangifera indica* L.). While the sub plots were concentrations of mango's leaf extract (K), that included 0, 500, 1000, 1500, and 2000 ppm. Results of the research showed that all parameters of weed growth (amaranth) were inhibited along with the increase of concentration of the mango's leaf extract. The results also showed the significant inhibition of amaranth's dry weight. Among three species of mangoes, *M. indica* L. showed the best inhibition mechanism to the amaranth weed, which significantly suppressed the weed growth on just 1000 ppm concentration.

Keywords : *Amaranthus spinosus, bioherbicide, mango leaves extract*

Introduction

The application of synthetic chemical herbicide is still the main choice in controlling weeds due to its effectiveness to control the weeds. However, continuous application of such synthetic chemical herbicide tends to create negative impact on soil (Inderjit et al., 2011) and weeds resistant to herbicide (De-Martino et al., 2010). Other problems posed on the use synthetic herbicide are poison to non-targeted organisms, disturb ecology as a whole and leave chemical residues on environment (Khan et al., 2013). To mitigate this problem, it requires effective and efficient control as alternative ways by using bio-compound as organic herbicides (bio pesticide), which are environmentally safe (Okigbo and Osuinde, 2003). Application herbicide from bio compounds to soils is safer than synthetic one. Mangoes leaves are potential bioresources due to their capability to produce allelochemical compound that can inhibit weed development (Yulifrianti et al., 2015). Mango leaf contains phenol, flavonoid and terpenoid (El-Rokiek et al., 2010). It also contains bioactive glycoside xanthone, tannin and derivatives of gallic acid (Shah et al., 2010). Some compounds that are also found such as mangiferin, isomangiferin and homomangiferin, are identified as phenolic components (Tanaya et al., 2015). Phenol compound is one of phytotoxic compounds, which can inhibit the growth. *Cyperus rotundus* and *Cynodon dactylon* could be controlled by phytochemical compounds derived from extract of mango leaves (El-Rokiek et al., 2010). The objectives of this study were to analyze the potential of several mango leaves extract to control the dominant dry land weed (amaranth) and to determine the appropriate concentration of mango leaves extract to inhibit the amaranth.

Materials and Methods

Tools used in this research include a set of macerator, rotary evaporator, precision's analytic scales, separated funnel (100 mL), erlenmeyer (250 mL), calibrated beaker of 100 mL, intercepting bottle (1 L), leaf area meter (LAM), spectrophotometer, oven, hoe, roll meter, raffia, scissors, hand counter, ruler, and digital camera.

Materials used for the research included amaranth seeds, mango leaves of kweni *(Mangifera odorata* Griff.*)*, pakel *(Mangifera foetida* Lour*)* and gadung *(Mangifera indica* L.). The chemical materials used were distilled water and methanol. The research was conducted at Laboratory of Environmental Resources, Department of Agronomy, Faculty of Agriculture, Brawijaya University. The experimental field was located at Bedali Village, Ngancar Sub district, Kediri Regency. The research was conducted from December 2015 to June 2016. The research was done by planting amaranth in experimental pots (polybag). The application of mango's leaf extract was done when the amaranth was planted. This research used a split plot design with three replications. The main plot was mango species (S) that includes kweni mango (*Mangifera odorata* Griff.), pakel mango (*Mangifera foetida* Lour) and gadung mango (*Mangifera indica* L.). The sub plot was concentrations of mango's leaf extract (K), that included 0, 500, 1000, 1500 and 2000 ppm. Each combination of the treatment was repeated three times in order to obtain 45 units of combination plots. The obtainable data were analyzed using analysis of variance (F-test) at level 5%. If any significant difference was found (F-count > F-table 5%), it was then continued with Least Significant Difference (LSD) test at level 5%.

Results and Discussion

Root inhibition

Root length of the amaranth was inhibited by the application of mango's leaf extract through the soil. Parameter of root length at 7 weeks after planting (WAP) showed the inhibition of allelopathic compounds (Figure 1). Mango's leaf extracts by concentration of 1000 and 1500 ppm were effective in inhibiting root length of the amaranth for about 28.72 and 46.01% than without extract treatment. Roots will be affected by allelopathic compounds due to roots is the first organ, which interacts with allelopathy. Roots will absorb the allelopathic compounds that can weaken absorbability of the roots (Shao-Lin et al., 2004).

