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Effects of Cigarette Tobacco Infusion on Root Regeneration and Proliferation of Two Cultivars of Garden Croton (*Codiaeum variegatum*)

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Abstract: Garden croton, an ornamental shrub or tree with diverse beautifully colored leaves, shapes and sizes, is used as informal hedge or screen for landscape, interiorscape and bouquet. This study, investigated the effects of cigarette tobacco extract on plant mutation, root regeneration and proliferation in a plant-plant interactive hydroponic assay using two well known reciprocal mutant cultivars (Guinea broad leaf and Guinea narrow leaf) of garden croton. The results showed that $1 \times 10^{-4-6}$ logarithm dilution of a standard teacup (270 mL) cigarette tobacco-infusion enhanced root regeneration, elongation and proliferation above control level. Higher concentrations of extract inhibited root elongation and proliferation. Undiluted tobacco infusion extract (100%) delayed root initiation until four weeks. Overall, the extract enhanced root elongation and proliferation better in the Guinea broad leaf than the Guinea narrow leaf cultivar. There was no sign of induced mutation in the plant cultivars by the extract.

Key words: *Codiaeum variegatum*, garden croton, root regeneration, tobacco infusion

INTRODUCTION

The Garden croton (*Codeaum variegatum* L. Blume) belongs to the family Euphorbiaceae (Dutta, 2003) popularly called spurges. It is a native of the tropics from Java to Australia and the South sea islands. The Garden Croton is highly susceptible to cold injury hence, it does not survive beyond the subtropics. It is restricted to the Southern and warmer parts of central Florida (Bailey, 1949). It is a shrub or tree with great variety of impressively colored fleshy, glossy and leathery leaves of different shapes and sizes. The leaves start with green color but later produced diverse color and colour combination patterns as they mature. Garden crotons have been used as informal hedges or screen shrub boarder, landscape or interiorscope (Jensen and Salisbury, 1972).

Garden crotons, as a tropical plant cannot stand either too high or too low temperature (ideal temperature is around 20°C). Generally, if the temperature is either too low or too high or affected by too drastic water stress, the plant may drop all of its leaves quickly (Facciola, 1990). Garden crotons are easily propagated in water or soil either as rooted cuttings from greenwood cuttings, leaf bud cuttings, or if plants are desired quickly, by air layering (or marcots) (Dirr and Heuser, 2009). Without pruning, the plant may attain a height of about 3-3.7 m and tend to produce the heaviest foliage at the tip of the plant. When left unpruned, they could be

susceptible to attacks by epiphytes especially the mistletoes as well as show high tendencies for mutation (Taylor, 1990).

The tobacco plant (*Nicotiana tabacum* L.) is indigenous to North and South America. The leaves of the plant are the commercial product and raw material for factories. They are often cured by smoking and consumed in form of cigar or cigarette, or in a smoking pipe or a water pipe when smoked. They could be chewed, stewed, snuffed, brewed or fermented as a beverage in water (Adam, 2002). Tobacco contains the obnoxious alkaloid- nicotine, which is a psychoactive substance. This is a powerful neurotoxin. In addition to nicotine, tobacco contains over 19 carcinogens (most collectively known as tars) and more than 4,000 chemicals (Tso, 1972).

Nicotine ($C_{10}H_{14}N_2$), the active ingredient in tobacco is second only to caffeine as the most widely used central nervous system stimulant (Barry and Gleeson, 1997). In combination with the tars and carbon monoxide in cigarette smoke, nicotine represent a serious health risk factor for lung and cardiovascular diseases as well as cancer (Campaign, 2004). Nicotine, however, is approved by Food and Drug Administration (FDA) as an insecticide (Barry and Gleeson, 1997).

Nicotine is a colorless oil which is soluble in water and turns brown on exposure to air. It is also found in tobacco smoke and can be described as recreational stimulant smoke (Collins and Hawks, 1993).

Literature is replete with effects of tobacco on animal or human systems resulting in breathing problems, lung cancer and liver cirrhosis. This has led to the banning of cigarette smoking in public places, age limit on cigarette purchase and raising of tax on tobacco companies in many countries. However, there is paucity of information on the effects of tobacco on plant metabolism which necessitated the present study. In this study, we investigated the effects of cigarette tobacco extract on garden croton stem cuttings in a plant-plant interactive hydroponic assay. This was with a view to determining the capacity for root regeneration and proliferation and plant mutation/mutagenicity using well known reciprocal mutants-the Guinea broad leaf and Guinea narrow leaf garden croton cultivars.

