



Research Journal of
Phytochemistry

ISSN 1819-3471



Academic
Journals Inc.

www.academicjournals.com

Physiological Response of *Amaranthus cruentus* and *Oryza sativa* to Phytotoxins of *Tithonia diversifolia*

O.J. Ilori, O.O. Otusanya and A.A. Adelusi

Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract: The understanding of the inhibitory effect that *Tithonia diversifolia* (Hemsl) A. Gray can cause to nutrient accumulation of associated crops in the field is required to substantiate the need for effective control of the weed. The susceptibility of *Amaranthus cruentus* and *Oryza sativa* Linn. to phytotoxic effects of *Tithonia diversifolia*, the phytotoxicity of the plant parts of *T. diversifolia* and also the effect of drying on the phytotoxic activity of the weed were determined. The effects of the aqueous extracts prepared from the shoots and roots of *T. diversifolia* on *Amaranthus cruentus* and *Oryza sativa* were obtained by subjecting the seedlings to the different aqueous extract treatments. Results showed that chlorophyll accumulation and total protein contents of test plants 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. This study concluded that *Tithonia diversifolia* was an allelopathic weed with water-soluble allelochemicals in its plant parts and had such phytotoxic potency that could reduce the nutrient accumulation of associated crop plants.

Key words: Allelopathic, chlorophyll, *Tithonia diversifolia*, *Amaranthus cruentus*, *Oryza sativa*, phytotoxins

INTRODUCTION

Putnam (1988) observed that chemicals with allelopathic potential i.e., allelochemicals are present in the plant root, rhizome, stem, leaves, flowers, inflorescence, pollen, fruits and seeds. Allelochemicals are known to affect numerous physiological and biochemical processes in plants (Rice, 1984). *Tithonia diversifolia* (Hemsl) A. Gray commonly referred to as Mexican sunflower is a member of the family Asteraceae. *T. diversifolia* is an aggressive weed with high invasive capacity and the ability to compete successfully with agricultural crops (Ayeri *et al.*, 1997). This weed has been reported to contain some allelochemicals (Tongma *et al.*, 1998). *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 (Eze and Gill, 1992). Aqueous extract from invasive weed *Chemopodium album* reduced chlorophyll and protein content of *Cassia occidentalis* and *Phaseolus aureus* (Daizy *et al.*, 2006). Allelochemicals reduced the chlorophyll content, photosynthesis rates and protein synthesis in leaf cells of velvet leaf (*Abutilon theophrasti*) (Mersie and Singh, 1993). Chlorophyll reduction was observed in soybean plants treated with aqueous extract of velvetleaf (Cotton and Einhellig, 1980). Yang *et al.* (2002) observed that chlorophyll biosynthesis of *Oryza sativa* seedlings were inhibited by exogenously applied allelochemicals. Rice (1984) was of the opinion that a reduction in chlorophyll accumulation would result in impaired photosynthesis and finally diminished growth.

Corresponding Author: O.J. Ilori, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria

Allelochemicals have been reported to alter protein synthesis in soybean seedlings (Baziramakenga *et al.*, 1997). The contents of proteins and carbohydrates of *Ageratum conyzoides* were observed to decrease with the passage of time after treatments with allelochemicals from *Parthenium hysterophorus* (Singh *et al.*, 2002). *Tithonia diversifolia* is capable of posing a serious threat of phytotoxicity to agricultural crops. Most farmers find it difficult to manage *Tithonia diversifolia* infestation in most crop fields particularly rice and maize field (Imeokpara and Okusanya, 1994). Therefore, the specific objectives of this study are to determine the susceptibility of *Amaranthus cruentus* L. and DTPMFe⁺ *Oryza sativa* 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 Botany Department of the Obafemi Awolowo University, Ile-Ife, Nigeria, during August-November, 2004. The seeds of *Amaranthus cruentus* L., *Oryza sativa* L. and *Tithonia diversifolia* (Hemsl) A. Gray were collected from the National Horticultural Research Institute (NIHORT) Ibadan, Genetics unit of the Botany Department, Obafemi Awolowo University (OAU), Ile-Ife and along road 20 of the Senior Staff Quarters of OAU, Ile-Ife, respectively. Six weeks old plants of *Tithonia diversifolia* (Hemsl) A. Gray were harvested and separated into shoots and roots for preparation of fresh plant parts aqueous extracts 360 g of fresh shoots and 360 g of fresh roots were cut into small chips of about 4 cm lengths and finely ground separately with a mortar and pestle. The ground plant parts were soaked in 5 L of water for 12 h. The solution was filtered through cheese cloth to remove debris and then filtered through Whatman No. 1 filter paper. The filtrate obtained from the extraction of the fresh shoot served as the fresh shoot aqueous extract treatment while the filtrate obtained from the extraction of the fresh root served as the fresh root aqueous extract treatment. The extracts were stored in a refrigerator to prevent the degradation of the allelochemicals present in the extracts. Six weeks old plants of *Tithonia diversifolia* (Hemsl) A. Gray were harvested and separated into shoots and roots. Three hundred and sixty grams of fresh shoots and 360 g of fresh roots 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 plant parts were soaked in 5 L of tap water for 12 h (Ahn and Chung, 2000). Processing of extracts then followed the procedure described above for the preparation of fresh tissue aqueous extracts. The extract obtained from the dry shoot served as treatment for the seedlings in the dry shoot aqueous extract regime while the extract obtained from the dry root served as treatment for the seedlings in the dry root aqueous extract regime.

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 and 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. Good humus top soil collected besides the Faculty of Agriculture, OAU, Ile-Ife was put into plastic pots (28 cm diameter and 15 cm depth). Seeds of *Amaranthus cruentus* L. were sown in each of the pots and 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 layed out in a completely randomized design.

Seeds of *Oryza sativa* were sown in black polythene bags (9 cm diameter and 14 cm depth) which were filled with good top humus soil collected besides the Faculty of Agriculture, OAU, Ile-Ife. The

polythene bags containing the seeds were placed in the nursery. The bags were supplied with 50 mL of tap water early in the morning and late in the evening daily. At three weeks, four uniform seedlings were transplanted into each plastic pot (28 cm diameter and 15 cm depth) which had been filled with good top humus soil. The seedlings were thereafter raised in the open. The pots in the control regime were supplied with 400 mL of water every morning while those in the different treatment regimes were supplied with 400 mL of the appropriate aqueous extract every morning. For zero days, plants were harvested just before treatment started. Thereafter, harvesting of the seedlings was on a weekly interval for a period of six weeks. Chlorophyll contents were determined using the method of Comb *et al.* (1985). Total protein concentration was determined using the technique of Lowry *et al.* (1951). 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

Table 1 shows the chlorophyll *a* accumulation in the shoots of *Amaranthus cruentus* L. grown under control, fresh shoot, dry shoot, fresh root and dry root aqueous extract treatment regimes. Chlorophyll *a* accumulation in the shoot of *A. cruentus* seedlings in the control regime increased from the start of the experiment to the end. The change in the level of chlorophyll *a* in the shoot of the seedlings treated with the different aqueous extracts increased slightly in the first three weeks of the experiment and then increased sharply during the remaining part of the experiment. The change in the level of chlorophyll *a* in the shoot of the seedlings in the control regime was highest during most part of the experiment. Accumulation of chlorophyll *a* in the control seedlings was significantly higher than that of the seedlings in the different aqueous extract treatment regimes at $p < 0.05$. Significant differences were also observed between the chlorophyll *a* accumulation in the seedlings belonging to the FSE and DSE regimes and that in the seedlings belonging to the FSE and FRE regime at $p < 0.05$ during the last two weeks of the experiment.

The accumulation of chlorophyll *a* in the shoot of *Oryza sativa* L. grown under control, fresh shoot, dry shoot, fresh root and dry root aqueous extract treatment regimes is shown in Table 2.