The absorbed allelopathic compounds will inhibit the growth, particularly on the root part, which was directly affected by the extract. Allelopathy may reduce stomatal conductivity and reduce turgor pressure on leaf. Allelochemical affects both phloem and xylem tissues that may disturb water absorption, nutrient absorption and assimilate translocation. It may make the roots increase the accumulation of abscisic acid hormone/ABA and will affect on physiological mechanism of the plants in general (Zeng et al., 2008). The existence of allelochemical may disturb auxin transportation from the shoot to the root and disturb cytokinins hormone synthesis in the roots.

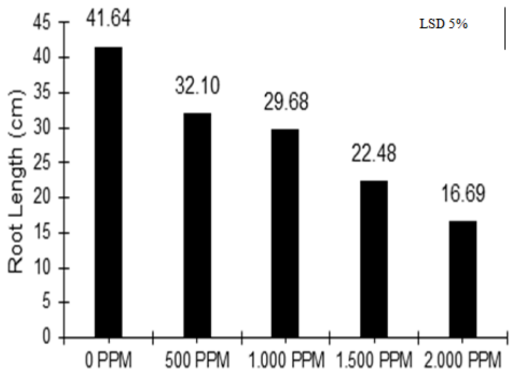

Figure 1. Effect of mango leaf extract concentration on root length of amaranth at 7 weeks after planting (WAP)

Cytokinin may trigger cell divisions (cytokinesis) in meristematic tissues, growth regulator and root's cell differentiation, as well as affect the shoot dominance, growth of terminal bud and ageing. In physiological process, cytokinin plays simultaneously with auxin as hormone to promote root elongation and cell enlargement. Allelochemical changes the growth or physiological functions of the recipient species. Because it causes mitochondrial disturbance by reducing activities of dehydrogenase enzyme, causes stomatal closing, cell membrane damages and disturb cell membrane permeability (Shabala, 2010). Allelochemical disturb nutrient absorption on root, photosynthesis, respiration, transpiration, and efficient photosystem II ATP synthesis (Omezzine et al., 2014).

Allelopathic inhibition is complex and may involves different interaction in each chemical, by different mix of compounds may have greater allelopathic effect from individual compounds. Allelopathic compounds inhibit cell division. In general, those compounds are phytotoxic against plants around them (El-Rokiek, 2007). The physiological process of plants could affect, such as elongation, ionic absorption of mineral, as well as metabolism of protein and function of membrane plasma (H^+ ATPase, NADH oxidase). All of those mechanisms could not take place by synthesis compounds from herbicide (Junaedi et al., 2006).

Permeability of cell membrane is highly affected by allelochemical. Membrane integrity is very important in maintaining structure and function of the cells because it regulates movement of different substances at the whole cells (Shao-Lin et al., 2004). In normal condition, cell membrane is selective in abnormal condition due to allelochemical, therefore, its permeability may be lost and it would not be selective anymore, so that the cellular mechanism could not be controlled (Farooq et al., 2013).

Height inhibition

The growth of amaranth might be inhibited by phenolic compounds by inhibiting activities of cell division and elongation. Parameter of the amaranth height at 7 WAP showed significant interaction. The application of extract concentration of 1500 ppm was effective to inhibit the height of amaranth. Such concentration might inhibit the height 57.27% by the application of leaf extract of *M. foetida* Lour and 72.06% by the application of *M. indica* L (Figure 2). However, on leaf extract of *M. odorata* Griff, the effective concentration is 1000 ppm because it might inhibit the height 59.95% from the treatment without the extract.

Height of the amaranth might have been inhibited by henolic compounds that inhibited metaphase stage at mitosis division. Such mechanism took place due to the phenolic compounds destroyed the spindle threads during metaphase process. Phenol reduces the mitosis index by producing excessive prophasic condensation and metaphasic chromosomes, which causes metaphase accumulation and cell percentage increase significantly through chromosome deviations. The main effect is the change of microtubular axis formation, which causes the formation of some spindle rods and asymmetric convergence from the chromosomes (Reigosa et al., 2006). If the cell proliferation process is inhibited, the cell propagation in organs of the plants may be inhibited as well, so that the growth may be inhibited and stopped and of course, both amounts and size of the cell would not increase. The phenolic compounds inhibit the metaphase stage on mitosis, which inhibit the mitosis process. Such inhibition may not increase amount and size of cell, so that the plant's elongation becomes inhibited (Yulifrianti et al., 2015).

