Full Length Research Paper

Inhibitory effect of mango (*Mangifera indica* L.) leaf extracts on the germination of *Cassia occidentalis* seeds

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The allelopathic effects of dry and fresh aqueous leaf extracts of *Mangifera indica* at 5, 10, 15 and 20% were investigated on the seed germination percentage and seedling growth rate of C*assia occidentalis* seeds in a greenhouse experiment. Both dry and fresh leaf extracts exhibited different degrees of inhibition in *C. occidentalis* germination percentage and growth rate of seedlings at varying concentration. Dry leaf extract at 15 and 20% completely inhibited the emergence of *C. occidentalis* while the aqueous fresh leaf extract at these concentrations yielded some degree of seed germination and growth rate*.* The seedling radicle and plumule length were adversely affected as the treatment level increases in both extracts. The concentration dependent responses of *C. occidentalis* to both dry and fresh leaf extracts confirmed that both extracts contain allelochemicals which are likely to be phenolic compounds. On the basis of overall toxicity potency of *M. indica* leaves, our result showed that 15 and 20% dry leaf extract exerted the highest germination percentage and growth inhibition on *C. occidentalis* seeds. The result from our study suggests that compounds from *M. indica* leaves especially the dry ones possess a strong phytotoxic potential and can serve as lead molecules for the synthesis of bioherbicides.

Key words: Allelochemicals, phytotoxic, radicle, growth inhibition, bioherbicides.

INTRODUCTION

Weeds are defined as unwanted plants growing in agricultural lands, gardens, road sides and mainly disturbed areas where they do not depend on human intervention for their reproduction and survival (Cassas et al., 1996; Lewu and Afolayan, 2009). They are of little or no economic values and are considered plant pest due to their tendencies to reduce biodiversity and promote habitat loss for less competitive species within the same ecosystem (Mungoro and Tezoo, 1999; Singh et al., 2003). A number of weedy species have competitive advantage over other species found growing in the same habitat due to their efficient utilization of mineral resources, adaptability to environment and comparative high fecundity (Lewu and Afolayan, 2009).

In agroecosystems, weeds and crops have co-evolved together right from the prehistoric times as revealed by pollen analysis studies (Cousens and Mortimer, 1995). Their co-evolution also gets substantiated from the fact that five major (Poaceae, Solanaceae, Euphorbiaceae, Convolvulaceae, and Fabaceae) families constitute the main world's weed contributors (Holm et al., 1977). Weeds cause a number of harms in agro-ecosystems due to their interference with crops (both competitive and allelopathic); they reduce crop yields leading to huge losses on a global scale. Weeds also affect crop quality, clog waterways, cause health problems in humans, and look unsightly in amenity areas such as garden, parks, pathways, and pavements. Despite the progress made in weed science over 40 years, weed remains one of the

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major constraints in agricultural production in developed and developing nations of the world (Adebayo and Uyi, 2010). Losses caused by weeds in developing worlds are at least 15% annually (Labrada, 1992). Furthermore, the costs of weed eradication could be enormous for example; the USA weeds cause about a 12% loss in crop yield and it costs nearly US$35 billion to control them (Pimentel et al., 2001). The costs are even more in developing countries.

In Nigeria, some methods of weed control have however been so difficult and time consuming, manual weeding by hand and simple tools used may consume as much as 70% or more of the farmer's time and energy during cropping season (Wrigley, 1969). A number of management practices are available in managing weeds. In the earlier times, before synthetic chemicals were known, crop rotations, polyculture, and other cultural management practices were tried that were low input but sustainable. These have enhanced crop production by minimizing competition between crops and weeds and reduced interference risk from weeds (Parry, 1989).

Cassia occidentalis is a common weed known worldwide with different names depending on where it is growing. It is a weed that grows throughout the tropics and subtropics including United States, Africa, Asia and Australia. It is an opportunist annual herb that grows along roadsides, fence lines and over heaps of waste material. The species is capable of growing on all kinds of soil including coastal sands. Like other species of *Cassia,* it also loves full sun but also capable of growing in partially shaded places. It requires frequent watering but tolerates drought once established.

