Chapter 1

Phytotoxic activity of water extract from *Tithonia diversifolia* (Mexican sunflower)

Introduction

Mexican sunflower (*Tithonia diversifolia*) is a herbaceous perennial plant of the genus Tithonia that originated in the central America region (Nanakorn, 1995; Schuster *et al.*, 1992). It was introduced to many tropical countries and becomes a dominant plant in its growing area (Ayeni *et al.*, 1997a, 1997b; Radanachales and Maxwell, 1992). Zungsontiporn and Harada (1995) reported that Mexican sunflower is widespread in the mountainous area of Mae Hong Son province, in the northern part of Thailand. Since it has succeeded to establish itself in that area, other plants cannot be grown, and it was hypothesized that Mexican sunflower may contain allelopathic substance(s).

Plants of the genus Tithonia were reported to contain a wide range of secondary metabolites that are considered to be the defensive substances against herbivores and possible allelopathic agents (Baruah *et al.*, 1979; Bordoloi *et al.*, 1996; Chowdhury *et al.*, 1980, 1983; Kuo and Chen, 1998; La Duke 1982; Pereira *et al.*, 1997; Schuster *et al.*, 1992). The production and distribution of the metabolites vary both qualitatively and quantitatively

between plant organs (Dutta *et al.*, 1993). Chemical compounds isolated from Mexican sunflower plant had been found to be insect feeding deterrents and plant growth inhibitors (Dutta *et al.*, 1993). Baruah *et al.* (1994) found that some chemical compounds isolated from Mexican sunflower leaves by methanol extraction could inhibit seed germination and seedling growth of test plants in petri dish bioassays. However, the phytotoxic activity of water extract from this plant has not been examined. Under natural conditions, phytotoxic compounds would be extracted by rain and by water in the soil. The purposes of the study in this chapter were to test the hypothesis that Mexican sunflower contains some water soluble substance(s) having inhibitory activity on germination and growth of other plant species, and to determine whether the contents of phytotoxic compounds vary between the parts of this plant.

Materials and Methods

Whole plants of six month-old Mexican sunflower (140-160 cm height) grown in a greenhouse were harvested and divided into 4 portions; 1) green leaves (fully expanded including blade and petiole), 2) senescent leaves, 3) stems and 4) roots. Plant materials were air dried, ground with an electric grinder, then passed through a 0.5 mm screen and kept in glass bottles at -20℃ until used. Twenty grams of dry powder was extracted with

1,000 ml of distilled water and shaken at room temperature for 12 hr. The extracts were filtered through a layer of glass fiber in vacuum. The filtrate solutions were considered to be water extracts with the concentration of 20 mg dry matter equivalent (DME)/ml. The initial extracts were diluted with distilled water to concentrations of 15, 10 and 5 mg DME/ml. It is considered that these concentrations are within the range normally used to bioassay the phytotoxic activity of water extract from dry materials of allelopathic plants (An *et al.*, 1997; Martin and Smith, 1994; Mersie and Singh, 1987; Wardle *et al.*, 1996). The pH and electrical conductivity (EC) values of each extract were then measured just before bioassay.

Seventeen plant species were bioassayed; barley (*Hordeum vulgare* L. cv. Kashimamugi), cabbage (*Brassica oleracea* var. *capitata* cv. Irodori F1), cucumber (*Cucumis sativus* L. cv. Shimoshirazujibae), lettuce (*Lactuca sativa* L. cv. Great Lakes 366), mung bean (*Vigna radiata* (L.) R. Wilczek. cv. Blackmap), oat (*Avena sativa* L. cv. Victoria), onion (*Allium cepa* L. cv. Senshukogane 1), radish (*Raphanus sativus* L. cv. Comet), rice (*Oryza sativa* L. cv. Nipponbare), sorghum (*Sorghum bicolor* (L.) Moench. cv. First Sorgo), tomato (*Lycopersicon esculentum* Mill. cv. Big Fukuiyu), wheat (*Triticum aestivum* L. cv. Norin No. 61) Southern crabgrass (*Digitaria* sp.), itchgrass (*Rottboellia exaltata* L.), jointvetch (*Aeschynomene americana* L.), rice flatsedge (*Cyperus iria* L.) and slender amaranth (*Amarathus viridis* L.).

Five ml of water extract from the green leaves at the concentration of 10 and 20 mg DME/ml were separately poured over a sheet of filter paper (Whatman No.1) into 9 cm diameter petri dishes (hereinafter referred to as petri dish bioassay). The same volume of distilled water was used as the control treatment. Fifty seeds of each test species (except 25 seeds for cucumber, 100 seeds each for crabgrass, rice flatsedge and slender amaranth) were grown on the paper. The dishes were placed in an incubator at 25℃ for 4 days in the dark (except for onion, rice, tomato and rice flatsedge which was 7 days). The percentage of seed germination was determined by counting the number of germinated seeds having a coleoptile or radicle of at least 2 mm and compared with the control. Shoot and root lengths were recorded and the percentage of inhibitory effect calculated.