Existence of the phenolic compounds may disturb the auxin transportation from shoot to root and disturb cytokinin synthesis at the root part. Cytokinins hormone functions for cell division and cell differentiation of the root and auxin is a compound, which promotes root elongation (Aamir and Ibrahim, 2013). Phenolic compounds disturb the phosphorilation channel or inhibit activation of Mg^{2+} and ATPase activities, as well as reduce total carbohydrate synthesis, protein and nucleic acid (DNA and RNA), inhibit ion absorption and disturb biosynthesis process. Besides that, it causes osmotic inhibition (Krisna et al., 2005).

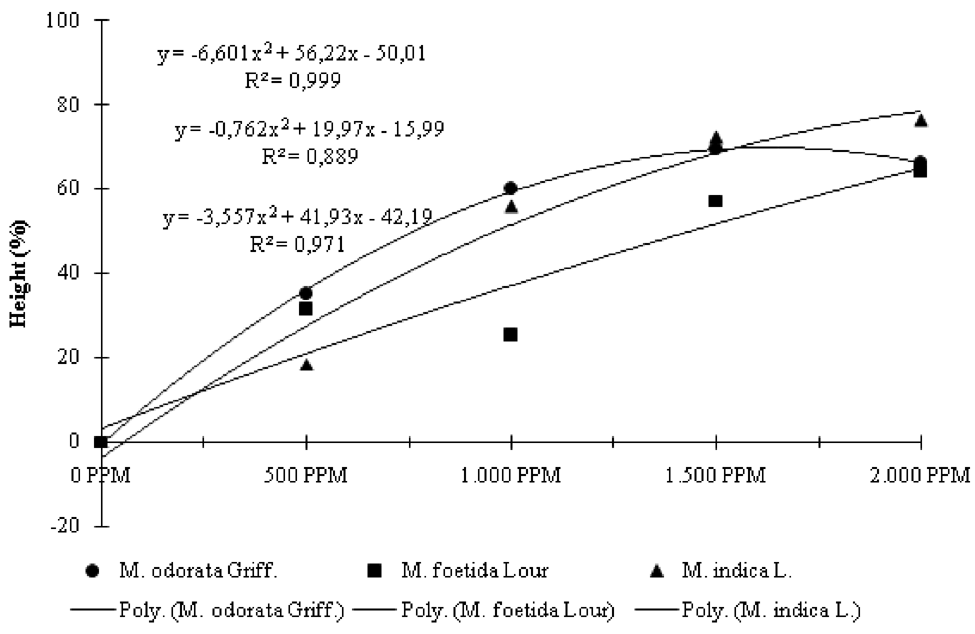

Figure 2. Effect of three species of mango leaf extract on inhibition (%) height of amaranth at 7 weeks after planting (WAP)

Terpenoid compounds are phytotoxic, which may cause anatomical and physiological changes of the seeds. It occurs due to decrease of some organelles, such as mitochondria, accumulation of lipid in cytoplasm, inhibition of DNA synthesis and disturbance in cell membrane (De-Martino et al., 2010). Monoterpene (1,8-cineole) inhibits mitochondria respiration from organelle and inhibits the whole mitosis stages at the end of the root. Sesquiterpene compounds (dehydro zaluzanin) inhibit peroxidase synthesis and cause division of the plasma membrane from the cell wall of the tissues (Knight, 2009). Besides that, it may inhibit the photosynthesis process (Sanchez-Munoz et al., 2012). Meanwhile, diterpene compounds cause the end of the root (apex radicis) swell, inhibit the chlorophyll synthesis, inhibit induction of the growth hormone, besides that triterpene compounds inhibit plasma membrane of NADH oxidase and loss of membrane integrity (Macias et al., 2004).

Cell elongation may be inhibited by phenolic compounds which affecting gibberelline hormone. Elongation of the stem internodes may be affected by activity of the gibberelline hormone, which promotes cell division, cell enlargement and stem elongation. The inhibition of such hormone may disturb cell division at the meristem tissue, which inhibit elongation of the stem internodes (Ashafa et al., 2012).

