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The Effect of Different Concentration of Glyphosate on the Growth of Coconut Seedlings

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ABSTRACT Coconut (*Cocos nucifera* L) is one of the predominant plantation crops in Sri Lankan economy which is known to have existed for over thousands of years. During the past decades coconut production had been reduced by a significant quantity. The usage of poor quality planting materials is a major reason for the low coconut production. Thus much attention needs to be paid in coconut nurseries. Weed management is a critical management practice in the nursery. Though glyphosate application is becoming popular in nurseries it can affect weeds as well as coconut seedlings growth. Therefore the effects of glyphosate were evaluated by determining the growth of shoot and root of coconut seedlings. Poly bag nursery was prepared and three treatments were used. Treatments were no glyphosate and manual weeding (T_1), application of glyphosate 1.08 ai kg ha^{-1} at 2 monthly interval (T_2) and application of glyphosate 1.44 ai kg ha^{-1} at 2 monthly interval (T_3). Application of glyphosate at early stage of seedling growth had a no significant effect on growth parameters tested. However, the concentrations of glyphosate negatively affected numbers, volumes and dry weights of secondary, tertiary and quaternary roots at the latter stage of seedling growth. The leaf area and the height of seedling were significantly reduced by the highest concentration of glyphosate. Among the growth parameters tested, seedling girth and shoot dry weight were not affected by the application of glyphosate. These results revealed that the usage of glyphosate at both concentrations negatively affected root growth of coconut seedlings. Based on these results, the both concentration levels of glyphosate should be applied to coconut nurseries before sprouting the seed nuts.

Key words: coconut; glyphosate; shoots and root growth; weeds.

INTRODUCTION

Coconut (*Cocos nucifera* L.) is by far the most extensively cultivated major plantation crop in Sri Lanka (Senarathne and Perera 2009). To maintain a proper coconut plantation, high quality coconut seedlings must be used as it helps to establish a healthy and uniform plantation and its one of the critical points of coconut cultivation. Even though, it is critical, production of quality seedlings is an essential and important manage-

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ment practice, the use of poor quality seedlings, will result in huge economic losses in the future. These facts will emphasize the necessity of using quality planting material that will ultimately give good yields (Menon and Pandalai 1958). By using standardize nursery technique high percentage of quality seedlings can be produced at relatively cheap cost (Menon and Pandalai 1958).

Among the cultural practices in coconut nurseries, irrigation and weeding are considered very important. Weed management is an important as well as expensive agronomic input in a coconut nursery. Especially at the seedling stage such weeds consistently compete with coconut seedlings for soil moisture, nutrients and some time light. To overcome these effects, seed beds are to be kept in weed free condition to avoid competition. Most popular weeding method in coconut nurseries is manual weeding. But it is a labor intensive and time-consuming method (Senarathne and Perera, 2005). This hasn't been found to be effective, especially for weeds with underground stolons (Pethiyagoda, 1980). However, chemical weeding too is a very popular method among coconut nurseries. It's superior to mechanical and cultural methods because of their effectiveness in terms of time, cost and labour (Ghosh *et al.* 2007).

Glyphosate is a non-selective, broad-spectrum herbicide that is widely used for weed management but also injures or kills crops when in direct contact with foliage. Glyphosate competitively inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme in the shikimate pathway, which leads to synthesis of aromatic amino acids and other ring-containing metabolites (Duke 1988). It is systemic within the plant, generally undergoes little or no metabolism in most plants, is readily translocate into metabolic sinks including plant roots (Duke, 1988) and is eventually released into the rhizosphere (Coupland and Caseley 1979), likely via a diffusion process along with sugars, amino acids and other low molecular weight compounds (Curl and Truelove, 1986). There were studies about the effects of glyphosate on the growth of some other crops. Cole (1985) found that glyphosate had little effect on the soluble protein content of maize (*Zea mays*) roots; increase in free amino acids was more notable in leaves than roots, although root growth was more sensitive to glyphosate. This application significantly inhibited root biomass production, root elongation, and lateral root formation of the glyposate resistant soybean variety (*Glycine max*) (Bott *et al.* 2008). The application of glyphosate on wheat foliage was exuded from the root into the soil and it has caused root inhibition and foliar injury symptoms in maize (*Zea mays*) seedlings growing in the same soil (Torstensson 1985).