MATERIALS AND METHODS

Test plants: The two cultivars of garden croton cultivars used are: The Guinea broad leaf (Gold dust or Aureomaculatum) and Guinea narrow leaf cultivars. The two cultivars of the garden croton plant were collected from the Babcock University Chapel premises, Ilishan-Remo, Ogun State, Nigeria in August and November, 2008. The fresh green stems were cut using secateurs and razor blade and were enveloped tightly in transparent polythene sachet bags to prevent desiccation. The two cultivars are well known reciprocal mutants.

Preparation of tobacco infusion extract: The procedure for the preparation of the tobacco infusion (extract) was adopted with slight modification from breakfast tea practice. Consequently, the London King-size tobacco cigarettes, a product of the British America Tobacco Company (BAT) Ibadan, Nigeria, was used for this study. Three cigarette sticks (0.7 g each), (an equivalent of one cup (one lipton) teabag which makes 270 mL brewed tea (standard England teacup) was brewed with Babcock University sachet water and brought to boil under bunsen burner flame for 30 min. A serial dilution in logarithm units of ten and in percentage was prepared from the original concentration of the infusion and the pH of each was read.

Experiment on garden croton cultivars: Fifty milliliter of each logarithm and percentage serial dilution was taken by a syringe and put in each beaker. Three stem cuttings of both Garden Croton plant cultivars (Guinea broad-leaf and Guinea narrow-leaf) were placed in each beaker and covered with a sachet size polythene bag. Equal amount of distill water was used as the control.

Statistical analysis: The means of the various treatments and the control were compared using the General Linear

Model (GLM) analysis of variance software package by Statistical Analysis System (SAS, 1999). When there is significance, treatment means were compared using the Duncan Multiple Range Software Option.

RESULTS

Low concentration of cigarette tobacco infusion (1-20% dilution) stimulated early root initiation under one week in the Guinea broad leaf garden croton stem cuttings. Higher concentration (30-90% dilution) delayed rooting by a week. Undiluted (100%) tobacco infusion delayed root generation further until four weeks (Fig. 1, 6).

Root elongation was concentration dependent under percentage dilution of the tobacco infusion. Root length increases as the concentration of the extract increases except the undiluted extract (Fig. 1). However, the highest tobacco-treated root length value (c. 1.7 cm) was below the control (c. 2.5 cm).

The root number was optimum (c. 16) at 50% extract dilution surpassing the control (c. 8) but decreases as extract concentration increases (Fig. 6).

Under logarithm dilution, both root length and number increased beyond the control values. Optimum root length (3.3 cm) and number (c. 9) were supported by low to medium tobacco infusion concentration ($1 \times 10^{-6.3}$ dilution) in the Guinea broad leaf cultivar. The Guinea narrow leaf cultivar required above average extract concentration ($1 \times 10^{-4.5}$ dilution) to yield optimum root length (3.1 cm) and number (5). Higher concentration in both cultivars inhibited root elongation and proliferation (Fig. 2-5).

Overall, tobacco infusion enhanced growth (root length and proliferation) better in the broad leaf cultivar than the narrow leaf. The pH for percentage dilution was more acidic (5.27-5.43) than the logarithm dilution (5.32-6.89). The acidity decreases as the concentration of the tobacco infusion increases. The percentage dilution has more of brown color infusion while the logarithm dilution was more or less colorless.

DISCUSSION

B-vitamins are essential component of enzymes or enzyme co-factors promoting plant growth. Their discovery (White, 1937) impacted positively on *in vitro* plant growth assay and led to the rapid development of Plant Tissue Culture (PTC).