One of the recent trends in weed management is to reduce heavy reliance on synthetic herbicides and to move towards low-input sustainable agriculture (LISA) as a part of integrated weed management (Kupatt et al., 1993). This is as a result of the increasing awareness of the adverse toxicological effects of synthetic herbicide on environment quality, public health, wildlife and overall ecology.

Additionally, resistance of weeds to synthetic herbicide has grown tremendously which has further renewed interest in LISA. Hence, these chemicals are under scrutiny to reassess their toxicological impacts on the environment especially in the developed countries like USA (Singh et al., 2002a).

Plants produce a number of secondary metabolites that are not directly involved in metabolism or have no obvious role in cell functioning. While first thought to be waste products, in reality these compounds serve various important roles within the plant. Some of these organic molecules protect the plant against herbivores and insect or pathogen attack, while others attract pollinators or function allelopathically to counter the competition of other plants (Hadacek, 2002). Advancement in technologies regarding identification, purification, evaluation of biological activity of plant natural products

has simplified protocols for using them in weed control (Duke et al., 2000a,b).

In the continual search for natural herbicides from plant products, leaf extracts from *Mangifera indica* were investigated for inhibitory effect on the seed germination of the popular weed, that is, *Cassia ocidentalis*. Earlier reports have shown that *M. indica* leaf from Nigeria possessed compounds like saponin, steroids, tannin, flavonoid, reducing sugars and cardiac glycosides (Aiyelaagbe and Osamudiamen, 2009). It also contains phenolics like ferulic, cumaric acid, benzoic, vanelic, chlorogenic, caffeic, gallic, hydroxybenzoic and cinnamic (El-Rokiek et al., 2010). Some of these compounds have been reported to be active medicinal and allelophatic chemicals. The present study therefore, reports on the effect of dry and fresh leaf extracts of *M. indica* on the seed germination of *C. occidentalis*.

MATERIALS AND METHODS

Plant collection and identification

Leaves of *M. indica* were collected in July, 2010 from two specific trees (Ojo Campus of Lagos State University) and washed under a running tap water then air-dried to a constant weight. For the fresh leave extract, leaves were collected fresh from the trees when needed.

The dried leaf materials were pulverized into powder using electric blender and then stored in an airtight container at 4°C until extraction.

Extract preparation

Dried and fresh mango leaf extracts were prepared according to the method described by Saeid et al. (2010) with slight modifications. Briefly, 10 g of both dry and fresh pulverized leaf materials were carefully transferred into cleaned, sterilized and labeled jars, and 200, 100, 66.5 and 50 ml of sterile, deionised distilled water was added to the corresponding labeled jars. The mixture was shaken and the jars were covered and left for 24 h at room temperature and filtered through a cheese cloth to remove debris and finally filtered using Whatman No.1 filter paper to give 5, 10, 15 and 20% concentrations, respectively. The extracts were then transferred into sterilized bottles and kept in the refrigerator. New extracts were usually prepared after every 5 days.

Seeds collections and viability test

C. occidentalis seeds were collected from a single population on a dump site at Jagun Street, Ojo Campus Lagos. Seed viability test was done by depoding seeds and soaking in sterile, deionized distilled water for 24 h. Twenty soaked seeds were picked into a 60 mm diameter Petri-dish and dissected into two equal halves avoiding the destruction or damage of the embryo using a pair of forceps and a surgical blade. A solution of 0.2% P-iodonitrotetrazolium salts was prepared in distilled water and applied on the Petri-dish containing dissected seeds. After some minutes, about 95% of the halved seeds turned red after absorbing the salt hence confirming their viability.