At present most coconut nurseries are having a tendency to use chemical weeding. Nevertheless glyphosate can be harmful to both weeds and coconut seedlings. There might be instances where the chemical is mistakenly sprayed to the coconut seedlings as well, at times of application, as seed nuts are laid at close spacing in the nurseries. Hence the objective of this study was to evaluate the effects of different concentration of glyphosate on growth of coconut seedlings.

MATERIALS AND METHODS

The experiments were carried out in the Bandirippuwa Estate in Coconut Research Institute located in the Low county Intermediate Zone of the North Western province of Sri Lanka from May to November in 2010. Coconut seed nursery received photo synthetically active radiation (PAR) ranging from 500～1150 μ mol m^{-2} s^{-1} and the average day and night temperatures were in the range of 30～34°C and 26～30°C, respectively. Relative humidity varied between 35～60% during the day and 20～27% during the night. The rainfall at the site during the period of study was uneven with dry spells.

All the coconut seed nuts were selected from high yielding palms (mother palms) in a seed garden. Before establishing the experiment, just sprouted seed nuts (3 months old) were laid in poly bags of 75 cm in height and 45 cm in width made of 500 gauges of black polythene with gussets. Few punch holes were pierced on all sides close to the bag bottom to drain out the excess water. Poly bags were filled with potting mixture which has coir dust and sand in a ratio of 3 : 1. High ratio of coir dust facilitates the uprooting of coconut seedlings with minimum damages to roots. Ploy bags were kept in a plot spaced 30cm between rows and 15 cm within rows in May 2010. Each experimental plot was 5.01 m × 2.04 m dimension containing 60 poly bags with just sprouted seed nuts. All the plotting area was watered when required to maintain adequate soil moisture. The following treatments were applied for the experiment in a Complete Randomized Design with three replicates.

Treatments of the experiment

T_1. Without application of Glyphosate (manual weeding)

T_2. Application of Glyphosate at 1.08 ai kg ha^{-1}

T_3. Application of Glyphosate at 1.44 ai kg ha^{-1}

Treatments were applied according to the schedule by using a hand sprayer on the entire surface of the plots. The treatments were applied at two monthly intervals, starting from June 2010 and ending in November 2010.

Sampling and sample preparation

Destructive samples were taken at three weekly intervals. Three coconut seedlings were uprooted randomly from each treatment plot. First the shoot measurements like seedling girth and height were measured. Then poly bags were removed and the potting mixture was washed by using flown water out without damaging roots. Then the coconut husk was carefully removed in to small strips by using knife. Almost all the roots in husk were taken and separated and measured. De-husked nut was removed and then the roots were separated in to primary, secondary, tertiary and quaternary groups.

Root measurements

The roots were separated from the shoot and grouped into primary, secondary, tertiary and quaternary roots. Numbers of roots in each group were counted. Root volume (cm^3) was taken by using Archimedes theorem. There was a specific measuring cylinder to facilitate the measuring of displaced water volume. First it was required to measure the displaced water volume by dipping some weight in the water filled measuring cylinder. Then the separated root samples, primary, secondary, tertiary and quaternary roots were dipped with known volume of water in order to measure the displaced volume of water respectively. Root samples were oven dried at 80°C temperature to a constant weight. Dry weights (g) of the roots were taken.

Shoot measurements

Final seedling girth (cm) was measured by using a measuring tape at the point of shoot is emerged from the nut. Final seedling height, the distance between shoot sprouted point from the nut and the tip of the longest leaf was measured by using a measuring tape as seedling height (cm). Leaves were separated from the petiole cut into pieces and areas of the leaf were measured by Leaf Area Meter (USA, Li COR 3100). After the measuring of leaf area, those leaf pieces were oven dried at 80°C to a constant weight. Then the leaf petioles were cut into small pieces and oven dried at 80°C temperature to a constant weight. Then the dry weight (g) of the shoots (leaf + petiole) was taken.

