Chapter 3

Effect of soil factors on phytotoxic activity of water extract from Mexican sunflower leaves

Introduction

Once the allelochemicals have been produced and released into the environment, they are immediately influenced either by biotic and abiotic factors (Rice, 1984). These factors may have direct or indirect, positive or negative effects on viability and activity of allelochemicals (Inderjit and Dakshini, 1995b). Many documents have reported that soil factors such as soil texture, organic matter, clay content and soil microorganisms affect quantitative and qualitative availability of plant allelochemicals in soil (Blum, 1995; Cheng, 1995; Dalton *et al.*, 1983; Inderjit, 1996b; Inderjit and Dakshini, 1994a; Teasdale, 1993; Yun and Kil, 1992; Zhang, 1993). The study in chapter 2 demonstrated that soil applied with Mexican sunflower leaf water extract and with leaf powder have phytotoxic activity on the growth of test plants. The phytotoxic activity of the leaf powder and water extract of Mexican sunflower in soil decreased with time, suggesting that this decrease may be due to the effect of soil factors. Therefore, it is necessary to clarify the effects of soil factors on the phytotoxic activity of Mexican sunflower in soil. The objective of this study was to determine the effect of soil type, soil absorption and soil microorganisms on the phytotoxic activity of water extract from Mexican sunflower leaf powder applied to soil.

Materials and Methods

Phytotoxic activity of Mexican sunflower leaf extract in three different soils

To determine if soil type affects the phytotoxic activity of Mexican sunflower leaf water extract, the phytotoxic activity of water extract from Mexican sunflower leaf powder at various concentrations was examined on rice seedling growth in three selected soils, namely Yawara soil, Kannondai soil and Ryugasaki soil. These soils are of different texture, organic matter and clay content and their physical and chemical characteristics are shown in Table 3.1. The water extract from Mexican sunflower leaf powder at the concentrations of 0, 5, 10, 15 and 20 mg DME/ml were prepared as described in chapter 1. Ten grams of air-dried, sieved Yawara soil, Kannondai soil and Ryugasaki soils were separately put in glass bottles (8 cm in height and 4 cm in diameter) and moistened with 7 ml of the leaf extract. Five uniformly germinated rice seeds were grown in each bottle and allowed to grow in the growth chamber at 20/25℃ and 12/12 hr day/night period for 4 days. Shoot and root length of the seedlings were measured.

Effect of soil microorganisms and soil adsorption on phytotoxic activity of Mexican sunflower leaf extract

To determine whether or not soil reduces the phytotoxic activity of Mexican sunflower leaf extract, the phytotoxic activity of water extract from Mexican sunflower leaf powder at concentrations ranging from 0 to 20 mg DME/ml on the growth of rice seedling was compared with the phytotoxic activity of the soil-water separated from the soil previously applied with water extract at the same concentration. Seventy milliliters of the water extract of each concentration was mixed with 100 grams of air-dried Kannondai soil and the soil-water was immediately separated from the treated soils by centrifugation (Kobayashi *et al.*, 1994). Seven milliliters of each soil-water was separately poured into glass bottles containing 20 grams of sea sand and bioassayed with five germinated rice seeds as the same procedure described in chapter 1.

To determine the effect of soil microorganisms on the phytotoxic activity of water extract from Mexican sunflower leaves, the phytotoxic activity of the sterilized water extract on the growth of rice seedlings in autoclaved and non-autoclaved soils were compared. The sterilized water extract used for bioassay was prepared by passing the initial water extract from Mexican sunflower leaf powder at the concentration of 20 mg DME/ml, through the sterile filter membrane (Millipore 0.45 μm) and then diluted to the concentrations of 0, 5, 10, 15 and 20 mg

DME/ml with autoclaved distilled water. Forty glass bottles were filled with 10 grams of air-dried, sieved Kannondia soil. Twenty of them were autoclaved three times for 20 min at 120℃ at 24 hr intervals. Both autoclaved and non-autoclaved soils were treated with 7 ml of the filter-sterilized extracts at the desired concentrations and bioassayed with germinated rice seeds in the growth chamber for 4 days. Shoot and root length of the rice seedlings were measured.

