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Allelopathic Effects of *Tithonia diversifolia* (Hemsl) A. Gray on Germination and Growth of *Amaranthus cruentus*

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Abstract: This study examined the susceptibility of *Amaranthus cruentus* Linn. to phytotoxic effects of *Tithonia diversifolia* (Hemsl) A. Gray, compared the phytotoxicity of the plant parts of *T. diversifolia* and determined the effect of drying on the phytotoxic activity of this weed. Results showed that the germination, growth parameters and fresh and dry matter production of *Amaranthus cruentus* were retarded by all the four different aqueous extracts applied. The retardation was more pronounced in older plants. The degree of retardatory effects of the aqueous extracts were found to follow this order: fresh shoot aqueous extract (FSE) > dry shoot aqueous extract (DSE) > fresh root aqueous extract (FRE) > dry root aqueous extract (DRE). There was a significantly higher phytotoxic potency at $p<0.05$ in the fresh tissue aqueous extract compared with the dry tissue aqueous extract as well as in the shoot aqueous extract compared to the root aqueous extract. *Tithonia diversifolia* was found to be an allelopathic weed with water-soluble allelochemicals in its plant parts and had such phytotoxic potency that could suppress the growth and nutrient accumulation of associated crop plants.

Key words: Phytotoxicity, *Tithonia diversifolia*, aqueous extract, *Amaranthus cruentus* allelopathic

INTRODUCTION

Tithonia diversifolia has been reported to contain some allelochemicals and therefore suggested as being capable of posing a serious threat of phytotoxicity to agricultural crops (Tongma *et al*., 1998). Detrimental effects of allelochemicals on plant germination and growth have been reported (Bogatek *et al*., 2006; Zahida *et al*., 2004; Bais *et al*., 2003; Ismail and Tet-Vun 2002; Onwugbuta-Enyi 2001). *Chromolaena odorata* L. also a weed in the family Asteraceae contains a large amount of allelochemicals especially in leaves which inhibit the growth of many plants (Ese and Gill, 1992).

Water extracts from several species of the family Asteraceae and the soil on which they were grown have been shown to inhibit germination and growth of other plant species (Inderjit and Dakshini, 1994; Kil and Yun, 1992; Macias *et al*., 1993; Menalaou *et al*., 1993; Sahid and Segan, 1993). Aqueous extract of root of *Helianthus annus* delayed and inhibited the germination and seedling growth of linseed (*Linum usitatissium* L.) and mustard (*Brassica juncea* L.) (Rawat *et al*., 2002) Extracts from the leaves of *Helianthus tuberosus* L. *Xanthium occidentale*, *Luctuca sativa* and *Cirsum japonica* all in the Asteraceae family inhibited the root growth of lucerne (Chon *et al*., 2003). Water extract of *Cirsium cirvense* (L) Scop reduced the growth of *Amaranthus retroflexus* L. *Cucumis sativa* L. and *Hordeum vulgare* L. (Stachon and Zindel, 1980). Leaf area can be reduced as a result of the application of some synthetic allelochemicals (Patterson, 1981). Reduced leaf area could result in lower photosynthetic capacity for a plant and ultimately limit growth (Sin Clair, 1990; Frederics and Comberato, 1995). Allelochemiclas from *Wedelia trilobata* L. reduced germination, plant height, fresh or dry root weight and dry weight per plant of rice (Chengrong, 2005). Aqueous leachates of

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Chenopodium album plant parts (roots, whole plant and leaves inhibited the germination, plant height growth and biomass of *Cassia occidentalis* and *Phaseolus aureus.* (Daizy, 2006). Tithonia diversifalia is a weed that grows in association with cultivated crops in Western Nigeria. This weed has been reported to contain some allelochemicals (Tongma *et al*., 1998). Therefore it is capable of posing a serious threat of phytotoxicity to agricultural crops. It is reasonable to investigate the phytotoxic effect of this weed. The objectives of this study are to determine the susceptibility of *Amaranthus cruentus* L. to phytotoxic activity of *Tithonia diversifolia* (Hemsl) A. Gray., compare the phytotoxicity of plant parts of *Tithonia diversifolia* and determine the effects of drying on the phytotoxic activity of *Tithonia diversifolia*.