Seed germination test

Depoded healthy seeds were washed in 95% alcohol then rinsed in

Dry leaf extract

Fresh leaf extract

A

B
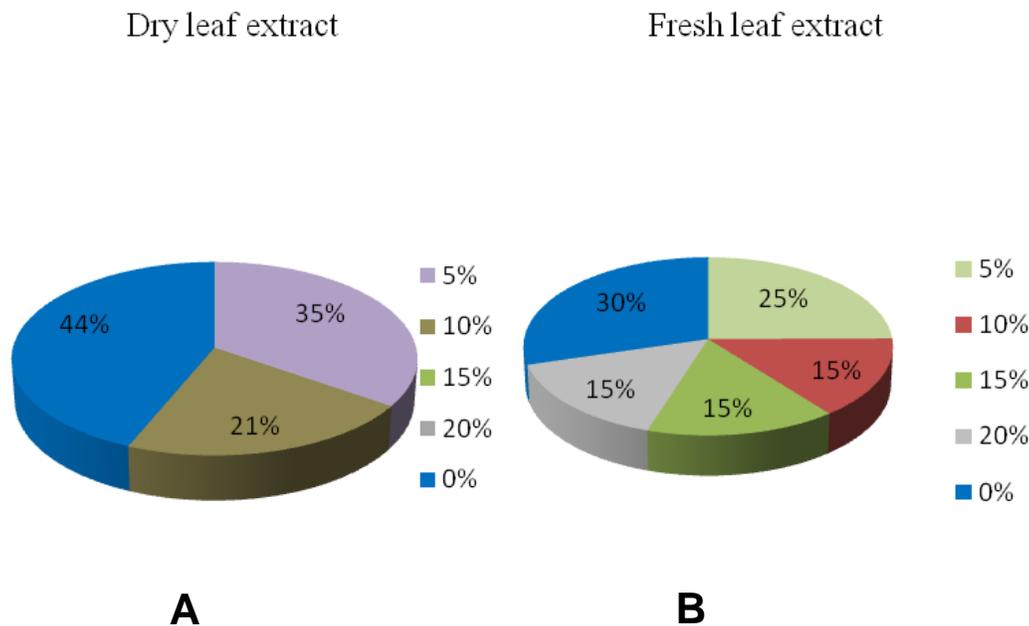
Figure 1. Effect of fresh (A) and dry (B) *Mangifera indica* leaf extracts on the germination percentage of *Cassia occidentalis* seeds.

double distilled water. Twenty seeds each were germinated in sterilized 90 mm diameter Petri-dishes lined with two layers of Whatman filter paper (No. 1) and moistened initially with 5 ml of respective leaf extract concentration treatments and 5 ml of distilled water for the control. All treatments including the control were replicated four times. Subsequently, extracts and distilled water were added *ad libitum.* Readings were taken at intervals from the onset of germination and emergence of radicle and plumule in each experimental treatment. Also, after complete germination of the seeds, the radicle and plumule length was measured by picking the seedling with a pair of forceps and a white thread was used to determine the length whose corresponding value was read on the meter scale ruler and recorded.

Experimental design and statistical analysis

The experiment was arranged as a completely randomized block design with three treatments and 4 replicates (block) each 4 units. Data were subjected to two - way ANOVA and test at 0.05 and 0.01 levels of probability and the Student and Newman-Keuls test was used to separate the treatment means.

RESULTS

Radicle emergence was found to be earliest in the control (no extract), followed by 5% fresh and 5% dry extract concentrations during 24, 36 and 48 h, respectively. In other concentrations of 10 and 15% fresh extract and 10% dry extract, concentrations radicle emergence occurred at 72 h. The radicle lengths of the germinating *C. occidentalis* seeds after 72 h of receiving treatment was also highest in the control standing at 1.67 cm compared to 1.0, 0.62, 0.4 and 0.2 cm for 5, 10, 15 and

20% extract concentrations respectively for fresh leaf extract concentrations. In sharp contrast to results obtained from fresh leaf extracts, emergence of radicle in 15 and 20% dry leaf extract was observed after 72 h. Generally, radicle lengths were higher at lower concentrations of both fresh and dry leaf extracts. Also, the radicle lengths in fresh leaf extracts were higher than that of dry leaf extracts (Figure 1a and b).