Table 1: Effect of aqueous extract of *Tithonia diversifolia* on chlorophyll *a* content of *Amaranthus cruentus*

| Weeks | Treatments | | | | | LSD $p < 0.05$ |
|-------|------------|---------------------|-------------------|--------------------|------------------|----------------|
| | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | |
| 0 | 53.2 | 50.03 | 42.0 | 46.02 | 48.12 | 3.48 |
| 1 | 72.6 | 52.80 | 51.3 | 52.02 | 51.60 | 0.36 |
| 2 | 82.8 | 57.70 | 59.8 | 62.70 | 63.00 | 3.00 |
| 3 | 113.8 | 59.80 | 77.2 | 80.80 | 82.00 | 2.09 |
| 4 | 141.6 | 132.80 | 134.2 | 138.00 | 142.80 | 2.26 |
| 5 | 245.6 | 245.80 | 137.6 | 211.00 | 155.00 | 4.39 |
| 6 | 510.2 | 376.00 | 312.8 | 322.80 | 328.00 | 4.08 |

Note: Chlorophyll *a* content: μg

Table 2: Effect of aqueous extract of *Tithonia diversifolia* on chlorophyll *a* content of *Oryza sativa*

| Weeks | Treatments | | | | | LSD $p < 0.05$ |
|-------|------------|---------------------|-------------------|--------------------|------------------|----------------|
| | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | |
| 0 | 20.25 | 25.10 | 22.13 | 20.13 | 26.05 | 5.64 |
| 1 | 51.76 | 32.00 | 23.76 | 31.46 | 31.80 | 0.67 |
| 2 | 87.60 | 41.76 | 37.60 | 42.10 | 86.70 | 0.69 |
| 3 | 89.20 | 72.20 | 74.60 | 77.20 | 77.80 | 2.58 |
| 4 | 123.80 | 121.60 | 65.60 | 79.40 | 113.40 | 6.33 |
| 5 | 210.00 | 182.00 | 72.20 | 184.80 | 192.60 | 5.87 |
| 6 | 303.00 | 154.20 | 165.20 | 115.20 | 113.80 | 6.55 |

Note: Chlorophyll *a* content: μg

Table 3: Chlorophyll *b* accumulation in *Amaranthus cruentus* as affected by aqueous extract treatments of *Tithonia diversifolia*

| Treatments | | | | | | |
|------------|---------|---------------------|-------------------|--------------------|------------------|------------|
| Weeks | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | LSD p<0.05 |
| 0 | 24.11 | 28.0 | 34.8 | 14.51 | 24.02 | 6.40 |
| 1 | 31.60 | 26.3 | 25.2 | 24.58 | 25.50 | 0.65 |
| 2 | 41.50 | 28.5 | 31.3 | 21.00 | 31.30 | 0.92 |
| 3 | 54.60 | 42.4 | 26.8 | 70.60 | 24.00 | 3.98 |
| 4 | 65.80 | 64.8 | 67.4 | 69.80 | 71.40 | 3.24 |
| 5 | 184.20 | 100.2 | 109.4 | 121.40 | 125.20 | 5.10 |
| 6 | 215.20 | 173.0 | 150.0 | 165.20 | 173.00 | 8.76 |

Note: Chlorophyll *b* content: μg

Table 4: Chlorophyll *b* accumulation in *Oryza sativa* as affected by aqueous extract treatments of *Tithonia diversifolia*

| Treatments | | | | | | |
|------------|---------|---------------------|-------------------|--------------------|------------------|------------|
| Weeks | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | LSD p<0.05 |
| 0 | 10.23 | 16.58 | 15.21 | 12.31 | 10.3 | 3.90 |
| 1 | 23.30 | 11.60 | 12.40 | 15.32 | 15.5 | 0.69 |
| 2 | 32.90 | 18.02 | 25.70 | 36.60 | 21.5 | 3.97 |
| 3 | 82.20 | 42.20 | 41.40 | 44.80 | 44.4 | 2.27 |
| 4 | 103.20 | 28.00 | 30.00 | 57.00 | 63.0 | 11.19 |
| 5 | 113.20 | 82.60 | 84.40 | 93.40 | 101.6 | 2.97 |
| 6 | 123.20 | 83.60 | 83.80 | 60.80 | 56.8 | 4.18 |

