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The Allelopathic Impact of *Psidium guajava* L., Leaf Extracts on the Germination and Growth of *Cassia occidentalis* L.,Seeds

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Abstract:*Weeds have been termed plant pests because of the damage they haveimposed on plants. The use of synthetic chemicals to control weeds has elicited doubts amongst various stakeholders because of environmental pollution and increased weeds resistance to them. Allelopathy, an interaction in which one plant causes suffering to another, has been considered environmental friendly method to control weeds and enhance crop production. Green fresh and brown dry Psidium guajava leaf extracts were used to investigateP. guajava allelopathic potential against seeds germination and growth of Cassia occidentalis. The leaves were concentrated at 20%, 10% and 5% for each category with distilled water being used as control.The experiment was replicated four times in a germination chamber. The green fresh and brown dry leaf extract both exhibited significant inhibition on seed germination (F(3,12)=44 p=0.001) and (F(3,12)=50.9 p=0.02), shoot length F(3,12)=52 p=0.002) and (F(3.12)=120 p=0.002) and root length elongation (F(3,12)=129 p=0.001) and (F(3.12)=209.1 p=0.002) respectively on C.occidentalis. A Post Hoc analysis reveal that inhibition was more pronounced at higher concentrations, 10% and 20% concentration, and the effect increased with an increase in concentration. A comparision between the two leaf extracts revealed that the brown dry leaf extract had more inhibition than green fresh extract at 20% concentration. Therefore, P. guajava leaves have a strong phytotoxic effect against C. occidentalis. The allelochemicals in itcan be used as lead molecules to synthesis bioherbicides for weed control.*
Keywords*:Allelochemicals, phytotoxic, growth inhibition, guavas*

I. Introduction

Weeds are unwanted plants growing in gardens, agricultural fields, road sides and other disturbed areas where their survival and reproduction is not dependent on human intervention(Singh et al., 2003;Siddiqui et al., 2010). Most weeds have been documented to have a competitive edge against other species within the same ecosystem due their tendencies to adapt to environmental changes, ability to proficiently utilize available minerals resources,profuse seeding and rapid growth rate (Lewu and Afolayan, 2009;Dawood et al.,2012).They reduce biodiversity and promote habitat loss for the less competitive species within the same habitat (Kowthar et al., 2010). Because of their threats to other plants, they are considered as plant pests (William and Hirase, 2004).

In agricultural fields, weeds cause harm to crops by serving as alternate hosts to insects, nematodes and pathogenic fungi, theyout-compete crops for water, minerals and space and reduces their quality and yields(Siddiqui et al., 2010).The damage it imposes on crops is more important than insects, fungi and other pest organisms (Milberg & Hallgren, 2004). According to Labrada, (1992) and Adebayo and Uyi, (2010), weedsstill remainthe most significant constraints in agricultural production in developed and developing countries, despite the progress that has been witnessed on weed science in the recent past years. Indeveloped countries the average yield loss in food production annually brought about by weeds is at least 12% while in developing countries it is at least 25% (Ashafa et al., 2012).

Cassia occidentalis Linn. is a member of Fabaceaefamily that grows in tropics and subtropics (Nwodo et al., 2015). It's an opportunistic herb that grows on farms,fence lines, roadsides, and over heaps of waste material. It can tolerate drought conditions and is also capable of adapting and growing in all kinds of soil including coastal sands. It has been classified as a weed due to its threat on crops (Ashafa et al., 2012).

In Kenya, weed control has been reported to consume more than 50% of farmers' total input into crop production (Mwanda, 2000). Some of the management practices that have been use to control weeds, before the introduction of synthetic chemicals included crop rotation, polyculture, mulching, intercropping, hand weeding and other cultural practices. These methods gave low input, but were sustainable as they promoted crop production by minimizing competition between crops and weeds(Esilaba, 2006).During the 20th century, a lot of attention was paid to the use of synthetic chemicals (chemical herbicides) to control weeds (Hussein, 2001). However, in the past 50 years, the use of chemical herbicides has raised doubts about their continuous use (Kowthar et al., 2010). These doubts have been occasioned by the toxicological effects of chemical herbicides on public health, environmental quality, wildlife and overall ecology. Additionally, weeds have shown resistance to most herbicides making them less effective (Ashafa et al., 2012). Scientists have therefore, opted to look for alternative ways to manage weeds and enhance crop production such as allelopathy(Dawood et al., 2012).

