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SURVEY OF FACTORS (SODIUM CHLORIDE, ACID ACETIC, SHADOW, CUTTING) AFFECT TO THE SPREADING OF WATER HYACINTH EICHHORNIA CRASSIPES (MART.) SOLMS

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ABSTRACT:

Water hyacinthEichhornia crassipes (Mart.) Solms is a weed species that causes a lot of harm to the environment and humans. Treatment of sodium chloride (salt),acid acetic, shadow or cutting is applied effectively in spreading of Eichhornia crassipes (Mart.) Solms. Sodium chloride 3% control widespread of Eichhornia crassipes (Mart.) Solms and shadow reduces the number of shoots and leaves stronger than acetic acid 5% and sodium chloride 3%, and acetic acid 5% caused leaf damage more strongly than sodium chloride 3%. Other while, if stolon is cut each week, the shoots and the stolon will reappear each week after cutting, but could not appear at week 6.

Keywords: water hyacinth Eichhornia crassipes (Mart.) Solms, sodium chloride, acid acetic, shadow, cutting.

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INTRODUCTION:

Water hyacinth Eichhornia crassipes (Mart.) Solms is a species of weed that grows strongly and continuously throughout the year, by forming lateral shoots from axillary buds below the apical meristem of the parent plant. Each lateral shoot at the tip of a stolon connects to the parent plant and then becomes a new shoot unit then spread into a large, dense carpet. A number of mechanical (salvage), chemical (processing 2-4 D herbicides) and biological (using insects and plant pathogens) measures are used to prevent the invasion of Eichhornia crassipes (Mart.) Solms, but ineffective, and more or less affect people and the environment. Treatment of sodium chloride (salt) was applied effectively in Giant sensitive plant (Kiet Thuong Vo, 2012) as well as in Pennisetum purpurrerum (Nghi Huu Vo, 20202) with controlled concentrations. This article presents the shoot formation results and some preliminary treatments, prior to application of sodium chloride to inhibit shoot formation and elongation of stolon in Eichhornia crassipes (Mart.) Solms.

MATERIALS AND METHODS:

MATERIALS:

Eichhornia crassipes (Mart.) Solms are collected along rivers and canals or grown in experimental gardens of the University of Natural Sciences and the University of Food Industry in Ho Chi Minh City.

METHODOLOGY:

Observe the spreading pattern, morphology, and structure of Eichhornia crassipes (Mart.) Solms:

The development of Eichhornia crassipes (Mart.) Solms on rivers and canals was observed in several places in Ho Chi Minh City, Viet Nam. Shoot formation was observed from plants grown in the experimental garden, in direct sunlight, in plastic containers containing 150 liters of water, and water was changed weekly. The mother plant was initially selected as a plant with 10 leaves, 1 bud at the top of stolon 1 cm long. The anatomical structure of leaves, buds and stolon cuttings was observed under an optical microscope (Olympus CX23) after bicolor staining (carmin red and iodine blue). The Eichhornia crassipes (Mart.) Solms buds in the cluster were denoted by four digit numbers representing the first, second, third and fourth level buds. At each level, 0 indicates the lateral shoot that has not appeared yet, 1 indicates the first lateral shoot, 2 indicates the second lateral shoot, 3 indicates the third lateral shoot, and 4 indicates the fourth lateral shoot. For example, 0000 is the first parent plant; 2000 is a 1st level second shoot from the first mother plant, without secondary, third, and quadruple shoots; 1121 is 4th level first shoot from 3rd level second shoot (3rd level secondary shoot from 2nd level first shoot, and 2nd level first shoot from a 1st level first shoot).

Eichhornia crassipes (Mart.) Solms and treatment of sodium chloride and acetic acid on plants *in vitro*:

In vitro plants 2 weeks old after transplanting having 1-2 leaves treated by dripping (by 1ml pipette) 0.5ml NaCl 3% with Tween 20 1 ‰ supplemented or acetic acid 5% onto leaves through a membrane filter with pore size 0.3 µm. Observe the number of leaves and shoots formed during 4 weeks of culture under 3000 lux light, 70 – 80% humidity and 25^{o}C temperature.