Note: Chlorophyll *b* content: μg

During the first week of treatments, there was a sharp increase in the accumulation of chlorophyll *a* in the control seedlings. This was followed by a lag after which the change in the level of chlorophyll *a* increased until the end of the experiment. Chlorophyll *a* contents in the shoot of the seedlings in the FSE, FRE, and DRE regimes decreased sharply at the end of the experiment (Table 2). During the first three weeks of the experiment, chlorophyll *b* accumulation in the *A. cruentus* seedlings in the DSE treatment regime decreased and then increased until the end of the experiment. Chlorophyll *b* accumulation in the DRE regime seedlings was constant initially and then decreased from week two to three. After the third week, chlorophyll accumulation in these seedlings increased until the end of the experiment. (Table 3). Chlorophyll *b* accumulation in the control seedlings of *Amaranthus cruentus* was significantly higher than that of the seedlings in the different aqueous extract treatment regimes at $p < 0.05$.

Accumulation of chlorophyll *b* in the control seedlings of *Oryza sativa* increased gradually from the beginning of the experiment to week two and then increased until the end of the experiment (Table 4). The content of chlorophyll *b* in *Oryza sativa* seedling in the DSE, FRE, DRE regimes decreased at the end of the experiment. Chlorophyll *b* accumulation in the seedlings in the FSE and DSE regimes decreased during the fourth week. The accumulation of chlorophyll *b* in the control seedlings was significantly different from that in the seedlings in all the treatment regimes at $p < 0.05$. Significant differences were also observed between chlorophyll *b* accumulation in the seedlings belonging to DSE regime and that in the seedlings belonging to the DRE regime and between FSE and FRE regimes at $p < 0.05$ during the last 3 weeks of the experiment.

The level of total chlorophyll in the control seedlings increased from the beginning to the end of the experiment. Accumulation of total chlorophyll in the seedlings belonging to the fresh shoot aqueous extract (FSE), dry shoot aqueous extract (DSE) and dry root aqueous extract (DRE) regimes increased slightly from the start of the experiment to week three and then increased gradually until the end of the experiment (Table 5). The total chlorophyll accumulation in the control seedlings was significantly higher than that of the seedlings treated with the different aqueous extracts at $p < 0.05$. Significant differences were also observed between the total chlorophyll accumulation in the seedlings belonging

Table 5: Effect of aqueous extract of *Tithonia diversifolia* on total chlorophyll content of *Amaranthus cruentus*

| Treatments | | | | | | |
|------------|---------|---------------------|-------------------|--------------------|------------------|------------|
| Weeks | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | LSD p<0.05 |
| 0 | 77.31 | 78.03 | 75.80 | 60.53 | 72.14 | 8.69 |
| 1 | 104.10 | 79.10 | 76.50 | 76.60 | 76.90 | 1.30 |
| 2 | 94.30 | 86.20 | 91.06 | 63.70 | 84.30 | 2.00 |
| 3 | 194.40 | 101.40 | 104.00 | 151.40 | 106.00 | 6.00 |
| 4 | 206.20 | 197.20 | 201.60 | 207.60 | 214.20 | 4.43 |
| 5 | 429.80 | 346.80 | 247.05 | 332.00 | 280.20 | 6.56 |
| 6 | 725.40 | 549.00 | 462.80 | 488.00 | 501.40 | 2.45 |

Note: Total chlorophyll content: μg Table 6: Effect of aqueous extract of *Tithonia diversifolia* on total chlorophyll content of *Oryza sativa*

| Treatments | | | | | | |
|------------|---------|---------------------|-------------------|--------------------|------------------|------------|
| Weeks | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | LSD p<0.05 |
| 0 | 30.84 | 41.68 | 37.34 | 32.44 | 36.35 | 8.75 |
| 1 | 75.00 | 43.60 | 36.20 | 46.78 | 47.30 | 7.01 |
| 2 | 97.48 | 59.78 | 63.30 | 78.70 | 107.80 | 7.13 |
| 3 | 171.40 | 144.40 | 116.00 | 121.00 | 122.20 | 2.84 |
| 4 | 226.80 | 149.60 | 95.60 | 136.40 | 176.60 | 7.56 |
| 5 | 323.20 | 265.60 | 156.00 | 278.20 | 191.60 | 3.26 |
| 6 | 432.20 | 237.80 | 249.00 | 172.40 | 170.60 | 52.84 |