Weed biomass

The weed biomass was collected at the same frequent four randomly placed, 0.25 m × 0.25 m quadrats per plot from May to November in 2010. Weed samples were dried at 80°C for five days and weighed.

Data analysis

Data analysis was done by using SAS computer software. CRD ANOVA with repeated measures used under SAS General Liner Model procedure. GLM repeated measure is a procedure used to model dependent variables measured at multiple tines using analysis of variance (online 5). Mean separation was done by using Least Significant Differences Test. This mean separation was controlled the type one comparison wise error rate and not the experimental wise error rate and effective mean comparison method if the number of comparisons are small.

RESULTS AND DISCUSSION

Effect of different treatments on number of roots of coconut seedlings

Primary root number was not affected by the usage of both glyphosate irrespective of the concentration, during the experimental period (Table 1). Secondary root number was not significantly different during the first two samplings. However, after third sampling onwards application of glyphosate has significantly reduced the number of secondary roots compared to control (Table 2).

Although there was no significant effect of glyphosate application on tertiary root number at the first sampling but (T_2) 1.08 ai kg concentration of glyphosate reduced tertiary roots at second sampling (Table 3). Nevertheless from third sampling onwards application of glyphosate has reduced the number of tertiary roots significantly irrespective of the concentration compared to control. The initiations of tertiary roots were highly sensitive to the application of glyphosate after the third sampling.

During the first and second sampling both concentration of glyphosate did not affect the number of quarternary roots (Table 4). However, from third sampling onwards glyphosate reduced the number of quaternary roots in T_3 and T_2 significantly, compared to control (T_1).

By the stage of fourth sampling, the experimental plots had been received two applications of glyphosate. Thus the initiation of quaternary roots was reduced by a significant quantity. Further, there was a significant difference between number of quaternary roots in treatments (T_2 and T_3).

Table 1. Effect of different treatments on number of primary roots of coconut seedlings.

Treatments	Primary root number				
	1^{st} sampling	2^{nd} sampling	3^{rd} sampling	4^{th} sampling	5^{th} sampling
T_1- Control (manual weeding)	3.2a	3.7a	6.2a	8.8a	9.8a
T_2- Glyphosate 1.08 ai kg ha^{-1}	3.4a	4.2a	6.5a	8.5a	9.9a
T_3- Glyphosate 1.44 ai kg ha^{-1}	4.1a	4.3a	6.4a	8.6a	10.9a

In each column, values with the same letter are not significantly different.

Table 2. Effect of different treatments on number of secondary roots of coconut seedlings.

Treatments	Secondary root number				
	1^{st} sampling	2^{nd} sampling	3^{rd} sampling	4^{th} sampling	5^{th} sampling
T_1- Control (manual weeding)	128.7a	123.0a	342.0a	339.3a	537.7a
T_2- Glyphosate 1.08 ai kg ha^{-1}	124.0a	109.3a	114.0b	153.3b	206.3b
T_3- Glyphosate 1.44 ai kg ha^{-1}	142.0a	167.7a	106.3b	197.0b	235.7b

In each column, values with the same letter are not significantly different.

Table 3. Effect of different treatments on number of tertiary rootsof coconut seedlings.

Treatments	Tertiary root number				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	237.7a	158.7ab	842.3a	1584.3a	1848.3a
T_2- Glyphosate 1.08 ai kg ha^{-1}	71.3a	59.0b	100.3b	179.7b	274.7b
T_3- Glyphosate 1.44 ai $kgha^{-1}$	127.0a	195.7a	53.6b	310.3b	308.0b

In each column, values with the same letter are not significantly different.

Table 4. Effect of different treatments on number of quaternary roots of coconut seedlings.

Treatments	Quaternary root number				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	27.7a	71.7a	104.3a	670.3a	953.0a
T_2- Glyphosate 1.08 ai kg ha^{-1}	22.0a	49.3a	20.3ab	18.0b	59.7b
T_3- Glyphosate 1.44 ai kg ha^{-1}	17.7a	36.0a	4.3b	37.7b	68.7b

In each column, values with the same letter are not significantly different.