Currently, no research information on the effects of the tobacco raw extracts on any aspect of plant growth was found in literature. However, nicotinic acid (niacin) has been routinely used *in vitro* as a mandatory beneficial vitamin component of most plant nutrient media

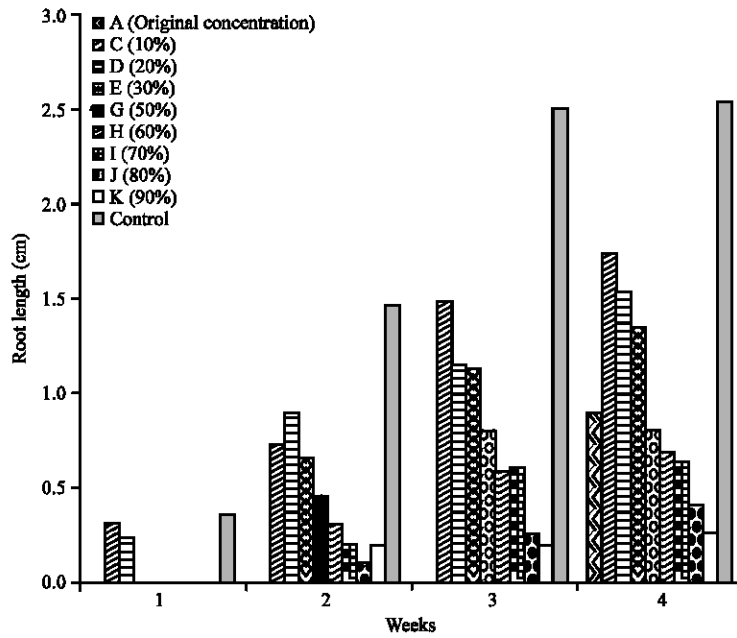


Fig. 1: Root length of stem cuttings of Guinea broad leaf garden croton grown in tobacco infusion percentage dilution series

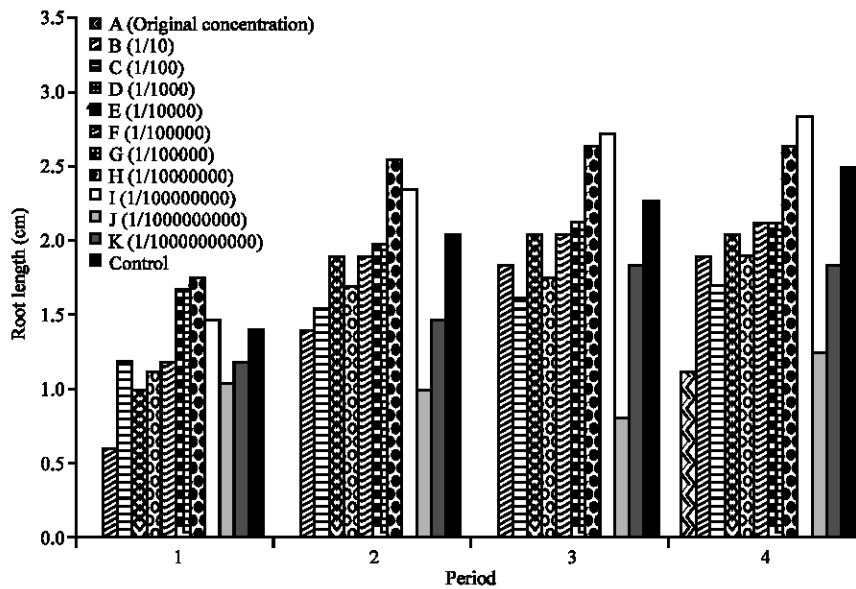


Fig. 2: Root length of stem cuttings of Guinea broad leaf garden croton grown in tobacco infusion logarithm dilution series

in plant tissue cultures (Hartmann *et al.*, 2002; Klein and Klein, 1970; Caponetti *et al.*, 2005; Dubey, 2006). Niacin (Vit B₃) in the form of nicotinic acid has also been routinely used *in vitro* as an essential (mandatory) component of most plant nutrients in growth media.

Tobacco plant cells, tissues or organs have featured severally and prominently in the development of PTC.

