Effect of Ethephon (2-chloroethylphosphonic acid) on Leaves, Flowering, Fruiting and Seed of Local *Jatropha curcas* L.

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Abstract

As a potential petro-diesel substitute, low number of fruit production becomes the main constrain to develop *J. curcas* as a second plant oil source after palm oil in Malaysia. Utilization of plant growth regulators has been scientifically proven to increase plant growth under certain concentrations. This study was conducted to investigate the effect of different concentrations of ethephon on leaves, flowering, fruit size and number of fruit seed of *J. curcas*. Different concentration of ethephon (0 mg L^{-1}, 150 mg L^{-1}, 300 mg L^{-1} and 600 mg L^{-1}) was sprayed once on foliar. Application of ethephon induced leaves discoloration and necrosis 24 hours after treatment. Number of leaves on plant extremely reduced in 72 hours. All ethephon treated plant fastened flower buds production, approximately 15 days in average after shoot emergence. Highest concentration (600 mg L^{-1}) reduced 26.95% flower stalk compared to control. 150 mg L^{-1} and 300 mg L^{-1} promoted female flower up to 8.4 compared to control. 300 mg L^{-1} of ethephon reduced 32.6% number of fruit and 20.54% number of seed, while 150 mg L^{-1} slightly increase 8.4% of fruit size compared to control. Ethephon has potential in promoting flower buds and increasing number of female flowers in *J. curcas* but not an effective growth regulator in increasing number of fruits.

Keywords: *Jatropha curcas*, Ethephon, Plant growth regulators

Introduction

Depleted resources of fossil fuel and air pollution have leading search of new alternative renewable energy as a substitute of petro-diesel. The new source must be environmental friendly, affordable and easily available (Abdullah et al., 2009). Researchers and investors have aroused their interest in an oil-producing seed plant known as *Jatropha curcas*. Uniqueness of *J. curcas* is that this tropical plant can live in arid and semi-arid climates because of its ability to survive in the dry season (Heller 1996; Augustus et al. 2002; Azam et al. 2005; Achten et al. 2008; Hafiz, 2009). The characteristic makes the plant survive well in Malaysia which experience rain and dry climate throughout the year. It's matured fruit content oil which can be extracted and processed to form biodiesel. In its life cycle, the plant requires approximately six month to fruits after planting. However, the biggest problem of this plant is uneven ripening time among fruits on the same inflorescence and low number of fruits production (Camellia et al., 2011). In Lucknow India, only 50% of flowers were reported to fruits (Bhattacharya et al., 2005). In this case, utilizing plant growth regulator (PGR) is considered would help as in many others plants that showed positive response upon application (Rahim, 1988; Rahim and Alamgir, 1995; Ud-Deen et al., 2009). It has been suggested that application of PGR improved plant growth under stress depends on application method and concentration used (Joshi et al., 2011).

Ethylene is a PGR that may promote or inhibit flowering. It also plays important roles in plants process of growth and development (Arshad & Frankenberger, 2002). Muromtsev et al., (1990) reported that ethylene increased yield production of tomatoes 30-50% together with ripening acceleration and quality improvement. In *J. curcas*, ethylene showed a significant benefit in promoting growth and yield (Joshi et al., 2011). Augustus et al., 2002 reported that ethephon has induced hydrocarbon content inside the fruit 5.0%. It was found that performance and effect of PGR vary under different climatic zones. It also has been reported higher concentration of PGR leads to prominent effect of plant morpho-physiology. The aim of this study was to investigate the effect of different concentrations of ethephon on leaves, flowers and fruits of local *J. curcas*.