Experiment of cutting stolon:

Eichhornia crassipes (Mart.) Solms with 9-10 leaves and 1 bud were collected and continued to be cultured in the experimental garden, in plastic containers (35x20x25cm) containing 8 liters of ionized supplemented water with MS medium 1/10 (Murashige Skoog). Cut the stolon with shoot at the tip (1 cm from the parent shoot) every week, from week 1 to week 6 and record the number of shoots formed from the parent plant after each cut. The experiment was repeated 3 times with 1 plant each time.

Shadow and treatment of sodium chloride and

acetic acid:

Eichhornia crassipes (Mart.) Solms with 6-10 leaves, 1-2 flowers and 1 bud with 3-5 leaves at the tip of stolon was collected in the experimental garden, then put 3 plants into plastic containers containing 10 liters of MS 1/10. The experiment had 4 treatments including control, treatment of sodium chloride 3% solution with Tween 20 1 ‰ supplemented, acetic acid 5% and shadow. Spray 500ml of treatment over all leaves once at 9am. Photograph and track lateral shoot formation and leaf count over 75 days. The experiment was repeated 3 times, each time 1 box.

Treatment of sodium chloride on leaves that remain on plants:

Eichhornia crassipes (Mart.) Solms with 10 leaves were collected along the river and placed in plastic containers (35x20x25cm) containing 8 liters of MS 1/10 (with ionized water) 48 hours prior to the experiment. Spray on leaves 9 with sodium chloride 3% solution (supplemented with Tween 20 1‰). Observe leaf damage, indicated by discoloration and withering, over time after treatment.

Treatment of sodium chloride and acetic acid onto separated leaves:

Leaves 8 and 9 (from the base) of a Eichhornia crassipes (Mart.) Solms with 10-11 leaves in the experimental garden were cut from the parent plant, plugged in water and sprayed once on both leaf surfaces with 5 ml of NaCl 3% solution supplemented with Tween 20 1 ‰ and acetic acid 5% (without Tween supplementation). The treated leaves were left in the growth room under led light at an intensity of 2500 ± 200 lux. Leaf damaged surface was recorded 12 hours after treatment. Each treatment was repeated 5 times, one leaf at a time.

The sign of leaf surface damage is the discoloration of the leaf blade from green to yellow and withering, and the leaf damage rate (R) is calculated using the formula (Curriculum for Plant Physiology, University of Food Industry):

$$\mathrm{R} = \frac{S1}{S} \times 100\%$$

where S and S1 are the areas of the leaf and the damaged area respectively.

S and S1 are calculated by placing the leaf and damaged area on a grid paper, then drawing, cutting, and weighing the paper, and using the following formulas:

$$\mathrm{S} = \frac{\mathrm{a} \times 100}{0.76}\ (\mathrm{cm})$$

$$\mathrm{S1} = \frac{\mathrm{b} \times 100}{0.76}(\mathrm{cm})$$

where a and b are the weight (g) of the leaf-shaped paper and the damaged leaf part respectively, and 0.76 g is the weight of the paper with an area of 100 cm^2.

Treat sodium chloride once on all leaves:

Cluster of Eichhornia crassipes (Mart.) Solms with 10 leaves and 5 shoots (level 1,2,3) at the tip stolon grown in the experimental garden, in plastic containers containing 10 liters of MS 1/10, sprayed with sodium chloride 3% solution supplemented with Tween 20 1‰ once or twice at 6 hours and 18 hours a day on all leaves. Photograph and monitor lateral shoot formation over 4 weeks. The experiment was repeated 3 times, each time 1 box

Treatment of sodium chloride several times on all leaves:

The Eichhornia crassipes (Mart.) Solms in this treatment were planted in the experimental garden, spread and covered the water surface of the 8 m^2 experimental plot, sprayed 12 L of NaCl 3% solution supplemented with Tween 20 1‰ once every week, in the morning, for 5 consecutive weeks. Monitor the number of new shoots (lateral shoots) appeared each week after treatment.