Allelochemical regulates production of the plant's hormones. The growth hormones, gibberelline and auxin, are affected by secondary metabolite, which significantly affect cell enlargement of the plant. The growth hormones play important roles in physiological process. Indole Acetic Acid (IAA) is found in plant, both in active and inactive forms. Inactive enzyme of IAA-oxydase may inhibit cell enlargement and growth of the plant. Some allelochemicals inhibit IAA-oxidase enzyme, which functions to activate IAA. Through this mechanism, allelochemical may affect roles of the plant's hormones. Allelochemical also affect induction of the growth hormone of gibberelline (Farooq et al., 2013). Inhibition in physiological process may occur in the formation of nucleic acid and ATP synthesis. Reduced ATP may inhibit almost the whole processes of cell metabolism, so that synthesis of the required substances may reduce as well. The phenolic compounds may disturb the phosphorilation channel or inhibit activation of Mg^{2+} and ATPase activities. It is due to the decrease of synthesis on total carbohydrate, protein and nucleic acid (DNA and RNA) or disturbs the biosynthesis process. Parameter on numbers of leaf showed significant interaction of the treatment at 7 WAP. For leaf extract of *M. odorata* Griff, its effective concentration is 1000 ppm that could reduce numbers of leaf by pressing percentage for about 54.15% in comparison with no extract treatment (Figure 3). However, the leaf extract of *M. foetida* Lour by concentration of 2000 ppm could inhibit 72.68% numbers of leaf and *M. indica* L. could inhibit 75.66% by concentration of 1500 ppm. Mango's leaf extract has reduced the numbers of leaf and leaf area as well. Significant interaction was found on parameter of leaf area at 7 WAP. Extract concentration of 2000 ppm was effective to reduce leaf area 95.32% on *M. odorata* Griff and 96.27% on *M. foetida* Lour (Figure 4). While on *M. indica* L., the effective concentration was 1000 ppm by inhibition percentage 81.52% from the treatment without extract.

High concentration of allelochemical may have acted as photosynthetic inhibitor because it blockades the electron acceptance and becomes antagonist energy, reduces activities of photosynthetic pigment and enzyme by disturbing biosynthesis of porfirin chlorophyll precursor and reduces Mg-chelatase, which decrease the chlorophyll accumulation level (Macias et al., 2004). Photosynthesis is a biochemical process to produce energy in ATP form, in which carbon dioxide and water, by the assistance of radiation, will produce carbon and energy (Teixeira et al., 2012). Photosynthesis takes place when radiation ionizes the chlorophyll molecule in photosystem II and the released electrons will be transferred along the electron transfer chain. However, it takes place in a complex membrane system (photosynthetic membrane), which comprises of complex protein, electron carrier, pigment (chlorophyll) and lipid molecules.

Clear reaction concentrates on photosystem II and photosystem and both of them work simultaneously. Products (yield) of the electron are transferred from water molecules for NADP+ produce NADPH, meanwhile NADPH and ATP are produced by clear reaction that provides energy for dark reaction of photosynthesis, which is well known as calvin cycle/carbon decomposition cycle. Calvin cycle takes place at watery phase from chloroplast and involves a set of enzymatic reactions. It will be catalyzed firstly by rubisco protein (cribulose 1,5 – biphosphate carboxylase/ oxygenase). CO_2 is absorbed through stomata for five carbon compounds and finally produces two molecules from three carbon compounds.

The next biochemical process involves some enzymes, such as fapase and phosphate triose (Reigosa et al., 2006). Photosynthesis is one of carbon assimilation due to photosynthesis process and free carbon from CO_2 will be bound into glucose as energy keeper molecule.