To determine the effect of soil adsorption on the phytotoxic activity of the extract, an additional treatment using autoclaved sea sand as the culture medium was included. Twenty grams of sea sand was placed in glass bottles and autoclaved three times. Seven milliliters of the sterilized extract was applied to each of the bottles containing sea sand and germinated rice seeds were bioassayed as described above.

All experiments were carried out twice with four replications each time.

Results and Discussion

Phytotoxic activity of Mexican sunflower leaf extract in three different soils

Shoot and root growth of rice seedlings grown in the soils treated with water extract from Mexican sunflower leaves are shown in Fig. 3.1. Shoot and root growth of rice seedlings were inhibited by the extracts applied into soils. The phytotoxic activity increased with increasing concentration of the extract but the degree of inhibition differed among the three soil types. The highest inhibitory effect on growth was observed in Ryugasaki soil, followed by that in Kannondai soil, and the lowest was in Yawara soil. This demonstrated that the phytotoxic activity of Mexican sunflower extract varied among the different soil types. The difference in the phytotoxicity of Mexican sunflower leaf water extract in the soils may relate to their physicochemical properties. It was reported that clay particles and organic matter were able to adsorb plant allelochemicals (Inderjit, 1996b). Blum *et al.*, (1993) reported that the concentration of phenolic allelochemicals was correlated with soil pH, soil moisture, total soil carbon and total soil nitrogen. Dalton *et al.*, (1983) reported that the adsorption of phenolic compounds in soil increased with increasing soil organic matter. Yawara soil and Kannondai soil have more clay and organic matter content than that of Ryugasaki soil (Table 3.1). The lesser phytotoxic activity of Mexican sunflower leaf water extract in Yawara soil and Kannondai soil compared to that of Ryugasaki soil indicated that the amount of available phytotoxic compound(s) in Yawara soil and Kannondai soil was lower than that in Ryugasaki soil. This suggests that phytotoxic substance(s) in the applied soil were partially adsorbed and the degree of adsorption may be dependent on the amount of clay and/or the organic matter content in the soil.

Effect of soil microorganisms and soil adsorption on phytotoxic activity of Mexican sunflower leaf extract

The phytotoxic activity of Mexican sunflower leaf water extract and of soil-water separated from the applied soil on the growth of rice seedlings were compared (Fig. 3.2). Shoot and root growth of rice seedlings in sea sand applied with the water extract and with the soil-water were inhibited, but the degree of inhibition in the soil-water was less than those by the initial water extract treatments. This demonstrated that the phytotoxic activity of Mexican sunflower water extract decreased when the extract was applied to the soil. The reduced phytotoxic activity of water extract in soil was probably due to the adsorption of phytotoxic compound(s) onto the soil particles. As a result, the concentration of the phytotoxic compounds in soil-water might be less than that of the initial water extract.

To ascertain the effect of soil microorganisms and soil adsorption on the phytotoxic activity of Mexican sunflower leaf water extract, rice was grown in autoclaved and non-autoclaved soils and autoclaved sea sand treated with sterilized leaf water extract at various concentrations. Shoot and root elongation of rice seedlings in autoclaved soil were more inhibited than in non-autoclaved soil (Fig. 3.3). This suggested that microorganisms in non-autoclaved soil degraded the phytotoxic compound(s), resulting in less phytotoxicity. Shoot and root growth was less inhibited in autoclaved soil than in autoclaved sand, suggesting that soil adsorbed the phytotoxic substance(s).

