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Germination and early growth performance of *Persea americana* in soils amended with extracts from different parts of *Annona muricata*

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ABSTRACT

In agro-ecosystems, several weeds, crops, agroforestry trees including fruit trees are known to exert allelopathic influence on crops thus affecting their germination and growth. This study evaluated the effect of *Annona muricata* Lin. extracts on the germination and seedling growth of *Persea Americana* Mill. The experiment was laid out in a Completely Randomised Design (CRD) separately for leaf, root and leaf/root extracts of *A. muricata*. Data were collected on germination emergence, germination duration, germination percentage, seedling height, collar diameter, leaf production, biomass and moisture content. A one-way analysis of variance (ANOVA) was used to test for significant difference ($p \leq 0.05$) in the measured growth attributes of *P. americana* among soils treated with different volumes (0ml, 25ml, 50ml, 75ml and 100ml) of *A. muricata* extract, separately for leaf, root, and root/leaf extracts. Plumule emergence of *P. americana* was earliest in soils treated with 25ml/50ml/100ml of *A. muricata* leaf extract; earliest at 50ml in soils treated with root extract and earliest at 50ml/75ml/100ml in soils treated with leaf/root extract. Duration of emergence was shortest with 25ml of leaf extract, 100 ml of root extract and 50ml/100ml of leaf/root extract. Germination percentage improved with soil amendment using *A. muricata* extract especially the leaf extract and a combination of leaf/root extracts. In majority of the cases, there was no significant difference ($p > 0.05$) in seedling height and collar diameter but the leaf extract increased the mean values of both number of leaves and seedling height. Mean values of seedling biomass and moisture content of *P. americana* were enhanced with the application of leaf extract especially. Soil amendment with extracts from different parts of *A. muricata* enhanced plumule emergence and seedling growth of *P. americana* while the leaf and a combination of leaf/root extracts enhanced germination of its seeds. It is therefore recommended that *A. muricata* leaf extract and combination of leaf/root extracts, be used to enhance germination while the leaf extract alone can be used to promote seedling growth of *P. americana*. The above results are also suggestive of the possibility of both species growing successfully in an integrated system though field trials are needed to authentic this.

Keywords: Plant extracts, Soil amendment, *Persea americana, Annona muricata,* Early growth,

INTRODUCTION

It has been reported that the extracts from all parts of *Annona muricata* (leaf, root and stem) inhibit the growth and development of other plants species such as *Phyllantus spp, Panicum maximum,* and *Tridax procubens* (Trupti and Rajendra, 2014); a phenomenon known as allelopathy. The concept of allelopathy was first studied in the forest ecosystems where many of the species investigated had negative effect on food and fodder crops (Olofsdotters, 1998). Allelopathy is defined by the International Allelopathy Society as any process involving plants or other organisms' secondary metabolites that influence the growth and development of surrounding biological systems. These metabolites causing allelopathic effect are called allelochemicals. The term derives from allelochemicals coined by Whittaker and Fenny in 1971 and was first used by Chou and Waller in 1983 dealing with interspecific chemical interaction between organisms (Reigosa et al. 2006). Allelochemicals indeed are defined as bio-communicators, suggesting the possibility of active mixtures because of the increasing number of findings in which single compounds are not active as a mixture (Khalid et al. 2002). Some studies have suggested that allelochemicals may be present in the mucilage around a germinating seed, in leachates from the aerial parts of plant, in exudates from plant roots, in volatile emission from growing plants and also among decomposing plants residues where microorganism may also be involved (Naylov, 2002).

Plant allelopathy has been studied more than other organisms and is regarded as a natural strategy protecting plants against environmental enemies and competing plants. In the last decades, application of synthetic toxins for control of weed, pests and plant disease caused serious environmental problems thus allelopathic interaction between plants and other organisms may become an alternative to synthetic herbicides and other pesticides (Anaya et al. 2005).