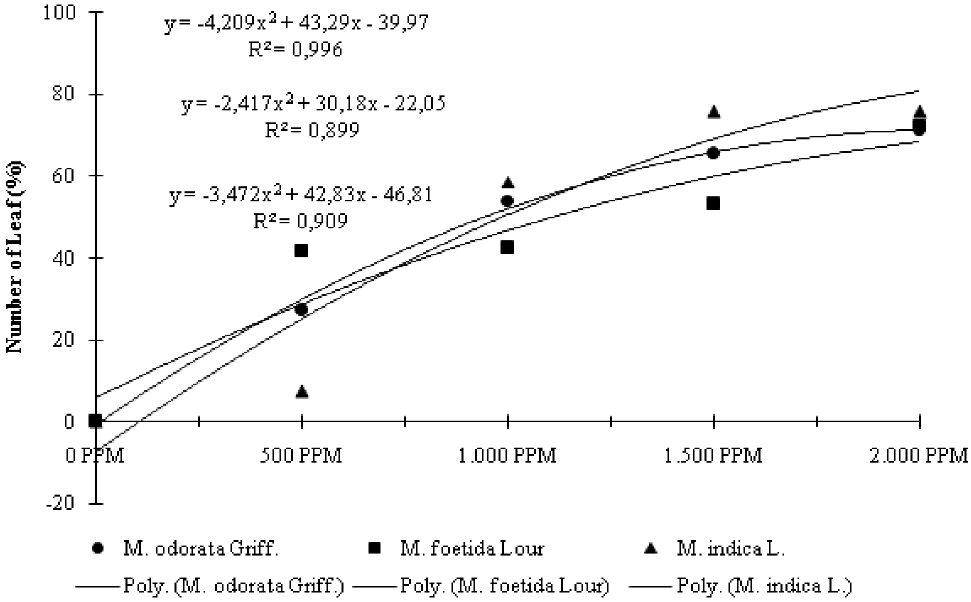

Figure 3. Effect of three species of mango leaf extract on inhibition (%) of amaranth number of leaf at 7 weeks after planting (WAP)

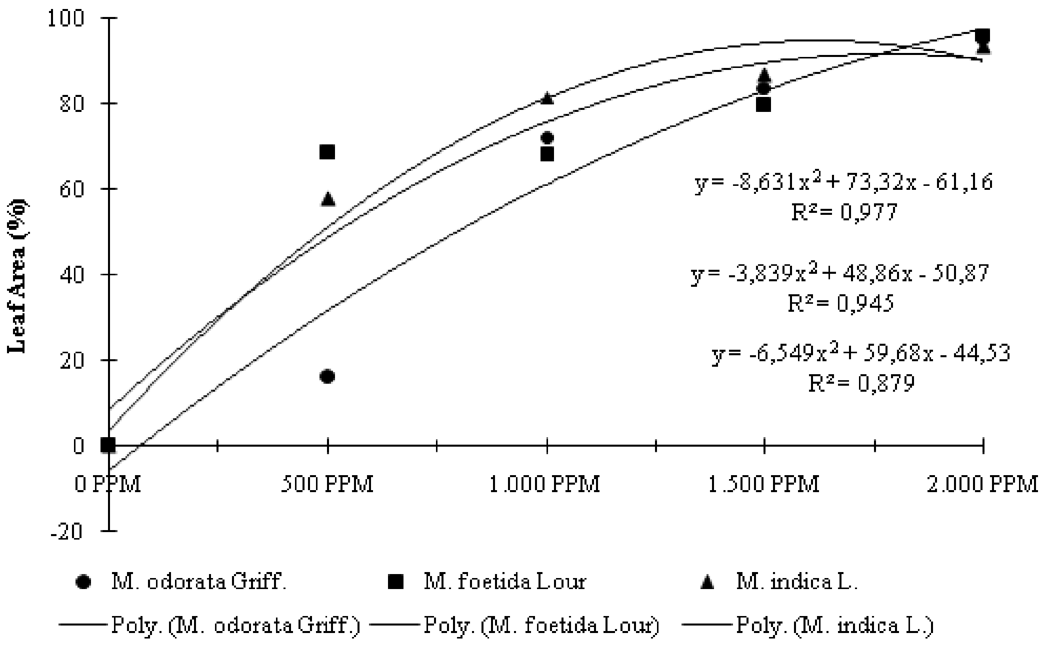

Figure 4. Effect of three species of mango leaf extract on inhibition (%) leaf area of amaranth at 7 weeks after planting (WAP)

Glucose is used to form other organic compounds, such as cellulose. This process takes place through cellular respiration. In respiration, glucose and other compounds will react to oxygen and produce carbon dioxide, water and energy. The plants absorb light using the chlorophyll pigment. Chlorophyll is in organelle, which is so called chloroplast where the photosynthesis takes place, in the stroma. Even though the whole green cell parts of the plant contain chloroplast, but most of energy is produced in leaves.

