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Allelopathic effects of Mexican sunflower [*Tithonia diversifolia* (*Hemsl*) A. Gray] on germination and growth of Spiderplant (*Cleome gynandra* L.)

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Abstract

Allelopathy refers to both beneficial and harmful effects of one plant on another plant by the release of chemicals from plant parts through leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems. *Tithonia diversifolia* contains allelochemicals that inhibit or stimulate growth of agricultural crops. A study was conducted to investigate the allelopathic effects of fresh shoot aqueous extracts of *T. diversifolia* on the germination of seeds and growth of young seedlings of Spiderplant (*Cleome gynandra*) under laboratory and glasshouse conditions. Seeds of *C. gynandra* were germinated in sterilized petri-dishes lined with two layers of whatman no.1 filter papers moistened with 10ml of fresh shoot aqueous extracts of *T. diversifolia* (0%, 25%, 50%, 75%, and 100%) in Department of Botany laboratory of Maseno University. Number of seeds germinating each day was recorded and germination percentage calculated for each treatment at the end of the experiment. Plumule and radicle lengths were also measured. In the glasshouse, individual plants were grown in 4.5 litre plastic pots containing soil. The treatments allocated to the pots involved 4 different concentrations: 400ml aqueous shoot extracts of *T. diversifolia* at 25%, 50%, 75% and 100% and tap water (control). The treatments were replicated five times and the pots laid out in a completely randomized design. The fresh shoot aqueous extracts of *T. diversifolia* did not show significant ($p > 0.05$) allelopathic effect on germination of seeds of *C. gynandra*. However, higher shoot extracts concentration stimulated the germination of the seeds. Plumule and radicle lengths were significantly ($p < 0.05$) inhibited by higher concentrations of the shoot extracts. The aqueous shoot extracts of *T. diversifolia* significantly stimulated shoot height, leaf area, number of leaves, fresh weight and dry weight of *C. gynandra*. The study indicated that aqueous fresh shoot extracts of *T. diversifolia* have both stimulatory and inhibitory effects on spiderplant *(C. gynandra,)* germination and growth.

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Introduction

Allelopathy refers to both beneficial and harmful effects of one plant on another plant (Khanh *et al.,* 2005; Hossain and Alam, 2010), by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and agricultural systems (Kruse *et al.,* 2000). Several crops including alfalfa, buckwheat, maize, rice, rye, sorghum, sunflower, wheat, etc. are affected either by their own toxicity or phytotoxin exudates when their residues decompose in the soil (Narwal, 1994; Khanh *et al.,* 2005). Organic chemicals released as leaf leachates, affect the desired crop plants (Hossain and Alam, 2010). Allelochemicals may be water soluble substances that are released into the environment through leaching, root exudation volatilization and decomposition of plant residues. Weed species such as wild sunflower, are known to be rich in secondary metabolites (allelochemicals). These chemicals modify the environment of the plants and of other plants growing in their vicinity (Nandal *et al.,* 1994). These chemicals released are known as allelochemicals, are toxic and they include phenolics, terpenoids and alkaloids and their derivatives. They may inhibit shoot and root growth, nutrient uptake, or may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of a nutrient. Detrimental effects of allelochemicals on plants germination and growth have been reported (Narwal, 1994; Bogatek *et al.,* 2006).

Delayed seed germination and slowed root growth by an autotoxic extract could be confounded with osmotic effects on rate of imbibition, delayed initiation of germination, and especially cell elongation (Sang-Uk Chon and Coutts, 2004). However, the effect of these compounds on crops in agroforestry systems is likely to be complex, and crops have been shown to vary greatly in their response to allelochemicals from the same tree species. Sundaramoorthy and Kalra (1991) found that allelochemical concentrations were greatest in tree leaves and concentrations in the soil were sufficient to exert a significant inhibitory effect on crops.