Persea americana Mill originated from the rainforest of central America. It is an evergreen tree in the family Lauraceae which grows for its nutritious fruits, the avocado. Its germination period is between 2 to 6 weeks. The avocado tree is large and dome shaped with oval or elliptical leaves arranged in a spiral on the tip of branches. The leaves have a red pigmentation when they first emerge and turn green as they mature (Berladi and Maguire, 1998). Avocado is usually consumed fresh as a fruit or as an ingredient in salads or savoury dishes. It has a markedly higher fat content than other fruit and is a staple in diets that has limited access to foods with high content of monosaturated fats. The fresh leaves have been consumed in form of aqueous infusion or decoction for menstruation pain, diabetes in Ecaudor and the leaves have been employed as anti-malaria in Nigeria (Armiyos et al. 2007; Adebiyi et al. 2012). Avocado leaves contain pepsin which is a toxin for lactating livestock (Oelrichs et al. 1995).

Information on the allelopathic effect or otherwise of *A. muricata* on important fruit tree species that have the potential to be integrated in agroecosystems for sustainable land management and improved productivity is lacking. This study specifically aimed at ascertaining if *P. americana* can be effectively integrated on the same land management with *A. muricata* considering the allelopathic effect of the latter. Allelopathy includes both positive and negative effect of one plant on the other though most of the studies seem to focus on its deleterious impacts. In agroecosystems, several weeds, crops, agroforestry trees including fruit trees have been shown to exert allelopathic

influence on crops thus affecting their germination and growth adversely. A replant problem has shown that some agricultural and horticultural crops affect their own seedling growing in succession (Kohli et al. 1997). Available literature indicates that allelochemicals act via bringing certain changes in physiological functioning like respiration, photosynthesis and ion uptake. These in turn result in visible changes in seed germination, further growth reduction and overall performance of the target plants.

Considering the dwindling arable land, environmental degradation and the increasing population, there is the need to develop integrated land use systems that are multispecies, multistructural and multifunctional in order to enhance productivity as well as conserve the environment. However, the success of such agroecosystems as described above will depend to a large extent on the ability of species to grow together. Although, the allelopathic effect of *A. muricata* has been reported especially on weeds (Mbagwu, 2006), no studies have been carried to ascertain its effect on the germination and early growth of very important fruit species like *P. americana.* This study therefore, was conducted to fill this gap in knowledge. Specifically, the objective of this study was to evaluate the germination and seedling growth performance of *P. americana* in soils amended with *A. muricata* extracts. It is hoped that the knowledge of the allelopathic effect of *A. muricata* on the germination and growth of *P. americana* will provide knowledge on the possibility of growing them on the same land management unit or not.

MATERIALS AND METHODS

Study Location: This research was carried out for a period of four months at the Research and Experiment Nursery of the Department of Forestry and Wildlife Management, Faculty of Agriculture, University of Port Harcourt. The University of Port Harcourt is located on a land area of about 400 hectares in Obio/Akpor Local Government Area of Rivers State at latitude 4.90794 and 4.90809N and longitude 6.92413 and 6.92432E (Chima et al. 2017).

Seed Collection, Processing and Viability Test

Fruits of *P. americana* were collected from a single mother tree in Aluu Community, Rivers State. The fruits were depulped to get the seeds. Seed viability test was done using the floatation method. Seeds that sank to the bottom of the container were regarded as being viable while those that floated were regarded as non-viable and discarded.

Experimental Design: The experiment was laid out in a Completely Randomized Design (CRD) separately for leaf, root and leaf/root extracts of the *A. muricata*. Five different volumes (0 ml, 25 ml, 50 ml, 75 ml and 100 ml) of the extracts were applied as treatments. Each of the treatments was replicated 5 times. One polypot with seedling was considered as an experimental unit and served as a replicate. The polypots (75) were placed inside a propagator to conserve moisture, control temperature and to protect seeds and seedlings from rodent attacks.

Preparation of the *Annona muricata* extracts: Leaves and roots of *A. muricata* were collected from the pathway to the Arboretum of the Department of Forestry and Wildlife Management, University of Port Harcourt, Nigeria. The leaf extract was prepared by chopping 10kg of *A. muricata* leaves collected from 10 sample trees and washed with distilled water into small pieces and squeezing out the extract using 10 litres of distilled water. For the

root extract, 10kg of *A. muricata* roots roots collected from 10 sample trees and washed with distilled water was crushed, put into a container with 10 litres of distilled water and allowed to stay for 48 hours before filtering the extract. The leaf/root extract was prepared by mixing the leaf and root extracts in a ratio of 1:1litre.