Chlorophyll inhibition

Chlorophyll determines the synthesis of organic compounds, which is used for physiological process during growth and development. Allelochemical may cause chlorophyll degradation. Parameter on amounts of the chlorophyll at 7 WAP showed a decrease due to concentration of the mango's leaf extract. Concentration of the extract 1000 ppm is effective to reduce 53.81% chlorophyll a, 49.85% chlorophyll b and 53.15% of total chlorophyll (Figure 5).

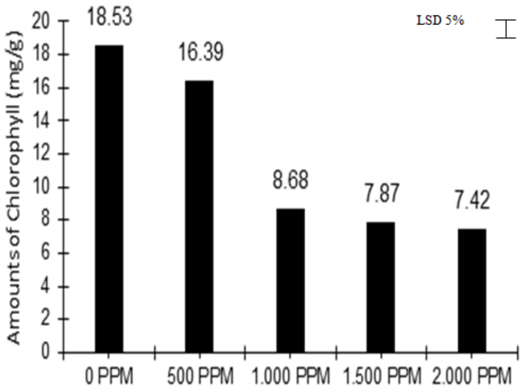

Figure 5. Effect of mango leaf extract concentration on amounts of chlorophyll total in amaranth leaf

The process of photosystem II locates at chloroplast tilakoid membrane and get involved in photosynthetic electron transportation. When it is illuminated by light, water complex oxidation from photosystem II changes the molecule of water into $1/2O_2$, $2H^+$ and 2 electrons. Then, the electron will cross a set of electron, which finally leads to ATP formation. Most of compounds, which will inhibit photosystem II, may compete to bind plastoquinon (PQ) on protein from photosystem II. PQ is benzaquinon lipophilic, which accepts 2 electrons from protein of photosystem II and $2H^+$ from stroma. Then, it moves and cross the tylacoid membrane and contributes 2 electrons for cytochrom, which finally transfer the electron to photosystem I. if the electron transfer channel is inhibited, of course, it will make the plants start to die (Reigosa et al., 2006).

Weed biomass synthesis inhibition

Effectiveness of the mango's leaf allelopathy application is visible in accordance with parameters of dry weight of the amaranth. Data on results of the research showed significant interaction at 7 WAP against dry weight of the amaranth. Concentration of 2000 ppm is effective to reduce dry weight of the amaranth on leaf extracts of *M. odorata* Griff and *M. foetida* Lour. The concentration reduces dry weight 97.93% on extract of *M. odorata* Griff. and 98.80% on extract of *M. foetida* Lour (Figure 6). Meanwhile, on extract of *M. indica* L., the effective concentration 1000 ppm could reduce dry weight 83.16% from the treatment without extract (Table 1). The results were supported by research of Yulifrianti et al. (2015), which showed that fresh weight and dry weight of *Cynodon dactylon* weeds may reduce due to mulch extract of mango's leaf (*M. indica* L.). Fresh weights reduce 21.28 and 69.76% from the treatments without extract due to concentration of mulch extract of mango's leaf for about 35 and 45%. By equivalent concentrations, dry weight of *Cynodon dactylon* reduces 82.81 and 95.79% in comparison with without extract treatment. It conforms to results of the research by El-Rokiek et al. (2010), in which mango's leaf extract could keep down dry weight of *Phalaris minor* Retz. and inhibit the growth of *Cyperus rotundus* L. by concentration 25%.

Based on results of the research by El-Rokiek et al. (2010) mango's leaf extract with concentration 25% could inhibit numbers of shoot, dry weight and growth of purple nuts edge (*Cyperus rotundus* L.). Besides that residue of mango's leaf in powder form has also showed significant decrease of dry weights for leaf and rhizome of the purple nuts edge. The highest decrease occurred by the application of 100 g/kg soil of the mango's leaf powder. Results of analysis on leaf tissues of the purple nut sedge showed that phenol content in leaf of the purple nut sedge increases along with the increase concentration. Ashafa et al. (2012) showed that monoterpene compounds also inhibit the germination of *C. occidentalis*.