The wild Mexican Sunflower *(Tithonia diversifolia* (Hemsl.) A. Gray) is an impressive member of the family, Asteraceae, and it is a perennial native of Mexico and Central America. The plant's flowers are a favorite of bees and African farmers have many uses for the plant, the most popular use being as an organic fertilizer for vegetable crops in compost form. Extracts from *Tithonia* plant parts have been reported to protect crops from termites and to contain chemicals that inhibit plant growth and control insects (Jama *et al.,* 2000). Extracts of *Tithonia* also have medicinal value for treatment of hepatitis and control of amoebic dysentery (Jama *et al.,* 2000).

Green leaf biomass of *Tithonia* is high in nutrients, averaging about 3.5% N, 0.37% P and 4.1% K on a dry matter basis. In some cases, maize yields were higher with incorporation of *Tithonia* biomass than with commercial mineral fertilizer at equivalent rates of N, P and K (Jama *et al.,* 2000). *Tithonia* is known to contain allelochemicals which may inhibit germination and growth of some plants as demonstrated by Otusanya *et al.* (2007). *Tithonia diversifolia* has been reported as being capable of posing serious threats of phytotoxicity to agricultural crops (Tongma *et al.,* 1998; Otusanya *et al.,* 2007). *Tithonia diversifolia* has both stimulatory and phytotoxic plant inhibitory attributes (Tongma *et al.,* 1998).

The Mexican sunflower, among others like *Chromolaena odorata* and *Helianthus annus* are members of the Asteraceae family, and are aggressive weeds that have the ability to compete with agricultural crops (Illori *et al.,* 2010). The weed has been used as an organic fertilizer for vegetable crops. The use of tithonia green manure resulted in an increase in maize (*Zea mays*) yields (Jama *et al.,* 2000; Nziguheba *et al.,* 2002; Sangakkara *et al.,* 2002). Oyerinde *et al.* (2009) investigated the allelopathic effect *T. diversifolia* on maize and they found out that the stimulatory functions of *T. diversifolia* significantly enhanced the growth parameters (shoot height, fresh weight, dry weight and leaf area) of older plants. According to Oyerinde *et al.* (2009), *T. diversifolia* had no effect on germination of *Zea mays,* but inhibited the radical and the plumule lengths of the seedlings. Studies have indicated that there is a decrease in shoot and root growth of the test plant species when grown in soil previously planted with Mexican Sunflower (Tongma *et al.,* 1998; 1997). Allelopathic compounds may decrease cell turgor, photosynthesis rate, enzyme activity, metabolic energy for respiration, protein and hormone synthesis, mineral absorption and transmission from roots to other parts of plant (Yarnia *et al.,* 2009).

The spiderplant (*Cleome gynandra)* is an erect herbaceous annual herb with heavy, often purple stems and many branches (Mwai, 2001). The leaves are edible. The vegetable is an important food in rural areas (Chweya and Mnzava, 1997; Mwai, 2001; Woomer and Ibumi, 2003). In some countries, this leafy vegetable is available during the relish-gap period, and therefore plays a significant role in household food security during drought (Chweya and Mnzava, 1997; Mnzava and Chigumira, 2004). The leaves are utilized in fresh form or dried as powder. Sap from leaves of *Cleome gynandra* may be used as an analgesic, particularly for headaches. An infusion of seeds is administered to reduce coughing.

Spiderplant is an important traditional leafy vegetable crop grown and consumed in Kenya and most parts of Africa (Masinde *et al.,* 2005). Increased production of this vegetable can lead to increased food security. However, there is lack of information concerning the phytotoxicity activity of *T. diversifolia* on spider plant even though there are views that *T. diversifolia* can be an effective source of nutrients for some crops including vegetables (Jama *et al.,* 2000). *T. diversifolia* is capable of inhibiting germination and growth of many agricultural crops (Otusanya *et al.,* 2007). There are no studies in the literature that have reported the allelopathic potential of *T. diversifolia* on *Cleome gynandra*. This study was therefore designed to investigate the allelopathic potential of *T. diversifolia* on germination and growth of spider plant. The main objective of this study was to investigate the allelopathic effects of Mexican Sunflower (*T. diversifolia*) on germination and growth of seedlings of spiderplant (*Cleome gynandra*). It was hypothesized that *Tithonia diversifolia* extracts inhibit germination and growth of spider plant seedlings.

