Chapter 2

Phytotoxic activity of Mexican sunflower in soil

Introduction

A very important point in allelopathy is that the chemicals are released through the environment from one organism to another (Rice, 1984, 1987). The presence of phytotoxic substances in the plant tissue or in its extract is not yet a conclusive proof that the plant has allelopathic effect (Einhellig, 1995b). Certain phytotoxic compounds contained in plant which can be easily extracted with some organic solvents may not necessarily leach or exude from the plant; and they may have short residence time in soil or may not accumulate in a bioactive concentration under natural conditions (Inderjit and Dakshini, 1995b; Inderjit and Mallik 1996). Plants in the family Asteraceae such as *Pluchea lanceolata* have been reported to contain several chemical compounds which show phytotoxic activity on the growth of other plants in soil under natural field conditions (Inderjit and Dakshini, 1994a, 1994b; Inderjit *et al.*, 1996). Alternatively, some plants in this family such as *Solidago altissima* L. which contains dehydromatricaria ester, a phytotoxic compound, show inhibitory activity in petri dish bioassay but have little phytotoxic activity in soil (Ito *et al.*,1998). The study in chapter 1 suggested that the leaves, stems and roots of Mexican sunflower contain plant growth inhibitors. Water extract from air-dried leaf powder applied on filter paper could inhibit seed germination and seedling growth of test plant species. In order to prove the allelopathic potential of Mexican sunflower, it is thus necessary to find its phytotoxic activity in soil, as well as the release and persistence of its phytotoxic substances in soil. The objective of the study in this chapter was to investigate the allelopathic potential of Mexican sunflower by determining 1) the phytotoxic activity of soil previously planted with Mexican sunflower, 2) the phytotoxic activity of Mexican sunflower leaf water extract and leaf powder when applied to soil, and 3) the residual phytotoxic activity of water extract and leaf residue after application into soil.

Materials and Methods

Germination and growth of seedlings in soil previously planted with Mexican sunflower

Plastic pots (16 cm in diameter and 19 cm in height) containing 3,400 cm^3 of Kannondai soil [light colored andosol, total carbon 3.5%, total nitrogen 0.2%, cation exchange capacity 21.0 me/100g, clay content 22.3%, maximum water holding capacity (MWHC) 69.8% of dry soil weight] were each planted with a Mexican sunflower seedling (4-leaf stage). Soil from the pots where Mexican sunflower was grown for 6 months was air-dried at room temperature. Forty grams of dry soil was put into 9 cm petri dishes. Twenty eight ml of distilled water was added to reach MWHC. Bioassay was carried out using 50 seeds of radish, rice and sorghum or 100 seeds of crabgrass, rice flatsedge and slender amaranth. Incubation was done in the dark at 25℃ for 7 days (except radish and sorghum for only 4 days). Soils from the non-planted pots were used as control treatments. Seed germinations were recorded, and the shoot and root length of seedlings were measured.

A similar bioassay experiment was conducted by using soil-water separated from planted soil. Soils from the pots planted with Mexican sunflower for 6 months were sieved, and then centrifuged using double centrifugation tubes at 13,000 x g for 40 min to separate the soil-water from the soil (Kobayashi *et al.*, 1994). Five milliliters of soil-water was used to moisten a sheet of filter paper in petri dishes and bioassay was carried out following the same procedures described above. Soil-water separated from the non-planted pots served as the control.

Seedling growth as affected by Mexican sunflower extract applied to soil

Water extract of Mexican sunflower leaves was prepared following the same procedures described in chapter 1. Ten grams of air-dried, sieved Kannondai soil was put into small glass bottles (4 cm in diameter and 8 cm in height) and moistened with 7 ml of the leaf water extract at the concentrations of 5, 10, 15 and 20 mg DME/ml (35-140 mg dry powder/10 g soil). Five uniformly germinated seeds of radish, rice, sorghum, crabgrass, rice flatsedge and slender amaranth were separately grown at 2 mm depth from the soil surface. The bottles were covered with translucent plastic caps and placed in the growth chamber for 4 days. Shoot and root length of the test plants were recorded.

Seedling growth as affected by Mexican sunflower leaf residue mixed into soil

Mexican sunflower leaves or peat moss powder were mixed with air-dried, sieved Kannondai soil at the ratios of 10 and 20 mg/g dry soil. Ten grams of the mixed and non-mixed soil was put into glass bottles and moistened with 7 ml distilled water. Five uniformly germinated rice seeds each having a 1 mm coleoptile were planted and placed in the growth chamber for 4 days. Shoot and root length of the rice seedlings were measured.