Effect of different treatments on root volume of coconut seedling

During the first two sampling, both concentration of glyphosate did not affect the volume of primary, secondary, tertiary and quaternary roots volume (Table 5, 6, 7 and 8). Second sample was taken after 21 days of first glyphosate application and the effects of glyphosate was less at the beginning of the experiment. However, on the third, fourth and fifth sampling, glyphosate has reduced the primary, secondary, tertiary and quaternary root volume in both treatments T_2 and T_3. Primary roots were less responsible for water and nutrient absorption compared to other roots due to its maturity.

At the transplanting stage seedling should possess well developed root system for the better establishment in the field. Therefore it is required to have mass total root volume. Table 9 shows that total root volume was not affected by the glyphosate during first two weeks. Two concentrations of glyphosate applications resulted a significant reduction in total root volume at the last three sampling days. Thus it is critical in the stage of transplanting. Inhibition of protein synthesis would be an important consequence of the action of glyphosate on aromatic amino acids, as evidenced by a rapid decline in the protein content of wheat root tips (Cole 1985).

Table 5. Effect of different treatments on volume of primary roots (cm^3) of coconut seedlings.

Treatments	Primary root volume (cm^3)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	63.1a	49.6a	104.7a	118.6a	147.9a
T_2- Glyphosate 1.08 ai kg ha^{-1}	52.2a	54.3a	53.5b	77.0b	97.0b
T_3- Glyphosate 1.44 ai kg ha^{-1}	57.0a	71.7a	63.5b	64.9b	98.0b

In each column, values with the same letter are not significantly different.

Table 6. Effect of different treatments on volume of secondary roots (cm^3) of coconut seedlings.

Treatments	Secondary root volume (cm^3)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	12.5a	20.1a	43.1a	59.1a	83.1a
T_2- Glyphosate 1.08 ai kg ha^{-1}	05.9a	10.5a	13.1b	13.5b	26.3b
T_3- Glyphosate 1.44 ai kg ha^{-1}	11.9a	14.0a	13.1b	23.5b	26.9b

In each column, values with the same letter are not significantly different.

Table 7. Effect of different treatments on volume of tertiary roots (cm^3) of coconut seedlings.

Treatments	Tertiary root volume (cm^3)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	2.9a	3.5a	11.8a	39.5a	45.2a
T_2- Glyphosate 1.08 ai kg ha^{-1}	2.0a	2.3a	1.9b	3.6b	6.1b
T_3- Glyphosate 1.44 ai kg ha^{-1}	2.6a	5.2a	3.3b	4.5b	8.0b

In each column, values with the same letter are not significantly different.

Table 8. Effect of different treatments on volume of quaternary roots (cm^3) of coconut seedlings.

Treatments	Quaternary root volume (cm^3)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	0.7a	1.3a	3.6a	8.8a	9.3a
T_2- Glyphosate 1.08 ai kg ha^{-1}	0.6a	0.1a	0.4b	0.5b	0.5b
T_3- Glyphosate 1.44 ai kg ha^{-1}	0.2a	0.7a	0.1b	0.4b	0.4b

In each column, values with the same letter are not significantly different.

Table 9. Effect of different treatments on volume of total roots (cm^3) of coconut seedlings.

Treatments	Total root volume (cm^3)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	79.2a	74.5a	163.2a	226.0a	285.5a
T_2- Glyphosate 1.08 ai kg ha^{-1}	60.7a	67.2a	68.8b	94.8b	129.9b
T_3- Glyphosate 1.44 ai kg ha^{-1}	71.7a	91.6a	80.0b	93.3b	133.4b

In each column, values with the same letter are not significantly different.

Effect of different concentration of glyphosate on root dry weight

Primary root dry weight was not affected by the usage of both concentration of glyphosate (T_2 and T_3) during the experimental period except on the fifth sampling stage (Table 10).

During the first two sampling, both concentrations of glyphosate did not effect on the dry weight of secondary, tertiary and quaternary root dry weights (Table 11, 12 and 13). However, after third sampling onwards

Table 10. Effect of different treatments on dry weight of primary roots (g) of coconut seedlings.