Several studies on allelopathy have demonstrated that the phytotoxicity of plant allelochemicals is affected by soil factors (Inderjit and Dakshini, 1994b; Teasdale, 1993, Yun and Kil, 1992, Zhang, 1993). Inderjit and Dakshini (1994a) reported that phytotoxic activity of water extract from *Pluchea lancelolata* leaves on shoot and root growth of mustard and tomato seedlings varied in four different soils. The phytotoxic activity of the extract in sandy soil and sandy loam soil was greater than that in silt loam soil and clay loam soil. In the present study, it was found that the phytotoxic activity of Mexican sunflower extract in light clay soil, clay loam soil and sandy loam soil differed (Fig. 3.1). The phytotoxic activity is likely to decrease with increasing organic matter, clay content and CEC in the soil (Table 3.1). The result in Fig. 3.2 demonstrated that the inhibitory activity of the soil-water separated from the soil applied with Mexican sunflower extract on rice seedling growth was less than that in the sea sand applied with water extract. In addition, the inhibitory activity on the rice growth in autoclaved soil was also less than that in autoclaved sea sand (Fig. 3.3). These results suggested that a considerable amount of phytotoxic compound(s) from Mexican sunflower water extract was adsorbed in soil.

Microorganisms have been considered to be an important factor affecting allelopathic activity in soil (Mason-Sedun and Jessop, 1988; May and Ash,1990). Blum (1998) reported that soil microorganisms degraded and utilized some allelochemicals in soil. In contrast, some allelochemicals can be produced by microorganisms during the decomposition of plant residue in soil (Hegde and Miller, 1990; Hoffman *et al.*, 1996a, 1996b; Ismail and Mah, 1993; Jimenez-Osornio and Gliessman, 1987; Kitou and Yoshida, 1993; Martin and Smith, 1994). The study in chapter 2 showed that the phytotoxic activity of Mexican sunflower water extract applied in soil decreased with time (Fig. 2.1). In this chapter, it was found that phytotoxic activity of the extract in non-autoclaved soil was less than that in autoclaved soil (Fig. 3.3). These results suggested that soil microorganisms degrade the phytotoxic substance(s) in Mexican sunflower water leaf extract in soil, resulting in a reduction of the phytotoxic activity. These investigations indicated that soil factors such as physicochemical soil properties and soil microorganisms affect the allelopathic activity of Mexican sunflower in soil.

Summary

1. The inhibitory activity of Mexican sunflower water extract on the growth of rice seedlings varied among the soil types.
2. The phytotoxic activity of Mexican sunflower extract was reduced in soil.

3. The phytotoxic effect of Mexican sunflower extract on rice seedlings grown in non-autoclaved soil was less than in autoclaved soil, and the inhibitory effect to rice seedling grown in autoclaved soil was less than in autoclaved sand.
4. These results suggested that the decrease in the allelopathic activity of Mexican sunflower extract in soil is due to soil adsorption and microbial degradation.

Table 3.1 Physical and chemical characteristics of Yawara soil, Kannondai soil and Ryugasaki soil.

Soil	Texture	Sand	Silt	Clay	Total C	Total N	C/N	pH	CEC
		(%)	(%)	(%)	(%)	(%)			(me/100g)
Yawara	Light clay	43.9	25.4	30.3	2.7	0.2	12.4	5.6	16.0
Kannondai	Clay loam	44.0	33.7	22.3	3.5	0.2	14.3	5.6	21.0
Ryugasaki	Sandy loam	75.7	17.2	7.1	0.6	0.1	14.6	5.9	9.1