Historically, several studies have also used hydroponic plant growth system to develop or raise isolated plant cells (Haberlandt, 1902). Cytokinin was later found to induce organ formation in tobacco callus culture (Skoog, 1944). Appropriate combination of cytokinin with auxin in a specific ratio also induces root and shoot formation using this tobacco system. This has led to the

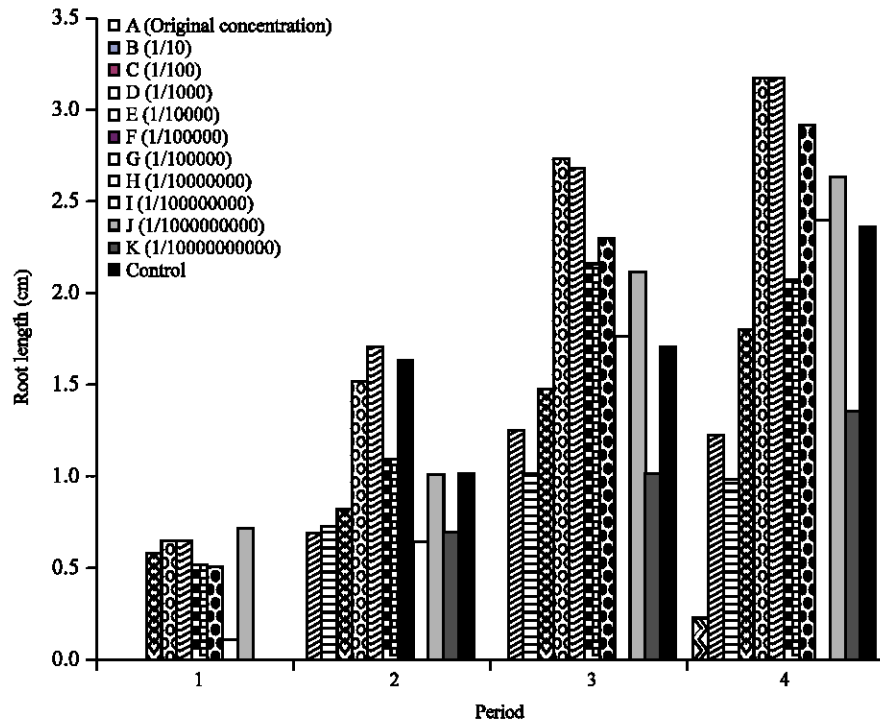


Fig. 3: Root length of stem cuttings of Guinea narrow leaf garden croton grown in tobacco infusion logarithm dilution series

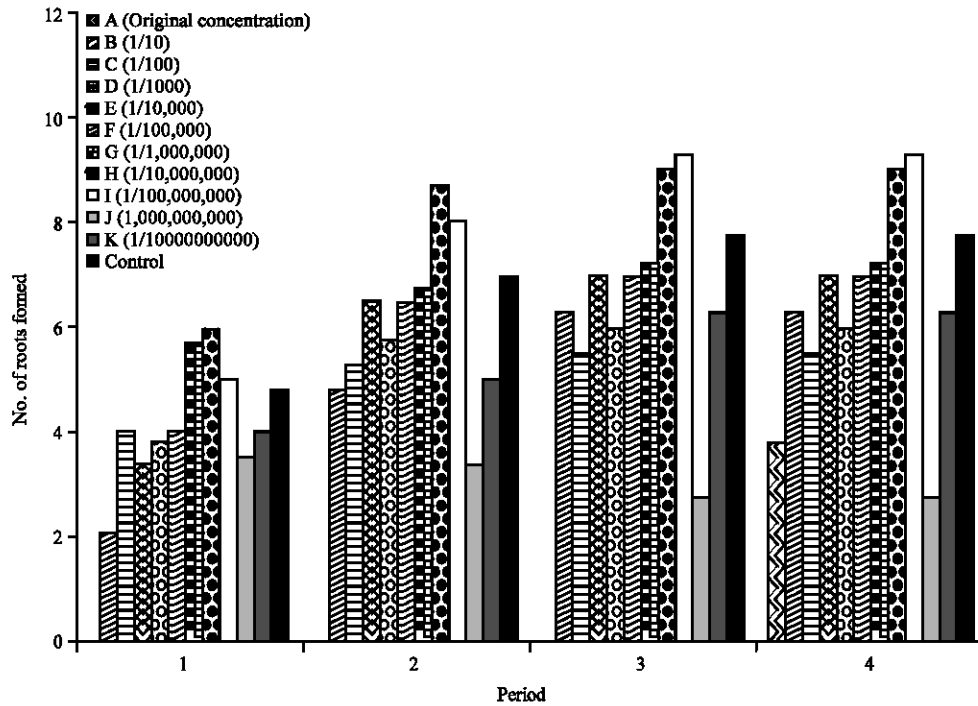


Fig. 4: Number of roots from stem cuttings of Guinea broad leaf garden croton grown in tobacco infusion logarithm dilution series

