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EFFECT OF ACETIC ACID AS PRE-PLANT HERBICIDE ON MAIZE GERMINATION

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ABSTRACT

The competition between weed and maize happened right after the early of growth, so that weed control could be done from the beginning through the application of pre-plant herbicide. Limited data shown that vinegar (acetic acid) may have potential as a natural herbicide. Therefore the study was conducted to determine the effect of acetic acid a pre-planting herbicide to the germination, early seedling growth and toxicity effect on maize. Pots experiment was conducted from July to August 2012. The Factorial Completely Randomized Design (CRD) was employed with 4 replications. The first factor was the concentration of acetic acid, i.e., control (untreatment) 0%, 10% acetic acid, and 20% acetic acid; the second factor was the timing of application, i.e., 4, 8 and 12 days before planting. The results showed that acetic acid treatment did not significantly inhibit maize germination, nor inhibit root growth. However, of the concentration at 10% and 20% inhibited the growth of shoot at the same level of inhibition. Pre-planting treatment generally does not inhibit the growth of shoot. The phytotoxicity effect to maize was categorized as mild, i.e., 9, 938 - 14, 635% which as compared with untreated will still allow the seedlings to grow normally.

Keywords: acetic acid, germination, maize (*Zea mays* L.), concentration, pre-plant herbicide.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops in the world of wheat and rice. The grain of maize is the main source of food and for feed. Its production is influenced by many factors. The presence of weeds influences the loss of yield, as much as up to 38% (Cerrudo *et al*., 2012).

The control of weeds using herbicides could be a good method as it will cut the costs, time, labor, and control the weeds. The ideal herbicides are expected to have not toxic to the plants, effective to control the weeds, inexpensive and leave no negatively impact to the environment. Organic herbicides are biodegradable and act as carbon source for soil microorganisms. In soil application acetic acid by spreadly will penetrate the soil surface. It will easily decompose by microorganism and shows no potential of biological accumulation or contamination (MSDS, Material Safety Data Sheet 2008).

Diaz (2002) explained that vinegar, with acetic acid (CH_3COOH) as its main component, is potential as a natural herbicide. Evant *et al*. (2011) added that acetic acid does not persist in the environment, but perishable with producing water as a by product. Fischer and Kuzyakov (2010) showed that the application of acetic acid found in soil (into microbial biomass or adsorbed to soil particles) of about 26% in the form of-COOH and 36% as-CH3. Microbes have the special use of the C-CH3 their growth, while the C-COOH groups tend to decarboxylation. Acetic acid in the soil provides a source of carbon for the decomposition process in producing carbon dioxide.

According to Webber and Shrefler (2006) that the acetic acid was absorbed into the plant and translocated to other parts plant of the inflict damage, therefore, it was considered to be a contact and as postemegence herbicide as glyphosate. Pujisiswanto, *et al*. (2012) found that the pre-emergence application at 10% and 20% of the glacial acetic acid solution on maize inhibited seed germination. No shoots and roots growth. This was due to the increase of electrical conductivity (EC) in growth medium electrolyte leakage caused by the high permeability of the damaged cell membrane of seed. Under control (no acetic acid treatment), the EC was 11µS/cm g, compared to 10 and 20% treatment of acetic acid were in 36 µS / cm g and 55 µS / cm g EC, respectively. It shows that no shoots and roots growth. Mathew and Powell (2006) found that the integrity of the cell membrane is determined by seed deterioration due to biochemical changes or physical damage. It can be considered as the cause of the differences in seed viability, which indirectly determines seeds leakage during EC test. Further Spancer and Ksander (1997) added that there is a possibility reason that application of acetic acid may cause degradation of the membrane protein. Johnson *et al*. (2004) reported that 10 % acetic acid applied as pre-planting in wheat could control broadleaf weed. However, it's no study on acetic acid as pre-planting on maize germination.

MATERIALS AND METHODS

The experiment was conducted in greenhouses started from July to August 2012. Factorial Completely Randomized Design (CRD) in 4 replications was used. The first factor was the concentration of acetic acid: control (acetic acid was replaced with water) 0%, 10% and 20% of acetic acid. The second factor was the time of the application: 4, 8, and 12 day before planting.

Pots with the diameter of 25 cm were filled with sand. Before application performed calibration in sand by flushing water through the field capacity, in order to obtain the volume of water used. These pots were watered every 4 days with 500 ml of water. The 10 and 20% acetic acid concentration were prepared by mixing 50 and 100 ml pure acetic acid in 500 of water respectively. Each treatment was poured into the pots on the 12, 8, and 4 days to planting. Ten seeds of maize were planted in each pot.

