Pak. J. Bot., 45(4): 1363-1368, 2013.

ROOT DEVELOPMENT OF *CHROMOLAENA ODORATA* STEM CUTTINGS ENHANCED BY INDOLE BUTYRIC ACID

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Abstract

A greenhouse study was conducted to determine the effect of rooting media in addition to Indole Butyric Acid hormone on root development of stem cuttings of *Chromolaena ododrata*. Stem cuttings of *C. Odorata* treated with equal quantities of the growth hormone (0.7%) were grown in 1 kgs of vermuculate, perlite, planting soil, sand, and equal mixture of all media. The set up were treated equally with water and organic manure. Control set up was made with a mixture of different rooting media without growth hormone. Data on shoot development were noted for 6weeks and root length was measured on the day of harvest. The parameters measured were analysed statistically using ANOVA. It was found out that root and shoot lengths were significant at p> 0.05 in the entire rooting media and the highest percentage development (49 and 51%) for root and stem respectively, was observed in the rooting media that had equal measure of each constituent (i.e. the mixed constituents). Germination rate among the media was 100, 70, 60, 50 and 40% for mixed, sand, planting soil and vermiculate, perlite and control respectively. Different sections of mature stem tested with Indole butyric acid hormone were not significant in their root and stem development, although basal cutting stems tend to mature faster than epical. This has proven that propagating *C. odorata* by stem cutting can be optimally achieved through mixture of 0.7% of Indole butyric acid in a collection of different rooting media.

Introduction

Chromolaena odorata (L.) King & Robinson (Asteraceae: Eupatoreae), commonly called chromolaena is referred to as Siam weed. This is sprawling shrub that has turned to be one of the world's invasive terrestrial weed in the humid tropics and some part of the sub-tropics. The plant is a shrub of the neotropical origin, but have been introduced to Africa and Asia in addition to other parts of the world (Singh *et al*., 2009). *C odorata* is a fast growing oppressive competitor occupying different kinds of arable land by forming dense strands that tends to prevent the growth of other flora. Therefore it affects plantation and other organisms in the ecosystem, by its suppressive effects to young plants as it is a growth inhibitor and has allelopathic capabilities. Review of literature revealed a lot of pharmacological and medicinal effects of extracts of *C. odorata*, and traditional uses of the plant especially in Asia to cure various disease ailments (Che Man 2010). These properties as exhibited by *C. odorata* are as a result of the phytochemical composition of the plant.

The medicinal properties of plants lies in their phytochemicals components like alkaloids, flavonoids, tannins in addition to other phenolics that co-opt as to producing definite physiological functions to the body of human being and animals (Taiwo *et al.*, 2000; Che Man 2010). However, analysis of extracts of *C. odorata* were found to be rich in flavonoid and flavonones, triterpene, terpenic compounds, chalcone, steroids as well as peroxidise isoenzymes (Anup *et al*., 2011). It is also rich in carbohydrates, high content of total proteins in addition to high percentage of nitrogen. All these contribute to the high phytochemical properties of the plant. *C. odorata* is therefore grown as a medicinal plant for the treatment of skin wounds especially in Indonesia (Metwally & Ekejuba 1981; Phan *et al*., 1996). In Nigeria for example, extracts from the leaf of C. odorata is used for the treatment of fresh wound, this is because of its antispasmodic, antiprotozoal, antibacterial, antihypertensive, analgesic, anthelmintic as well as antitrypanosomal activities as was reported by Taiwo *et al*., (2000) (Phan *et al*., 1996; Akinmoladun *et al*., 2007; Anup *et al*., 2011). *C. odorata* has also been implicated by its diuretic, hepatotropic and adstringent properties (Weninger & Robenean 1988; Iwu 1993).

Traditionally, boiled fresh leaves of *C. odorata* have been used in so many countries for the treatment of various skin ailments. For example in Vietnam, decoction of the plant is used to treat leech bite, burns, wounds of the soft tissue, as well as other skin infections (Phan *et al*., 2001; Che Man 2010). The leaf poultice of the plant has also been traditionally used in the treatment of fresh cut or wounds as an aid to stop bleeding. In other instances, *C. odorata* is implicated in the treatment of sting from a pine of sea catfish, its root used as analgesic and antipyretic remedy and the extracts from the leaves mixed with table salt could be used as gargle for the treatment of sore throat and flu (Che Man 2010). For some time now, C. odorata was just known for its medicinal properties but, recent studies has implicated the plants in biotechnology where it has been used in phytoremediation of organic and inorganic contaminants (Singh *et al*., 2009, Tanhan 2011). *C. odorata* can be propagated either by seed or stem, this is one of the significant agronomic properties of the plant (Tanhan *et al*., 2007). However, it has been reported that germination of seeds from weeds are most likely affected by soil compositions especially in the field study therefore propagation by cutting is preferred (Adebayo *et al*., 2005; Agbo & Obi 2007). Moreover, stem cuttings propagation prolongs hybridization of genome as segregation through recombinant gene is avoided.