During the experiment, the ambient temperature and the intensity of direct sunlight varied between 26.9 ± 0.4 to 30.6 ± 1.0 º C and 39.1 ± 1.5 to 48.4 ± 1.7 klux respectively. Photograph and count the number of new shoots after each week of treatment, from 15 homogeneous mother plants selected just before treatment, each of these mother plants had 7-10 leaves and 1 bud had 2 - 4 leaves.

RESULTS:

Spread of the Eichhornia crassipes (Mart.) Solms over the water surface:

In some parts of rivers and canals in Ho Chi Minh City, Eichhornia crassipes (Mart.) Solms form clusters floating on the water surface, can be scattered on the fast flowing river, or big clusters like a carpet on slow-flowing water, or dense and wide carpet walls in the parts of slack water (Figure 1).

Figure 1. Wide Eichhornia crassipes (Mart.)

Solms on slow-flowing river near Binh Thuan bridge (Binh Chanh district, Ho Chi Minh city) and dense on slack water in black water canal (Tan Phu district, Ho Chi Minh city) After 30 days of being grown in water, in the experimental garden, a Eichhornia crassipes (Mart.) Solms bush taken from the canal, consisting of the original mother plant (symbol: 0000) with 10 leaves, 1 lateral shoots with 2 leaves at the tip of a stolon 1 cm, created 25 new plant units consisting of 4 shoots level 1, 10 shoots level 2, 8 shoots level 3, and 3 shoots level 4. The stolon is below the 2000 and 1100 shoot tips extending to 30 cm (Figures 2 and 3).

Figure 2: A Eichhornia crassipes (Mart.) Solms bush of the original mother plant after 30 days of being grown in water in the experimental garden

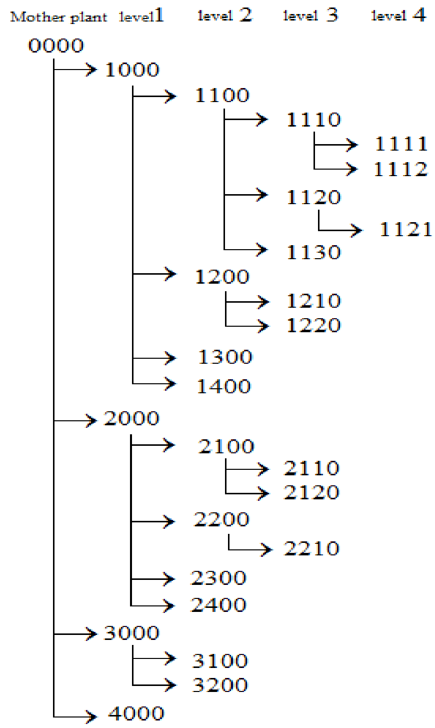

Figure 3: Diagram showing hyacinth branching in four tiers to add 25 new plant units from the original parent plant, after 30 days of hydroponics (as per figure 2).

<u>Eichhornia crassipes (Mart.) Solms morphology and structure</u>:

Observations of Eichhornia crassipes (Mart.) Solms at a growth stage with 5 leaves with extended lamellae revealed that spongy stems help the plant float above water and an abundant adventitious root system in the base region with numerous branch roots. Axillary buds appeared at the base of each leaf stalk, creating an elongated stolon to connect with the mother plant in the Eichhornia crassipes (Mart.) Solms bush. The apical meristem, at the apex or axillary of the leaves, is the place to create new shoot units, consisting of 2-3 layers of the outermost cell (tunica) with a perpendicular division pattern, followed by a group of cells that divide according to the random direction (corpus), and below that is the core meristem region surrounded by the procambium. The leaf blade, petiole and stolon have many large air chambers (Figure 4).