Observations were made at the age of 2 weeks after planting (wap), parameters measured were as follows:

$$\text{Germination percentage} = \frac{\text{number of normal seedlings}}{\text{number of seed germinated in test}} \times 100\%$$

Roots growth

Total root length and root surface area: measurements were done on three samples of plants with line intersection method (line intersection) is read by meter area (Richard *et al*., 1979 ; Collin *et al*., 1987 *cit* Indradewa, 2002). Total root length is read by meter area on the position of the "length and area". Root length was converted using the standard, Y = X. (0.8869) -0.0969, where X is the average length of the two notch roots. To get the root surface area, total root length was measured after extensive reading on the position of the projected root "area".

Plant height and leaves growth

Plant height: measured using a ruler on 3 plants sampled from the base of the stem to the longest leaves.

Leaf area: It is measured in all the leaves that have opened up perfectly on the 3 plant samples using a Leaf Area Meter by way of cut leaves, then placed over the area of the leaf area meter can be read.

Leaf greenness: measurements performed using a Minolta Chlorophyll Meter SPAD 502. Measurements were made at 3 plants per treatment sample. Leaves are measured from the top of the third leaf has opened up perfectly. The measurements were made by clipping leaves and the numbers appear is the value of green leaves.

Total chlorophyll: measurements of chlorophyll content is done by taking fresh leaves weighing 1 gram, then crushed in a mortar and then added 20 ml of 80% acetone. After stirring, the solution was filtered using Whatman filter paper 41. Measurement of chlorophyll is by using the Spectronic Spectrophotometer 21d, absorbance at wavelengths of 663 and 645 nm.
To get the total chlorophyll as follows:

$$\text{Total Chlorophyll} = 20,2 \times D_{645} + 8,02 \times D_{663} \times \frac{20\text{ ml}}{1000 \times 1\text{ g}}$$

(Arnon, 1949).

Root dry weight, shoot dry weight, and root/shoot ratio

Plant dry weight was done after on dried harvested crop on oven at a temperature of 80^{o}C observed. While the root/shoot ratio is calculated by dividing the dry weight of roots with dry weigh of shoots in each treatment.

Plant phytotoxicity

Plant phytotoxicity was observed visually by comparing the leaves color of the treated plants to the healthy one (control), which was expressed on a scale of 0-4 (Pesticide Committee, 2000), i.e.

0 = no poison, 0-5% shape and color of young leaves are not normal
1 = mildly poisoned, > 5-20% shape and color of young leaves are not normal
2 = moderately poisoned, > 20-50% shape and color of young leaves are not abnormal
3 = severely poisoned, > 50-75% shape and color of young leaves are not normal
4 = very severely poisoned, > 75% shape and color of young leaves are not normal, dry and fall off, the plant dies

Statistical analysis

Analysis of Variance (ANOVA) was performed using Completely Randomized Design employing SAS 9.1.3. Portable. Analysis was continued with DMRT test when significant differences existed. The data were analyzed using software SAS 9.1.3. Portable.

RESULTS AND DISCUSSIONS

Germination percentage

The analysis of seed germination test at 2 weeks after planting showed that there is no interaction between concentration of acetic acid and time of treatment, while the Inter treatment resulted in germination was not significant different. However, the concentration of acetic acid treatment produces a lower germination compared with untreatment. Under control (no acetic acid treatment), the germination was 90%, compared to 10 and 20% treatment of acetic acid were in 84 and 83 % respectively. While the pre-planting treatment on 4 day before planting produced the lowest germination was 80%, compared with 8 and 12 day before planting at 83 and 87%. This indicated that acetic acid up to 20% and the pre-plant application at 4 day before planting did not effect the seed germination since the good viability seed will germinated more that 80%. The raising of the pH in the treatment pots due to the nature of acetic acid which is easily evaporated from the ground, when the vapor reached the air and leaching, it will condensate to form water to normalize soil pH. One day after treatment the pH was 5.05. It is raised to about 6.12 on planting. However, the pH of the control was steady on 7, 43. Banteng (2010) reported that acetic acid is released into the soil will evaporate into the air and decompose naturally in the atmosphere by light.

Roots growth

Acetic acid treatment during pre-planting did not inhibit the total root length, and root surface area. Total length, and surface area of the root on the treatment was not significant different as $P < 0.05$. However, the rate of

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root growth was lower at 20% concentration and 4 day before planting. The presence of herbicides in the endosperm and sprout stem, resulted in limited glucose supply to support stem elongation. It is thought that poisoned roots utilized glucose at its own benefit (to grow more roots), while other part of the plant; especially stem will not be able to use it for its growth (elongation) (Ashton and Crafts, 1981; Suwarni *et al.*, 2000).

Plant growth

The all of plant growth were done on plant height and leaves growth. Acetic acid treatment as pre-planting application was significantly affected plant height Table-1. High concentration of acetic acid produced shorter plant height than plant height of untreatment (control), while pre-plant application of acetic acid at 12 and 8 day before planting produced plants with the same height, and these plants are higher compare to the plant height of the 4 day before planting. In general, the application of acetic acid as pre-planting treatment produces shorter plant compare to control. It is suspected that acetic acid causes the inhibition to plant growth.