The success of rooting of stem cuttings has been attributed amongst other factors to the rooting medium as well as the presence of rooting hormone and its concentration (Al-Saqri & Alderson 1996; Hartmann *et al*., 1997; Afzal *et al*., 2011; Ullah *et al*., 2012). Influences of rooting media and hormone on rooting in different kinds of plant have been documented and its effects in structure propagations were elucidated (Akwatulira *et al*., 2011). These media combination used in the rooting of cuttings provide physical support as well as oxygen and water to the cuttings (Larsen & Guse 1997). However, IBA, an auxin containing product was reported to stimulate adventitious roots in apical cuttings of some plants (Araya *et al*., 2007). This was buttressed by the study by Rao *et al*., (2005) which reported that IBA was the leading plants hormone used to promote the formation of roots in Tomatoes. Hence with the quest for a systematic search for specific useful factors from medicinal plants like *C. odorata*, even though most plants cuttings can root without hormone, there is need for an improved method of propagation and growth for such nutraceutical plants. This will therefore form a rational approach towards drug and nutritional research. The aim of this study was to evaluate the effect of Indole butyric acid hormone and rooting media for optimal rooting of propagated *C. odorata* stem cuttings.

Materials and Methods

Mature stems of *C. odorata* plants was collected from the greenhouse at the University of South Africa in Pretoria (25°46'1"S, 28°12'2"E) and 1439m above sea level. The stem was cut into pieces of 10cm each containing at least a node and a leaf bud. The experiment took place in polyvinyl chloride (PVC) pots with each dimensions 30x25x30 at a garden at the University of South Africa. The pots contain 1kgs of different rooting medium that has been mixed with equal volume of organic manure collected at the animal farm of the University of Pretoria in Onderstepoort. Inside each pot was made holes designed to contain equal weight of IBA hormone (0.7 %), for the plants. The set up was made in a complete randomized design containing a 3x4 factorial treatment model (Jeruto *et al*., 2008). The rooting media used here were vermiculate, planting soil, sand, perlite, planting soil and a mixture of equal volume of the four medium (w/w). About thirty six stem cuttings were used in each set of rooting media and IBA hormone concentration by volume percentage. Then sets of water treated cuttings were inserted into holes containing the IBA hormone in the rooting media and were replicated thrice. Random allocation of cuttings to the rooting media using random digit from a table was employed as to eliminate bias (Johnson & Bhattacharyya 2006).

Rooting media: The rooting media was homogenized by hand, mixed with equal volume of organic manure and air dried on an impervious polythene sheet for 24hours, there were pasteurized and fumigated, measured into the PVC pots with the hole containing about 0.7 % of IBA hormone. This concentration was chosen because of its optimum performance from other unpublished trials.

Propagation of the cuttings: The 10.0cm cuttings with lateral buds were about 360 collected at tale end of the afternoon when the weather was moist, and were kept in a bow containing water (Agbo & Obi 2007). The bases were made squared by the use of sharp sickle in order to spread the rooting. For each of the cuttings, about half of it was dipped into the IBA hormone that has been made into hole in each of the pots as to soak the powder. Excess of it was shaken off the base of the cuttings and the hormone was manually made to concentrate at the base. The cutting was then inserted into the soil to about 6 cm, watered and maintained to about 70 % humidity (Tanhan *et al*., 2007). Fungicides were sprayed onto the cuttings to control infections (Yeboah & Amoah 2009). The set up was allowed to grow for six weeks while monitored in between days to remove any invading weeds. Data on shoot development was taken at interval of weeks and root length was measured on the day of harvest after weeks.

Data collection: Data collection which commenced on the first week after propagation continued till the sixth week and the parameters measured were survived cuttings, length of shootings/buds, and the root length, number and weight of the cuttings on the day of harvest.