The duration for the complete germination of seeds (*C. occidentalis*) was found to be quickest in the control after seven days of germination period. In a similar pattern, there was complete germination at lower concentrations in both dry and fresh extracts as against the delay in higher concentrations. However, at 15 and 20% concentrations, the seeds never fully germinated despite initial radicle emergence in very few seeds. The final radicle length values after over 21 days of germination was highest in the control and 5% fresh extract with 2.20 and 2.24 cm, respectively. The 15 and 20% dry extract concentrations had the lowest values of 0.2 cm respectively (Figure 2a and b). Generally, with increase in both extract concentrations, there was corresponding decrease in radicle length values except in 15% fresh extract concentration where a slight surge was observed.

The final plumule length value was highest (5.06 cm) in 5% fresh leaf extract concentration which was higher than that of the control at 3.82 cm. Across the fresh leaf extract concentrations, it was the higher the concentrations the lower the plumule lengths. On the other hand, the dry leaf extract concentrations at 5 and 20% had no values and 3.67 cm was recorded at 10%
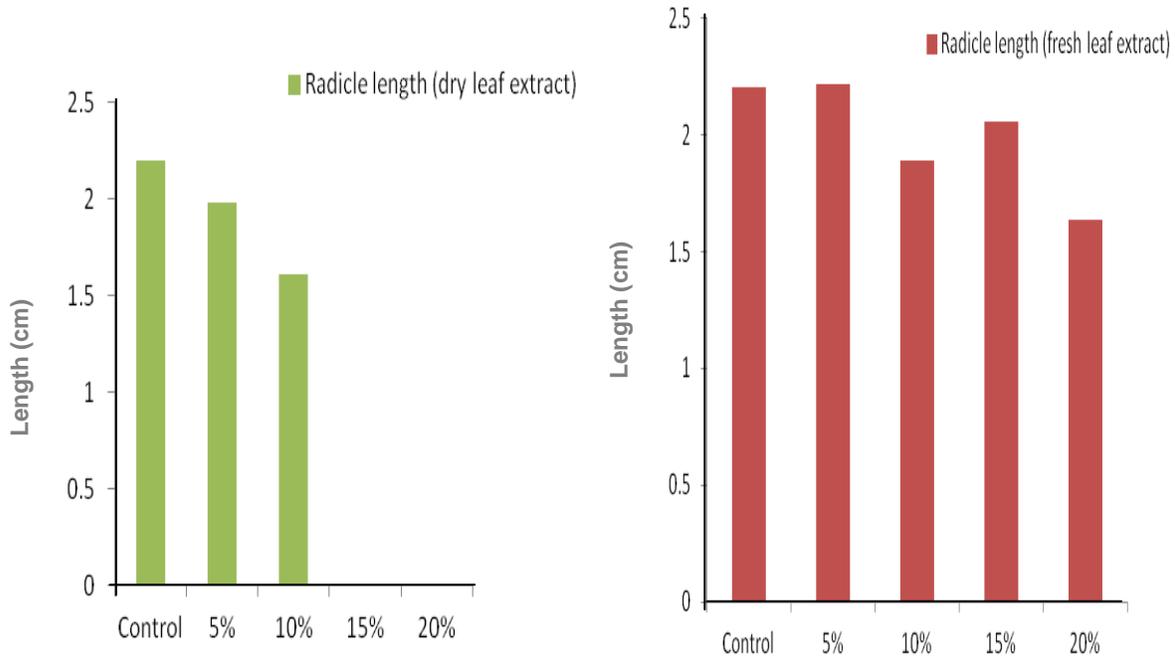
Figure 2. Effect of fresh (A) and dry (B) *Mangifera indica* leaf extracts on radicle length (cm) of *Cassia occidentalis* seeds.
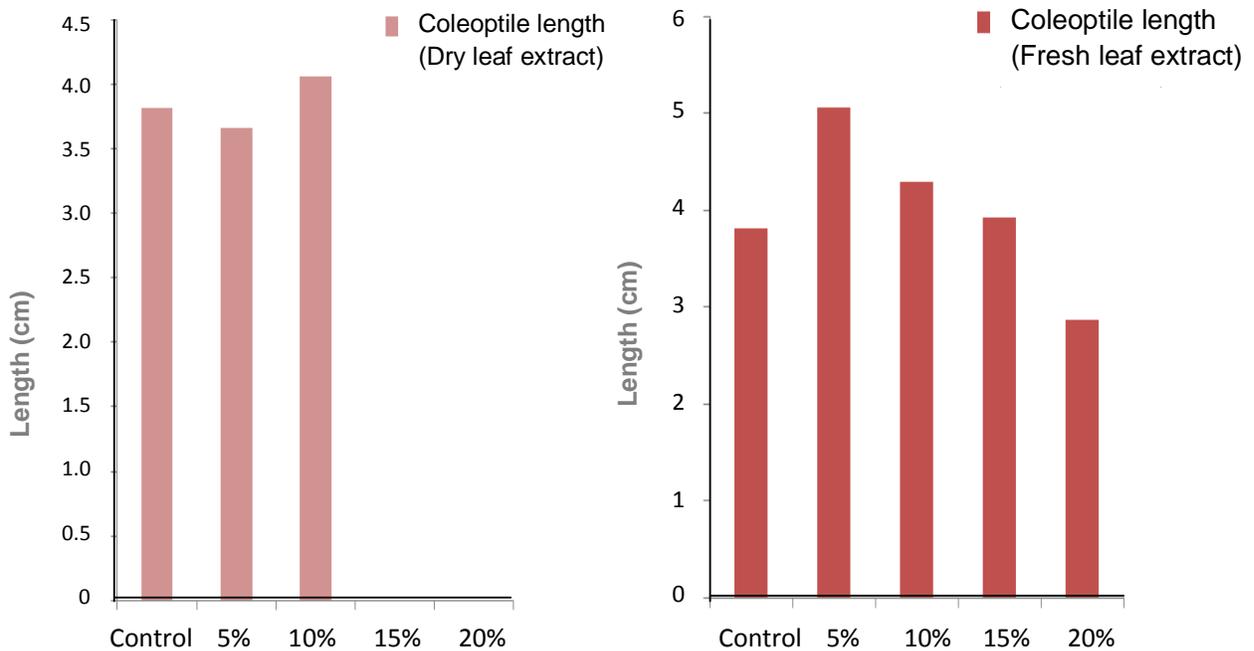
Figure 3. Effect of fresh (A) and dry (B) *Mangifera indica* leaf extracts on plumule length (cm) of *Cassia occidentalis* seeds.