It is assumed that the acetic acid absorbed by germinated seed and its roots was transported to the elongated area of the stem. This herbicide will inhibit the synthesis of amylase, and reduces the hydrolysis of starch into sugar inside the endosperm. This condition will result in the reduction of the amount of glucose deliver to the shoots and later on stunting the plants (Suwarni *et al.*, 2000).

Table-1. Effect of acetic acid concentration and its pre-planting application on plant height and growth of maize leaves at 2 wap.

Treatment	Plants height (cm)	Leaf area (dm^2)	Total chlorophyll ($mg.g^{-1}$)	Leaf greenness
Concentration of acetic acid				
Untreatment	29.000 a	680.50 a	25.975 a	0.927 a
10% acetic acid	22.167 b	469.17 b	22.716 b	0.802 b
20% acetic acid	21.000 b	429.92 b	22.575 b	0.771 b
Time of treatment				
4 day before planting	19.750 b	502.67 b	22.363 a	0.828 a
8 day before planting	22.125 ab	519.67 ab	22.588 a	0.829 a
12 day before planting	22.875 a	557.25 a	22.988 a	0.842 a
	(-)*	(-)*	(-)*	(-)*

*The sign (-) indicates no interaction between factors. The numbers followed by the same letter are not significantly different at 5% DMRT.

Leaves are the organs that play a critical role in the process of photosynthesis. Acetic acid treatment significantly influenced the leaf greenness and chlorophyll content, while the application of acetic acid as pre-planting treatment significantly affect maize leaf area Table-1. Ten and 20% of acetic acid treatment resulted in the reduction of the leaf area, leaf greenness, and chlorophyll content compared with the untreated control. Acetic acid was thought to inhibit the growth of the leaves. The application of acetic acid at 12 day before planting produced the widest leaf area, but not significantly different than the 8 day before planting treatment. Acetic acid treatment in general inhibited the growth of leaves, while its pre-planting application was more obvious to reduce the spreading of leaf area at 4 day before planting.

The lower of total chlorophyll and the reduction of leaf area will decrease photosynthetic rate, since it is not enough the chlorophyll pigment available in chloroplasts, while it is not enough light energy can be captured by the leaves. Increasing the concentration of acetic acid lowers total chlorophyll at all time pre-plant applications Figure-1.

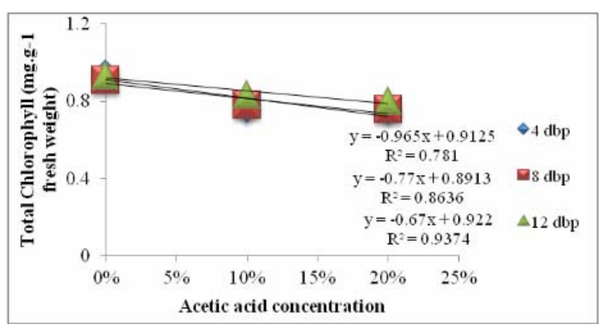

Figure-1. The relationships between of acetic acid concentration and total chlorophyll at the time of application 4, 8, and 12 day before planting.

The total chlorophyll decreases the concentration 20% of acetic acid higher than total chlorophyll decrease of concentration 10%. The total chlorophyll decrease at time of application 4 days before planting is higher than the decline in total chlorophyll at 8 and 12 day before planting. The total chlorophyll decrease seen from slope

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value each the time of application sequentially from lowest value is 12 day before planting (a = -0.67), 8 day before planting (a = - 0.86) and 4 day before planting (-0.96).

According to Taiz and Zeiger (2002), the green color of leaves due to the chlorophyll absorbs red and blue light while the green light is transmitted or reflected this suggests that the higher of chlorophyll content then the green leaves. There is a regression relationship between total chlorophyll and the leaf greenness in 4, 8, and 12 day before planting. Total chlorophyll relationship with leaf greenness showed linear equations with coefficient of determination R^2 = 0.816; 0.822; 0.824, respectively. The value of coefficient R^2 have the meaning that the total chlorophyll in each time of application contribute to leaf greenness of 81.6%, 82.2% and 82.4%, respectively Figure-2.

The leaf greenness levels associated with the control of leaf pigment content in green, which comprises chlorophyll a ($C_{55}H_{72}O_5N_4Mg$) which controls a dark green and chlorophyll b ($C_{55}H_{70}O_6N_4Mg$) that control light green color (Anonymous, 2012). It is seen that the timing of application of acetic acid were getting closer to planting corn contribute a smaller leaf greenness. The results Wong (2000) indicated that concentration at 2 mg/l or more of the concentration of the herbicide paraquat did not show an increase in chlorophyll from the growth phase, due to the inhibition of chlorophyll synthesis.