Data analysis: The data collected was analysed using Microsoft excel to obtain the mean number and percentage of cuttings that were able to form developed root and sprouted shoots from the series of stem cuttings. The data was then imported into SPSS version 13.0 and was analysed using analysis of variance (ANOVA) and standard deviation for the length of shoot and roots. The significance effects were determined at 5% level of significance.

Results

C. odorata cuttings treated with concentration of 0.7 % (w/w) IBA hormone propagated in different growth media recorded the highest mean number of cuttings that developed roots and sprouted shoots in the set up with equal mixtures of the four growth media with organic manure (Figs. 5-7). While the stem cuttings propagated in the perlite and control recorded the least mean rooted (2 each) and sprouted shoots (4 and 3) as recorded in Table 1 and Fig. 1. Amazingly, perlite and vermiculate maintained almost equal numbers of shoot developed (4 and 3 respectively) like in the root (2 each). However, significant difference existed among the different media with reference to their mean rate of development and formation of root and shoot. The efficacy of Indole butyric acid hormone to the development of root and shoots was noticed by the difference between the treated samples and the controls without the hormone (Fig. 2). There was significant difference between the two samples.

Table 1. Number of rooted and shooted cuttings within rooting media. Values are mean of replicates (Numbers with same superscript within same row are not statistically significant at P = 0.05).

Rooting media	Number of cuttings		Growth hormone concentration
	Rooted	Shooted	
Planting soil	4 ± 0.75^{a}	12 ± 2.55^{b}	0.7
Perlite	2 ± 0.71^{a}	4 ± 1.22^{a}	0.7
Sand	4 ± 0.71^{a}	6 ± 0.71^{a}	0.7
Vermiculate	2 ± 0.68^{a}	3 ± 0.71^{a}	0.7
Mixture	13 ± 1.41^{b}	29 ± 1.41^{c}	0.7
Control	2 ± 0.69^{a}	3 ± 0.00^{a}	0.7

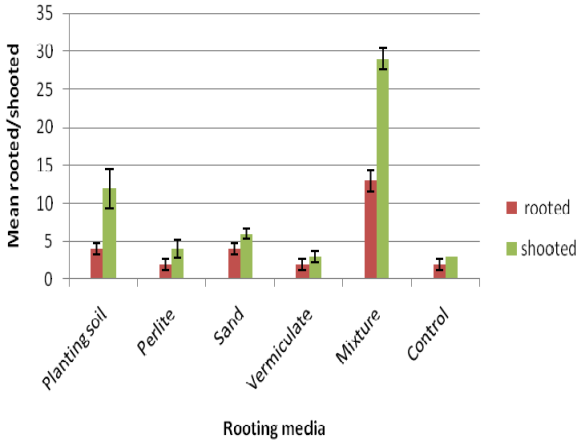

Fig. 1. Bar chart representation of the means values of rooted and shooted cuttings within rooting media (The error bars represent the standard error from the mean).

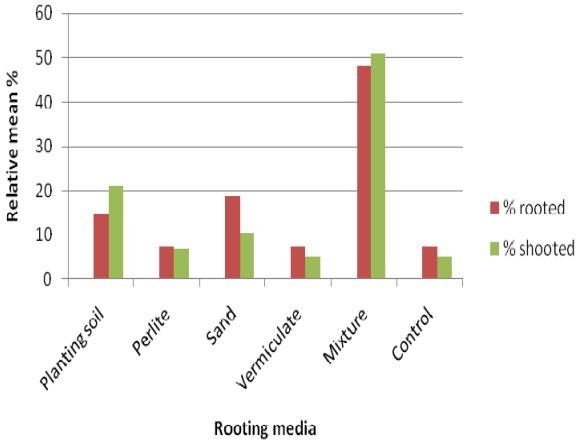

Fig. 2. Relative mean percentage of the rooted and shooted cuttings among the rooting media.

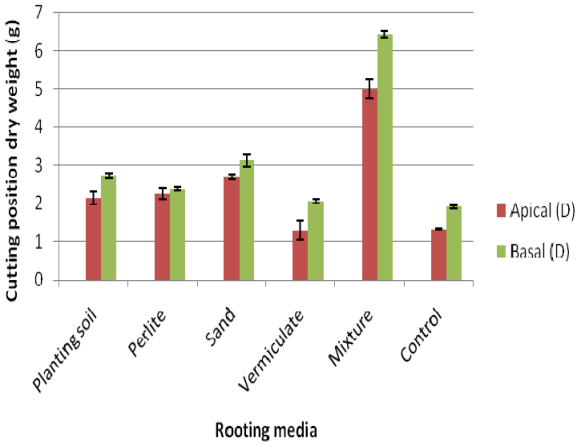

Fig. 3. Dry weight of Cutting positions within different rooting media (The error bars represent the standard error from the mean).