MATERIALS AND METHODS

The experiment was carried out at the Department of Botany the Obafemi Awolowo University, Ile-Ife, Nigeria during April-July, 2005. The seeds of *Amaranthus cruentus* L. and *Tithonia diversifolia* (Hemsl) A. Gray were collected from National Horticultural Research Institute (NIHORT) Ibadan and along road 20 of the Senior Staff Quarters of O.A.U., Ile-Ife, respectively. To prepare the extracts, 360 g each of the shoots and roots of six weeks old *Tithonia diversifolia* were cut into small chips of about 4 cm lengths and finally ground separately with mortar and pestle. Also 360 g each of these parts were oven dried separately in Gallenkamp (Model IH-150) incubator at 60°C for 5 days and ground with a Christy and Morris 8000 rpm lab mill to pass through a 2 mm screen. The ground plants parts were soaked separately in 5 L of distilled water for 12 h (Ahn and Chung 2000). The filtrates obtained serve as treatments for the seedlings in the different aqueous extract regimes. Experimental pots were randomly allocated to the following regimes control (No application but water) fresh shoot aqueous extract treatment (FSE) regime, dry shoot aqueous extract treatment (DSE) regime, fresh root aqueous extract treatment (FRE) regime, dry root aqueous extract treatment (DRE) regime. The seedlings in the control regime were supplied daily with 400 mL of water while the seedlings in the treatment regime were supplied daily with 400 mL of the appropriate extract.

The seeds of *Amaranthus cruentus* L. were soaked in 5% sodium hypochlorate to prevent fungal infection after which they were rinsed for about 5 min in running water. The seeds were washed in distilled water and 20 seeds were placed in clean oven dried Petri dishes which had been lined with a Whatman No. 1 filter paper. The filter paper in each Petri dish allocated to the control was then moistened with 10 mL of distilled water while the filter paper in each of the petri dishes allocated to the other four treatments was moistened with 10 mL of the appropriate aqueous extract. The Petri dishes were incubated at room temperature for 2 weeks. Emergence of 1 mm of the radicle was used as the criterion for germination experiment.

For growth, fresh and dry production, i.e., *Amaranthus cruentus* L. seeds were sown in pots (28×15 cm) containing good humus top soil collected besides the Faculty of Agriculture, OAU, Ile-Ife. Seeds of *Amaranthus cruentus* L. were watered with 400 mL of tap water every morning. At two weeks, seedlings in each pot were thinned down to 15 seedlings per pot. The pots were then allocated to the control and the four different treatments. Thereafter, the pots in the control regime were supplied with 400 mL of water daily while the pots with the different aqueous extracts were supplied with 400 mL of the appropriate aqueous extract daily. The pots were laid out in a completely randomized design. Plants were harvested just before treatment started. Thereafter, harvesting of the seedlings was on a weekly interval for a period of six weeks. Root length, shoot height, leaf area, fresh weight and dry weight of roots and shoots were determined. For the shoot height the distance between the base of shoot at soil level and the upper point of the terminal bud of the seedling was measured using a metric rule. Leaf area was determined using the formula according to Pearcy *et al*. (1989).

LA = 0.5 (L×W)
L = Length of leaf
W = Maximum width

Leaf Area Ratio (LAR) was calculated using the formula of West *et al.* (1920).

$$LAR = \frac{\text{Leaf area}}{\text{Total plant dry weight}}$$

The root system was carefully excavated. The root was then washed free of soil and the length of the root was measured as distance between the base of plant and root tip. Measurements were carried out on five seedlings and mean values were calculated. Five seedlings were randomly harvested in each regime. Each seedling was separated into shoot and root. The fresh plant parts were then weighed on a Meltler Toledo balance to obtain the fresh weight of the plant parts. Five seedlings were randomly harvested in each regime and each seedling was separated into shoot and root. The plants parts were then packaged separately in envelopes and dried to constant weight at 80°C in a GallenKamp (Model IH-150) incubator. The dried plant parts were weighed on a Meltler Toledo balance to obtain the dry weights and then mean weights were calculated. All experiments were conducted in five replicates and the data obtained was subjected to appropriate statistical analysis. Analysis of variance (ANOVA) was carried out for all the data. Treatment means were compared using least significant difference (LSD $p<0.05$).