Note: Total chlorophyll content: μg Table 7: Effect of aqueous extract of *Tithonia diversifolia* on total protein content of *Amaranthus cruentus*

| Treatments | | | | | | |
|------------|---------|---------------------|-------------------|--------------------|------------------|------------|
| Weeks | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | LSD p<0.05 |
| 0 | 7.3 | 8.4 | 9.2 | 6.9 | 7.11 | 0.94 |
| 1 | 11.8 | 17.2 | 7.6 | 12.0 | 14.50 | 0.66 |
| 2 | 16.8 | 12.7 | 13.1 | 17.2 | 18.50 | 0.58 |
| 3 | 23.0 | 19.4 | 22.8 | 26.5 | 25.00 | 1.03 |
| 4 | 24.2 | 18.4 | 18.6 | 15.0 | 18.30 | 0.87 |
| 5 | 24.9 | 17.3 | 18.0 | 19.6 | 19.20 | 0.67 |
| 6 | 19.7 | 17.5 | 18.7 | 20.6 | 21.10 | 1.02 |

Note: Protein content: μg Table 8: Effect of aqueous extract of *Tithonia diversifolia* on total protein content of *Oryza sativa*

| Treatments | | | | | | |
|------------|---------|---------------------|-------------------|--------------------|------------------|------------|
| Weeks | Control | Fresh shoot extract | Dry shoot extract | Fresh root extract | Dry root extract | LSD p<0.05 |
| 0 | 5.2 | 5.3 | 6.20 | 4.2 | 4.0 | 0.69 |
| 1 | 6.3 | 4.6 | 4.80 | 7.0 | 6.5 | 0.73 |
| 2 | 8.7 | 6.2 | 5.00 | 8.4 | 9.1 | 0.49 |
| 3 | 21.0 | 12.8 | 7.90 | 7.7 | 22.2 | 1.09 |
| 4 | 15.2 | 11.0 | 12.80 | 13.2 | 24.0 | 0.98 |
| 5 | 21.1 | 7.6 | 13.30 | 13.6 | 19.7 | 0.41 |
| 6 | 36.1 | 27.7 | 30.44 | 31.0 | 35.0 | 0.26 |

Note: Protein content: μg

to the FSE and DSE regimes and between that of the seedlings in the FRE and DRE regimes. Table 6 shows the change in the level of the total chlorophyll in the shoot of *Oryza sativa* L. grown under control, fresh shoot, dry shoot, fresh root and dry root aqueous extract treatment regimes. In the control seedlings, the level of total chlorophyll increased gradually from the start of the experiment to week two and then increased until the end of experiment.

Total chlorophyll contents in the shoot of seedlings in the FSE, FRE and DRE regimes decreased at the end of the experiment. Significant difference was observed between the total chlorophyll accumulation in the control seedlings and that in the seedlings belonging to the different aqueous extract regimes at $p<0.05$.

The total protein content of the shoot of the seedlings in the control regime increased during the first three weeks of the experiment and then increased gradually up to week five after which the total protein content in these seedlings decreased at the end of the experiment (Table 7). In the shoot of the seedlings belonging to the treatment regimes protein content increased at the end of the experience. Significant differences at $p < 0.05$ were observed between the total protein content of the shoot of the seedlings in the control regime and that of the shoot of the seedlings in all the aqueous extract treatment regimes.

At the end of the experiment, *Oryza sativa* seedlings in the FSE regime had the lowest protein content. Shoot aqueous extract treatment had a negative effect on protein content. Root aqueous extracts increased the protein content of the seedlings.