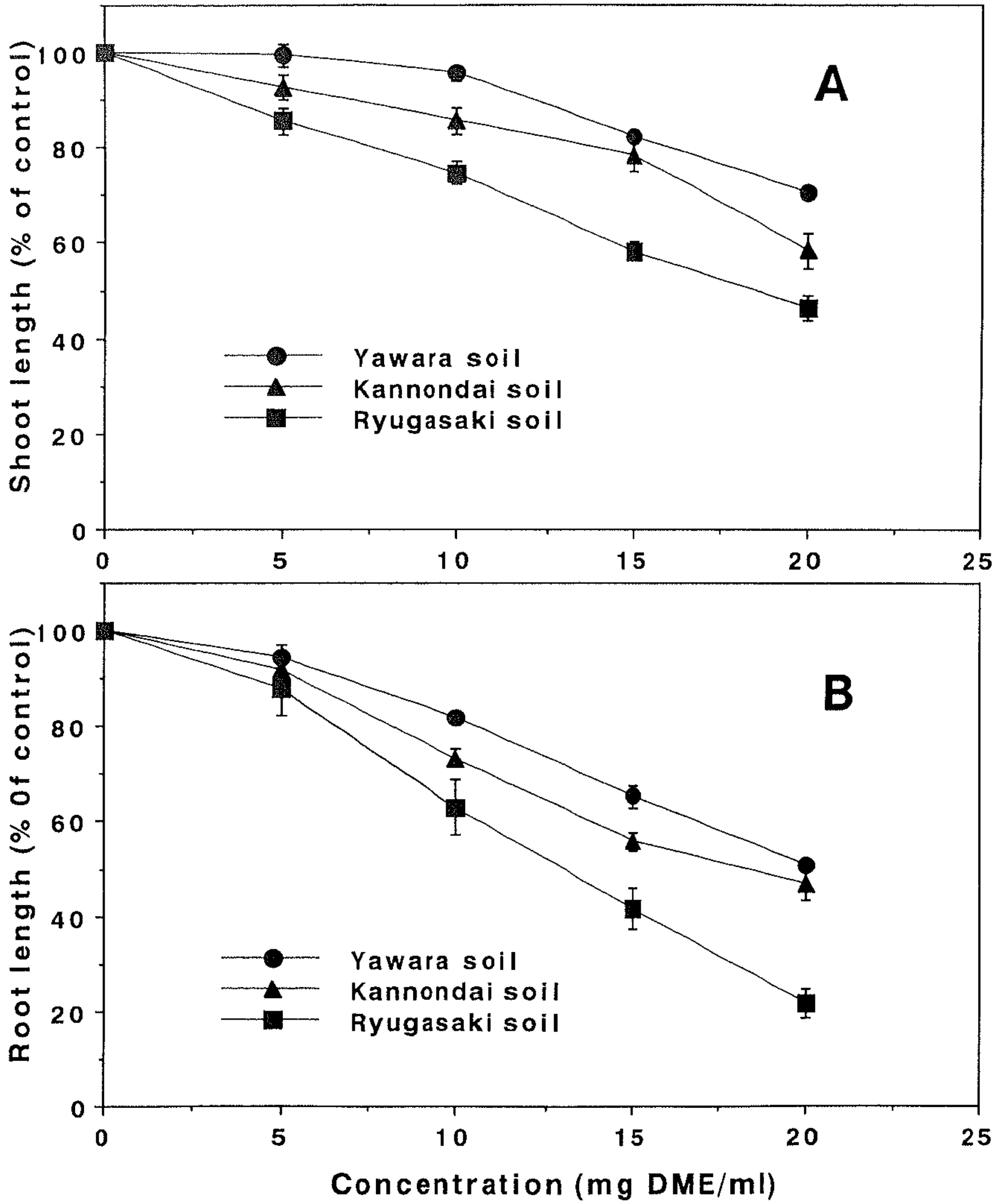

Fig. 3.1 Shoot growth (A) and root growth (B) of rice seedlings in Yawara soil, Kannondai soil and Ryugasaki soil applied with water extract from Mexican sunflower leaves. Shoot length of the control seedlings in Yawara soil, Kannondai soil and Ryugasaki soil were 40±3, 39±3 and 39±3 mm, respectively. Root length of the control seedlings in Yawara soil, Kannondai soil and Ryugasaki soil were 79±4, 67±5 and 66±6 mm, respectively.