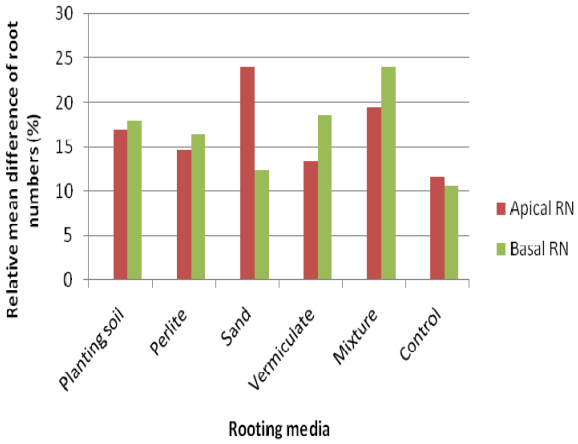

Fig. 4. Fresh weight of of Cutting positions within different rooting media (The error bars represent the standard error from the mean).

Number and length of roots developed among different rooting medium and the concentration of hormone were not significant at P = 0.05, but shoot lengths and number per sprouted stem was significant. Increase in shoot length occurred in this order: mixture> vermiculate> perlite> planting soil> sand> control while increase in root length were in the order: mixture> vermiculate> perlite> planting soil> sand> control. But it made a turnaround in the number that rooted and shooted within the rooting media, i.e. sand and planting soil had the same mean number of roots (4) and a little difference in Shoots (6 and 12). The mixture of the media had a high occurrence in root and shoot followed by planting soil, sand, perlite, vermiculate and control media maintained equal numbers (Table 2). In fresh weight of cutting positions (apical and basal), there were

higher weight measurement in the basal cutting than the apical ones, though not significant (Figs. 3 and 4). Equally, set up containing the mixture of the rooting media still maintained the highest weight followed by sand, perlite, planting soil, vermiculate and the control. The same sequence was also followed in their dry weights. In sand however, there were greater number of apical root numbers while in other rooting media basal cuttings had greater number of roots. Shoot and root length of mixture of rooting media and vermiculate were synonymous with each other and were almost significant with the rest of the medium. Apical root lengths were 34 and 33mm for mixture and vermiculate respectively, 39 and 37mm in basal. In apical shoot length, there were 41 and 41mm and 43mm all in basal respectively. The set up containing equal mixtures of all media had 100 % germination rate compared to 70, 60 50 and 40% for sand; planting soil/vermiculate; perlite and control respectively (Table 2). However, the rest of the media demonstrated improved significant effects in all the parameters measures compared to the control.

Table 2. Rate of germination of shoots among rooting media with 0.7% concentration of the growth hormone. Values are mean of replicates (Numbers with same superscript within same row are not statistically significant at P = 0.05).

Rooting media	Growth hormone concentration (%)	Rate of germination (%)
Planting soil	0.7%	60^{b}
Perlite	0.7%	50^{a}
Sand	0.7%	70^{b}
Vermiculate	0.7%	60^{b}
Mixture	0.7%	100^{c}
Control	0.7%	40^{a}

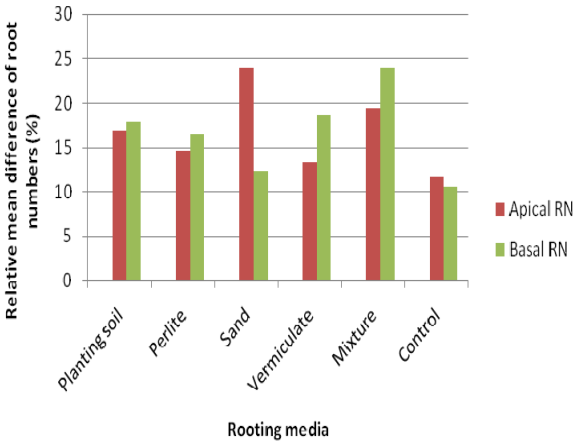

Fig. 5. Percentage relative mean difference of root numbers in cutting position among rooting media.