Materials and Methods

Field experiment was carried out in October 2011 on one year old plants at Unit Ladang, Universiti Teknologi MARA. The cuttings were collected from local village (Kg. Guar Nangka) in Perlis. Selected 25-30 cm in length cuttings were sown in polythene bags filled with soil-compost-sand (3:2:1) mixture. The cuttings were grown in nursery and irrigated daily for two months before transplanted at experimental sites. Transplanting was made during onset of the rainy season. Each treatment consists of 18 plants considering a single plant as one replicates and arranged in complete randomized design (CRD). The distance between plants was 2.5 m. Fertilizers were given once for every harvesting period. Ethephon solution was diluted at different concentration (0, 100, 300 and 500 mg L^{-1}) with distilled water. Plant was covered with perforated plastic bag to avoid ethephon from evaporates. 30 mL of ethephon was applied once on foliage via foliar sprays. Plastic bag was removed after an hour. Observations on leaves were made immediately after treatment. The number of flowers buds, length of flower stalk, number of female flower and number of fruits were recorded. Analysis of variance (ANOVA) on the data was analyzed using SPSS software. Significance difference among treatments was compared according to Least Significant Different (LSD) at $p < 0.05$.

Results

Effect on leaves discoloration and abscission

After 48 hour of ethephon treatment, the leaves discoloration started to change from green to yellowish green (Figure 1). After 72 hour, most of the yellowish green color leaves changed its color to yellow. The color was clearly visible covering most of the area on leaves surface. Leaves discoloration was observed within 24 to 48 hour after treated with ethephon at all concentrations. Early symptom of leave dry was also observed after treatment with visible brown spot occurs on leave surface. Highest concentration (600 mg L-1) shows numerous quantities compared to 150 mg L^{-1} and 300 mg L^{-1}. The application of ethephon also affected leaves tissue that caused necrosis. The effect was clearly visible at the edge of the leaves. Most of the treated leaves shrunk and bent upward after treatment. Figure 2 showed that treated plant experienced sudden loss of leaves 24 to 72 hours after treatment compared to control. Approximately 70% of leaves abscised after 72 hour in ethephon-treated plants.

Effect on flower and fruit development

Table 1 showed that in average, untreated plant produced the lowest number of flower buds (43.2) while plant treated with 150 mg L^{-1} produced the highest (57.2). The higher concentrations 300 mg L^{-1} and 600 mg L^{-1} produced 52.0 and 45.7, respectively. In length of flower stalk, highest concentration of 600 mg L^{-1} showed the shortest flower stalk (1.22 cm), followed by 300 mg L^{-1} (1.42 cm) and 150 mg L^{-1} (1.22 cm) compared to control (1.67 cm). However, there was no significance different detected in length of flower stalk for all treatments. Number of female flower was increased significantly to 8.4 when treated with 150 mg L^{-1} and 300 mg L^{-1} compared to control (4.7). However, number of fruits per plant was significantly reduced at all concentration of ethephon. Control showed the highest number of fruit (9.2) while 300 mg L^{-1} showed the lowest (6.2).

J. curcas starts producing unisexual flowers after leave abscission. The plant requires almost a month from vegetative stage to produce flower buds. In this experiment, ethephon tends to initiate flowering by inducing early flower buds production. In Figure 3, flower buds of ethephon treated plants were visible 15 days after leaves emergence as compared to control which required 25 days. The number of flower buds in control plant ranging from 21 to 80 per inflorescence while total number of flower buds in treated plant ranging from 18 and up to 204 per inflorescence.

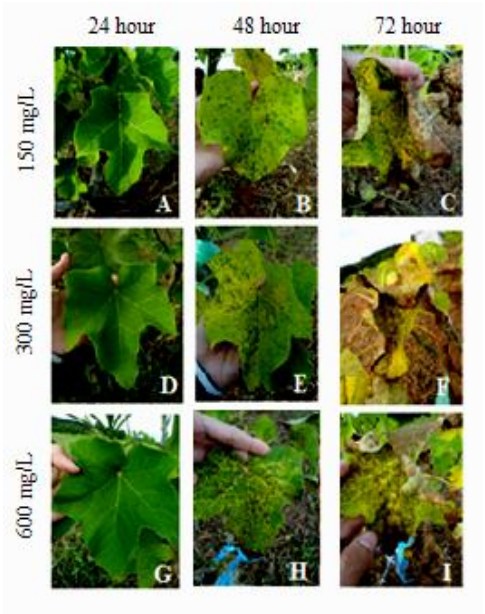

Figure 1 : Effect of different concentrations of ethephon on leave at 24, 48 and 72 hours after treatment.