Residual phytotoxic activity of Mexican sunflower leaf water extract and leaf residue in soil

To compare the phytotoxic activity of Mexican sunflower extract and leaf residue at different periods after treatment to soil, 10 g of Kannondai soil was treated with 7 ml of water extract from Mexican sunflower leaves at the rate being equivalent to the amount of Mexican sunflower leaf powder 10, 14 and 20 mg/g dry soil. The treated soils were incubated in the dark at 25℃ for 0, 7, 14, 21, 28, 35, 42, 56 and 70 days. Similar treatments were conducted to investigate the residual phytotoxic activity of Mexican sunflower leaf residue at different periods after incorporation into soil. Mexican sunflower leaf powder was mixed with air-dried Kannondai soil at the ratios of 10, 14 and 20 mg/g dry soil. Ten grams of soil mixed with the leaf powder was put into the bottle, moistened with distilled water and incubated in the dark at the same periods as described above. At the end of the incubation period, 5 germinated rice seeds were planted in each bottle and placed in the growth chamber for 4 days. Shoot and root length of rice seedlings were measured and compared.

All experiments were carried out twice with four replications each time. The data were combined and presented as the pooled mean values. The *t* test was used to determine the difference between treatment means in the study of the effect of soil previously planted with Mexican sunflower and of the soil-water on the germination and seedling growth of test plants. In the study of the effect of leaf water extract and leaf residue on seedling growth, data were analyzed by analysis of variance and the Duncan's Multiple Range Test (DMRT) was used to determine the difference among means.

Results and Discussion

Germination and growth of seedlings in soil previously planted with Mexican sunflower

Germination and growth of test plants in soil previously planted with Mexican sunflower is shown in Table 2.1. Seed germination of all test plant species grown in Mexican sunflower planted soil and non-planted soil were similar. Shoot growth of radish, sorghum, crabgrass and slender amaranth were significantly reduced in soil previously planted with Mexican sunflower. Root growth of radish, rice, sorghum and slender amaranth were markedly reduced. This indicated that the soil planted with Mexican sunflower contains some growth inhibitory substance(s) in amounts sufficient to suppress shoot and root growth of the seedlings in the early stage, although these amounts have no inhibitory effect on seed germination. The phytotoxic activity differed among the test plant species.

Shoot growth of most test plants was inhibited by soil-water separated from soil previously planted with Mexican sunflower (Table 2.2). Root growth of all test plants was inhibited. Plant responses observed in this experiment were generally similar to the results of the bioassay using previously planted soil. This suggested that the growth inhibition observed in the soil previously planted with Mexican sunflower was actually induced by the compound(s) present in the soil-water which might be readily absorbed by roots. In this study, shoot growth of sorghum on filter paper applied with soil-water separated from the non-planted soil was limited because of the narrow space in the petri dish. The difference between shoot of sorghum treated with soil-water from the planted and non-planted soil should be greater than that shown in Table 2.2.

Seedling growth as affected by Mexican sunflower extract applied to soil

Shoot and root growth of radish, rice, sorghum, rice flatsedge and slender amaranth were inhibited in the soil treated with water extract from Mexican sunflower leaves (Table 2.3). Shoot and root growth of all test plants decreased with increasing concentration of the extract. Sorghum shoot growth was the most sensitive to the extract, followed by radish, rice and slender amaranth. Root growth of radish, sorghum and rice were more susceptible to Mexican sunflower water extract than those of crabgrass, rice flatsedge and slender amaranth.

The result agreed with that described in chapter 1, in which water extract from Mexican sunflower leaves applied on filter paper has the capacity to reduce seed germination and seedling growth of bioassay plants, and that the inhibitory activity of water extract depended on plant species (Table 1.1). The result also showed that the phytotoxic activity of Mexican sunflower extract was present in the soil and the degree of inhibition was dependent on the plant species. However, the activities of water extracts applied into soil seemed to be less than that in the petri dish bioassay. This suggests the effects of soil factors on the phytotoxic activity of the extract.