The total protein content of the shoot of *Oryza sativa* seedlings in the control regime remained highest when compared with that of the seedlings in the other treatment regimes while that of the shoot of the seedlings in the fresh shoot aqueous extract treatment regime was lowest when compared with that of the seedlings in the other treatment regimes in the last two weeks of the experiment (Table 8). At the end of the experiment, the protein contents of seedlings in the dry tissue aqueous extract regimes were higher than the protein content of the seedlings in the fresh tissue extract regimes. There was a significant difference between the total protein content in shoot of the seedlings in the control regime and that of the shoot of the seedlings in the different aqueous extract treatment regimes at $p < 0.05$. Significant differences at $p < 0.05$ were observed between the total protein content of the seedlings in the FSE and FRE regimes and that of the seedlings in the DSE and DRE regimes, respectively, between the total protein content of the seedlings in the FSE and FRE regimes and finally between that of the seedlings in the DSE and DRE regimes.

DISCUSSION

Result presented in this study indicated that chlorophyll *a* chlorophyll *b* and total chlorophyll content in the control shoots of *Amaranthus cruentus* L. and *Oryza sativa* L. seedlings were significantly different at $p < 0.05$ and higher than those of the shoot of the seedlings treated with the different aqueous extracts. Some workers have earlier obtained similar result. Ahmed *et al.* (2004) reported that that root and shoot aqueous extract of *Chenopodium murale* reduced pigments content and protein content of *Melilotus indicus*, *Trifolium alexandrinum*, *Triticum pyramidal*, *Lycopersicon esculentum* and *Cucumis sativa*. They further stated that inhibition was a function of extract concentration and plant tissue type. Kirby and Sheaku (1994) were of the opinion that the content of chlorophyll in the seedlings treated with allelochemicals may give some idea about the mode of action of allelochemicals. Reports given by these earlier workers have shown that extracts from allelopathic plants are capable of impairing chlorophyll synthesis thereby reducing chlorophyll accumulation.

Heji *et al.* (1993) showed a correlation between photosynthetic alteration and the action of juglone an allelochemical from walnut. According to St. John (1982), Al-Khatis *et al.* (1992), Nambat *et al.* (1996), Gonzalez *et al.* (1998), Muller and Niyogi (2001) and Masura *et al.* (2002), the alteration of photosynthesis by allelochemicals could be as a result of the disruption of electron transport chain and alteration in chlorophyll biosynthesis. Yang *et al.* (2002) stated that allelochemical substances may reduce chlorophyll accumulation in three ways; the inhibition of chlorophyll biosynthesis; the stimulation of chlorophyll degradation or both. The allelochemicals present in all the aqueous extracts must therefore have exerted growth inhibitory effects primarily through reduction in chlorophyll synthesis or stimulation of chlorophyll degradation and consequently reduction in net photosynthesis. Some studies have shown a positive relationship between leaf photosynthesis rate and dry matter accumulation in sorghum (Peng *et al.*, 1991). Therefore, any reduction in chlorophyll contents of plants

would be expected to naturally diminish photosynthetic rate and ultimately total plant growth. Also this reduction in chlorophyll accumulation in the treated seedlings could be related to the reduced biomass accumulation observed at the latter weeks of the experiment in these seedlings.

Seedlings of *Oryza sativa* belonging to the control regime had the highest chlorophyll *b* accumulation while the seedlings treated with the aqueous extract prepared from the shoot had the lowest chlorophyll *b* accumulation from week three till the end of the experiment. These observations could be probably related to the higher concentration of allelochemicals in the shoot of *Tithonia diversifolia* compared to the root of the plant which imparted a more pronounced inhibition on chlorophyll *b* synthesis in these seedlings. Though Rice (1984) found that allelochemicals are present in plant root, rhizome, stem, leaves, flower, inflorescence, pollen, fruits and seeds of plants, he emphasized that the leaves are the major sources of these allelochemicals. He was of the opinion that the concentration of the allelochemicals could vary with age, season and plant parts. These observations supported the result obtained in this study which showed that the extracts prepared from the shoot of *Tithonia diversifolia* had more retardatory effects on the accumulation of chlorophyll in the treated seedlings than the extracts prepared from its roots during the latter weeks of the experiment.