Allelopathy is the interaction in which one plant causes suffering to another plant (Khan et al., 2014). It's an environment-friendly technique which may prove to be a tool for weed management and thereby increase crop yields (Rice, 1984). The main principle upon which allelopathy is based arises from the fact that plants produces chemical compounds known as allele chemicals which alter the growth and physiological functions of the receiving species(Kowthar et al., 2010). These chemicals are released through leaching, exudation or decomposition process(Namkeleja et al., 2014). Allelochemicals are eco-friendly and free from the problems associated with synthetic chemicals. Once they are released into the environment, allelochemicals are short lived and therefore do not disastrously upset the balance as the synthetic chemicals would do (Dawood et al., 2012).

In this study, green fresh leaf and brown dry leaf extracts from *Psidium guajava* L. (Myrtaceae) were used. Previous study by Chapla & Campos, (2010) documented that *P. guajava* contain allelochemicals such as terpenoids, flavonoids, coumarins, cyanogenic acids. All these allelochemicals possess actual or potential phytotoxicity (Bhadoria, 2011). This study therefore attempt to find out if the leaves of *P. guajava* have the capacity to suppress germination and growth of *C. occidantalis* seeds. The results of this study will come in handy to farmers and other agricultural management authorities as it will help provide solutions to the weed menace, in Kenya and beyond.

II. Materials And Methods

2.1 Collection of *P. guajava* leaves

Psidium guajava leaves were collected from Kakamega tropical forest, Western Kenya. The forest lies between longitudes 34° 40' and 34°57' 30" East and 0° 15" South. It has a varied topography with altitudes ranging from 1250 to 2000 m above sea level(Vuyiya *et al*., 2014).Temperature range from 18^0C to 29^0C with minima of 11^0C to 12^0C (Kiefer & Bussman, 2008). The annual rainfall show high variation averaged at 2007mm over a recent 19-year period (Mammides *et al*., 2008).

2.2 Preparation of the extracts of *P. guajava* leaves

The plant materials consisted of green fresh leaves attached to the plant and the brown dry leaves fallen to the ground. Each category of leaves was washed separately in running water and 100 grams of whole leaves blended with 500 ml distilled water. The two different categories of leaves extracts obtained were filtered through a cotton cloth and concentrated to 20, 10 and 5% extracts as described by Chapla & Campos, (2010).

2.3 C. occidentalis seeds collections and viability test

The seeds of *C. occidentalis* were collected from agricultural fields located at Kenya Agricultural and Livestock Research Organization (KALRO), Kakamega branch. The viability test for the seeds was done as described by Ashafa *et al*., (2012). The seeds were depoded and soaked in sterile, deionized distilled water for 24 h. The soaked seeds were picked into a 60 mm diameter Petri-dish and dissected into two equal halves to avoid 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.

2.4 Germination Bioassays to evaluate the allelopathic effects. *guajava* leaf extracts on germination rate, shoot and root length elongation of *C. occidentalis*

Thedepoded and healthy*C.occidentalis* seedswere washed in 95% alcohol solution and rinsed in distilled water.Ten 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 (control). All the treatments were replicated four times. The seeds were allowed to geminate in germination chamber at 30ºC and 12-hour photoperiod for a duration of 21 days. This is the duration (21 days) taken by seeds of *C. occidentalis* to germinate and develop their shoots and roots to measurable levels. Subsequently, extracts and distilled water were added when their volume reduced in the respective petri dishes. Readings were taken at intervals of 12 hours from the onset of germination and emergence of radicle and plumule in each treatment recorded. 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. New extracts were prepared readily kept in a refrigerator for refilling the petri dishes (Ashafa *et al*., 2012).

2.5 Statistical analysis

All the data was subjected to normality test before analysis. Since all the data were normal, a parametric test was used. SPSS, (Version 22) was used for analysis. One-way analysis of variance (ANOVA) was used to compare means and Tukey Post hoc test values were used to separate the significant different means at $p \leq 0.05$.