Preparation of the Growth Media and Sowing of Seeds: Topsoil was collected from 0 – 10 cm after removing litter from the surface. The topsoil was bulked, sterilized and used to fill 5 kg polypots. The treatments (0 ml, 25 ml, 50 ml, 75 ml and 100 ml) of the leaf, root, and leaf/root extracts) were applied as appropriate to the respective polypots in the three sets of the experiment representing the three extract types (leaf, root, and leaf/root) two days before sowing. Viable seeds were sown singly in each polypot. The polypots were monitored and watered daily (early in the morning) with 35cl of water per polypot, to maintain adequate moisture content. Seed germination was considered to have occurred once the plumule emerges from the soil.

Data Collection: Data collected on germination was used to calculate Inception of plumule emergence, Duration of germination and germination percentage for each extract type (leaf, root and leaf/root) using the formula below.

Inception of plumule emergence = time (number of days) for plumule emergence after sowing.

Duration of germination = period of germination emergence to the end of germination.

Germination Percentage = $\frac{Number\ of\ germinated\ seeds}{Number\ seeds\ sown} * \frac{100}{1}$

Seedling growth data were collected on the following variables monthly for a period of 3 months. Seedling height was measured in centimetre (cm) using a meter rule; stem collar diameter was measured in millimetre (mm) using a digital caliper while leaf production was assessed by visual observation and counting. The seedlings were weighed using a digital weighing scale calibrated in grams (g) to determine plant wet and dry weight and moisture content was calculated using the following formula.

Moisture Content = Fresh weight – dry weight

Data Analysis: A one-way analysis of variance (ANOVA) was used to test for significant differences in the measured growth attributes among the different concentrations of *A. muricata* extracts separately for the leaf, root, and root/leaf of *P. americana*. The ANOVA was performed using Statistical Package for Social Sciences (SPSS version 18, SPSS Inc.). Duncan multiple range test was used to indicate levels of differences/compare means.

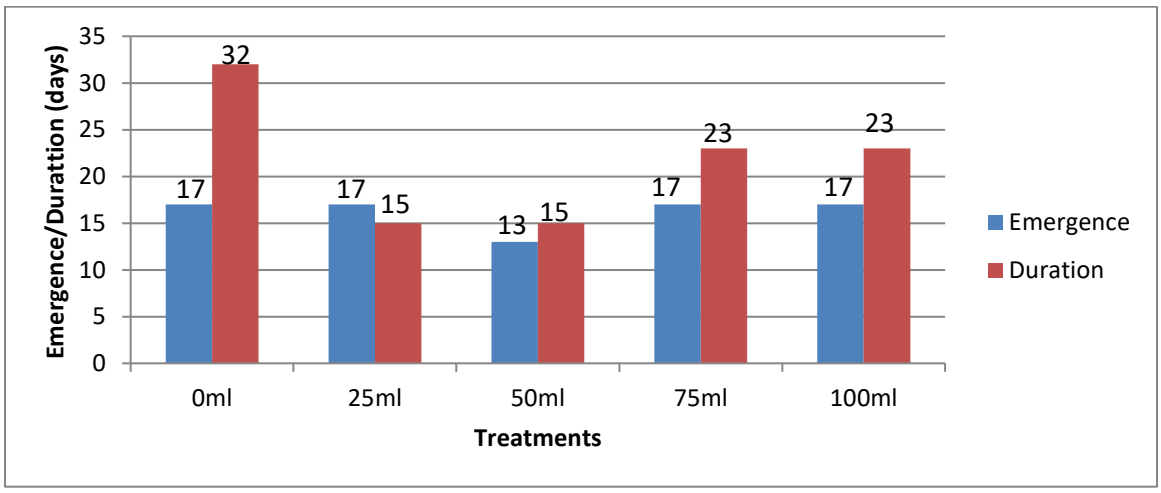
RESULTS

Effect of extract from different parts of *Annona muricata* on the Inception of plumule emergence and duration of germination of *Persea americana* seeds