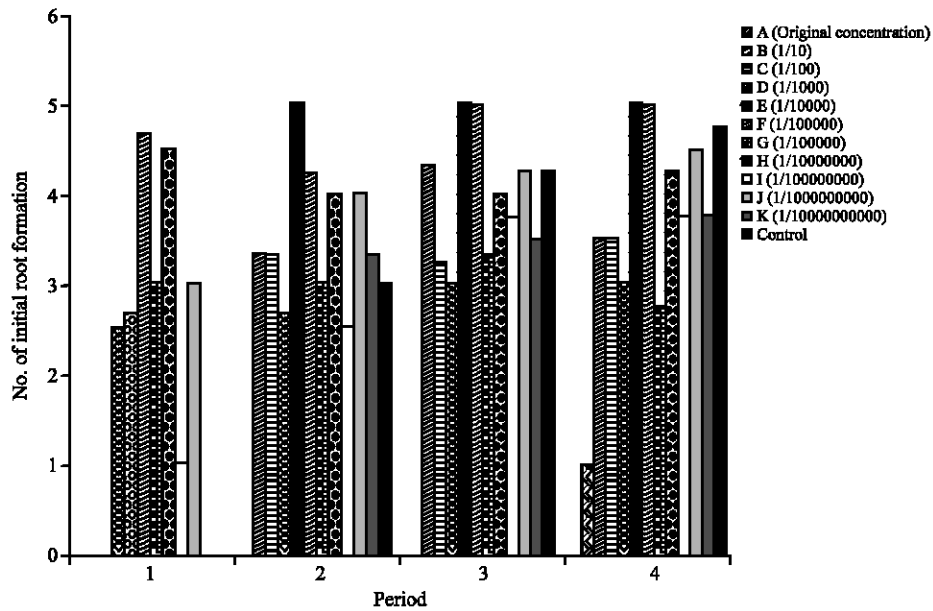


Fig. 5: Number of roots from stem cuttings of Guinea narrow leaf garden croton grown in tobacco infusion logarithm dilution series

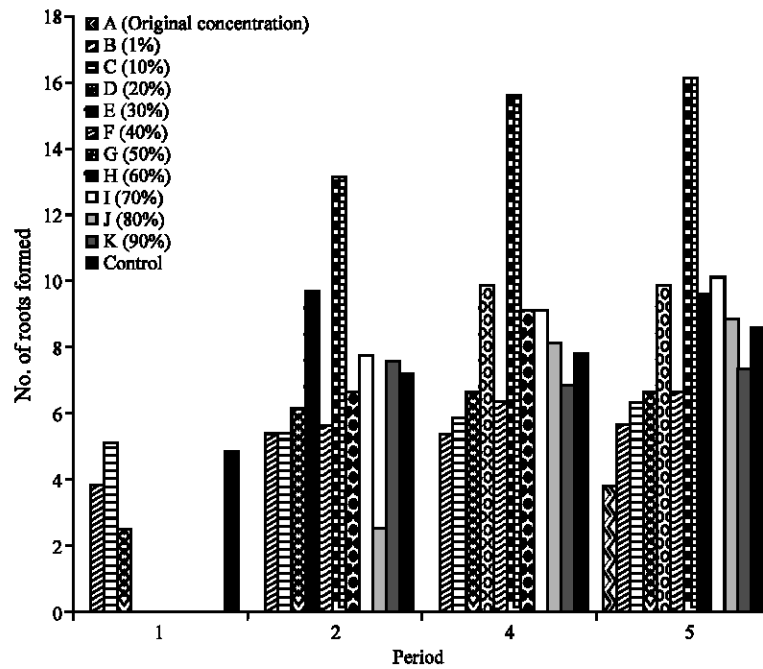


Fig. 6: Number of roots from stem cuttings of Guinea broad leaf garden croton grown in tobacco infusion percentage dilution series

development of the universally accepted Murashige and Skoog (1962) plant growth medium.

