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Full Length Research Paper

Allelopathic effect of *Tithonia diversifolia* on the germination, growth and chlorophyll contents of maize (*Zea mays* L.)

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The allelopathic effects of fresh shoot aqueous extract of *Tithonia diversifolia* (Hemsl.) A. Gray was investigated on the germination of seeds and growth of young seedlings of *Zea mays* L. Although the fresh shoot aqueous extract did not show significant allelopathic effect on the germination of *Z. mays,* however, the radicle and plumule lengths of the seedlings were significantly inhibited by the fresh shoot aqueous extract. Also, the fresh shoot aqueous extract of *T. diversifolia* was found to significantly stimulate the growth of older plants of two weeks old and above. The application of fresh shoot aqueous extract of *T. diversifolia* was observed to significantly enhance the following growth parameters: fresh weight, dry weight, leaf area and ratio. In addition, chlorophyll a, chlorophyll b and total chlorophyll accumulation were reduced in these young plants. The study was able to reveal that fresh shoot aqueous extract of *T. diversifolia* could have differing effects (inhibitory and stimulatory), on seedling growth of this test crop, depending on plants growth stage.

Key words: Allelochemicals, allelopathy, fresh shoot aqueous extract, inhibitory, stimulatory, *Tithonia diversifolia, Zea mays*.

INTRODUCTION

Chemicals that are released from plants which impose allelopathic influence on other plants are called allelochemicals or allelochemics (An et al., 1996). According to them, allelochemicals that are toxic may inhibit shoot/root growth, nutrient uptake, or may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of a nutrient. The consequent effects may be inhibited or retarded germination rate, reduced root or radicle and shoot or coleoptile extension, lack of root hairs, swelling or necrosis of root tips, curling of the root axis, increased number of seminal roots, discolouration, reduced dry weight accumulation and lowered reproductive capacity (Ayeni et al., 1997).

Plants in the Asteraceae family e.g. *Tithonia diiversifolia* and *T. rotundifolia* are known to exhibit allelopathy. *T. diversifolia* (Mexican sunflower), an aggressive weed with high invasive capacity, is a native of Mexico and Central America and has been introduced to West Africa as an ornamental plant and possibly with imported grains (Akobundu and Agyakwa 1987; Ayeni et al., 1997). *T. diversifolia* as reported by Taiwo and Makinde (2005) has both stimulatory and phytotoxic plant inhibitory attributes. It has been used as an organic fertilizer for vegetable crops, it is use as green manure resulted in an increase in maize (*Zea mays*) yield and it proved as an effective source of nutrients for lowland rice (*Oryza sativa*) (Jama et al., 2000; Nziguheba et al., 2002; Sangakkara et al., 2002).

However, Tongma et al. (1998; 1997) investigated the allelopathic effect of *T. diversifolia*; and they found out that there was a decrease in shoot and root growth of the test plant species when grown in soil previously planted with *T. diversifolia* though seed germination was not affected. They also found out that soil-water separated from the same soil and water extract from *T. diversifolia* leaves applied to soil also reduced shoot and root growth. Till date, the dual role of allelopathic substances on a

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single species has not been fully investigated, hence the aim of this study was to elucidate on the possibility of this phenomenon occurring at different level of development of a single species.

MATERIALS AND METHODS

Collection of plant materials

The seeds of *Z. mays* were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State of Nigeria. Fresh shoots of six weeks old of *T. diversifolia* were collected near the screen house of Botany Department, Obafemi Awolowo University, Ile-Ife, Osun State of Nigeria.

Preparation of Fresh Shoot Aqueous Extract (FSE) of *T. diversifolia*

The extraction procedure followed that of Ahn and Chung (2000). Seventy-two gram of fresh shoot of *T. diversifolia* were harvested and cut into small chips of about 4 cm length and finely ground with mortal and pestle. The ground plant material was soaked in one Litre of distilled water in a large beaker for 12 h. The solution was filtered through cheese cloth to remove debris and finally filtered using Whatman No. 1 filter paper. The final filtrate served as the Fresh Shoot Aqueous Extract (FSE) of *T. diversifolia*. This filtrate was prepared daily and kept in the refrigerator to prevent the degradation of its allelochemicals.