RESULTS

The mean percentage germination of the seeds in the DRE regime was comparable to that of the control regime. The germination of seeds treated with root aqueous extracts was slightly higher than that of the seeds in the two regimes treated with shoot extracts (Table 1). The plumule length of the seedlings in the fresh shoot aqueous extract regime was lower and significantly different from that of the seedlings in the two regimes treated with the root aqueous extracts at $p<0.05$ (Table 2). The radicle lengths of Seedlings in the dry shoot aqueous extract (DSE), fresh root aqueous extract (FRE) and dry root aqueous extract (DRE) regimes were found to be slightly variable from that of the seedlings in the control regime and were found not to be statistically different at $p<0.05$. The seedlings treated with fresh shoot aqueous extract (FSE) had a radicle length which was lower and significantly different from that of the seedlings in the control, dry shoot aqueous extract, fresh root aqueous extract and dry root aqueous extract regimes at $p<0.05$ (Table 2).

The fresh weight of the shoot of the seedlings in the control and dry root aqueous extract (DRE) regimes increased gradually from the start of the experiment to week four and then increased sharply until the end of the experiment (Table 3). The shoot fresh weight of the seedlings in the control regime remained highest throughout the duration of the experiment when compared with that of the seedlings in all the aqueous extract treatment regimes. There was a significant difference between the fresh weight of the shoot of the seedlings in the control regime and that of the seedlings in all the aqueous extract treatment regimes at $p<0.05$. The root fresh weight of the seedlings in the control regime was significantly different and higher than of the seedlings treated with the aqueous extracts. Significant differences were observed between the fresh weights of the root of the seedlings in the FSE and FRE regimes which were significantly different from that of the root of the seedlings in the DSE and DRE regimes at $p<0.05$ (Table 4). The dry weight of the shoot of the seedlings in the control regime was slightly higher than that of seedlings in all the extract treatment regimes from week two until the end of the experiment (Table 5). Significant difference was observed between the shoot dry weight of the

Table 1: Effect of aqueous extract of *Tithonia diversifolia* on seed germination of *Amaranthus* cruentus

Treatments	Percentage germination
Control	63.2
FSE	52.8
DSE	61.5
FRE	56.2
DRE	63.4

Table 2: Effect of the application of fresh shoot, dry shoot, fresh root and dry root aqueous extract treatments on the plumule length and radicle length of the germinating seedlings of *Amaranthus cruentus* L

Treatments	Plumule length (cm)	Radicle length (cm)
Control	2.6±0.5	1.4±0.2
FSE	2.2±0.5	1.0±0.1
DSE	2.4±0.4	1.3±0.2
FRE	2.7±0.5	1.3±0.2
DRE	2.7±0.2	1.4±0.3
LSD p<0.05	0.24	0.09

Table 3: Effect of aqueous extract of *Tithonia diversifolia* on shoot weight (g) of *Amaranthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD p<0.05
0	1.02	1.08	1.08	1.10	0.602	0.22
1	1.79	1.74	1.59	1.37	0.707	0.46
2	2.17	1.08	0.94	0.89	0.930	0.43
3	4.72	4.04	3.06	4.23	1.840	1.41
4	5.48	4.82	3.02	5.09	2.650	1.59
5	12.37	6.27	4.81	5.45	4.370	0.53
6	13.19	10.36	8.22	10.73	6.290	0.31