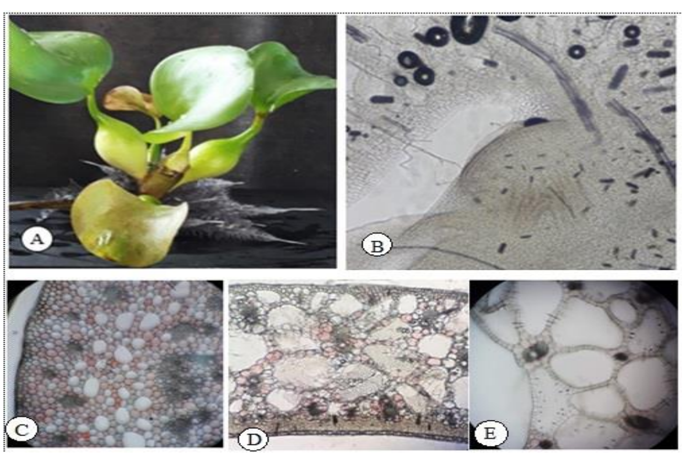

Figure 4. Hyacinth at the growth stage (A) and structure of the apical meristem (B), stolon (C) leaf blade (D) and petiole (E)

Effects of sodium chloride and acetic acid on plants *in vitro*:

After 4 weeks of culturing, in vitro plants produced more leaves and lateral shoots, while treatments of sodium chloride 3% (supplemented Tween 20 1 ‰) and reduced acetic acid 5% for leaf and lateral shoot formation. (Table 1, Figure 5).

Table 1. Effects of sodium chloride and acetic acid after 4 weeks of treatment on leaf and shoot formation of *in vitro* 2 weeks old plants

Treatment	Number of leaves	Number of shoots
Control	$7,3^{c} \pm 0,5$	$2,6^{c} \pm 0,5$
CH3COOH 5%	$4,6^{b} \pm 0,5$	$2,0^{b} \pm 0,0$
NaCl 3%	$3,0^{a} \pm 0,0$	$1,6^{a} \pm 0,5$

$^{a, b, c}$: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

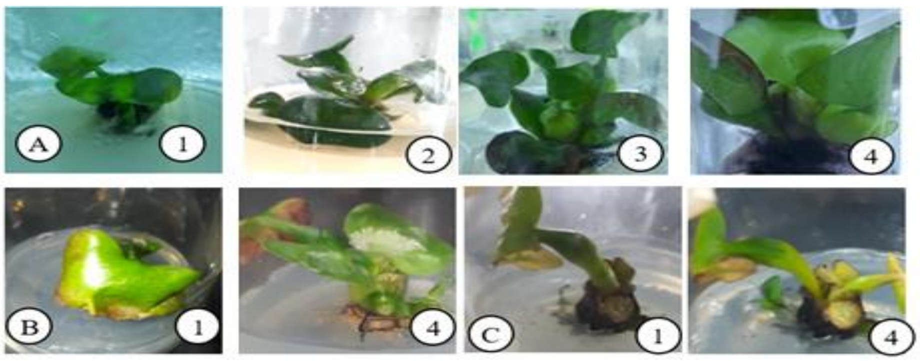

Figure 5. *In vitro* plants at 1, 2, 3 and 4 weeks old (A), and at 1 and 4 weeks after acetic acid 5% (B) and sodium chloride 3% (C) treatment on 2 weeks old plants

Stolon cutting:

In the experimental garden, after the removal of all the stolon (week 0), the mother plant (with 9-10 leaves and 1cm stolon with shoots at the tip) regenerated the shoots and the stolon after 1 week (week 1). If cutting continued each week, the shoots and the stolon reappear each week after cutting, but could not appear at week 6. Thus, a total of about 8 shoots appeared from mother plants within 6 weeks of observation (Table 2, Figure 6).

Table 2. Number of water hyacinth shoots grown in experimental garden after the stolon removal treatment, respectively at each week

Time (week)	Number of new shoots
0 (right after the 1st cutting)	$0,0 \pm 0,0^{d}$
1 (right before the 2nd cutting)	$1,5 \pm 0,2^{bc}$
2 (right before the 3rd cutting)	$2,3 \pm 0,3^{a}$
3 (right before the 4th cutting)	$2,0 \pm 0,3^{ab}$
4 (right before the 5th cutting)	$1,3 \pm 0,3^{bc}$
5 (right before the 6th cutting)	$1,0 \pm 0,1^{c}$
6 (1 week after the 6th cutting)	$0,0 \pm 0,3^{d}$