The total protein content of the *Amaranthus cruentus* L. seedlings belonging to the fresh shoot aqueous extract treatment regime was higher than that of the control seedlings and seedlings in the other aqueous extract treatment regimes in the first week. However, continuing application of this extract to the soil must have led to the accumulation of allelochemicals in the soil resulting in the inhibition of protein synthesis and causing the protein content in these seedlings to drop as from the second week until the end of the experiment. This observation supported the statement of Hall and Henderlong (1989) that the allelochemicals have to accumulate in sufficiently high quantity in the soil to cause allelopathic effects. The drop in total protein content in the control seedlings at the end of experiment could be attributed to the diversion of the total protein into other biosynthetic pathway in the matured seedlings.

Oryza sativa L. seedlings in the control regime had a total protein level that was higher than the total protein level of the seedlings belonging to the fresh and dry shoot aqueous extract treatment regimes throughout the duration of the experiment. Earlier researchers such as Singh *et al.* (2002) and Mersia and Singh (1993) had similarly reported reduction in protein synthesis due to the use of allelochemicals in treating *Ageratum conyzoides* and leaf cells of velvet leaf (*Abutilon theophrasti*), respectively. Chengrong *et al.* (2005) stated that aqueous extract derived from *Wedelia trilobata* reduced leaf chlorophyll content and the activities of some enzymes. Reduction in protein content as a result of treatment with allelochemicals may be due to the inhibition of the incorporation of many amino acid into protein (Van Sumere *et al.*, 1971). Therefore the observed retardatory effects of allelochemicals on protein accumulation could be a result of such interference in the protein biosynthetic pathway i.e., either by the inhibition of the action of some relevant enzymes or alteration of the activity of the enzymes in the biosynthesis of amino acid.

In this study, the total protein content of seedlings of *Amaranthus cruentus* L. and *Oryza sativa* L. treated with dry root aqueous extracts was found to be higher than that of the seedlings in the control regime during the first 3 weeks and four weeks of the experiment, respectively. This indicated that the dry root aqueous extract either enhanced or did not affect protein synthesis in the seedlings during this period. This observation might be due to low concentration of allelochemicals present in the dry root aqueous extract.

This study suggests the importance of allelopathy in weed crop relations. The reduction in the chlorophyll contents and total protein contents of *Amaranthus cruentus* and *Oryza sativa* by aqueous extracts of *Tithonia diversifolia* treatments suggest the allelopathic effect of this weed on the test crops. Therefore the infestation of this weed should be controlled to reduce its association with cultivated crop in the field.