Table 4: Effect of aqueous extract of *Tithonia diversifolia* on root fresh weight (g) of *Amaranthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD p<0.05
0	0.060	0.0660	0.042	0.053	0.050	0.00
1	0.087	0.0780	0.076	0.073	0.054	0.00
2	0.158	0.0914	0.140	0.084	0.090	0.06
3	0.389	0.3440	0.251	0.357	0.206	0.15
4	0.722	0.6330	0.426	0.464	0.265	0.13
5	2.700	2.5900	1.940	2.410	1.480	0.11
6	2.820	2.7200	2.240	2.560	1.820	0.10

Table 5: Effect of aqueous extract of *Tithonia diversifolia* on shoot dry weight (g) of *Amaranthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD p<0.05
0	0.105	0.200	0.080	0.080	0.055	0.04
1	0.183	0.145	0.114	0.121	0.067	0.00
2	0.269	0.243	0.157	0.231	0.117	0.01
3	0.843	0.752	0.440	0.760	0.254	0.25
4	1.135	0.816	0.456	0.947	0.393	0.26
5	2.140	1.100	0.820	0.920	0.840	0.10
6	2.680	2.590	1.820	2.190	1.310	0.09

seedlings in the control regime and that of the seedlings in the aqueous extract treatment regimes at $p<0.05$. Significant differences were observed between the shoot dry weights of the seedlings in the FSE and FRE regimes and between those of the seedlings in the DSE and DRE regimes. Also the shoot dry weights of the seedlings in the FSE and FRE regimes were significantly different from those of the DSE and DRE respectively. The effect of different aqueous extracts of *Tithonia diversifolia* (Hemsl) A. Gray on the dry weight of the root of *Amaranthus cruentus* L. is presented in Table 6. The dry weight of the root of the control seedlings and that of the root of the seedlings belonging to all the other regimes showed the same pattern with that of control being slightly highest.

The shoot height of the seedlings in the control regime and all the four treatment regimes followed essentially the same trend. The height of the shoot of the control seedlings remained slightly higher than that of the treated seedlings throughout the duration of the experiment. The same applied to the

shoot height of the seedlings treated with root aqueous extracts (FRE and DRE) which remained slightly higher than that of the seedlings treated with shoot aqueous extracts (FSE and DSE) from the second week until the end of the experiment (Table 7). The shoot height of the seedlings treated with dry shoot and dry root aqueous extracts remained higher than that of the seedlings treated with fresh shoot and fresh root aqueous extracts respectively throughout the experiment. The shoot height of the seedlings in the control regime was statistically significantly different from the shoot height of the seedlings in all the treatment regimes at $p<0.05$.

The root length of the seedling in the control regime and all the four treatment regimes were similar in the first week of growth after which the root length of the seedling increased steadily until the end of the experiment. The root length of the seedlings in the control regime was slightly longer than that of the seedlings in all the treatment regimes. Seedlings in the two root aqueous extract treatment regimes (FRE and DRE regimes) had a root length that was longer than that of the seedlings in the two shoot aqueous extracts (FSE and DSE) treatment regimes (Table 7). The root length of the seedlings in the control regime was significantly different when compared with that of the seedlings treated with the FSE, DSE and FRE at $p<0.05$. The root length of the seedlings treated with fresh shoot aqueous extract was significantly different from that of the seedlings treated with dry shoot extract at $p<0.05$. Significant difference was also observed between the root length of the seedlings treated with fresh root aqueous extract and that of the seedlings treated with dry root aqueous extract at $p<0.05$ (Table 8). The leaf area of the seedlings in the FSE regime remained lowest throughout the duration of the experiment while the leaf area of the seedlings in the control regime was continuously higher than that of the seedlings in the other regimes throughout the duration of the experiment (Table 9). The leaf area ratio of the seedlings treated with dry root aqueous extract was higher than that of the seedlings in the other treatment regimes while that of the seedlings is the fresh shoot aqueous extract was lowest (Table 10).