Seven ml of water extract from each plant part at the concentration of 5 mg DME/ml was separately poured into small glass bottles (8 cm in height and 4 cm in diameter) containing 20 grams of sea sand (hereinafter referred as sea sand bioassay). Five uniformly pre-germinated rice seeds having a 1 mm coleoptile and radicle were grown in the sand. The bottles were placed in a growth chamber at 20/25℃ and 12/12 day/night period for 4 days, after which the shoot and root length of seedlings were measured. All treatments were conducted with four replications.

Results and Discussion

Effect of water extract from Mexican sunflower leaves on germination and seedling growth

Plant species differed in their response to the water extract from mature leaves in filter paper bioassay (Table 1.1). The extract at the concentration of 10 mg DME/ml showed significant inhibitory activity on the seed germination of cabbage, oat, onion, tomato, wheat, itchgrass, jointvetch, rice flatsedge, and slender amaranth, while the germination of barley, cucumber, lettuce, mung bean, radish, rice sorghum and crabgrass was not affected. However, at the concentration of 20 mg DME/ml the germination percentage of most plant species except that of cucumber and mung bean was markedly decreased. At this concentration, the germination of rice flatsedge and slender amaranth were completely inhibited, because the whole seeds sank completely and absorbed the inhibitory substance(s) more than larger seed plants, perhaps due to their small seed size.

The water extract at the concentration of 10 mg DME/ml also significantly reduced the shoot and root growth of most plants, but the shoot growth of mung bean and radish and the root growth of barley, cucumber and mung bean were not inhibited. Shoot and root growth of all plant species were more strongly inhibited by the extract at a 20 mg DME/ml concentration.

The result obtained here was in agreement with that reported by Baruah *et al.* (1994), that seed germination or seedling growth of radish, cucumber and onion were reduced by a single treatment of tagitinin A, tagitinin C and hispidulin isolated from Mexican sunflower leaves by methanol extraction. However, in the present study, water extracts from Mexican sunflower parts were directly used as the materials for bioassay without isolation, and the results showed that the inhibitory activity of the water extract was similar to those of the isolated compounds. This suggested that the phytotoxic activity of water extract may be induced by the combined effect of the isolated compounds and/or other water soluble substance(s).

In this study, there was a general trend toward greater inhibitory activity of the water extract on seedling growth than on germination. This was considered that seed germination may not be the primary site for phytotoxic activity. The results demonstrated that the root was more sensitive to the water soluble phytotoxic substance(s) than the shoot. This was probably due to the fact that the roots of the test plant seedlings were in direct contact with the water extract, or it could have been caused by the different physiological susceptibility between shoot and root.

Phytotoxic effect of water extract from each part of Mexican sunflower

The effect of water extract from green leaves, senescent leaves, stems and roots of Mexican sunflower on seedling growth was compared using germinated rice seeds. In the sea sand bioassay, water extract from leaves and stems significantly reduced the shoot and root growth of rice seedlings compared to the extract from roots (Fig.1.1). There was no statistical difference in phytotoxic activity on shoot and root growth among the extracts from green and senescent leaves and stems. These activities were also higher than that of water extracts from roots. Rice shoot elongation was less affected by Mexican sunflower root extract in the concentrations tested (Fig. 1.1). This suggests that the leaves and stem of Mexican sunflower contain a higher amount of water soluble plant growth inhibitor(s) than the roots.

It was reported that extract from fresh and dry leaves of Mexican sunflower contains tagitinin A, tagitinin C and hispidulin, but none of these compounds were found in the stem and root extracts (Dutta *et al.*, 1993). In the present study, however, the extract from both the stems and roots showed inhibitory activity on the root growth of rice seedlings. It might, therefore, be hypothesized that the stem and root of Mexican sunflower also contain some other plant growth inhibitor(s).

Table1.2 shows the pH and EC values of water extracts from Mexican sunflower plant parts at the concentrations of 5, 10, 15 and 20 mg DME/ml. The pH of the extracts was in the range of 6.8 to 8.7. The extracts from roots and stems were neutral while

the extracts from green and senescent leaves were somewhat basic. The EC value of water extract from the stems was higher than those from the leaf extract and root extract, respectively. The EC values decreased as the dilution of the extracts increased. A preliminary experiment showed that shoot and root elongation of rice seedlings was not influenced in the pH range 4.0 to 9.0. The results of another preliminary study using ethylacetate to separate the initial water extract of Mexican sunflower leaves at various concentrations showed that the ethylacetate soluble fraction with low EC value inhibited shoot and root growth of test plants to a similar extent as the initial water extract; whereas the water soluble fraction with high EC value showed little inhibition of seedling growth (data not shown). This suggested that the reduction of seedling growth was affected by the soluble phytotoxic compound(s), and was not due to the influence of the pH or EC of the extracts.