Materials and methods

Collection of plant materials

Fresh shoots of *T.diversifolia* were collected from the field near botanic garden of Maseno University, Kenya.

Preparation of fresh shoot aqueous extracts of T. diversifolia

108 grams of fresh shoots of *T. diversifolia* were harvested at vegetative stage and cut into small chips of about 4cm length, and finely ground with pestle and mortar, and then soaked in 1litre of tap water in a 2 litre bucket for 24hrs. The collected extract was filtered through cheese cloth to remove debris and finally filtered using Whatman No.1 filter paper to have 100% concentration. Aqueous extracts of 25%, 50% and 75% concentrations were made by diluting the original extract with distilled water.

Germination Experiment

Germination tests were carried out in botany laboratory in department of Botany of Maseno University at room temperature. Twenty uniform seeds of *Cleome gynandra* were soaked in 5% sodium hypochlorate to prevent fungal infection after which they were rinsed for about 5 minutes in running water. The seeds were then washed in distilled water, placed in clean oven dried Petri-dishes lined with two layers of Whatman No.1 filter papers and moistened with 10 ml of the respective aqueous shoot extracts and distilled water (control). The treatments were replicated five times. Data on number of seeds germinating each day were recorded and germination percentage determined at the end of experimental period.

Growth Experiment

Growth tests were carried out in a glasshouse in the university Botanic Garden. 4.5 litre plastic pots with dimensions 21cm in height and 19cm in diameter were filled with top soil collected from Maseno University botanic garden. Maseno soils are classifield as acrisol deep reddish brown clay and well drained with a pH range of 4.5- 5.4 (Mwai, 2001). The pots were perforated at the bottom to avoid water logging. Fifteen seeds of *Cleome gynandra* were sown in each of the pots. Watering was done every morning with 400ml of tap water per pot up to the end of two weeks. After two weeks, the seedlings in each pot were thinned down to five plants per pot. The pots were then allocated to five different concentrations of extracts from leaves of *Tithonia diversifolia* (0% (tap water), 25%, 50%, 75% and 100%) and they were laid out in a completely randomized design. Thereafter, the control pots were supplied with 400ml of tap water daily and the pots with the different aqueous extracts were supplied with 400ml of the appropriate aqueous extracts daily for four weeks. The treatments were replicated five times. Data was collected at the end of the experimental period. Plants were grown under naturally illuminated glasshouse conditions of 30/25 ^{o}C day/night temperature, 70% relative air humidity, and a photon flux density of 230µmol m^{-2} s^{-1} of photosynthetically active radiation at plant height.

Measurement of parameters

Radicle and plumule length

The lengths of the plumule and the radicle were measured using a transparent meter rule.

Germination percentage

The number of seeds germinating every day after treatments was counted and the total used to calculate the final germination percentage in each treatment.

Shoot height

Shoot heights were measured from the soil level to the upper point of the terminal bud of the seedling using a meter rule.

Number of leaves

Number of fully expanded mature leaves per plant was counted and recorded on each plant.

Leaf area

Leaf area was obtained by measuring the length of the leaf and width of the leaf and calculated following the formula of Otusanya *et al.* (2007) as shown below:

$LA=0.5(L_l \times W_l)$, where L_l is the leaf length and W_l is the maximum width measured for each leaf on each plant.

Root and shoot fresh weights

At the end of the experiment, the plants were carefully uprooted from the soil, cleared off the debris, separated into root and shoot and then measured separately using an electronic weighing balance.

Root and shoot dry weights

Fresh plants (roots and shoots) were packaged separately in envelopes and dried to constant weight at 80^{o}C in an oven. Root and shoot dry weights were then weighed on an electronic weighing balance; and then mean weights calculated.

Statistical analysis

Data obtained from the study was subjected to analysis of variance (ANOVA) in SAS statistical package. Treatment means were compared using Least Significance Difference (LSD at 0.05).