Table 6: Effect of aqueous extract of *Tithonia diversifolia* on root dry weight (g) of *Amaranthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD $p<0.05$
0	0.005	0.008	0.005	0.005	0.006	0.00
1	0.009	0.007	0.007	0.007	0.007	0.00
2	0.017	0.013	0.0122	0.009	0.010	0.00
3	0.075	0.069	0.044	0.072	0.034	0.00
4	0.108	0.092	0.043	0.099	0.036	0.00
5	0.320	0.310	0.290	0.290	0.240	0.04
6	0.430	0.370	0.300	0.310	0.270	0.00

Table 7: Effect of aqueous extract of *Tithonia diversifolia* on plant length (cm) of *Amaranthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD $p<0.05$
0	7.26	7.20	7.10	7.22	7.25	1.30
1	8.92	8.40	8.76	8.70	8.80	1.62
2	16.76	12.34	14.98	15.68	16.16	0.87
3	25.64	18.08	20.34	21.10	22.40	0.92
4	27.66	18.78	22.36	24.92	26.22	3.61
5	28.46	22.60	23.44	24.96	25.52	2.00
6	37.94	27.86	28.92	32.60	34.64	1.60

Table 8: Efect of aqueous extract of *Tithonia diversifolia* on root length (cm) of *Amaranthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD $p<0.05$
0	4.00	3.80	3.82	3.92	4.02	1.03
1	4.60	4.12	4.24	4.36	4.56	0.84
2	8.80	6.26	7.26	8.18	8.48	1.01
3	9.34	7.62	8.58	9.00	8.94	1.44
4	11.04	7.72	9.70	9.88	10.66	1.04
5	12.14	9.82	9.92	10.94	11.60	2.59
6	18.00	14.64	15.70	17.30	17.68	1.60

Table 9: Effect of aqueous extracts of *Tithonia diversifolia* on the leaf area (cm^2) of *Amarabthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD p<0.05
0	6.01	6.200	6.60	7.00	7.30	0.51
1	12.60	6.600	7.73	8.16	11.56	0.88
2	21.04	11.480	12.46	18.43	18.60	1.69
3	28.86	12.158	12.81	19.06	22.73	1.33
4	38.73	16.785	22.31	27.60	33.20	1.49
5	71.13	41.570	45.72	53.22	60.91	0.94
6	86.22	52.190	68.71	72.51	79.80	1.33

Table 10: Effect of different extracts on leaf area ratio ($cm^2\ g^{-1}$) of *Amaranthus cruentus*

Weeks of treatments	Control	Fresh shoot extract	Dry shoot extract	Fresh root extract	Dry root extract	LSD p<0.05
0	60.24	41.06	58.45	58.08	142.12	1.29
1	65.63	43.42	63.88	63.75	156.22	5.71
2	73.57	44.84	73.72	76.79	146.46	3.74
3	31.44	14.81	26.47	22.90	78.92	1.18
4	31.16	18.48	44.71	29.18	77.38	1.97
5	28.91	29.48	41.18	43.98	56.40	2.19
6	27.72	17.63	32.41	29.00	50.51	1.99

DISCUSSION

Extensive studies have been carried out on the colonizing plants of the Asteraceae family and it has been suggested that these plants could compete effectively and suppress other plants in the same habitat as a result of their allelopathic activity. From this result, the extracts from the fresh shoot, fresh root and dry shoot tissues of *Tithonia diversifolia* had slight inhibitory effect on the germination of seeds of *Amaranthus cruentus* L. This observation, however was contrary to that of Brucker *et al*., (2003) who found that allelochemicals from the inflorescence extract of *Ambrosia artemisifolia* did not significantly reduce the germination of seeds of *Amaranthus hypochondriacus* L. However, it was found to be consistent with that of Rawat *et al*. (2002) who observed that allelochemicals from aqueous extracts of sunflower (*Helianthus annus* L.) inhibited germination in some other species like linseed (*Linum usitatisium*) and mustard (*Brassica juncea* L.). Indergit and Darkshini (1994) also found that the water extracts from the roots of *Phichea lanceolata* in the family Asteraceae inhibited the germination of tomato and mustard. A significant difference was observed between germination of seeds treated with the fresh tissue aqueous extracts and those treated with the dry tissue aqueous extracts. In fact, the percentage germination of seeds in the dry root regime was actually almost equivalent with that of the control regime. This indicated that the amount or potency of allelochemicals present in the dry tissue aqueous extracts were considerably lower compared to that of the fresh tissues aqueous extracts.