Seedling growth as affected by Mexican sunflower leaf residue mixed into soil

The effect of Mexican sunflower leaf and peat moss residue incorporated into soil on the growth of radish, rice, sorghum, crabgrass, rice flatsedge and slender amaranth seedlings is shown in Table 2.4. Peat moss powder at 10 or 20 mg/g dry soil did not inhibit shoot and root growth of test plant seedlings, whereas Mexican sunflower leaf powder at 10 and 20 mg/g dry soil caused a reduction. This demonstrates that the inhibitory effect of Mexican sunflower is induced by phytotoxic substances released from the leaf residue in soil.

Residual phytotoxic activity of Mexican sunflower leaf water extract and leaf residue in soil

The residual phytotoxic activity of Mexican sunflower leaf water extract and leaf powder applied into soil was investigated at various periods of incubation. Shoot and root growth of rice seedlings in soil applied with Mexican sunflower leaf water extract and mixed with the leaf powder were remarkably inhibited in the first 4 weeks of incubation (Fig. 2.1). However, the phytotoxicity decreased with the incubation period elapsed. The reduction of phytotoxicity of water extract was greater than that of the leaf powder residue. This was probably due to the continuous release of phytotoxic compound(s) from the leaf residue into soil during the incubation period, while no phytotoxic substance was additionally released in the water extract treatment. This result suggested that the phytotoxic substance(s) are released from the leaf residue in soil but are influenced by soil factors such as microbial degradation with time. Heisey (1990) reported that the phytotoxicity of *Ailanthus altissima* root bark and leaflets decreased rapidly with time in soil. Shilling *et al.* (1992) found that incubation of celery (*Apium graviolens* L.) root residue in soil at 1% (v/v) for 1 to 4 weeks reduced growth of lettuce (*Lactuca sativa* L.) but there was no inhibition of lettuce growth after an eight week incubation period. They suggested that it was probably due to the activity of soil microorganisms or degradation of phytotoxic compounds. The result obtained here also showed that the phytotoxic activity of Mexican sunflower extract and leaf powder were maintained in the soil for at least 4 weeks, and the performance period of phytotoxic activity was dependent on the amount of water extract or leaf powder application.

Under natural conditions, plants produce several bioactive compounds in the tissue and may release these compounds to surrounding soil through the process of root exudation, water leaching and decomposition of plant residue (Chung and Miller, 1995c). In chapter 1, it was found that leaves, stems and roots of Mexican sunflower contain plant growth inhibitor(s) inhibiting seed germination and seedling growth of other plants. In this study, it has been demonstrated that soil previously planted with Mexican sunflower for 6 months has the ability to inhibit seedling growth of plants tested. This suggested that phytotoxic compound(s) contained in the roots could be released into the soil and accumulate in amounts sufficient to affect the growth of other plants. The residual phytotoxic activity of water extract and the leaf residue in soil roughly mimicked the phytotoxic activity of water leachate from Mexican sunflower leaves and that of plant residue in naturals fields. The finding regarding the release and the residual phytotoxic activity of Mexican sunflower in soil suggests that there exists an allelopathic potential of this plant under natural conditions.

Regarding the shoot and root growth of rice seedlings in sea sand treated with soil-water from the soil previously planted with Mexican sunflower, in the soil applied with Mexican sunflower extract and in the soil mixed with the leaf powder, they were inhibited to a similar extent as those grown directly in the treated soils (Table 2.1, Table 2.2 and Fig. 2.2). This demonstrated that the phytotoxic activity of Mexican sunflower extract and leaf residue in soil was well correlated with that in the soil-water. Little information is known about the relationship between the concentration of allelochemicals in soil-water and their bioactivity in soil. With respect to the activity of herbicide applied to soil, the phytotoxic activity of herbicide is influenced by soil characteristics and the availability of herbicide to the plant is dependent on its concentration in soil-water (Kobayashi *et al.*, 1994, 1996; Nakamura *et al.*, 1996). In the case of plant allelochemicals it has been reported that the phytotoxic activity of dehydromatricaria ester, a phytotoxic compound isolated from *Solidago artissima* L., in soil was related to its concentration in the soil-water but not to the total amount in soil (*Ito et al.*, 1998). In the present study, it was found that the phytotoxic activity in soil caused by Mexican sunflower was related well to the activity of soil-water separated from the soil. It thus suggested that the phytotoxic activity of Mexican sunflower in soil was induced actually by the concentration of active compounds in soil-water.