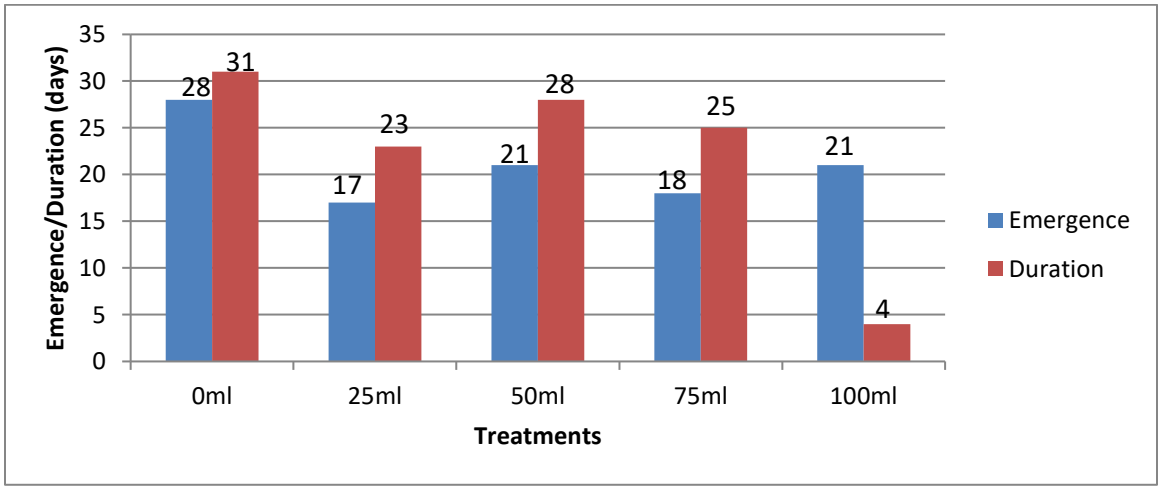
Plumule emergence and duration in soils treated with extracts from different parts of *A. muricata* are shown in Figure 1 (a, b, and c) for the leaf, root and leaf/root extracts respectively. In soil treated with *A. muricata* leaf extract (Figure 1a), plumule emergence of *P. americana* was earliest in 0ml/25ml/75ml/100ml, followed by 50ml

while the duration of emergence was shortest in 25ml/50ml, followed by 75ml/100ml and 0ml respectively. In soil treated with *A. muricata* root extract (Figure 1b), plumule emergence of *P. americana* was earliest in 25ml, followed by 75ml, 50ml/100ml and 0ml while the duration of emergence was shortest in 100ml, followed by 25ml, 75ml, 50ml and 0ml, respectively. In soil treated with *A. muricata* leaf/root extract (Figure 1c), plumule emergence of *P. americana* was earliest in /75ml/100ml, followed by 0ml, 25ml and 50ml, while the duration of emergence was shortest in 100ml, followed by 0ml, 75ml, 50ml and 25ml, respectively.

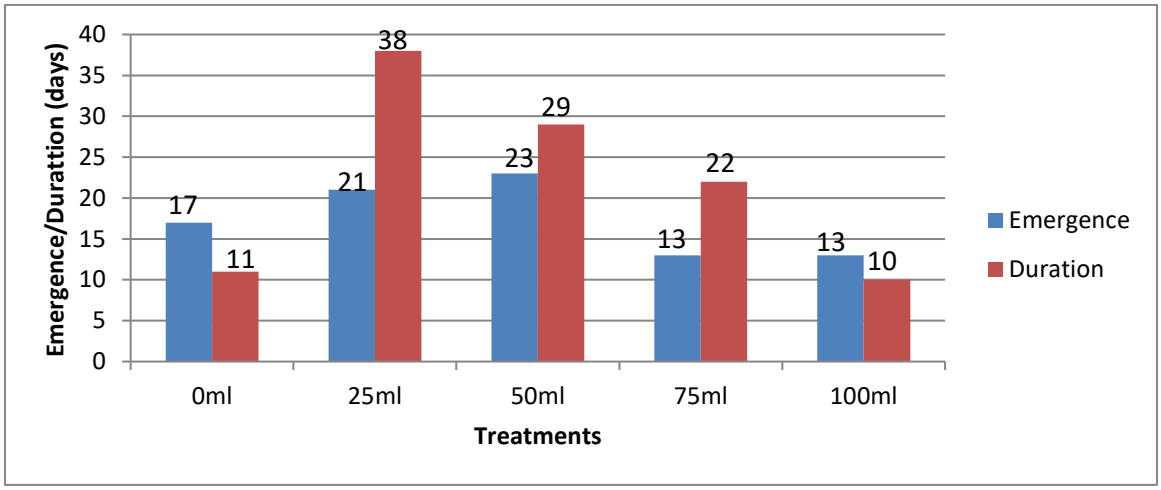
Soil amendment using *A. muricata* leaf extract had greater impact on the duration of plumule emergence of *P. americana* than on the inception of emergence as the application of 25 to 100 ml of the extract shortened the duration of emergence especially at 25 and 50ml. The inception of plumule emergence was similar between the Control (0ml) and the other treatments except at 50ml where it was enhanced. On the other hand, *A. muricata* root extract generally enhanced the inception of germination and shortened the duration of emergence (especially at 100ml). Also, a combination of leaf/root extract of *A. muricata* enhanced inception of emergence at 75 and 100ml and shortened the duration of emergence at 100ml in *P. americana*.