Results

The study examined the allelopathic effects of *Tithonia diversifolia* extracts on germination and growth of *Cleome gynandra*. Table 1. shows the effects of *tithonia diversifolia* extract on germination and seedling growth of *Cleome gynandra*. The germination percentage of spiderplant seeds increased with increasing concentration of shoot extracts of *Tithonia diversifolia* (Table 1). However there were no significant differences ($p<0.05$) in germination percentages among the treatments. Plumule length and radicle of *Cleome gynandra* in control were significantly higher than in *Tithonia diversifolia* extract treatments (Table 1). The plumule length and radicle length was 80% and 21% of control treatment in 100% aqueous shoot extract of *Tithonia diversifolia* respectively.

The shoot heights increased significantly ($p<0.05$) with the increase in concentration of the extracts (Table 2). The shoot height increment was 365% of control treatment at 100%, *Tithonia diversifolia* shoots extracts concentration. The mean shoot height of *Cleome gynandra* in control was 3.7cm compared to the mean height in the 100% extract concentration which was 11.92cm.

The number of leaves per plant significantly ($p<0.05$) increased with increasing shoot extract concentration. The control treatment had 4.2 leaves, while 100% extract concentration had 11.4 (Table 2). The number of leaves per plant at 100% shoot extract concentration was 271% of control treatment.

The leaf area significantly ($p<0.05$) increased in all treatments (Table 3). The control had the lowest leaf area (1.608cm^2), followed by 25% concentration (3.872cm^2), 50% concentration (6.248cm^2), 75% concentration (8.256cm^2) and finally 100% concentration which had the highest value (10.136cm^2). The individual leaf area at 100% shoot extract concentration was 629% of control seedlings. The fresh weight of the shoot of the *Cleome gynandra* seedlings increased significantly ($p<0.05$) with increase in shoot extract concentrations (Table 3).The shoot fresh weight in control treatment was lowest when compared with that of seedlings treated with aqueous shoot extracts of *Tithonia diversifolia*. The root fresh weight of *Cleome gynandra* seedlings in control treatment was significantly ($p<0.05$) higher than of the seedlings treated with shoot extracts of *Tithonia diversifolia* (Table 3). The dry weight of the shoot of *Cleome gynandra* seedlings was slightly higher at 100% shoot extract concentration compared to all other treatments (Table 3). Significant ($p<0.05$) differences in shoot dry weight were observed between control treatment and the various extract concentration levels. The root dry weight was generally negligible at the lowest concentration levels, 0 and 25% (Table 3). However, there were significant ($p<0.05$) increases in root dry weight of *Cleome gynandra* seedlings with increase in shoot extract concentration of *Tithonia diversifolia*.

Table 1. Effects of aqueous extracts of *Tithonia diversifolia* on germination, plumule and radicle lengths of *Cleome gynandra* seeds and seedlings after four weeks of extracts treatments.

Treatment	Germination %	Plumule length (cm)	Radicle length(cm)
0	50a	2.516a	4.240a
25	51a	2.364ba	2.368b
50	53a	2.232bac	1.664c
75	54.5a	2.152bc	1.340c
100	55a	1.992c	0.868d

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of *Tithonia diversifolia*.

Table 2.Effects of aqueous extracts of *Tithonia diversifolia* on growth parameters of *Cleome gynandra* after four weeks of extracts treatments.

Treatment	Shoot height (cm)	No. of leaves	Leaf area (cm^2)
0	3.27a	4.2a	1.608a
25%	4.40a	7.2b	3.872b
50%	6.72b	8.2c	6.248c
75%	8.0b	8.6c	8.256d
100%	11.92c	11.4d	10.136e

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of *Tithonia diversifolia*.

Table 3. Effects of aqueous extracts of *Tithonia diversifolia* on shoot and root fresh weights and dry weights of *Cleome gynandra* after four weeks of extracts treatments.

Treatment	Shoot fresh weight(g)	Shoot dry weight(g)	Root fresh weight(g)	Root dry weight(g)
0	0.062a	0.01a	0.006a	0.0a
25%	0.154b	0.016a	0.012b	0.0a
50%	0.528c	0.046b	0.032c	0.002b
75%	0.532c	0.05b	0.042d	0.004b
100%	1.086d	0.088c	0.06e	0.006b

Means followed by the same letters within each column are not significantly different according to LSD ($p < 0.05$). (0) control, 25, 50, 75 and 100 denote the concentration of extract of *Tithonia diversifolia*.