Today, the most commonly used vitamins (biocatalyst/growth stimulator) in PTC include: Thiamine (Vit B₁), Nicotinic acid (Vit B₃), Pyridoxine (Vit B₆), Glycine,

Inositol, Panthothenic acid (Vit B₅) and Biotin (Vit H). However, thiamine is often considered essential while nicotinic acid and pyridoxine are usually additive (Dubey, 2006). Whereas thiamine is involved in carbohydrate metabolism and the biosynthesis of some

amino acids, nicotinic acid (niacin) is a respiratory coenzyme (Beyl, 2005).

Despite the extensive use of tobacco plant system in PTC, there is no information yet in literature from our extensive search on the effects of synthetic tobacco extracts on plant growth. The indispensable role of leaves as primary photosynthetic light-capturing and atmospheric gas (CO₂, O₂, H₂O) exchange organ (Trigiano and Gray, 2005) cannot be overemphasized. However, leaves can undergo extensive often unusual, modifications due to the nature of their environment, its genetic system and evolutionary history. These attributes are extraordinary characteristic of the *Codiaeum variegatum* cultivars. They exhibit variety of beautiful leaf color combinations which could be up to fourteen on a single leaf, such that no two leaves on any of its branches could display the same color pattern on the same plant. Notwithstanding, the leaves could be devoid of visible variegation. In addition stem cuttings and detached leaves possess the capacity to root readily in either polluted or ordinary water-system within fourteen days.

Above all, some members of the cultivars characteristically produce specific mutant(s) of varying leaf color and/or shape (Ogunwenmo *et al.*, 2007) spontaneously, which are very stable or sometimes reversible. Consequently, this choice of the garden croton as the test plant for regeneration in nicotine polluted water.

So far, no mutant, carcinogenic growth or even death was observed over the range of concentration used and during the duration of this study. Nevertheless, tobacco cigarette stick infusion enhanced rooting in garden croton test plants used. Lower concentrations of the cigarette infusion (1-20% dilution) enhanced early root initiation and root elongation. However, at higher concentration (30-90% dilution), rooting was delayed by a week and root length reduction was observed. Consequently, it appears some cultivars of garden crotons have the capability to catabolize nicotine to nicotinic acid which has been found to stimulate root regeneration. This might justify the routine use overtime of nicotinic acid as a mandatory component of plant tissue culture media (Dubey, 2006). The pathway for this catabolic reaction has, however, not been established and therefore, yet unknown. Nevertheless, it is possible that the plant produces enzymes which are capable of catalyzing the conversion of nicotine to nicotinic acid, a reverse process of the biosynthesis of nicotine from nicotinic acid which is catalyzed by nicotine synthase (Caroline *et al.*, 2008; Nicholson and Dagley, 1970; Salisbury and Ross, 2002). The results of this study suggests that low concentration of tobacco infusion may be a good medium not only for

garden croton root regeneration from leaf and stem cuttings, but also an appropriate native source and substitute for niacin vitamin requirement in *in vitro* assays.

The responses of the two cultivars (Guinea broad and narrow leaf) to logarithm and percentage dilution differed. The broad leaf was superior to the narrow leaf in root formation (Fig. 2, 3) and length in the logarithm and percentage dilution. Generally, root lengths were significantly higher ($p < 0.05$) in the logarithm than the percentage dilution for both broad and narrow leaf cultivars. These varied responses are not unexpected as the two morphotypes have demonstrated natural reciprocal mutation (Esan *et al.*, 2008). However, the more frequent mutant is the broad leaf which mutates to produce the narrow leaf morph thus making the latter probably, the more stable form. This is a natural phenomenon in this cultivar.

All leaf cuttings eventually produced roots in all concentrations, except the undiluted extract. Thus, diluted tobacco infusion was not lethal or adversely inhibitory. Indeed, it enhanced growth at low concentrations.

CONCLUSION

This result is important in the biodegradation of nicotine in the soil as a result of environmental pollution from tobacco manufacturing companies, producing farmers and tobacco users especially cigarette smokers. Garden croton, *Codiaeum variegatum*, can be used to control and breakdown nicotine into harmless but useful component like niacin, a plant growth promoter easily metabolized by plants.

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