REFERENCES

- Ahmed, A.E., K.H. Ahmed and K.G. Hanna, 2004. Does allelopathy have a role in the ecology of *chemopodium murale*? *Anna Bot. Fennici*, 41: 37-45.
- Ahn, J.K. and M. Chung, 2000. Allelopathic potential of rice hulls on germination and seedling growth of barnyard grass. *Agron. J.*, 92: 1162-1167.
- Al-Khatis, K., G.I. Mink and R. Parker, 1992. Detection and tracking of air borne herbicide by using bio-indicator plants. *Proc. Western Soc. Weed Sci.*, 45: 27-31.
- Ayeni, A.O., D.T. Lordbanjou and B.A. Majek, 1997. *Tithonia diversifolia* (Mexican sunflower in South-western Nigeria. Occurrence and growth habit. *Weed Res.*, 37: 443-449.
- Baziramakenga, R., G.D. Levoux, R.R. Simard and P. Nadeau, 1997. Allelopathic effects of phenolic acids on nucleic acid and protein levels in soybean seedlings. *Can. J. Bot.*, 75: 445-450.
- Chengrong, N., L. Shiming,, Z. Rensen, M. Meihua, L. Huashou and L. Chuxia, 2005. Allelopathic potential of *Wedelia trilobata* L. effects on germination, growth and physiological parameters of rice. 4th World Congress on Allelopathy.
- Comb, J.H., S.I. Long and J. Scurlock, 1985. *Techniques in Bio Productivity and Photosynthesis* Pergamon Press. Oxford. New York, pp: 233-225.
- Cotton, C.E. and F.A. Einhellig, 1980. Allelopathic mechanisms of velvet leaf (*Abutilon theophrasti*) Medic. Malvaceae on soybean. *Am. J. Bot.*, 67: 1407-1413.
- Daizy, R., P.S. Harminder, R. Nipunika and K.K. Ravinder, 2006. Assessment of allelopathic interference of *Chenopodium album* through its leachates, debris extracts, rhizosphere and amended soil. *Arch. Agron. Soil Sci.*, 52: 705-715.
- Eze, S.M.O. and L.S. Gill, 1992. *Chromolaena odorata*-a problematic weed. *Compositae Newslett.*, 20: 14-18.
- Gonzalez, V.M., J. Kazimir, C. Nimbai, L.A. Weston and G.M. Cheniae, 1998. Inhibition of a photosystem II electron transfer reaction by sorgoleone a natural product. *J. Agric. Food Chem.*, 45: 1415-1421.
- Hall, M.H. and Henderlong, 1989. Alfalfa autotoxic fraction characterization and initial separation. *Crop Sci.*, 29: 425-428.
- Heji, A.M., F.A. Einhellig and J.A. Rasmussen, 1993. Effects of juglone on growth photosynthesis and respiration. *J. Chem. Ecol.*, 19: 553-568.
- Imeokpara, P.O. and B.A. Okusanya, 1994. Relative effectiveness economics of cultural and chemical weed control methods in low land rice (*Oryza sativa*) in southern guinea savanna of Nigeria. *Nig. J. Weed Sci.*, 10: 35-47.
- Kirby, M.F. and D.A. Sheahan, 1994. Effects of atrazine, isoproturon and mecoprop on the macrophyte *Lemna minor* and the alga *Scenedesmus subspicatus*. *Bull. Environ. Contam. Toxicol.*, 53: 120-126.
- Lowry, O.H., A.L. Rosebrough and R.J. Ranall, 1951. Protein measurement with the phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Masura, T., J.E.W. Polle and A. Melis, 2002. Biosynthesis and distribution of chlorophyll among the photosystem during recovery of the green algae (*Dunaliella salina*) from irradiance stress. *Plant Physiol.*, 128: 603-614.
- Mersie, W. and M. Singh, 1993. Phenolic acid affect photosynthesis and protein synthesis by isolated leaf cells of velvet-leaf. *J. Chem. Ecol.*, 19: 1293-1301.
- Muller, P. and K.K. Niyogi, 2001. Non-photochemical quenching. A response to excess light energy. *Plant Physiol.*, 125: 1558-1566.

- Nimbat, C.I., L.A. Weston, C.N. Yerkes and S.C. Weller, 1996. Herbicidal activity and site of action of the natural product sorgoleone. *Proc. Weed Sci. Soc. Am.*, 36: 56.
- Peng, S., D.R. Krieg and F.S. Girma, 1991. Leaf photosynthetic rate is correlated with biomass and grain production in grain sorghum lines. *Photosynthesis Res.*, 28: 1-7.
- Putnam, A.R., 1988. Allelochemicals from plants as herbicides. *Weed Technol.*, 2: 510-518.
- Rice, E.L., 1984. *Allelopathy* 2nd Edn., Academic Press, New York, pp: 422.
- Singh, H.P., D.R. Batish, R.K. Kohli, D.B. Saxena and V. Arora, 2002. *J. Chem. Ecol.*, 28: 2169-2179.
- St. John, J.B., 1982. Effect of herbicides on the lipid composition of plant membranes. In: *Biochemical Responses Induced by Herbicides*, pp: 97-109.
- Tongma, S., K. Katsuchiro and U. Kenji, 1998. Allelopathic activity of Mexican sunflower (*Tithonia diversifolia*) in soil. *Weed Sci.*, 46: 432-437.
- Van Sumere, C.F., J. Cottence, J. De Greef and J. Kint, 1971. Biochemical Studies in relation to the possible germination regulatory role of naturally occurring coumarins and phenolics. *Res. Adv. Phytoch.*, 4: 165-221.
- Yang, C.M., C.N. Lee and C.H. Chou, 2002. Allelopathic phenolics and chlorophyll accumulation. Effect of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: Inhibition of supply-orientation. *Botanical Bulletin of Academia Sinica*, 43: 299-304.