III. Results

3.1 Effects of aqueous *P. guajava* leaf extracts on germination, shoot and root longation of *C. occidantallis*

The results revealed that both the green fresh and brown dryleaf extract had significant impact on seed germination of *C. occidantallis* ($F(3,12)=44$ $p=0.001$) and ($F(3,12)=50.9$ $p=0.02$) respectively. The inhibitory effect was much more pronounced at higher concentrations, and it increased with the increase in concentration (Table 1)

On shoot length elongation, the green fresh leaf extract had a significant impact on *C. occidantalis*($F(3,12)=52$$p=0.002$). The same inhibition was seen with the brown dry leaf xtracts ($F(3.12)=120$$p=0.002$). This same pattern of inhition was also observed in root length elongation with both fresh green and dry brown leaf extracts inducing significant influence on the roots in comparison with the control,($F(3,12)=129$$p=0.001$)and extracts ($F(3.12)=209.1$$p=0.002$) respectively. The inhibition were concentration dependant and increased with an inreased in the concentration of the extract (Table 1).

Table 1: The effects of *P. guajava* leaf extracts on seed germination, shoot and root length elongation of *C. occidentalis*

***P. guajava* leaf extracts**	**Treatments**	***C. occidantalis* Seed germination**	***C. occidantalis* Shoot length**	***C. occidantalis* Root length**
Green fresh leaves	T1 (0%)	$8.0 \pm 0.8_a$	$4.0 \pm 0.4_a$	$3.1 \pm 0.8_a$
	T2 (5%)	$5.5 \pm 0.6_b$	$3.1 \pm 0.1_b$	$2.7 \pm 0.3_b$
	T3 (10%)	$4.0 \pm 0.8_c$	$2.3 \pm 0.2_b$	$2.0 \pm 0.1_b$
	T4 (20%)	$2.5 \pm 0.7_d$	$1.7 \pm 0.2_c$	$1.0 \pm 0.1_c$
Brown dry leaves	T1 (0%)	$8.0 \pm 0.8_a$	$4.0 \pm 0.1_a$	$3.0 \pm 0.1_a$
	T2 (5%)	$4.5 \pm 0.6_b$	$2.9 \pm 0.3_b$	$2.4 \pm 0.2_b$
	T3 (10%)	$3.0 \pm 0.7_b$	$2.3 \pm 0.1_c$	$1.8 \pm 0.1_c$
	T4 (20%)	$1.3 \pm 0.9_c$	$1.1 \pm 0.4_d$	$0.5 \pm 0.1_d$

Mean values of germinated seeds are presented as mean ± standard deviation per treatment. Means having different letters in the same column differ significantly from each other at p=.05

3.2 Comparison of the inhibitory prospective of green fresh leaf and brown dry leaf extracts on seed germination.

The inhibitory comparisons of the brown dry leaf and green fresh leaf extracts revealed that there was significant difference among the two categories of extracts (F (7, 24) =42.4 p=0.01). A post hoc, Tukey HSD analysis revealed that it was the brown leaf extract that had more inhibitory impact on the germination percentage of *C. occidantalis* at 20% concentration (Figure 1).

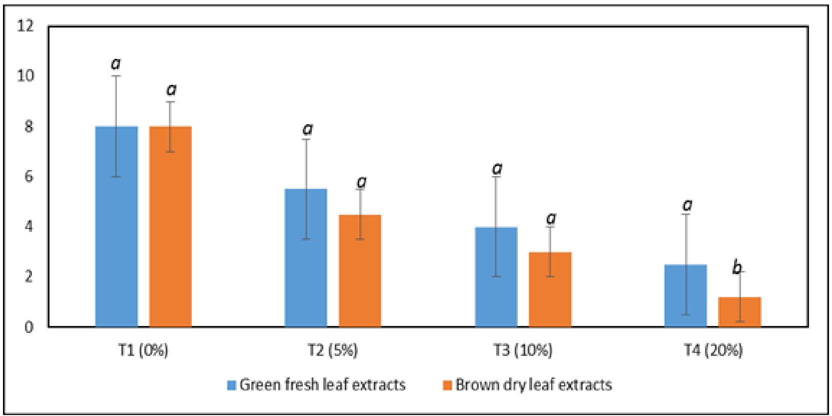

Figure 1: Inhibitory potential of the two leaf extracts.