Summary

1. The soil previously planted with Mexican sunflower for 6 months and its soil-water have the capacity to inhibit shoot and root growth of test plant seedlings.
2. Mexican sunflower leaf water extract at the concentration of 10-20 mg DME/ml applied to soil, and Mexican sunflower leaf powder incorporated in soil at the ratio 10-20 mg/g dry soil, significantly inhibited shoot and root growth of the plants

tested.

3. The phytotoxic activity of leaf water extract and leaf powder applied in soil on the growth of rice seedlings could be maintained in soil for several weeks depending on the application rate.
4. The dynamic pattern of the phytotoxic activity of soil treated with Mexican sunflower extract or leaf powder was well correlated with that in soil-water.
5. These results suggested that Mexican sunflower could release phytotoxic compound(s) to soil and may have phytotoxic activity under natural conditions.

Table 2.1 Seed germination and seedling growth of test plant species grown in soils collected from the pots planted and non-planted with Mexican sunflower.

Plant species	Seed germination		Shoot length		Root length	
	Planted soil	Non-planted soil	Planted soil	Non-planted soil	Planted soil	Non-planted soil
	%		mm			
Radish	92 NS	96	50 *	57	41 *	60
Rice	92 NS	96	24 NS	27	55 *	88
Sorghum	89 NS	93	40 *	81	38 *	74
Crabgrass	56 NS	58	30 *	34	7 NS	8
Rice flatsedge	68 NS	63	16 NS	18	7 NS	9
Slender amaranth	57 NS	59	13 *	18	6 *	9

An asterisk (*) represents means in planted soil and non-planted soil are different according to paired *t* test at $p = 0.05$; NS = nonsignificantly different.

Table 2.2 Seed germination and seedling growth of test plant species grown on filter paper moistened with soil-water separated from the soil planted and non-planted with Mexican sunflower.

Plant species	Seed germination		Shoot length		Root length	
	Planted soil	Non-planted soil	Planted soil	Non-planted soil	Planted soil	Non-planted soil
	%		mm			
Radish	81 NS	79	40 *	44	41 *	52
Rice	94 NS	93	23 NS	25	28 *	39
Sorghum	86 NS	87	82 *	98	57 *	71
Crabgrass	40 NS	43	25 *	47	3 *	13
Rice flatsedge	69 NS	71	6 *	11	1 *	7
Slender amaranth	46 NS	45	19 *	31	6 *	14

An asterisk (*) represents means in planted soil and non-planted soil are different according to paired *t* test at $p = 0.05$; NS = nonsignificantly different.

Table 2.3 Shoot and root growth of test plant species in soil applied with water extract from Mexican sunflower leaves.

Plant species	Concentration (mg DME/ml)				
	0	5	10	15	20
	Shoot length (mm)				
Radish	20 a	17 ab	15 b	12 b	12 b
Rice	28 a	27 ab	26 abc	22 bc	21 c
Sorghum	55 a	39 b	34 b	28 b	28 b
Crabgrass	17 a	17 a	16 a	16 a	14 a
Rice flatsedge	15 a	13 b	13 b	12 b	12 b
Slender amaranth	24 a	22 ab	21 b	18 c	16 d
	Root length (mm)				
Radish	78 a	62 b	42 c	32 c	29 c
Rice	62 a	57 b	45 c	31 d	24 e
Sorghum	79 a	53 b	44 bc	42 bc	33 c
Crabgrass	22 a	20 a	18 ab	15 b	15 b
Rice flatsedge	13 a	9 b	7 c	7 cd	6 d
Slender amaranth	14 a	13 a	12 ab	11 b	10 b

Means in a row followed by the same letter are not significantly different at $p < 0.05$ as determined by Duncan's Mutiple Range Test.

Table 2.4 Shoot and root growth of test plant species in soil mixed with Mexican sunflower leaf and peat moss powders.

Plant species	Nonmixed soil	Peat moss (mg/g dry soil)		Mexican sunflower (mg/g dry soil)	
		10	20	10	20
	Shoot length (mm)				
Radish	20 a	21 a	20 a	19 a	15 b
Rice	34 a	34 a	34 a	26 b	18 c
Sorghum	55 a	55 a	55 a	35 b	33 c
Crabgrass	17 a	18 a	18 a	16 b	14 bc
Rice flatsedge	16 a	16 a	16 a	14 b	12 c
Slender amaranth	25 a	25 a	25 a	18 b	16 c
	Root length (mm)				
Radish	80 a	80 a	80 a	63 b	31 c
Rice	69 a	68 a	67 a	36 b	28 c
Sorghum	76 a	76 a	77 a	46 b	35 c
Crabgrass	23 a	23 a	24 a	19 b	15 c
Rice flatsedge	14 a	14 a	14 a	11 b	7 c
Slender amaranth	16 a	16 a	16 a	13 b	11 c

Means in a row followed by the same letter are not significantly different at $p < 0.05$ as determined by Duncan's Mutiple Range Test.