Germination experiment

The seeds of *Zea mays* were decontaminated by soaking for 10 min in 5% Sodium hypochlorite, rinsed for five minutes in running water and finally washed in distilled water. Ten uniform seeds of this test crop were randomly selected and placed in each of 12 clean, oven dried Petri dishes which have been lined with cotton wool and Whatman No. 1 filter paper. The cotton wool served as an absorbent for water or the FSE used as treatment so as to keep the seed moistened always and avoid the imposition of water stress. Six Petri dishes moistened with 15 ml distilled water served as the control while the remaining six Petri dishes were allotted to the FSE regime and each was moistened with the 15 ml of the extract. The Petri dishes were incubated at room temperature (28˚C) with good lightning for two weeks. Emergence of 1 mm radicle was used as the criterion for germination. Daily measurements of the plumule and radicle were taken.

Raising of plants for growth experiment

Twenty plastic pots each with dimension 30 × 13 cm were prepared by perforating the base of the pots and filled with top humus soil. The perforation was to prevent the pots from water logging. Fifteen seeds of *Z. mays* were sown in each of the pots and watering was done morning and evening with 800 ml per pot.

The pots were kept at the screen house of Botany Department, Obafemi Awolowo University where good ventilation was ensured and at least eight hours of full sunlight passing through the transparent, corrugated roofing sheet was available. The temperature range was 30 ± 2˚C in the day and 26 ± 2˚C at night.

The seedlings in each pot were thinned down to 12 seedlings after one week; separation of the pots to control and FSE regimes was done after two weeks with ten pots in each regime. The control and FSE regimes were then supplied with appropriate volume of water and FSE of *T. diversifolia* respectively on daily basis. Initial harvest of the plants was done at two weeks and it continued on a weekly interval for a period of five weeks.

Measurement of growth parameters

The following parameters were measured weekly: plant shoot height, leaf length and leaf breadth with the latter two parameters used to calculate the Leaf Area (LA) using the formula of Hoyt and Bradfield (1962); Leaf Area Ratio (LAR) was determined using the formula of West et al. (1920); fresh and dry weight were also measured. All measurements were done in five replicates and the mean calculated.

Determination of chlorophyll content

Determination of the chlorophyll contents followed the method of Combs et al. (1985). Chlorophyll a, chlorophyll b and total chlorophyll were calculated as follows:

Chlorophyll a = $13.19A_{664} - 2.57A_{647}$ µg/g dry weight.
Chlorophyll b = $22.10A_{647} - 5.26A_{664}$ µg/g dry weight.
Total chlorophyll = $7.93A_{664} + 19.53A_{647}$ µg/g dry weight.
Where A_{664} = absorbance at wavelength 664 nm.
A_{647} = absorbance at wavelength 647 nm.

Statistical analysis

Comparison of means was carried out using the One way Analysis of Variance (ANOVA) at $P \leq 0.05$.

RESULTS

The allelopathic effect of *T. diversifolia* was investigated throughout the germination and growth stages of *Z. mays*. On this test species, a dual effect of the allelochemicals in *T. diversifolia* was displayed. Figure 1 shows the effect of FSE of *T. diversifolia* on percentage germination, radical length and plumule length of *Zea mays.* In both the control and FSE-treated plants, steady increases in germination were observed with time. The differences observed between the germination percenttage of the FSE-treated and control plants, these differences were not observed to be significant ($p \leq 0.05$). After 14 d period, germination percentages for the FSE-treated and control plants were observed to be 98.9 and 97.78%, respectively (Figure 1).

In the case of radical and plumule lengths, steady increases in lengths were observed with an increase in time (14 d period) in the control plants, while in the FSE-treated plants, radical length was observed to increase up to the sixth day, after which there was a cessation in growth (Figure 1). A comparison of the radical growth (79.4:105 mm) and plumule lengths (77.8:148 mm) of the FSE-treated and control plants, respectively, revealed significantly higher lengths in the control plants ($p \leq 0.05$).