[a, b, c]: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

Figure 6. Hyacinth shoot generation from mother plant in experimental garden after removal of all stolon in turn each week

A, Immediately after 1st cutting (mother plant cut the eel lines with the lateral buds)

B, One week after 2nd cutting (mother plant with 4 lateral shoots)

C, One week after 5th cutting (mother plant with 2 lateral shoots)

D, One week after the 6th cutting (mother plant no longer produces lateral shoots)

Shadow and sodium chloride and acid acetic treatment:

The experiment had 4 treatments including control, treatment of sodium chloride 3% solution supplemented with Tween 20 1 ‰, acetic acid 5% and shadow. Spray 500ml of treatment over all leaves once at 9am. All three treatments of shadow, acetic acid 5% and sodium chloride 3% reduced the number of shoots and leaves compared to the initial control (with 1 lateral shoot and 6-10 leaves), especially with shade treatment, when observed on day 75 after treatment in the experimental garden (Table 3).

Table 3. Effects of shade treatments, sodium chloride and acetic acid on water hyacinth leaf and shoot formation in experimental garden (observed on day 75)

Treament	Number of new shoots	number of mother plant's leaves
Control	$3,3^{c} \pm 0,2$	$11^{c} \pm 0,6$
Shadow	$1,3^{a} \pm 0,1$	$4,3^{a} \pm 0,4$
NaCl 3%	$2,3^{b} \pm 0,1$	$7,3^{b} \pm 0,5$
CH_3COOH 5%	$2,0^{b} \pm 0,2$	$6,0^{b} \pm 0,3$

[a, b, c]: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

Spray sodium chloride 3% or acetic acid 5% onto separated leaves:

12 hours after treatment, spraying sodium chloride 3% solution (with Tween 20 1‰ supplementation) and especially acetic acid 5% on the outer surface (top and

bottom) of separated leaves (from experimental garden) increased the rate of leaf damage: leaves changed color from normal green to yellow and withered (Table 4, Figure 7).

Table 4. Damage of water hyacinth leaves in experimental garden at 12 hours after spraying the leaf surface with sodium chloride 3% solution (with Tween20 1‰ supplementation) or acetic acid 5%

Treament	The rate of leaf damage (%)
Control	$0,0^{a} \pm 0,0$
NaCl 3%	$8,7^{b} \pm 0,9$
CH_3COOH 5%	$25^{c} \pm 3,2$

a, b, c: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

Figure 7. Control hyacinth leaf (A) and separate leaf surface damage at 12 hours after sodium chloride 3% (B) and (C) acetic acid 5% treatment.

Treatment of sodium chloride on leaves on plants:

Spraying a sodium chloride solution 3% (with Tween 20 1 ‰ supplemented) on leaf 9 (on a 10-leaf plant in the experimental garden) damaged this leaf, while the other leaves on the plant remained green and in normal shape as before treatment (Figure 8). 1 hour after treatment, leaf 9 became greyish-yellow from the edge to about half of the leaf area and bent at the edge. However, leaf 9 gradually turned green again after 6 - 24 hours, only curl and burn yellow at the edge.

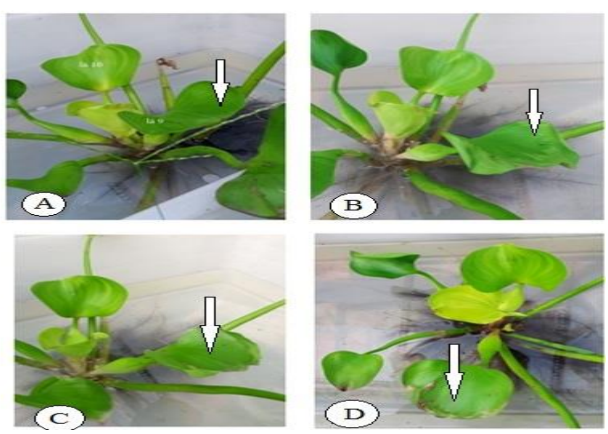

Figure 8. Effects of spraying sodium chloride 3% on 9th leaves on 10-leaf plants: right before treatment (A), 1 hour after treatment (B), 6 hours after treatment (C) and 24 hours after treatment (D)