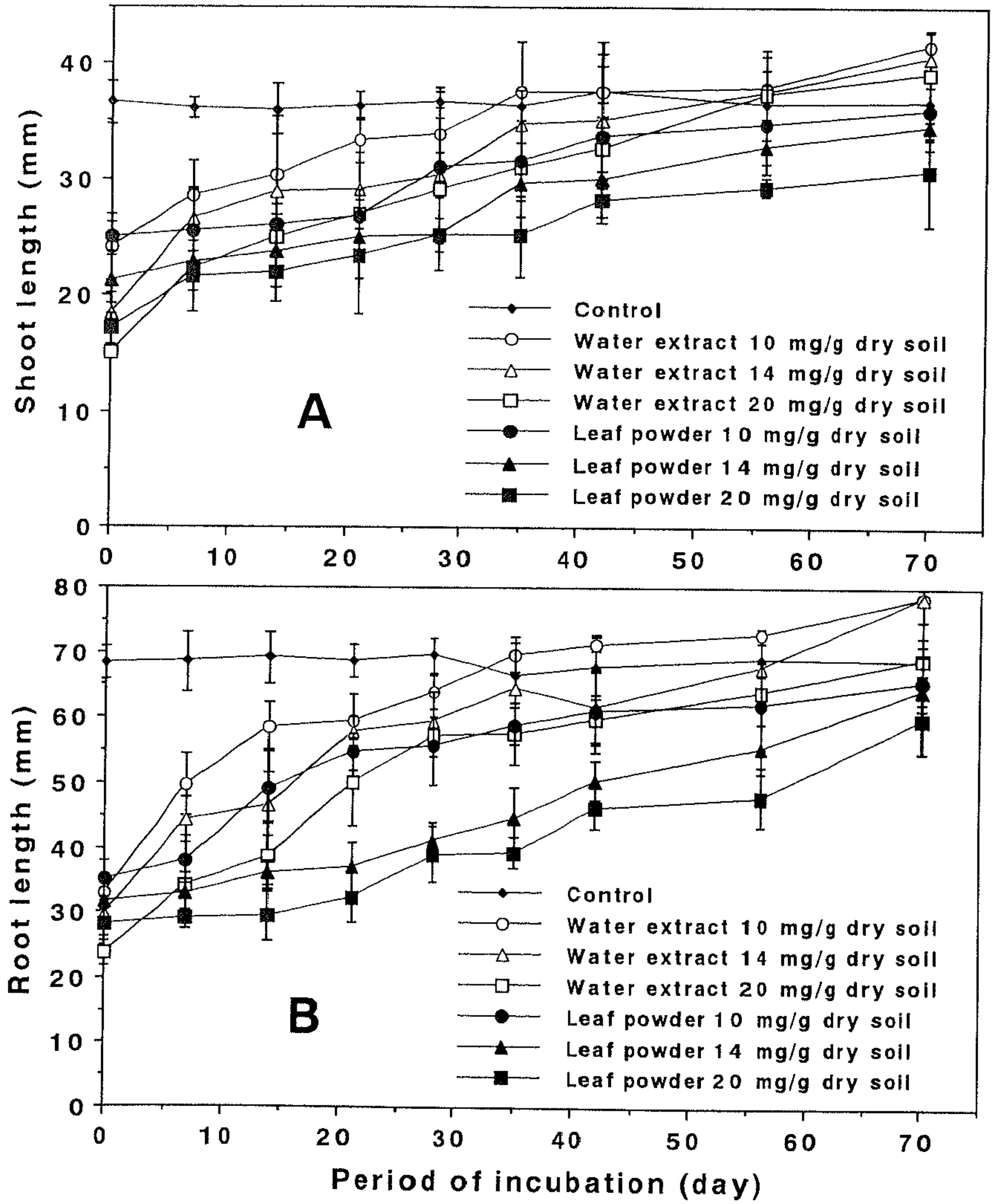

Fig. 2.1 Shoot growth (A) and root growth (B) of rice seedlings in soil applied with water extract from Mexican sunflower leaves and mixed with Mexican sunflower leaf powder at different period of incubation.