The radicle growth of germinating *Amaranthus cruentus* L. seedlings treated with the aqueous extract prepared from fresh and dried shoot of *Tithonia diversifolia* was observed to be inhibited. A similar result was obtained by Rahman (1998) on the effect of aqueous extract derived from the inflorescence, stem and leaves of *Barthenium hysterophorus* L. on the growth of radicle and plumule of *Cassia sophera* L. However, in this study, the aqueous extracts prepared from the fresh and dried root of *Tithonia diversifolia* did not affect the radicle growth of germinating seeds of *Amaranthus cruentus*. This probably could be attributed to low concentration of allelochemicals in the two root aqueous extracts. In support of this was the finding of Miller (1996) who stated that water extract of top growth of *Medicago sativa* L. produced more allelopathic effect on seedlings than extracts from the roots.

The fresh weight and dry weight of the shoot of the control seedlings of *Amaranthus cruentus* remained highest in most parts of the experiment and was significantly different from that of the shoot

of the seedlings in the different aqueous extract treatment regimes. This result agreed with that of Ahn and Chung (2000) who found that aqueous extract of rice hull inhibited the shoot fresh weight of Barnyard grass (*Echinochloa crusgalli*). The root fresh weight of aqueous extract treated seedlings of *Amaranthus cruentus* were observed to be significantly reduced when compared to that of the control seedlings. Huber *et al.* (2002) had earlier observed that exogenously applied phenolic acids reduced root fresh weight and dry weight of soybean. Although the aqueous extracts prepared from the shoot and root of *Tithonia diversifolia* were observed to retard the shoot height of *Amaranthus cruentus* it was however evident that the shoot extracts were more phytotoxic and had more inhibitory effect on the shoot height of the treated seedlings than the root aqueous extracts. The shoot height of *Amaranthus cruentus* seedlings treated with the dry shoot aqueous extract and dry root aqueous extract were higher than those treated with the fresh shoot and fresh root aqueous extracts respectively. The drying process could have reduced the amount of volatile allelochemical in these plant tissues hence the low inhibitory effect of the extract prepared from the dried tissue. It has been fairly well established that root length was more sensitive to phytotoxic compounds than either seed germination or shoot elongation in many crops (Hall and Henderlong, 1989; Kuiters, 1980; Luu *et al.,* 1989; Hedge and Miller, 1990). Huber *et al.* (2002) showed that exogenously applied phenolic acids reduced root length of soybeans. In this work, the root length of the treated seedlings of *Amaranthus cruentus* was reduced by aqueous extract treatments applied. This indicated that the extracts applied contain some growth inhibitory substances in amount sufficient to suppress the growth of the root of these seedlings. Variation in the root length of the control and treated seedlings followed the same pattern as observed for the shoot height. The shoot aqueous extract regimes had seedlings with shortest root length during most part of the experiment. This observation was supported by the findings of Eze and Gill (1992) who stated that *Chromolaena odorata* L. a related weed also in the family Asteraceae had a high concentration of allelochemicals especially in its leaves.

Canston and Venus (1981) were of the opinion that leaves are the most important photosynthetic producers of the plant. According to these workers, light interception and photosynthetic rate depend to a large extent upon the available leaf area. Therefore, the amount of light intercepted is assumed to be directly proportional to the leaf area. In this study, the leaf area of seedlings in the control regime was significantly higher than that of seedlings in all the aqueous extract treatment regimes. That is, the application of the different aqueous extract was observed to have reduced the leaf area of these seedlings. This observation was consistent with the findings of Patterson (1981) who detected that the application of some synthetic allelochemicals reduced the leaf area of soybean.

The growth of *Tithonia diversifolia* in association with cultivated crop may lead to reduction in growth of these crops. It is therefore required that the weed be controlled in the crop fields.

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