Chlorophyll a contents were observed to range from 9.8 – 21.3 µg/g and from 9.8 – 20.4 µg/g, for the control and

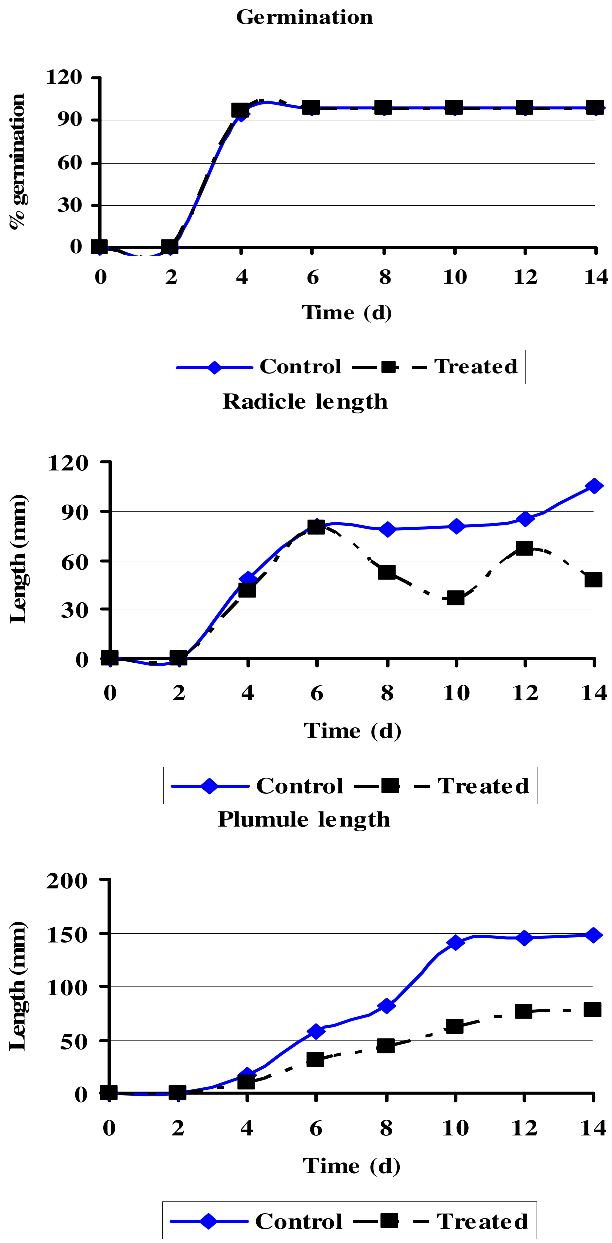

Figure 1. Effect of fresh shoot aqueous extract of *T. diversifolia* on the germination, radical length and plumule length of *Zea mays*