Error bars denote the standard deviation and the letters above the bars represent significant differences for one way ANOVA conducted for the particular extracts.

IV. Discussion

Despite the progress that has been made recently in weed management, weeds still remain one of the most important constraints in crop production (Adebayo and Uyi, 2010). This is because of their ability to out-compete crops for water, nutrients and space, thereby lowering their productivity (Dawood, 2012). Owing to the toxicological effects of synthetic herbicides on environment and crops and the resistance of weeds to the herbicides, efforts have been made to get eco-friendly means to manage weeds (Namkeleja et al., 2014). The results from this study revealed that has allelochemicals inherent in *P. guajava* had an inhibitory impact on germination, growth and development of *C. occidantalis* seeds. This findings were in agreement with (Kawawa et al., 2016) where *P. guajava* leaf extracts inhibited germination and growth in *Croton magalocarpus*. Similar cases of allelopathic inhibition have also been reported in other studies(Barbosa et al., 2008;Kowthar et al., 2010;Ashafa et al., 2012;Namkeleja et al., 2014).

The allelochemicals inherent in *P. guajava* include terpenoids, flavonoids, coumarins, cyanogenic acids(Chapla & Campos, 2010). These allelochemicals inhibit seed germination and growth by blocking hydrolysis of nutrients reserve and cell division thus causing significant reduction in germination percentage and in the growth of plumule and radical of various plant species (Khan et al., 2014). In some cases, they change membrane permeability, interfere with chlorophyll formation, inhibit protein synthesis and inactivate the activity and functions of certain hormones and enzymes (Namkeleja *et al*., 2014). Germination in *C. occidantalis* was significantly affected by *P. guajava* leaf extracts. Namkeleja *et al*.,(2014) in his study of '*Allelopathic effects of Argemone mexicana to growth of native plant species*' documented that during the process of seed germination, a rapid increase in glycolytic activity which is trigered by an increased rate of respiration is experienced. This process (Glycolytic activity) is necessary for the assemblage of stored carbohydrates to provide the seed with ATP and carbon products required for the biosynthesis of the radicles and plumule. The ability of the allellochemicals, to disrupt activity of metabolic enzymes that are involved in glycolysis Muscolo *et al*., (2001), coud therefore be the reason for the significant reduction in germination of seeds in *C. occidantalis*.

Shoot and root length elongation were equally significantly suppress. Regulation of the concentrations of hormones such as auxins and gibberellins is essential for growth in plant cell and morphogenesis (Karuppanapandian *et al.*, 2011). Allelochemicals such as terpenoids have been documented to disrupts hormone equilibrium (Namkeleja *et al*., 2014). This disruption is brought about by the ability of the allelochmicals to inhibit polar auxin transport leading to a disturbance in normal auxin levels and resulting in the induction of lateral shoots and roots and subsequent suppression of growth (Brunn *et al.*, 1992).

The inhibitory effect was concentration dependent and was more suppressive at higher concebtrations. Such phenomena has been repoted by Siddiqui*et al*., (2009), Yarnia*et al*., (2009) and Ghorbanli*et al.*,(2011) where inhibition impact of various plant extracts increased with increase in concentration. Comparison between the two extracts reveled that brown dry fallen leaves had a significant inhibitory effect on the native species at 20% concentration than the green fresh leaf extracts at the same concentration. Ashafa *et al*.,(2012) attributes this to the fact that as leaves dry up, they pull together compounds (allelochemicals) that make them more suppressive than when they are fresh.

V. ConclusionAnd Recommendations

The results from this study that *P. guajava* leaf extracts (more so, the brown dry leaves) have allelochemicals inherent in them which significantly inhibited germination and growth of *C. occidentalis* seeds. The allelochemicals can be used as lead molecules to synthesis bio-herbicides which will benefit farmers to control weeds, improve crop production and enhance environmental conservation. Theauthors recommend that more studies should be carried out to verify if *P. guajava* could also inhibit germination and growth in other destructive weeds.

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