Discussion

Allelopathy is the direct influence of a chemical released from one living plant on the development and growth of another (Bano *et al.,* 2012). Plants may favorably or adversely affect other plants through allelochemicals, which may be released directly or indirectly from live, dead plants or organic residues. The main goal of this study was to examine inhibitory and stimulatory nature of interference of aqueous leaf extract of *Tithonia diversifolia* on *Cleome gynandra* seedlings. The results of this study indicated that the allelopathic effects of fresh shoot aqueous extracts of *Tithonia diversifolia* on *Cleome gynandra* are both inhibitory and stimulatory. The fresh aqueous extract of *Tithonia diversifolia* stimulated the germination of *Cleome gynandra* seeds. The results do not agree with that of Oyerinde *et al.* (2009) who found no effect of aqueous extracts of *Tithonia diversifolia on Zea mays*. Inhibition of seed germination and seedling growth of some herbaceous plants by leaf extracts of eucalyptus have been reported (Siddiqui *et al.,* 2009).

The extracts inhibited plumule length and radical length of the seedling. This could be due to the presence of water soluble allelochemicals in the shoots of *Tithonia diversifolia*. This has also been demonstrated by Otusanya *et al.* (2007) on the inhibitory effect of shoot extract of *Tithonia diversifolia* on germination and growth of *Amatanthus cruentus*. Similar report on reduced plumule and radicle length has been made by Abu – Roman *et al.* (2010) on the allelopathic effect of Spurge (*Euphorbia hierosolymitana*) on wheat (*Triticum durum*). These results are also in agreement to the findings of Hussain (1985) who reported that *Azadirachta indica* leaf extract reduced radical growth of wheat, millet, maize, lettuce and mustard. The radicle and root lengths were more sensitive to phytotoxic compounds than plumule and shoot height in agreement with the results reported by Otusanya *et al.* (2007) on growth of amaranthus plants. These results may be associated to the observation that the radicle and roots were in continuous contact with the extracts. The allelochemicals may have reduced cell division. Seedling root growth is sensitive to allelochemicals which inhibit cell division and elongation in the root apical meristems (Zhang and Fu, 2009). Previous studies have shown that extracts from various plants tend to inhibit germination and seedling growth of a number of crop species (Sundaramoorthy and Kalra, 1991). Allelochemicals might inhibit seed germination by suppressing synthesis of gibberellins and indole acetic acid (Zhang and Fu, 2009). Many phytotoxic allelochemicals have been isolated, identified, and found to influence a number of physiological reactions, for example, water utilization, photosystem II (PSII) efficiency, nutrient uptake, ATP synthesis, cell division, and gene expression (Blum, 1996; 2005).

The aqueous extracts of *Tithonia diversifolia* generally stimulated growth of *Cleome gynandra* seedlings in agreement with the results reported by Neelamegam, (2011), where the leaf extract of *Ixora coccinea* showed more stimulatory (negative allelopathic) effect on seed germination and most of the seedling growth parameters of paddy. This stimulation in growth suggests that the functional allelochemicals in *Tithonia diversifolia* were broken down and transformed into plant nutrients. The green leaf biomas of *Tithonia diversifolia* is known to be rich in nutrients, averaging about 3.5% N, 0.37%P and 4.1%K (Jama *et al.,* 1998; Taiwo and Makinde, 2005). Sangakkara *et al.* (2003) reported in their investigation that *Tithonia diversifolia* is a potential green manure and organic fertilizers for vegetable crops. Sorghum extracts with 2-20% concentration decreased seed germination, complete plant and root growth of *Amaranthus spinosus*, *Yamopsis tetragonoloba* and *Vigra unguiculata* (Yarnia *et al.,* 2009).