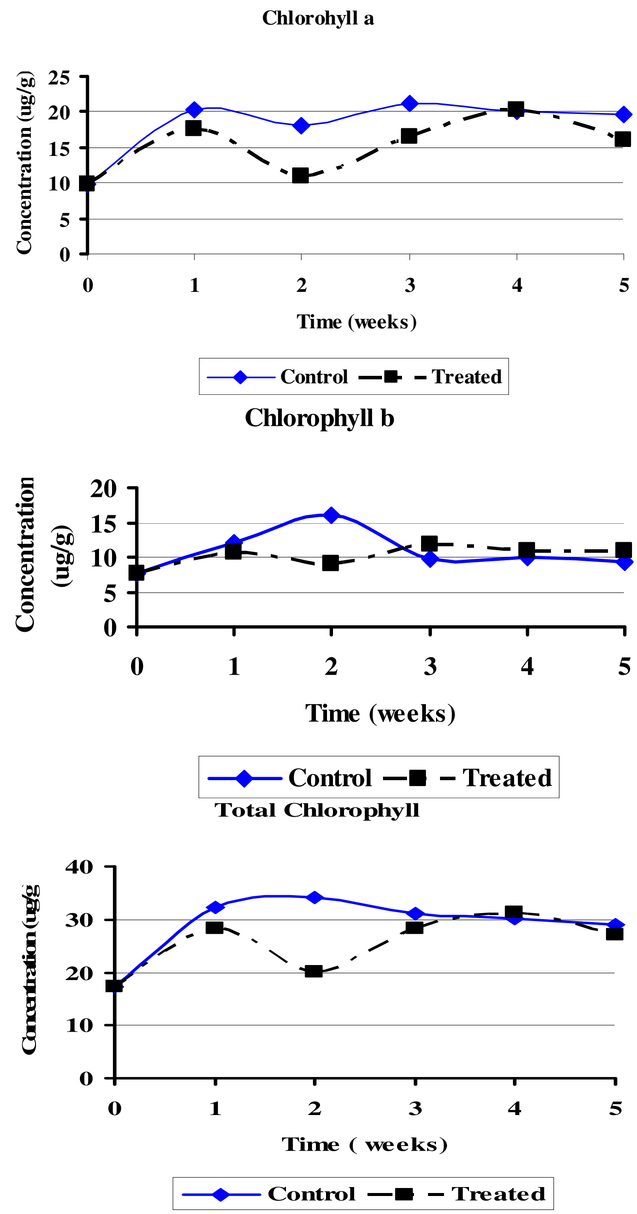

Figure 2. Effect of fresh shoot aqueous extract of T. diversifolia on chlorophyll a, b and total chlorophyll contents of Zea mays.

FSE-treated plants, respectively (Figure 2), with maximum values attained a week earlier in control compared to FSE treated plants. In the case of chlorophyll b, concentrations were observed to range from 7.6 - 16.1 µg/g and from 7.6 - 11.8 µg/g, in the control and FSE treated plants, respectively. Maximum chlorophyll b concentrations were observed in the second and third weeks, for the control and FSE-treated plants, respecttively (Figure 2). Also, total chlorophyll content in the control plant was observed to range from 17.4 - 34.1 µg/g with the highest as well as significantly different in the the second week. In the FSE treated plant, total chlorophyll content ranged from 17.4 - 31.2 µg/g, with the highest in the fourth week (Figure 2). In all the chlorophyll types, the accumulation in the control plant was higher than that in the FSE-treated plants, except for chlorophyll b where the accumulation in the FSE-treated plant was higher in the last two weeks of the experiment.

Shoot height, wet weight and dry weight in the control plant and FES-treated plants were observed to increase steadily with time (Figure 3). In the control plant, shoot height, fresh weight and dry weight of the control plant ranged from 8.7 – 35.68 cm, from 2.15 – 8.26 g and from

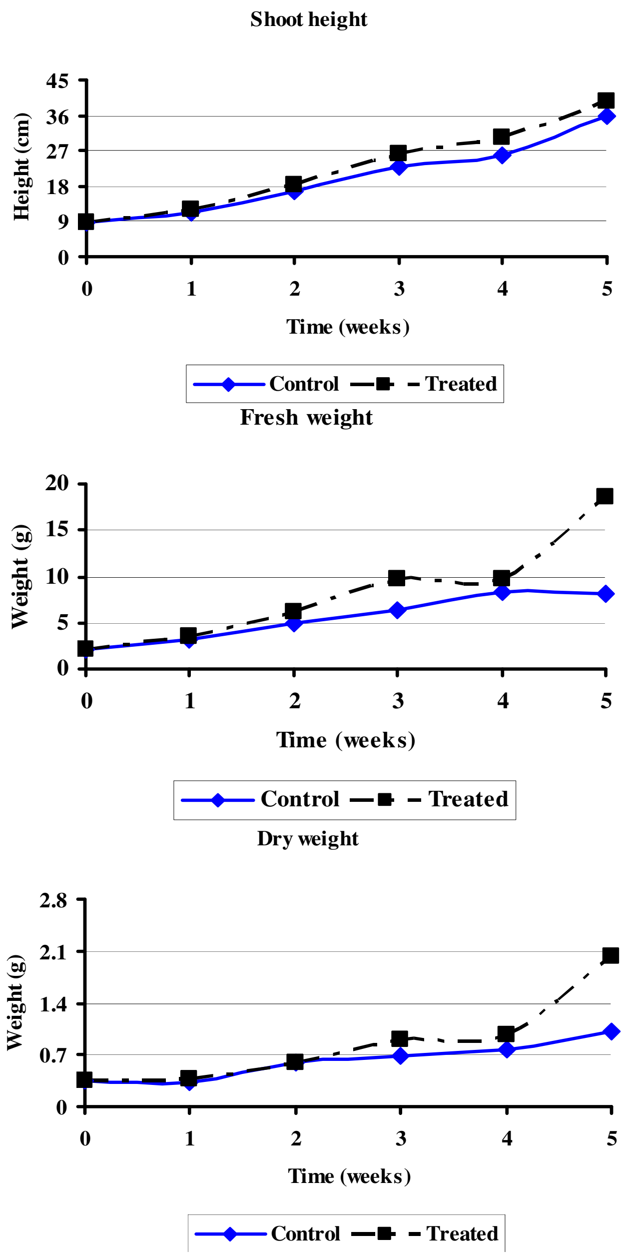

Figure 3. Effect of fresh shoot aqueous extract of *T. diversifolia* on shoot height, wet weight and dry weight of *Zea mays*.