(a)



(b)



(c)

Figure 1: Inception of plumule emergence/duration of germination of *P. americana* in soils treated with *A. muricata* (a) leaf (b) root and (c) Leaf/root extracts

Effect of extract from different parts of *Annona muricata* on the germination percentage of *Persea americana* seeds: The germination percentage of *P. americana* seeds in soils amended with extracts from different parts of *A. muricata* are shown in Figure 2 (a, b, and c) for leaf, root, and leaf/root extracts, respectively. Soil amendment using different volumes of *A. muricata* leaf extract, improved the percentage of germination except at 50ml volume where the same germination percentage was recorded with the control (0ml). In soils amended with *A. muricata* root extract, germination percentage was 100% for the Control (0ml) and the other treatments except at 100ml where a reduction of 20% was observed. However, soil amendment using a combination of *A. muricata* leaf/root extracts enhanced germination percentage of *P. americana* seeds by 20% in 50 and 100ml to 40% in 25 and 75ml.

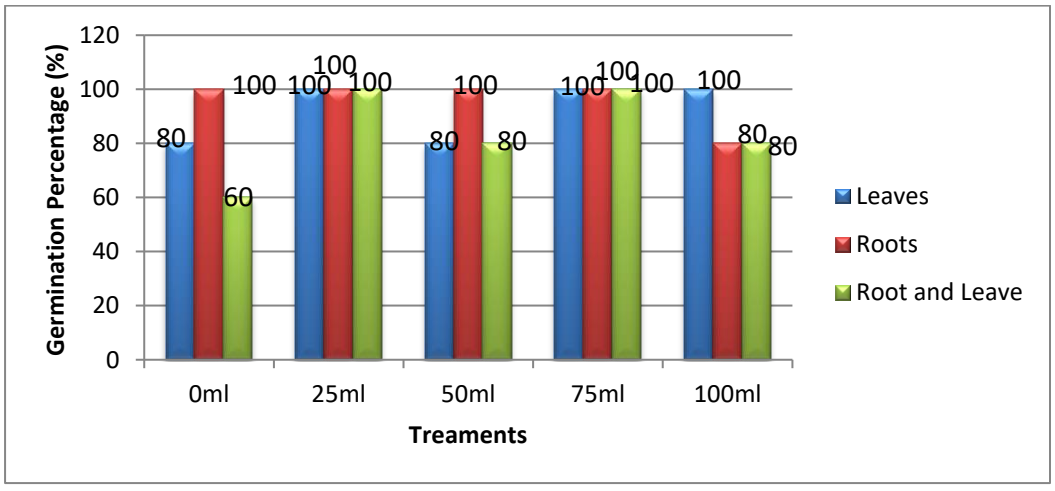


Figure 2: Germination percentage of *P. americana* seeds in soils treated with *A. muricata* extracts

Effect of extracts from different parts of *Annona muricata* on early growth of *Persea americana:* The effect of extracts from different parts of *A. muricata* on the seedling growth attributes of *P. americana* is shown in Table 1.

In soil treated with leaf extract, seedling height was highest in 50 ml treatment with no significant difference ($p > 0.05$) among the treatments. Collar diameter was highest at 25 ml treatment with no significant difference ($p > 0.05$) among the treatments. Number of leaves was highest in 75 ml with no significant difference among treatments.

In soil treated with root extract, seedling height of *A. muricata* was highest in 25 ml with no significant difference ($p > 0.05$) with 0, 50 and 75 ml but varied significantly with 100 ml. Collar diameter was highest in 0 ml with no significant difference ($p > 0.05$) observed among treatments. Number of leaves was highest in 50 ml treatment with no significant difference ($p > 0.05$) among treatments.