In this study, distilled water was selected to be used as a medium for extraction of the phytotoxic compound(s) from Mexican sunflower plant, because under natural conditions the phytotoxic compounds contained in the plant would be eluted by rain and soil-water. Inderjit and Dakshini, (1995b) suggested that the use of organic solvents should be avoided because there are qualitative and quantitative differences between the compounds extracted in laboratory bioassay and those occurring in natural conditions.

In this chapter, two bioassay methods; the petri dish bioassay and the sea sand bioassay, were used to determine the phytotoxic activity of Mexican sunflower water extracts. The petri dish bioassay may be appropriate for the investigation of the effect of water extracts on seed germination because the paper to which the extract was applied provided a uniform moisture condition. However, the paper might also have absorbed some of the chemicals. The sea sand bioassay may be more suitable for investigation of the effect of water extract on seedling growth, because sea sand helps to support the seedling stand and could scarcely adsorb the chemical compounds.

It was noted that the phytotoxic effect of water extract from Mexican sunflower leaf powder on seedling growth was evident in the reduction of shoot and root elongation. No injurious symptom was observed during the bioassay period. Other plant growth parameters such as fresh weight and dry weight of the treated seedlings were reduced similarly to the seedling length. For practical purposes, therefore, shoot and root length were selected to be used as the growth parameters to determine the phytotoxic activity of water extract from Mexican sunflower in all experiments of this study. In the study on the phytotoxic effect of water extract from each part of the Mexican sunflower plant and in following experiments, rice was selected to be used as the bioassay plant because it is sensitive to the substance(s) contained in the water extract. Rice is also practically easy to

grow and provides better uniform seedling growth compared to other test plant species.

The results in this study demonstrated that water extract from Mexican sunflower leaves as well as stem and root powders contain water soluble phytotoxic substance(s), reducing seed germination and seedling growth of other plants. The degree of phytotoxic activity varied among plant species and depended on the part of plant material used and concentration of the water extract.

Summary

1. Water extract from mature leaves at the concentration of 10 mg/ml showed a slight effect on seed germination but caused a significant reduction in the seedling growth of most of the plants tested. The extract at the concentration of 20 mg DME/ml remarkably inhibited seed germination and seedling growth.
2. The degree of inhibition of plant growth varied depending on the plant species, and root growth was more sensitive than shoot growth.
3. The inhibitory activity of water extract from green leaves, senescent leaves and stems on the growth of rice were similar, and the activity of leaf water extracts were greater than that of the extract from roots.

4. These results suggested that leaves, stems and roots of Mexican sunflower contain some water extractable growth inhibitory substance(s) which could inhibit seed germination and seedling growth of other plants, but the activity and /or the amount of these substance(s) was dependent on the part of Mexican sunflower.

Table 1.1 Effect of water extract from Mexican sunflower leaves on germination and growth of some crops and weeds in filter paper bioassay.

Plant species	Seed germination		Shoot length		Root length	
	10 mg DME/ml	20 mg DME/ml	10 mg DME/ml	20 mg DME/ml	10 mg DME/ml	20 mg DME/ml
	% of control					
Crops						
Barley	93 NS	43 *	25 *	47 *	93 NS	65 *
Cabbage	84 *	43 *	82 *	65 *	39 *	17 *
Cucumber	105 NS	99 NS	82 *	78 *	103 NS	73 *
Lettuce	97 NS	42 *	102 NS	34 *	28 *	9 *
Mung bean	99 NS	100 NS	51 *	35 *	94 NS	78 *
Oat	65 *	34 *	57 *	36 *	45 *	23 *
Onion	62 *	27 *	69 *	15 *	68 *	19 *
Radish	91 NS	15 *	101 NS	28 *	69 *	26 *
Rice	92 NS	75 *	49 *	32 *	42 *	8 *
Sorghum	91 NS	87 *	81 *	75 *	53 *	45 *
Tomato	70 *	17 *	15 *	1 *	19 *	6 *
Wheat	87 *	72 *	53 *	37 *	52 *	38 *
Weeds						
Crabgrass	98 NS	48 *	77 *	32 *	59 *	16 *
Itchgrass	58 *	58 *	35 *	32 *	32 *	26 *
Jointvetch	68 *	68 *	66 *	41 *	61 *	40 *
Rice flatsedge	9 *	1 *	36 *	19 *	27 *	0 *
Slender amaranth	7 *	0 *	58 *	0 *	24 *	0 *

Data are presented as % of the control, NS and * denote nonsignificantly different and significanly different at $p < 0.05$ as determined by LSD between values of control and the treated plant, respectively.