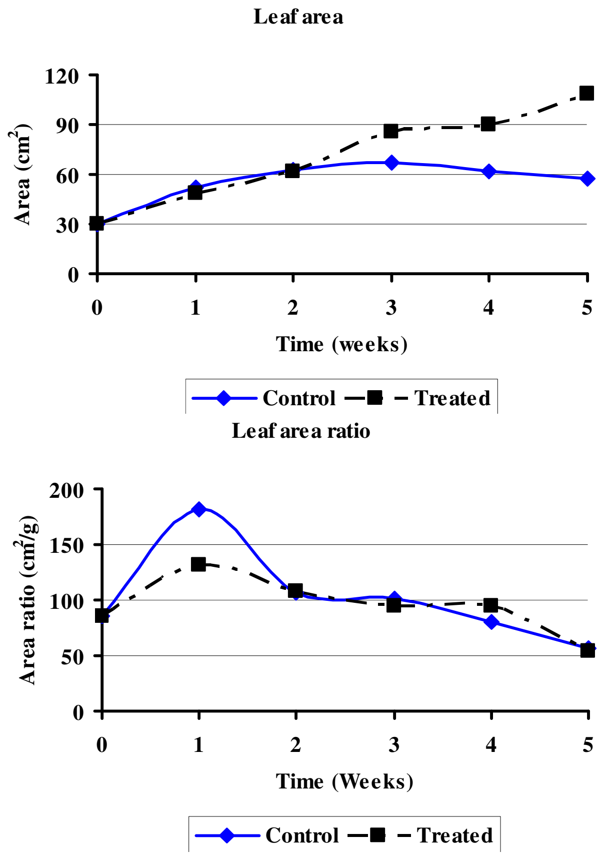

Figure 4. Effect of fresh shoot aqueous extract of *T. diversifolia* on leaf area and leaf area ratio of *Zea mays*.

0.36 – 1.02 g, respectively. In the FSE-treated plant, shoot height, wet weight and dry weight values ranged from 8.7-39.7 cm, from 2.15 – 18.62 g and from 0.36 – 2.04 g, respectively (Figure 3). The shoot heights of the control and the FSE-treated *Z. mays* plant followed the same pattern throughout the duration of the experiment. A comparison of the shoot heights, fresh weights and dry weights of the control and FSE-treated plants revealed significantly higher values in the FSE-treated plants.

In case of leaf area and leaf area ratio (LAR), the control and FSE-treated plants were observed to have values ranging from 30.20 – 67.43 cm^2 (leaf area) from 30.20 – 108.87 cm^2/g (LAR) in the control and from 30.20 – 108.87 cm^2 (leaf area), from 54.48 - 131.74 cm^2/g (LAR) in the FSE-treated plant (Figure 4). In both the

control and FSE-treated plants, a steady increase with in time was observed in the leaf area. There was a steady increase in the leaf area of the FSE-treated plant throughout the experiment, whereas, after the third week there was a gradual decrease in leaf area of control. The leaf area of the FSE-treated plant became higher than that of the control plants in the last three weeks of the experiment and this difference was found to be significantly different ($p \leq 0.05$). There was an initial sharp increase in the leaf area ratio of the control and FSE-treated plants in the first week of the experiment followed by a sharp decrease.

DISCUSSION

In the study, the allelopathic effects of fresh shoot aqueous extract of *T. diversifolia* (Hemsl.) A. Gray on the germination of seeds and growth of young seedlings of *Zea mays* L was investigated. The choice of germination and growth of the young seedlings was due to the fact that known sites of action for some allelochemicals have been reported to include cell division, pollen germination, nutrient uptake, photo-synthesis and specific enzyme function, with commonly cited effects being reduced seed germination and seedling growth (Ferguson and Rathinasabapath, 2009).