Apply sodium chloride by spraying once over all leaves:

Spraying sodium chloride 3% (with Tween 20 1‰ supplemented) on all Eichhornia crassipes (Mart.) Solms leaves (with 5 shoots at the tips of the stolon) in the experimental garden, in three treatments (once at 6 o'clock, once at 18 o'clock, and twice a day at 6 and 18 o'clock) did not inhibit the shoot formation (the number of shoots was still increasing), but significantly decreased shoot formation compared to the control after 2 weeks, and the effects lasts up to 6 weeks. Treatment in the afternoon (18 o'clock) or twice (6 o'clock and 18 o'clock) reduced shoot formation more strongly than the morning treatment (6 o'clock) at weeks 3 and 4, but the difference was not significant at week 6 (Table 5). All plants, which were normal (control) with leaf damage and reduced number of shoots due to the treatments, had green leaves and flowered after 6 weeks (Figure 9).

Table 5. Effects of spraying sodium chloride 3% solution (with Tween 20 1‰ supplemented) on shoot formation of water hyacinth in experimental garden

Time (week)	Number of new shoots			
	Control	Morning treatment	Afternoon treatment	Twice a day
0	$5\pm0,0^{a,1}$	$5\pm0,0^{a,1}$	$5\pm0,0^{a,1}$	$5\pm0,0^{a,1}$
1	$5\pm0,0^{a,1}$	$5\pm0,0^{a,1}$	$5\pm0,0^{a,1}$	$5\pm0,0^{a,1}$
2	$7\pm1,0^{b,2}$	$5\ \pm0,0^{a,1}$	$5\pm0,0^{a,1}$	$5\pm0,0^{a,1}$
3	$11\pm0,3^{c,3}$	$7,6\pm0,5^{b,2}$	$5,3\pm0,5^{a,1}$	$5\pm0,0^{a,1}$
4	$14,3\pm0,3^{d,3}$	$10,6\pm0,3^{c,2}$	$8,2\pm0,3^{b,1}$	$8\pm0,3^{b,1}$
6	$17\ \pm0,3^{e,2}$	$13,6\pm0,3^{d,1}$	$13\ \pm0,3^{c,1}$	$13,6\pm0,3^{c,1}$

$^{a,\ b,\ c}$: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

$^{1,\ 2,\ 3}$: show differences in significant rows at confidence level $p \leq 0.05$ in the Duncan test.

Figure 9. Apply sodium chloride 3% (with Tween 20 1 ‰ supplemented) once to all leaves of the hyacinth bush grown in the experimental garden: control at the beginning of the experiment (A) and at week 3 (B) and 6 (C), and at 1 hour (D), week 3 (E) and week 6 (F) after treatment once at 6 o'clock.

Apply sodium chloride by spraying several times over all leaves:

NaCl 3% spray treatment with Tween 1ml/l supplemented over leaves surface leaves every week, for 4 consecutive weeks, at the experimental garden, strongly suppressed shoot generation from week 1 (1 week after treatment), and then new shoot formation cannot continue until week 4. The number of leaves is therefore also markedly reduced (leaves are waterlogged and submerged). This treatment caused almost no damage to surrounding plants in the same tank, and the control plants grew and flowered normally in week 4 (Table 6, Figure 10).

Table 6. Number of hyacinth shoots after NaCl 3% treatment consecutively per week, for 4 weeks, in experimental garden

Time (week)	Number of new leaves		Number of new shoots	
	Control	Treatment	Control	Treatment
0 (before treatment)	0,0 ^{a} ± 0,0	0,0 ^{a} ± 0,0	0,0 ^{a} ± 0,0	0,0 ^{a} ± 0,0
1	1,2 ^{b} ± 0,2	1,1 ^{c} ± 0,2	1,6 ^{b} ± 0,1	0,8 ^{b} ± 0,0
2	1,3 ^{b} ± 0,2	0,7^{bc} ± 0,2	1,9 ^{b} ± 0,3	0,9 ^{b} ± 0,1
3	1,5^{bc} ± 0,2	0,6 ^{b} ± 0,3	5,6 ^{c} ± 0,0	0,7 ^{b} ± 0,3
4	1,8 ^{c} ± 0,2	1,0^{bc} ± 0,3	10,3 ^{d} ± 0,3	0,7 ^{b} ± 0,3

$^{a, b, c}$: show differences in significant columns at confidence level $p \leq 0.05$ in the Duncan test.