concentration (Figure 3a and b).

The control had the highest germination percentage of 78.75% followed by 5% concentrations of both fresh and dry leaf extracts with 66.25 and 62.5% germination respectively. At 15 and 20%, dry leaf extracts no value was recorded.

Overall, the dry leaf extracts had the best inhibitory effects on the germination of *C. occidentalis* seeds.

DISCUSSION

Weeds have long been one of the major problems in agriculture in that they compete with commercially grown crops for nutrients and space which eventually leads to low productivity. The result of the present study revealed that the allelopathic influence of *M. indica* leaves reduced and inhibited radicle and plumule growth as well as percentage germination of *C. occidentalis* seeds. The result is consistent with the previous finding of El-Rokiek et al. (2010), where dried mango leaves extract reduced and inhibited growth of *Cyperus rotundus*. Our observation is also similar to the report of Singh et al. (2002b) where four monoterpenes (a class of allelochemicals) reduced and inhibited the growth and percentage germination of *C. occidentals*. This result also agree with the earlier findings of Ilori et al. (2010) where fresh extract of *Chromolaena odorata, Heliathus annus* and *Tithonia diversifolia* expressed inhibitory effects on germination of *Vigna unguiculata*. The result further agree with the study of Ilori et al. (2007) who stated that radicle growth of *Oryza sativa* was inhibited by aqueous extract of *T. diversifolia* and the report of Zoheir et al. (2008) who reported that *Azadiratcha indica* inhibited and reduced germination of six plant species. The allelopathic effect of *M. indica* against *C. occidentalis* seeds appears to be concentration dependent. This means that higher concentrations have higher allelopathic potential and this may be attributed to the relative amount of allelochemicals that was released by the extracts (El-Rokiek et al., 2011). Our result however contradicts the earlier findings of Bruckner et al. (2003) where extract from *Ambrosia artemisifolia* had no significant inhibition on the germination and growth of *Amaranthus hypochondriacus*.