In the germination experiment carried out in this work, two observations were made: Firstly, the fresh shoot aqueous extract of *T. diversifolia* did not have either inhibitory or stimulatory effect on the germination of *Z. mays*. This result is consistent with that of Sangakkara et al. (2003) who found that *T. diversifolia* did not have allelopathic effect on germinating maize. However, the radicle and plumule lengths of *Z. mays* were both significantly retarded. A similar trend was observed in the plumule length of the seedling. Some workers have also observed inhibitory effects of some plants on other test plants. Eze and Gill (1992), reported that *Chromolaena odorata* contains a large amount of allelochemicals especially in the leaves, which inhibit the growth of many plants in nurseries and plantations. Otusanya et al. (2007) have demonstrated that aqueous extract and shoot extract of *T. diversifolia* was inhibitory to the germination and growth of *Amaranthus cruentus*.

Similarly, Bhatt et al. (1994) have reported that the bark, leaf and leaf extract of *Quercus glauca* and *Q. leucotricophora* significantly reduced germination, plumule and radicle length of wheat (*Triticum* sp.) and mustard seeds. Although the principle of allelopathy was not investigate in the present study, earlier investigators have suggested that allelochemicals or toxins are released from the weed by the action of micro-organisms during decomposition, which may interfere with the plant growth processes (McCalla and Haskins, 1964; Pandya, 1975)

In the present study, growth parameters (shoot height, fresh weight, dry weight, leaf area and ratio) of *Zea mays* treated with FSE were observed to be significantly higher than the control plants in most weeks of the experiment. The plants in the FSE regime accumulated more materials in their development as it was reflected in the shoot height, fresh and dry weights compared to their counterpart in the control regime. A similar growth promoting effect on wheat seedlings is reported by Hussain et al. (2007), where application of Senna mulching was the allelochemical source. Kato et al. (2005), is also of the view that plants species that display allelopathic effects e.g. *T. diversifolia*, have species-selective allelopathic function.

In addition, the present study revealed leaves of the FSE-treated plants looking healthier and expanded in length and breadth than the leaves of the control plants, especially after two weeks of the treatment. This is obvious in the calculated values of the leaf areas. However, the LAR did not follow a consistent pattern like other parameters, except in the first 3 weeks, particularly, in the second week when the leaf area ratio of the plants in the FSE regime. These observations were not surprising as some authors were of the opinion that the green biomass of *T. diversifolia* can serve as an effective source of nutrients for *Zea mays* (Jama et al., 2000; Nziguheba et al., 2002).

This may however be an indication of stimulatory effect of allelopathy. Selective activity of tree allelochemicals on crops and other plants has also been reported in the past. For example, *Leucaena leucocephala*, the miracle tree promoted for revegetation, soil and water conservation and animal improvements in India, also contains a toxic, non-protein amino acid in leaves and foliage that inhibits the growth of other trees but not its own seedlings (Ferguson and Rathinasabapathi, 2009).

Sangakkara (2003) also reported in their investigation that *T. diversifolia* is a potential green manure and organic fertilizer for vegetable crops. This is further corroborated by Ilori et al. (2007) who reported the stimulatory effect of *T. diversifolia* on the germination and growth of *O. sativa*. The opinion and findings of those authors probably explain why the growth parameters were enhanced by the FSE. Although the growth parameters were enhanced by FSE of *T. diversifolia*, chlorophyll accumulation in the FSE-treated plants were observed to be slightly reduced than the control plants.

This present study showed that the fresh shoot aqueous extract of *T. diversifolia* may contain allelochemicals that performed both stimulatory and inhibitory functions. Although *T. diversifolia* was not observed to have any effect on the germination of *Zea mays*, it did inhibit the radicle and the plumule lengths of the seedlings. The stimulatory functions of these chemicals were evident in the significant enhancement of the growth parameters (shoot height, fresh weight, dry weight and leaf area) of older plants. A similar observation has been reported by Ilori et al. (2007). *T. diversifolia* can enhance the growth parameters of older plants after seedling establishment. It also suggests that the allelopathic function of *T. diversifolia* is not only species-selective, but also has selectivity on the developmental stages of the test plant.

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REFERENCES

Ahn JK, Chung IM (2000). Allelopathic potential of rice hulls on germination and seedling growth of Brnyard grass. Agron. J. 92: 1162-1167.

Akobundu IO, Agyakwa GW (1987). A handbook of West Africa weeds. International Institute of Tropical Agriculture, Ibadan, Nigeria.