Treatments	Primary root dry weight (g)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	5.3a	8.6a	12.4a	16.9a	18.3a
T_2- Glyphosate 1.08 ai kg ha^{-1}	4.7a	6.8a	10.4a	11.8a	13.3b
T_3- Glyphosate 1.44 ai kg ha^{-1}	5.5a	7.2a	11.8a	13.4a	15.0ab

In each column, values with the same letter are not significantly different.

Table 11. Effect of different treatments on dry weight of secondary roots (g) of coconut seedlings.

Treatments	Secondary root dry weight (g)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	2.3a	3.1a	7.7a	11.8a	14.6a
T_2- Glyphosate 1.08 ai kg ha^{-1}	1.3a	1.7a	2.4b	2.8b	5.3b
T_3- Glyphosate 1.44 ai $kgha^{-1}$	1.5a	2.0a	1.8b	4.4b	5.5b

In each column, values with the same letter are not significantly different.

Table 12. Effect of different treatments on dry weight tertiary roots (g) of coconut seedlings.

Treatments	Secondary root dry weight (g)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	65.9a	53.3a	115.0a	137.5a	173.9a
T_2- Glyphosate 1.08 ai kg ha^{-1}	53.7a	56.2a	56.4b	80.2b	103.4b
T_3- Glyphosate 1.44 ai kg ha^{-1}	58.7a	74.1a	65.5b	70.1b	105.1b

In each column, values with the same letter are not significantly different.

Table 13. Effect of different treatments on dry weight of quaternary roots (g) of coconut seedlings.

Treatments	Quaternary root dry weight (g)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	0.01a	0.013a	0.188a	0.713a	1.895a
T_2- Glyphosate 1.08 ai kg ha^{-1}	0.008a	0.011a	0.024b	0.027b	0.062b
T_3- Glyphosate 1.44 ai kg ha^{-1}	0.006a	0.011a	0.005b	0.009b	0.103b

In each column, values with the same letter are not significantly different.

both concentrations of glyphosate reduced the secondary, tertiary and quaternary root dry weights significantly compared to the control.

The results of the total root dry weight shows during the first two samplings both concentration of glyphosate did not affect on total root dry weight (Table 14).

However, with time after third, fourth and fifth sampling it shows at both concentrations of glyphosate significantly reduced the total root dry weight. In wheat, increases in free amino acids were more notable

Table 14. Effect of different treatments on dry weight of total root (g) of coconut seedlings.

Treatments	Total root dry weight (g)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	65.9a	53.3a	115.1a	137.5a	173.9a
T_2- Glyphosate 1.08 ai kg ha^{-1}	53.7a	56.2a	56.4b	80.2b	103.4b
T_3- Glyphosate 1.44 ai kg ha^{-1}	58.7a	74.1a	65.5b	70.1b	105.1b

In each column, values with the same letter are not significantly different.

in leaves than roots, although root growth was more sensitive to glyphosate (Cole 1985).

When consider about the root growth of the coconut seedlings, two concentrations of glyphosate has significantly affected secondary, tertiary and quaternary root numbers, root volumes as well as root dry weights from six weeks onwards after glyphosate application. Glyphosate mainly accumulates on the meristematic area of roots which is actively cell growing area. Secondary, tertiary and quaternary roots are highly responsible for the active growing area which is important to the absorption of soil water and nutrients compared to primary roots. Therefore, glyphosate seems to have caused damages on the growth of secondary, tertiary and quaternary root parameters (Menon and Pandalai 1958).

Effect of different treatments on shoot growth of coconut seedlings

Two concentration of glyphosate significantly affected leaf growth by reducing leaf area after third sampling onwards (Table 15). Nevertheless high concentrated glyphosate (T_3) reduced the leaf area significantly at the fifth sampling.

Effect of different treatments on final seedling girth (cm), height (cm) and shoot dry weight (g) of coconut seedlings

Seedling height was significantly reduced by the application of high concentrated glyphosate treatment

Table 15. Effect of different treatments on leaf area (cm^2) of coconut seedlings.