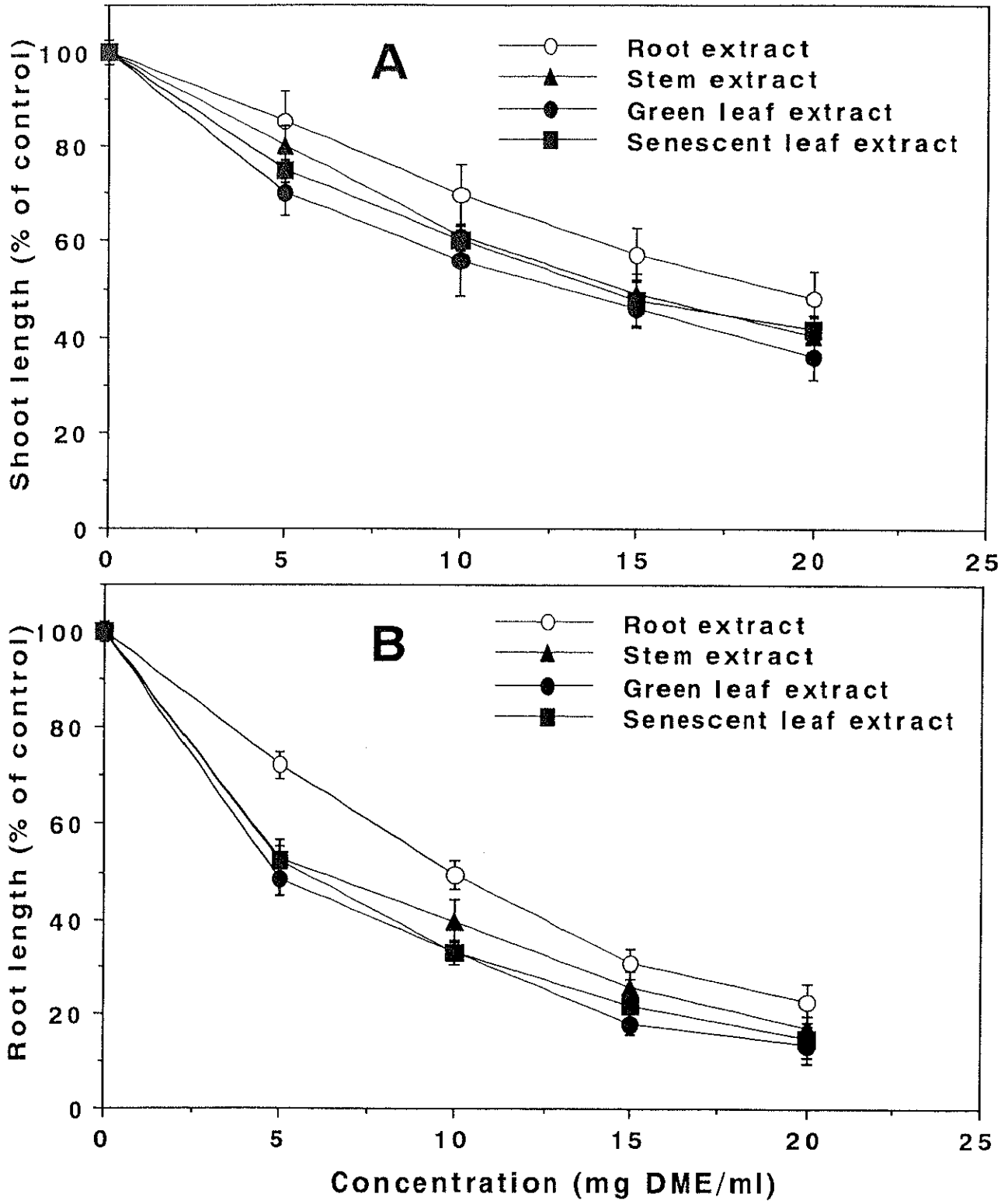

Fig. 1.1 Shoot growth (A) and root growth (B) of rice seedlings in sea sand treated with water extract from roots, stems, green leaves and senescent leaves of Mexican sunflower. Shoot and root length of the control were 23 ± 2 mm and 37 ± 2 mm, respectively.

Table 1.2 The pH and EC values of water extracts from roots, stems and leaves of Mexican sunflower at various concentrations.

Water extract	pH				EC (mS/cm)			
	Concentration (mg DME/ml)				Concentration (mgDME/ml)			
	5	10	15	20	5	10	15	20
Root extract	6.8	6.8	6.9	6.9	0.7	1.5	2.3	3.1
Stem extract	7.2	7.3	7.3	7.3	1.5	3.0	4.4	5.9
Green leaf extract	8.6	8.6	8.7	8.7	1.3	2.3	3.4	4.5
Senescent leaf extract	8.6	8.6	8.6	8.7	1.3	2.6	3.9	5.3