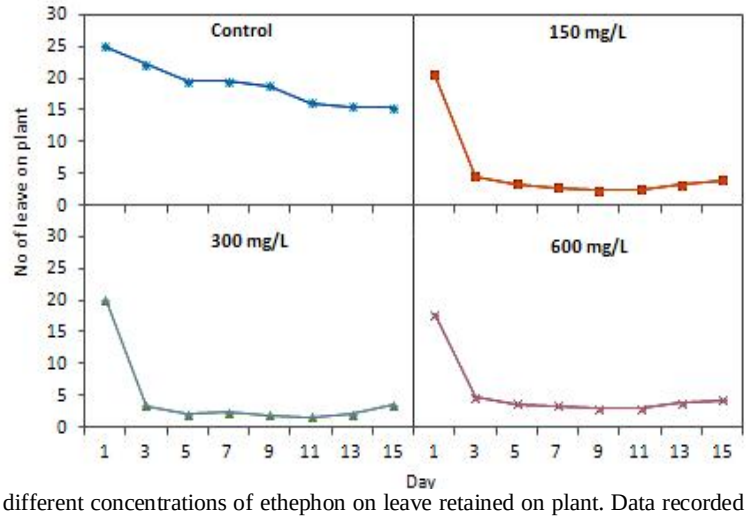

Figure 2 : Effect of different concentrations of ethephon on leave retained on plant. Data recorded every three days for 15 days. Number of leaves was counted per branches.

The number of flower buds in ethephon treated plant was three times higher than control. Fruit size in plant treated with 150 mg L^{-1} and 300 mg L^{-1} was slightly larger compared to control. However, the size was smaller in plant treated with the highest concentration of ethephon (600 mg L^{-1}). Number of seeds of all ethephon treated plant was less than control plant (22.4). Plant treated with 300 mg L^{-1} has the lowest number of seeds (17.8).

Discussions

It has been reported by Prameswara et al. (2009) that high concentrations of ethylene (300 mg L^{-1} and 500 mg L^{-1}) caused dry and necrosis or red coloration in *P. nobilis* plantlets in *in vitro* experiment. Leaves of *P. spicatus* responded by showing the effect of dry, chlorosis and abscised. Study done by Zhang et al., (2011) showed that exposure to 20µL L^{-1} ethylene for 24 hour also caused yellowing to postharvested *Brassica rapa*. The effect took place after three to five days of treatment. It was suggested that high concentration of ethylene may become toxic to plant (Prameswara et al., 2009).

In citrus leaf showed that abscission process increased about 10% after 48 hours after exposed from minimum (0.1 ppm) to maximum (10.0 ppm) concentration of exogenous ethylene. In presence of highest amount of ethylene (10.0 ppm), the abscission process starts within 24 to 28 hours (Ratner et al., 1969). In cotton plant, significant abscission observed after 12 hours with 78% of leave abscised after treated with 90 ppm of ethylene. The time interval for the abscission process to takes place within 24 to 48 hours after exposure if supplied ethylene exceed threshold of 0.08 ppm (Bayer and Morgan, 1971). Ethylene plays major roles in loosening abscission organ of plant. This induce-senescence regulator will cause leaves deterioration, which trigger chlorophyll degradation and accelerating the senescence process (Gergoff et al., 2010). In citrus leaf, time interval within 6 to 24 hours was detected where cellulase activity started to work progressively before separation of leave takes place. Leave separation started 12 to 24 hours and the process not ceasing until 72 hours when number of leave loss decreased. (Ratner, 1969; Agusti et al., 2008). The exposure to ethylene might cause flux of auxin level inside that plant that contributes to inhibition of cell separation at abscission zone in leaves (Mishra et al., 2008).

Table 1 : Effect of different concentration of ethephon on number of flowers, length of flower stalk and number of female flower and number of fruit.

Treatment (mg L^{-1})	No of flower buds / plant^{NS}	Flower stalk (cm)^{NS}	Female flower*	No of fruit / plant*
150	57.2	1.61	8.4b	6.8a
300	52.0	1.42	8.4b	6.2a
600	45.7	1.22	6.3a	7.2a
control	43.2	1.67	4.7a	9.2b

^{NS} No significance

* Means with different letter within column are significantly difference at p=0.05 by least significant difference test.