Treatments	Leaf area (cm^2)				
	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling
T_1- Control (manual weeding)	1531.6a	1826.8a	2322.8a	3136.0a	3270.3a
T_2- Glyphosate 1.08 ai kg ha^{-1}	1776.7a	1587.3a	1839.3a	2059.2b	2941.5a
T_3- Glyphosate 1.44 ai kg ha^{-1}	1661.5a	1668.1a	1735.7a	1918.4b	1904.5b

In each column, values with the same letter are not significantly different.

Table 16. Effect of different treatments on final seedling girth, height (cm) and shoot dry weight (g) of coconut seedlings.

Treatments	Seedling girth (cm)	Seedling height (cm)	Shoot dry weight (g)
T_1- Control(manual weeding)	11.0a	96.8a	66.8a
T_2- Glyphosate 1.08 ai kg ha^{-1}	10.4a	92.9ab	55.5ab
T_3- Glyphosate 1.44 ai kg ha^{-1}	10.2a	86.3b	51.7b

In each column, values with the same letter are not significantly different.

(T_3) compared to control and low concentrated glyphosate (T_2) during the experimental period (Table 16). Seedling girth was not shown significant differences at two concentrations of glyphosate during the experimental time, resulted same seedling girths in all treatments (Table 16).

However, high concentrated glyphosate treatment (T_3) has reduced final shoot dry weight significantly compared to control treatment (Table 14). The negative effect of treatment (T_3) was due to reduced leaf growth (leaf area) and the seedling height and it has reduced shoot dry weight. Rhizomes or stolon growths of perennial grasses have been shown to be inhibited by glyphosate, including *Imperata cylindrica* and *Panicum maximum*. Death of rhizomes buds has also been reported as well as the reduction of shoot growth and plant height is also often observed after the glyphosate treatments (Ashton and Craft 1981).

Effect of different concentration of glyphosate on weed biomass

Among the treatments, the lowest weed biomass was produced by T_2 (1.08 ai kg ha^{-1} Glyphosate) and T_3 (Glyphosate at 1.44 ai kg ha^{-1}) treatments (Fig.1). Manual weeding treatment was not so effective in suppressing weeds satisfactorily. However, T_2 and T_3 treatments were effective in reducing both monocotyledonous and dicotyledonous weeds.

Glyphosate is a widely used, non-selective, foliage applied herbicide known to be highly toxic to *Panicum repens* (Manipura and Somaratne 1974). Although, manual weeding treatment suppressed weed growth temporarily, fast re-growth was observed, especially with monocotyledonous weeds. Generally manual weeding causes more damage to dicotyledonous weeds and aerial parts of the monocotyledonous weeds and less damage to root systems or underground plant parts such as stolens, bulbs and rhizomes of monocotyledonous weeds. Hence, dicotyledonous weeds are much easier to control by manual weeding. Boyall (1979) showed that when glyphosate was applied, it gets translocate into the underground rhizomes and destroys all-viable buds. Therefore, this is the ideal method for controlling monocotyledonous weeds.

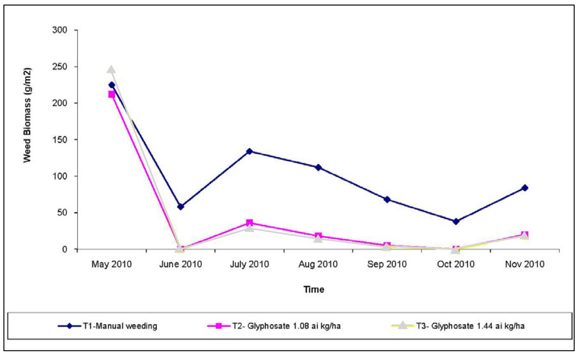

Fig. 1. Weed biomass (g m^{-2}) in different treatments in coconut nursery from May 2010 to Nov 2010.

CONCLUSION

The results obtained from the experiment revealed that the usage of glyphosate at both concentrations negatively affect the root growth of coconut seedlings. High concentration of glyphosate (1.44 ai kg ha^{-1}) has negative effects on the shoot growth of coconut seedlings. Based on these results, the both concentration levels of glyphosate seem to be unsuitable to control weeds in coconut nurseries when the seed nuts are at sprouting stage. However, it can be applied to coconut nurseries before sprouting the seed nuts specially to manage harmful monocotyledonous weed species.

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