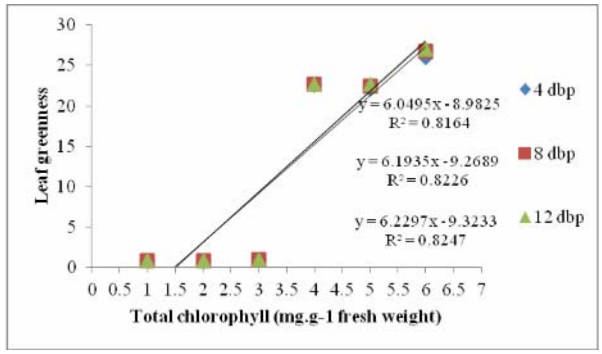

Figure-2. The relationships between leaf greennnes and the total chlorophyll on maize at the time of application of acetic acid at 4, 8, and 12 day before seed planting.

Root dry weight, shoot dry weight and root / shoot ratio

Acetic acid concentration significantly affects shoot dry weight and root / shoot ratio, but not to root dry weight. Ten and 20% acetic acid concentration yield in lower shoot dry weight compared to untreatment (control). The results of the analysis are presented in Table-2.

Table-2. Effect of acetic acid concentration and its pre-planting application on maize root dry weight, shoot dry weight and shoot / root ratio at 2 wap.

Treatment	Root dry weight (g)	Shoot dry weight (g)	Root / shoot ratio
Concentration of acetic acid			
Untreatment	0.495 a	0.902 a	0.548 a
10% acetic acid	0.480 a	0.565 b	0.849 b
20% acetic acid	0.450 a	0.500 b	0.900 b
Time of treatment			
4 day before planting	0.461 a	0.622 a	0.741 a
8 day before planting	0.471 a	0.617 a	0.763 a
12 day before planting	0.482 a	0.628 a	0.767 a
	(-)*	(-)*	(-)*

*The sign (-) indicates no interaction between factors. The numbers followed by the same letter are not significantly different at 5% DMRT

Shoot/root ratio showed was acetic acid concentration significant differences, there is a tendency on the poisoned plants to show higher root-shoot ratio than the non poisoned plants. It is assumed that when poisoned, glucose was not transported to the shoots but accumulated in the root to grow new roots. This condition is obvious in root growth parameter Table-2, where no inhibition observed, while the shoots growth was retarded. According to Barchok (1999), the hydrogen (H^+) ions as the cause of acetic acid acidity, to allow it to penetrate the membrane. Once inside the organell it will continue to break down the important molecules. It is also causes problems since the protein will perform better at normal pH. The ion H^+ lowers the pH, and thus it will be able to stop some of the reactions where proteins are involved. This will reduce carbohydrate formation and finally plant growth will be.

Plant phytotoxicity

Phytotoxicity scoring was obtained from green leaves acetic acid treatment compared with controls

Figure-3. The analysis indicates that maize was poisoned in various levels.

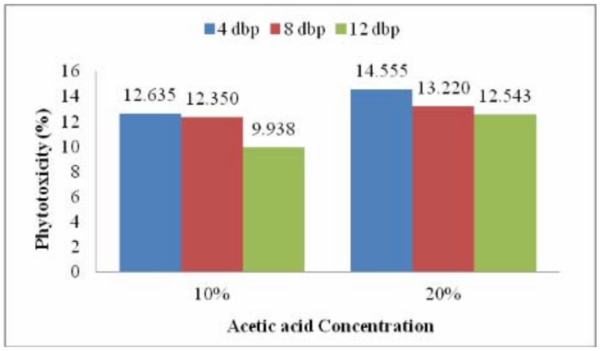

Figure-3. The relationships of acetic acid concentration with Phytotoxicity at the time of application 4, 8, and 12 day before planting.

Pre-planting application of acetic acid showed mild poisoning on maize, i.e., 9, 938 - 14, 555% which will allow the plants to grow back to normal. Johnson *et al*. (2004) found that the efficacy of vinegar at 10% concentration resulted in significant damage to wheat at early phase, however, 28 days after the treatment the plant barley shown the damage and could provide maximum grain harvest.

CONCLUSIONS

No significant inhibition on maize germination on pre-planting application of acetic acid. Its biodegradable nature (due to evaporation) enables the pH to regain its neutrality at 2 wap. Acetic acid treatment does not inhibit root growth, however reduces the growth of plant height and leaf area maize. Ten and 20% acetic acid concentration caused similar growth inhibition of the shoot, while pre-plant applications are generally not inhibit the growth of shoot. Maize poisoning after acetic acid treatment are categorized as mild poison i.e., 9, 938 - 14, 555 %, which as compared with untreatment (control) will still enable the plant to grow normally.

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