The allelochemicals contained in *T. diversifolia* extracts stimulated growth in shoot height, leaf area and number of leaves of *Cleome gynandra*. This is similar to observations made by Ilori *et al.* (2007) on the growth of *Oryza sativa*, using *T. diversifolia* shoot extracts. Increase in leaf area and number of leaves per plant can result to higher photosynthetic capacity for a plant and ultimately promote growth. Similar findings were reported by Oyerinde *et al.* (2009). The results are also in agreement with the findings of Nassir *et al.* (2005) on the stimulatory effect of *Stevia rebaundiana* on lettuce and cucumber. Taiwo and Makinde (2005) have equally reported similar effects when *Vigna unguiculata* was treated with extract from *Tithonia sp*. In this study, *T. diversifolia* leaf extracts stimulated shoot and root dry weights. These findings are in agreement with the results reported by Bano *et al.* (2012), where neem leaf extracts had significant stimulating effect on root growth of Wild oat seedlings. Increase in leaf area may have led to increased light interception hence increased photosynthesis which indirectly increased accumulation of biomass and shoot growth. Contrary to our findings, shoot and root length and dry weight were decreased by increasing concentration of extracts (Murthy *et al.,* 1995). The findings in study are in line with the work of Alam (1990) who reported that *Azadirachta indica* leaf extracts significantly increased shoot growth of wheat by increasing extract concentrations. Allelopathic chemicals such as phenolic compounds and alkaloids from trees have been observed to suppress yields in a variety of food and fodder crops (Rizvi *et al.*, 1999; Abu- Romman *et al.,* 2010). Previous work on *S. birrea* has identified a number of phenolic compounds, particularly concentrated in the leaves (Braca *et al.,* 2003). The response of allelochemicals may be concentration dependent and inhibit the growth of some species at certain concentrations might infact stimulate the growth of the same or different species at different concentrations (Azania *et al.,* 2003).

Allelopathic plants affect the physiological and biochemical processes in acceptor plants, by influencing energy circulation and enzyme activity in the acceptor plants (Zuo *et al.,* 2012). Previous studies revealed that when sorghum was amended as a green manure, weed biomass in succeeding alfalfa crops was significantly suppressed (Forney and Foy, 1985), indicating the possibility of allelopathic effects in situations, where green manures are used in agroforestry. Grain sorghum showed inhibitory effects on surrounding weed growth occurring through the following growth season (Einhellig and Rasmussen, 1989). Rice and Elroy (1984) inferred that chemicals that inhibit the growth of some species at certain concentration could stimulate the growth of the same or different species at lower concentration. In earlier studies, *Amaranthus retroflexus* dry matter accumulation in root, shoot and leaf was decreased 77.48, 73 and 64.51% by 5% sorghum shoot extracts (Yarnia *et al.,* 2009). The application of fresh shoots aqueous extract of *T. diversifolia* has been observed to significantly affect the fresh weight, dry weight and leaf area of *Amaranthus cruentus* (Oyerinde *et al,* 2009). Detrimental effects of allelochemicals of T. *diversifolia* on plant germination and growth have also been reported on some other crops including *Amaranthus cruentus* (Bogatek *et al.,* 2006; Otusanya *et al.,* 2007).

Conclusions

The results indicate that fresh shoot aqueous extracts of *Tithonia diversifolia* have both inhibitory and stimulatory effects on *Cleome gynandra*. Fresh shoot aqueous extracts of *Tithonia diversifolia* stimulated spiderplant seed germination percentage; this was evidenced by the increasing percentage of seed germination with increase in extract concentration. The shoot extracts also stimulated shoot height growth, number of leaves, individual leaf area and fresh and dry weight of shoots and roots of spiderplant. The plumule length and radicle length were inhibited by the shoot extracts of *tithonia diversifolia*. These results confirm both inhibitory and beneficial functions of the allelochemicals in *T. diversifolia* shoot extacts. The study clearly demonstrated that water soluble inhibitory and promotory substances were present within the leaf extracts of *Tithonia diversifolia*. The soluble substances have the potential to significantly affect germination and growth of spiderplants *(Cleome gynandra*), by leaching and decomposition of the leaf green manures. We therefore recommend *Tithonia diversifolia* leaf biomass to be used in agroforestry, which can be employed to achieve an increase in the germination and seedling growth of spider plants *(Cleome gynandra*). Further studies should be conducted to identify the exact allelochemicals in *Tithonia diversifolia* which inhibit or promote the growth of spiderplant.

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