In soil treated with a combination of leaf & root extracts, seedling height was highest in 75 ml with no significant difference ($p > 0.05$) among treatments. Collar diameter was highest in 100 ml with no significant difference ($p > 0.05$) among treatments. Number of leaves was highest in 0ml and varied significantly among treatments.

Table 1: Early growth performance of *Persea americana* seedlings sown in soils treated with extracts from different parts of *Annona muricata*

Growth Attributes	Treatments				
	(Leaf Extract)				
	0ml	25ml	50ml	75ml	100ml
Total Height (cm)	56.50±2.48a	56.60±3.06a	64.88±4.48a	59.30±2.94a	64.60±2.56a
Collar Diameter (mm)	8.15±0.78a	8.33±0.25a	8.24±0.37a	7.91±0.37a	6.65±1.04a
No. of Leaves	11.75±2.21a	15.50±2.29a	15.75±2.93a	17.00±1.52a	14.00±0.71a
			(Root Extract)		
Total Height (cm)	54.10±5.17ab	61.40±4.48a	59.80±4.27ab	58.60±5.09ab	44.88±5.23b
Collar Diameter (mm)	7.70±0.39a	7.33±0.12a	7.10±0.34a	7.08±0.29a	7.08±0.45a
No. of Leaves	13.40±1.29a	14.60±0.75a	14.80±1.32a	10.80±2.48a	13.00±1.29a
			(Leaf/Root Extract)		
Total Height (cm)	51.83±7.93a	47.10±2.09a	53.88±4.03a	56.70±1.34a	48.63±1.28a
Collar Diameter (mm)	6.47±1.56ab	5.74±0.39b	6.67±0.44ab	7.00±1.00ab	7.77±0.29a
No. of Leaves	14.67±0.88a	10.67±1.60b	10.25±1.11b	14.60±0.93a	12.75±0.48ab

Means with the same alphabet on the same row for each extract type are not significantly different (p>0.05)

Effect of extracts from different parts of *Annona muricata* on the biomass and moisture content of *Persea americana*: Seedling biomass and moisture content of *P. americana* in soils treated with extracts from different parts of *A. muricata* are presented in Table 2. In soil treated with leaf extract, there was no significant difference ($p > 0.05$) in wet weight and moisture content among treatments but the dry weight varied significantly. The highest wet weight and moisture content were recorded at 50ml while the highest dry weight was recorded at 75 ml. In soil treated with root extract, there was no significant difference ($p > 0.05$) in wet weight, dry weight and moisture content although highest wet weight, dry weight and moisture content were recorded at 0 ml. In soil treated with a mixture of leaf and root extracts, there was no significant difference ($p > 0.05$) in wet weight, dry weight and moisture content although the highest wet weight and moisture content were recorded at 25 ml. while highest dry weight was recorded at 100 ml.

Table 2: Biomass and moisture content of *Persea americana* seedlings sown in soils treated with extracts from different parts of *Annona muricata*

Attributes	Treatments				
	(Leaf Extract)				
	0ml	25ml	50ml	75ml	100ml
Wet Weight (g)	27.84±8.10a	38.40±2.28a	43.30±4.77a	37.32±2.57a	40.75±3.42a
Dry Weight (g)	8.06±2.53b	11.20±0.63b	12.08±0.49b	17.22±2.02a	16.45±2.39a
Moisture Content (g)	19.78±5.74a	27.20±2.39a	31.23±.4.32a	20.10±2.15a	30.85±5.55a
			(Root Extract)		
Wet Weight (g)	37.26±5.75a	30.50±4.03a	26.34±3.61a	29.64±2.64a	24.68±3.77a
Dry Weight (g)	13.52±3.24a	10.86±1.59a	6.26±0.87a	9.50±1.01a	8.38±1.87a
Moisture Content (g)	23.74±3.26a	19.94±3.15a	20.10±.3.78a	20.14±2.18a	16.30±2.03a
			(Leaf/Root Extract)		
Wet Weight (g)	27.77±3.28a	30.36±2.35a	29.68±0.95a	20.42±2.38a	28.75±5.45a
Dry Weight (g)	9.37±1.27ab	9.38±1.02ab	11.28±1.18ab	6.34±0.82b	12.13±2.92a
Moisture Content (g)	18.40±3.22a	20.98±2.04a	18.40±.1.21a	14.08±1.73a	16.63±3.34a