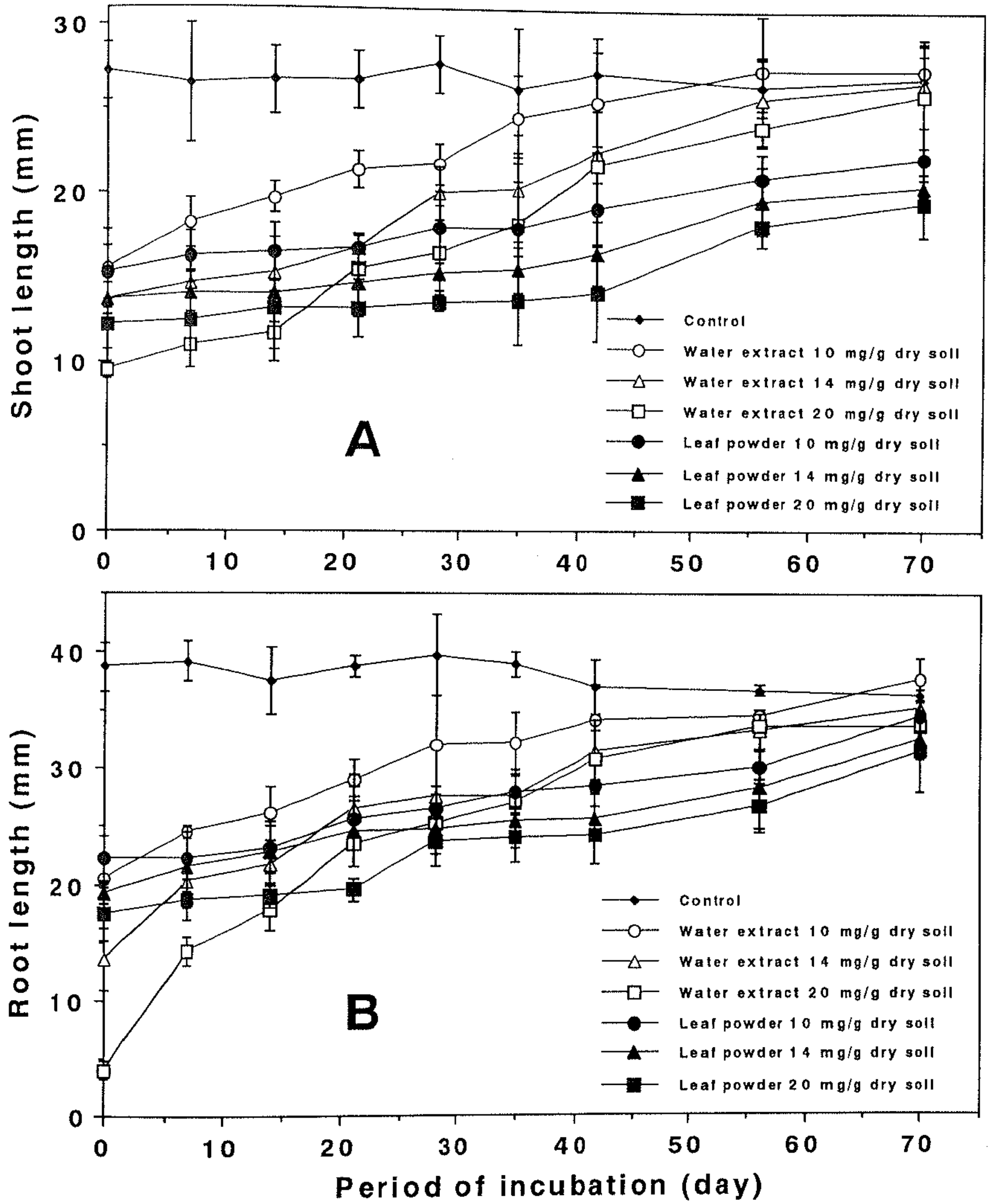

Fig. 2.2 Shoot growth (A) and root growth (B) of rice seedlings in sea sand applied with soil-water separated from the soil previously applied with water extract from Mexican sunflower leaves and mixed with Mexican sunflower leaf powder at different period of incubation.