Phenolic compounds may affect the cell membrane, which cause non-specific permeability changes and transform hydraulic conductivity of the roots, disturb the ionic balance, correlation between water-plant, stomatal function, respiration and photosynthetic rates (Shabala, 2010). Phenol reduces potential electrochemical transmembranes that depend on pH concentration and lipid solubility. If low pH transfers into and passes through the membrane in high concentration, phenol will induce the membrane depolarization and it will disturb the balance between anion and cation, therefore, it will increase permeability of the cell membrane. Finally, it inhibits the absorption of ion, phosphate, potassium, nitrate and magnesium. Benzoic acid and cinnamic acid destroy the cell membrane integrity by reducing the sulfhidril groups. The compounds induce lipid peroxidation, which is resulted from free radicals formation in membrane and catalase inhibition (Macias et al., 2004). In general, allelopathy may affects on plant development. The process begins at the plasma membrane when some damages occur on the structures, modification of membrane canal and lost of function of ATPase enzyme, which affects water and ionic absorption that finally lead to stomatal opening and photosynthesis process (Shabala, 2010). The next inhibitions occur in synthesis process of protein, pigment and carbon compounds, as well as activities of some phytohormones. Allelochemical disturbs cell divisions, biosynthesis of hormone, mineral absorption, membrane permeability, stomatal oscillation, photosynthesis, respiration, protein metabolism and water connection of the plant, which may reduce the growth (Farooq et al., 2013).

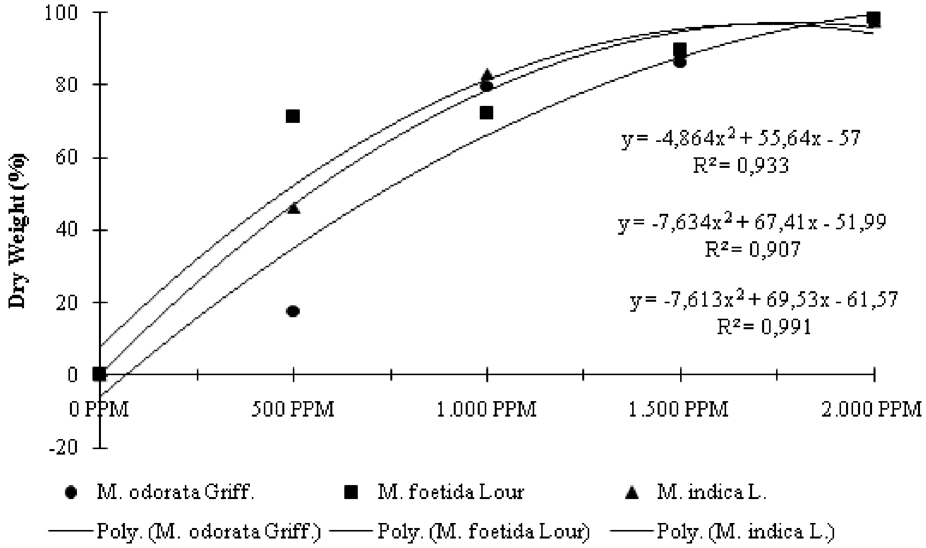

Figure 6. Effect of three species of mango leaf extract on inhibition of (%) dry weight of amaranth at 7 weeks after planting (WAP)

Table 1. Effect of three species of mango leaf extract on inhibit (%) dry weight of amaranth at 7 weeks after planting (WAP)

Mango Species	Dry Weight (g) at 7 WAP				
	Extract Concentration (ppm)				
	0	**500**	**1.000**	**1.500**	**2.000**
M. odorata Griff.	62.33 f	51,23 e	12,57 b	8,422 b	1,289 a
M. foetida Lour	79.38 g	23,01 c	22,22 c	8,378 b	0,956 a
M. indica L.	65.59 f	35,29 d	11,04 b	6,944 ab	1,522 a
LSD 5%	6.138				
CV (%)	14.00				

Conclusion

All species of mangoes (*Mangifera odorata* Griff., *Mangifera foetida* Lour and *Mangifera indica* L.) show the same effect inhibition to the root length and chlorophyll development. While *M. indica* L. shows the best inhibition effect to the weed biomass, leaves number and leaves area of weed. Each mangoes extract shows the similar trend of inhibition along with the level of applied concentration. Generally, the best inhibition effect is the application of 1000 to 1500 ppm mango leaves extract and *M. indica* L. shows the best inhibitions compare *M. odorata* Griff. and *M. foetida* Lour.

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