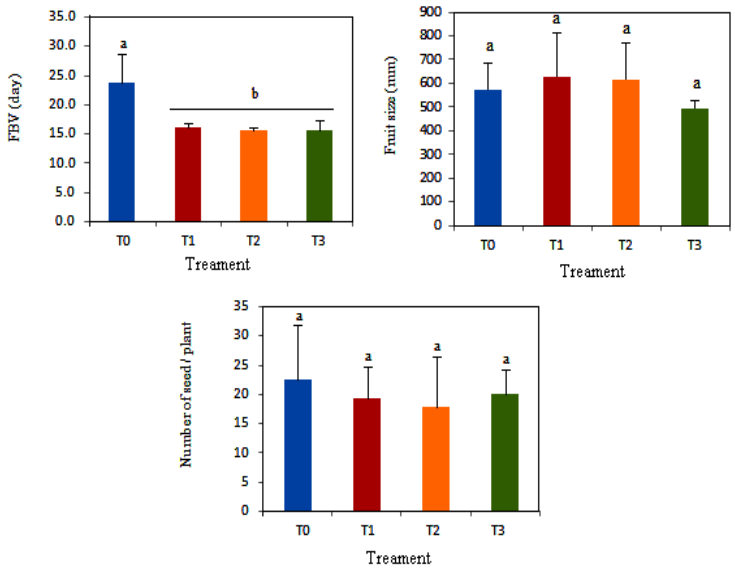

Figure 3: Effect of ethephon at different concentrations on different characteristics of *J. curcas*. (FBV: Flower bud visible, T0: control, T1: 150 mg L-1, T2: 300 mg L-1 and T3: 600 mg L-1).

Ethylene has been reported to affect plant growth and plant metabolism. Effect of flower stalk reduction was observed in *Curcuma alismatifolia* when exposed to ethephon at 300 mg L^{-1} and 500 mg L^{-1} (Khuankaew et al., 2009). Reduction of flower stalk may be due to reduction of dividing and expanding cell when exposed to high concentration of ethylene. Growth-inhibiting effect by high ethylene concentration caused cell expansion reduction in Arabidopsis (Stepanova and Alonso, 2005; Khuankaew et al., 2009). Effects of ethephon on flower characteristics were differed among plant species in various ways. High concentration of ethephon tends to induce flower abortion. In addition, ethylene also influence in sex reversal (Prameswara et al., 2009). Application of ethylene-releasing agent on shoot apices of androecious promoted the production of female flowers in cucumber. They found that ethrel regulated a gene in shoot apex and that gene maybe involved in formation of female flowers (Wang et al., 2005).

Study done in bromeliads showed that flowering was induced when exposed to ethylene-releasing compound (Dole and Wilkins, 1999). In contradictory, 500 mg L^{-1} and 1000 mg L^{-1} of ethephon sprays delayed flowering in *Achillea*, *Echinacea*, *Monarda* and *Physostagia* 1 to 7 days compared to untreated (Hayashi et al., 2001). It was suggested that the induction of flower buds is due to the reduction of auxin transport level which, therefore stimulate flower buds formation in plant (Sanyal and Bangerth, 1998).

Applying ethephon on pistachio nut showed that the number of fruitlets per cluster and number of nut were reduced compared to ethephon untreated plant but increased size of nut. From the result they suggested that the percentage of seedless nut was decreasing may be due to the fruit reduction (Rahemi and Ramezanian, 2007). Similar results showed in almond nut when applied with 75 mg L^{-1}, 150 mg L^{-1} and 300 mg L^{-1}. The number of fruit only 0.5% to 1.5% compared to control (10.4%). It may be due to defectiveness of megaspore during development stage or deficiency of carbohydrate in plant (Dennis, 1976; Contreras et al, 2011).

Conclusions

In this experiment, ethephon showed a good potential in promoting flower buds and increasing number of female flowers. However, it is not an effective growth regulator in increasing number of fruits in *J. curcas*. Further study on the effect of this ethylene-releasing compound on flower retention and application at different growth stages under control environment should be carried out in order to increase yield production of local *J. curcas*.

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