Allelopathy, the chemical warfare effect of a plant [part(s)] specie on the other specie through the release of natural plant chemicals (allelochemicals) into the environment usually allow the receiving plant species growth and physiological processes to be altered. Allelochemicals often decreases cell elongation, expansion and division which are growth prerequisite (Olofsdotter, 2001). Impaired metabolic activities caused by allelochemicals decreases root and shoot length (Saeid et al., 2010). In the present study, the radicle and plumule lengths were adversely affected when compared with results from the control experiment. This might be attributed to the allelochemicals present in the leaf residue of *M. indica.*

Researchers such as Chon et al. (2003), Singh et al. (2003) and Chon and Kim (2004) have attributed the significant allelopathic potency of certain plant extracts to the presence of phenolic acid which include coumarin, α-coumaric acid, p-coumaric acid, benzoic acid, p-hydroxybenzoic acid, ferullic acid and cinnamic acid. The indirect relationship between lower germination rate and allelopathic influence may be as a result of water uptake

inhibition (Tawaha and Turk, 2003), and the disturbance in the synthesis as well as the activity of gibberellic acid (GA_3) (Olofsdotter, 2001). The GA synthesis or activity disturbance may be due to the presence of some acidic compounds (Einhelling, 1996). The observed lower germination percentage in the dry leaf extract may be an indication that drying has pull together compounds (allelochemicals) that are capable of influencing the inhibition of water uptake by *C. occidentalis* seeds.

Phenolic acids have been reported to inhibit the synthesis of GA_3 which regulate de novo amylase production during seed germination and are abundantly found in leaf and litter leacheates of many angiosperms (Chandler et al., 1984; Abdul-Rahman and Habib, 1989). Phenolic acid and their derivatives are one of the most studied group of potential allelochemicals and their need give reasons to evaluate them further in the allelopathy phenomenon. Simple phenolic compound such as the cinnamic acid derivatives (ferullic acid and p-coumaric acid) and benzoic acid derivatives (vanillic acid, p-hydroxybenzoic acid) serve a variety of plant ecosystem functions and are wide spread in plant (Harborne, 1990). Ferrulic acid and p-coumaric acid are also known to inhibit radicle growth of plant such as radish *(Raphamus sativus)* while ferullic acid alone could be inhibitory as found in *Sida spinosa* (Liebl and Worshman, 1983) and caused lignifications in *Glycine max* root (dos Santosh et al., 2004). A number of phenolic acids and their derivatives have been reported to be present in *M. indica*'s leaf (El-Rokiek et al., 2010). The radicle growth inhibition observed in this study may be due to the phenolic acids in the *M. indica* leaf residues.

Nigeria is a country blessed with a variety of mango species. The availability is not limited to a particular region as all geo-political zones produce large amount of mango fruits every year. In most cases, the leaf drops are considered wastes and farmers gather the dry leaves in heaps and set ablaze.

The results from the present study have shown that *M. indica* dry leaves possesses strong germination and growth inhibitory properties that makes the plant a potential bioherbicide resource for the local farmers in Nigeria. Further study is required to ascertain these findings in the field against other weedy species. It is also imperative to isolate and characterise the allelochemicals of the Nigerian ecotypes of *Mangifera* species in order to ascertain the actual herbicidal compounds in them.

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