Means with the same alphabet on the same row for each extract type are not significantly different (p>0.05)

DISCUSSION

With particular reference to *P. americana* soil amendment with different volumes of *A. muricata* leaf and root extracts resulted in earlier plumule emergence than in the Control (0 ml), while leaf/root extract only improved emergence at 75 and 100 ml, and duration at 100 ml. This implies that extract from different parts of *A. muricata*

had varying degrees of effects on the duration of emergence in *P. americana,* with those of the leaf and root being more beneficial for shortening or reducing the period for plumule emergence and the duration of germination when applied separately than a combination of leaf/root extract. These findings agree with the report of Rai and Triputhi (1984) that allelopathy includes both promoting and inhibitory activities. Germination of *P. americana* seeds was also observed to have improved with the amendment of soil using different volumes of extracts from different parts of *A. muricata* except at 100 ml volume when the root extract alone was used. The silvicultural importance of these findings cannot be overemphasized.

Initial seedling growth is an important life stage critical for establishment and succession in forest (Jerzy et al. 2015). With respect to the different extracts from different parts of *A. muricata*, there was no significant difference in seedling height and collar diameter among the treatments. Leaf extracts of *A. muricata* enhanced seedling height at different concentrations. According to Talukder et al. (2015), the presence of some growth-regulatory substances in the leaf extracts may be the cause of the increased growth. Root extracts also enhanced seedling height, however it was found to have reduced with high volume (100ml). This is in line with Abu-Romman et al. (2010) who carried out a study on the effect of *Euphorbia hierosolymitana* on wheat and indicated that higher concentration of aqueous leachate of *E. hierosolymitana* reduced seedling growth. Also, seedling height varied in soil treated with a combination of root/leaf extract with some concentrations enhancing growth and others inhibiting it.

Collar diameter in soil treated with leaf extract was enhanced at lower concentrations and inhibited at higher concentration. This implies that lower concentrations can stimulate plant growth, while higher concentrations can cause inhibition. Root extracts of *A. muricata* reduced growth in collar diameter with increase in concentration. This shows strong inhibitory effect of root extract on *P. americana* and also implies that species are concentration dependent. Reduced collar diameter indicates the accumulation of toxic substances (allelopathic potential) of the donor plant, which is harmful to the growth of seedlings of receptor plants (Uddin et al. 2007). However, a combination of leaf/root extracts enhanced growth at higher concentration. According to Rizvi and Rizvi (1987), allelopathy can include both promoting and inhibitory effects on plants.

Soil treated with leaf extract enhanced leaf production at different concentrations when compared to the soil treated with leaf/root extract which inhibited leaf production at different concentrations with lower concentration having more inhibitory effect. Root extracts also enhanced leaf production at lower concentration.

Soil treated with leaf extract enhanced seedling biomass and moisture content at different concentrations. On the other hand, soil treated with root extract inhibited seedling biomass and moisture content at different concentrations while soil treated with leaf/root extract enhanced and inhibited biomass and moisture content at different concentrations.

CONCLUSION

Extracts of different parts of *A. muricata* (especially leaf and a combination of leaf/root) enhanced germination of *P*. *americana* while a combination of the leaf and root extracts reduced the time of germination

emergence and duration at lower concentrations (25 and 50 ml). The effect of extracts from different parts of *A. muricata* on the growth of *P. americana* varied. Different concentrations of leaf extracts enhanced seedling height and leaf production but only enhanced collar diameter at lower concentrations. Root extracts enhanced seedling height and leaf production at lower concentrations but reduced growth in collar diameter. Leaf/root extracts enhanced seedling height at 50 and 75ml, collar diameter at higher concentrations (50, 75 and 100 ml) and reduced leaf production. Also leaf extracts of *A. muricata* enhanced plant biomass and moisture content while root extracts reduced them. It is therefore recommended that *A. muricata* extract especially leaf extract and leaf/root extracts combined, be used to enhance germination of *P. americana* while the leaf extract in particular can be used to promote its seedling growth. The above results are also suggestive of the possibility of both species growing successfully in an integrated system though field trials are needed to authentic this.

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