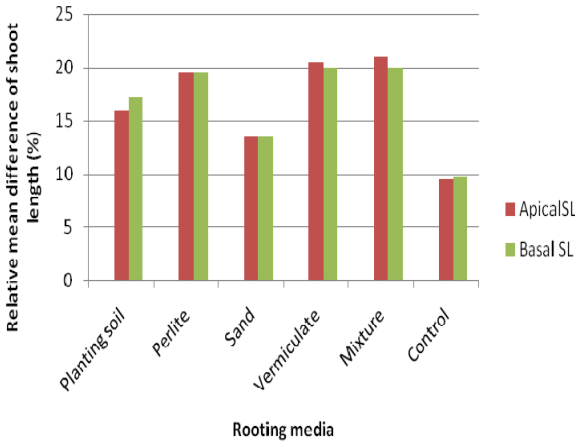

Fig. 6 Percentage relative mean difference of shoot length in cutting positions among rooting media.

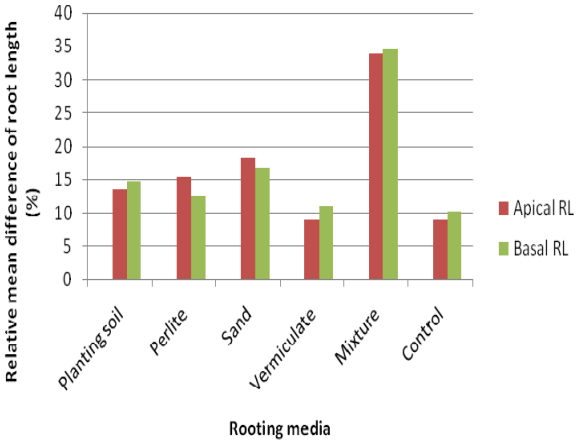

Fig. 7 Percentage relative mean difference of root length in cutting positions among rooting media.

Discussion

The rooting medium suplement that has mixtures of planting soil, perlite, sand and vermiculate at equal proportion enabled highest development of root and shoot in a cutting of *C. odorata*. This improved effect could be attributed to the fact that the combined nutrient supplement of the entire rooting media acted optimally with the Indole butyric acid hormone concentration added which created the enabling soil condition for example, optimum aeration and moisture level (Kalyoncu & Ozer 2000; Akwatulira *et al*., 2011; Nasim *et al*., 2012). Meanwhile dose application of Indole butyric acid hormone was shown to be an aid to improved moisture content in the soil for an optimal rooting and shooting development of cuttings (Milleton *et al*., 1980; Leakey *et al*., 1982; Aminah *et al*., 2006; Akwatulira *et al*. 2011; Ullah *et al*., 2012; Jaskani *et al*., 2013). However, most of

these past studies maintained their Indole butyric acid hormone concentration to about 0.7-0.8 % of the rooting media used while this study based its growth hormone measurement on the weight of the cuttings. Morever, the control sample of rooting medium supported low development of root and shoot than the auxin treated samples in the entire experiment. This could perhaps be becouse soil resistant to root penetration is dependent on amongst other factors on water content, structure and strength of soil as well as bulk densities though these were not measured in the rooting media (Amri *et al*., 2009). Soils do not posses the required aeration porosity for optimal gas exchange required for rooting of the cuttings resulting in poor rooting in the control. Poor rooting numbers in control could also be attributed to the feel of anoxia which is an effect of low oxygen in the soil (Hartmann *et al*., 1997). Low incidence of rooting was found in the control samples. Non-theless, there are instances where optimum concentration of the growth hormone in the soil has resulted in the failure of the development of roots by stem cuttings as was reported by Griffin & Shroeder (2004). That was an indication of the sensitivity of root formations to hormone formulations (Akwatulira *et al*., 2011). It has also been reported that rooting hormone to a certain instance could be inhibitory to the development of roots in the cutting especially during the initiation stage (Montesano & Orci, 1988; Akwatulira *et al*., 2011). This then means that different rooting media accomodates different concentration of auxin for optimal growth of plants.

Conclusions

Mixture of various rooting media in the right proportion with 0.7% of growth hormone has been proven to be effective in the root and shoot development of cuttings of *Chromolaena* odorata irrespective of the position of the cuttings. Although the individual medium did not have high incidences in most of the parameters measured, but there were able to support root and shoot development as well. Therefore, there is need for a study of Indole butyric acid concentration effects on the media as to be able to determine if concentration was not the factor responsible for the low root and shoot development. This will promote mass production of *C. odorata* especially in areas that the plants does not occur naturally.

Acknowledgements

Wish to express my appreciation to the the University of South Africa and National Research Foundation for their financial assistance in this project.

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(Received for publication 25 November 2011)