An M, Pratley J, Haig T (1996). Allelopathy: from concept to reality. Environmental and Analytical Laboratories and Farrer Centre for Conservation Farming, Charles Sturt University, Wagga Wagga.

Ayeni AO, Lordbanjou DT, Majek BA (1997). *Tithonia diversifolia* (Mexican sunflower) in South Western Nigeria; occurrence and growth habit. Weed Res. 37(6): 443-449.

Bhatt BP, Chauhan DS. Todaria P (1994). Effect of weed leachate on germination and radicle extension of some food crops. India J. Plant Physiol. 36: 170-177.

Comb JI, Long SI, Scurlock J (1985). Techniques in bioproductivity and photosynthesis. Pergamon press, oxford, New York, Toronto, Sydney, Frankfurt.

Eze JMO, Gill LS (1992). Chromolaena odorata- a problematic weed. Compositae Newsletter 20: 14-18.

Ferguson JJ, Rathinasabapathi B (2009). Allelopathy: How plants suppress other plants. University of Florida IFAS Extension, HS 944.

Hoyt P, Bradfield R (1962). Effects of varying leaf area defoliation and density on dry matter production of corn. Agron. J. 54: 523-525.

Hussain S, Siddiqui SU, Khalid S, Jamal A, Qayyum A, Ahmad Z (2007). Allelopathic potential of Senna (Cassia angustifolia Vahl.) on germination and seedling characters of some major cereal crops and their associated grassy weeds. PJ Bot. 39(4): 1145-1153.

Ilori OJ, Otusanya OO, Adelusi AA (2007). Phytotoxicity effects of *Tithonia diversifolia* on germination and growth of *Oryza sativa*. Res. J. Bot. 2(1): 23-32.

Jama B, Palm C, Buresh R, Niang A, Gachengo C, Nziguheba G, Amadalo B (2000). *Tithonia diversifolia* as a green manure for soil fertility improvement in western Kenya: A review. Agrofor. Syst. 49(2): 201-221.

Kato T, Tomita-Yokotani K, Kosemura S, Hasegawa K (2005). Allelopathy of fruits in sunflower (*Helianthus annuus* L.) and Mexican sunflower (*Tithonia diversifolia* (Hemsl.) A. Gray. Fourth world congress on allelopathy. Keio University, Japan. pp. 1-5.

McCalla TM, Haskins FA (1964). Phytotoxic substances from oil micro-organism and crop residues. Bacter. Rev. 28: 181-207.

Nziguheba G, Merckx R, Palm CA, Mutuo P (2002). Combining *Tithonia diversifolia* and fertilizers for maize production in a phosphorus deficient soil in Kenya. Agrofor. Syst. 55: 165-174.

Otusanya OO, Ilori OJ, Adelusi AA (2007). Allelopathic effect of *Tithonia diversifolia* (Hemsl.) A. Gray on germination and growth of *Amaranthus cruentus*. Res.J. Environ. Sci. 1(6): 285 – 293.

Pandya SM (1975). Effect of Celosia argentea extracts on roots and shoot growth of bajra seedlings. Geobios 2: 175-178.

Sangakkara UR, Richner W, Schneider MK, Stamp P (2003). Impact of intercropping beans (*Phaseolus vulgaris*) and sunhemp (*Cotalaria juncea*) on growth, yields and nitrogen uptake of maize (Ze*a mays*) grown in the humid tropics during the minor rainy season. Maydica 48: 233 – 239.

Sangakkara UR, Stamp P, Soldati A, Liedgens M (2002). Green manures stimulates root development of maize and mungbean seedlings. J. Agron.19: 225-237.

Taiwo LB, Makinde JO (2005). Influence of water extract of Mexican sunflower (*Tithonia diversifolia*) on growth of cowpea (*Vigna unguiculata*). Afr. J. Biotechnol. 4(4): 355-360.

Tongma S, Kobayashi K, Usui K (1997). Effect of water extract from Mexican sunflower (Tithonia diversifolia (Hemsl.) A. Gray) on germination and growth of tested plants. Weed Res. 42(4): 373-378.

Tongma S, Kobayashi K, Usui K (1998). Allelopathic activity of Mexican sunflower (*Tithonia diversifolia*) in soil. Weed Sci. 46(4): 432-437.

West C, Briggs GE, Didd F (1920). Methods and significant relations in the quantitative analysis of plant growth. New Phytologist, 19:200-207.