Figure 10. Weekly NaCl 3% treatment in experimental garden
A, Control area. B, Treatment area. C, Control plant. D, Treatment plant
1, 2, 3 and 4: photos taken at weeks 1, 2, 3 and 4.

DISCUSSION:

Eichhornia crassipes (Mart.) Solms is a weed species that causes a lot of harm to the environment and humans: affects irrigation systems and water transport, fishing, and increases diseases (such as malaria, tapeworms, worms and cholera) and reduce biodiversity (Gebregiorgis, 2017). The observations in this research are consistent with published results: mature Eichhornia crassipes (Mart.) Solms include a system of long roots, rhizomes, stolons, leaves, flower clusters and fruits (Ayanda et al. 2020); rapidly invasive, especially in static or slow-flowing freshwater (Reddy et al., 1991); have specialized structures for floating life such as thin epidermal cells, large air spaces, and little vessels (Qaisar, 2005), and there are many axillary shoots that generate stolon by internode to connect with the parent plant for rapid invasion (Richards, 1982). Sequential ablation of stolon from a mother plant with 9-10 leaves indicated that as many as 8 lateral shoots (level 1 shoots) appeared and extended from the axillary leaf of a mother plant of 9 - 10 leaves (Table 2, Figure 6).

Among experimental garden primary treatments, shadow reduced the number of shoots and leaves stronger than acetic acid 5% and sodium chloride 3%, and acetic acid 5% caused leaf damage more strongly than sodium chloride 3%. Solanki (2017) also found that acetic acid causes more damage for Eichhornia crassipes (Mart.) Solms than NaCl and KCl, causing leaves to change color from green to brown and affecting both the petioles and rhizomes, while NaCl and KCl only damage the leaf edges. However, sodium chloride is applied because it is convenient, inexpensive and less harmful to the environment and people. Furthermore, floating aquatic plants like Eichhornia crassipes (Mart.) Solms have underdeveloped vascular systems and few root hairs, and the absorption of water and minerals largely takes place through the stem (Nasir and Ali, 1977), so a strong leaf blade injury due to acetic acid treatment would not help the treatment agent follow the cell pathway down to the rhizomes to inhibit shoot formation. Spraying sodium chloride 3% (with Tween 20 1‰ supplemented) on all the leaves in the experimental garden, once or more, significantly reduced the shoots formation of Eichhornia crassipes (Mart.) Solms, a plant adapted to fresh water life. For one-time treatment of sodium chloride, whether spray in the morning (6 o'clock), afternoon (18 o'clock), or twice morning and afternoon, the shoot-forming suppression effect was observed only 2 weeks after treatment. This time is necessary for the migration of sodium chloride from the leaf blade to the rhizome to specifically inhibit the shoot formation up to week 6, but without damage to the leaves and not to prevent flowering. Treatment of sodium chloride 3% (with addition of Tween 20 1‰ supplemented) over all leaves surface every week, for 4 weeks, completely prevent new shoot formation (thus, leaves decreased significantly) from week 1, shows the applicability of sodium chloride to control widespread spread of Eichhornia crassipes (Mart.) Solms.

CONCLUSION:

Currently there are many methods of dealing with water hyacinth spread such as chemical, biological and mechanical methods, but these methods are quite expensive. A new method of combining existing treatment is needed. Sodium chloride, acid acetic, shadow or cutting affect to the spreading of water hyacinth eichhhonia crassipes (mart.) solms. However Sodium chloride is applied because it is convenient, inexpensive and less harmful to the environment and people.

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