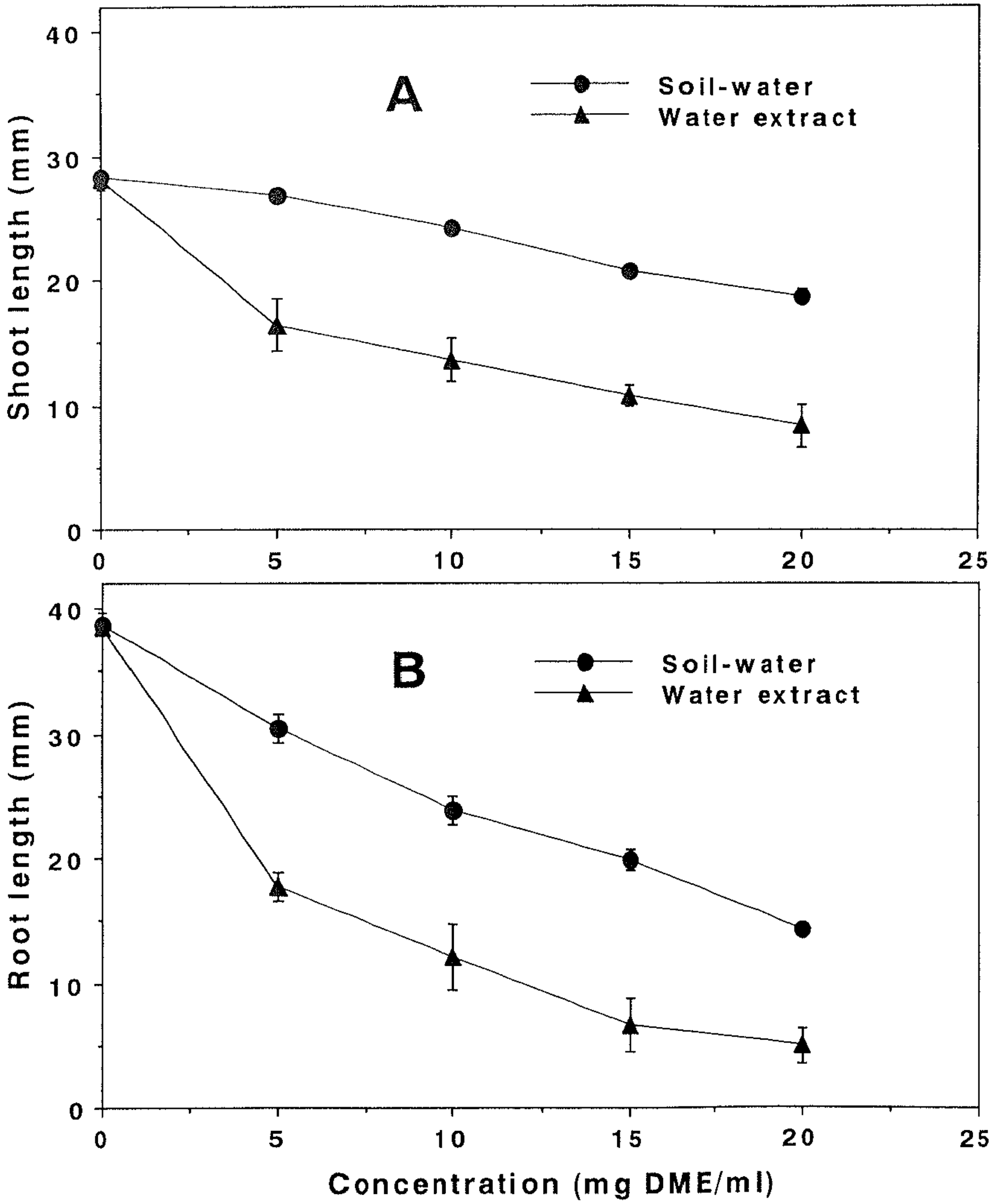

Fig. 3.2 Shoot growth (A) and root growth (B) of rice seedlings in sea sand treated with water extract from Mexican sunflower leaves and soil-water separated from the soil previously applied with the extract.

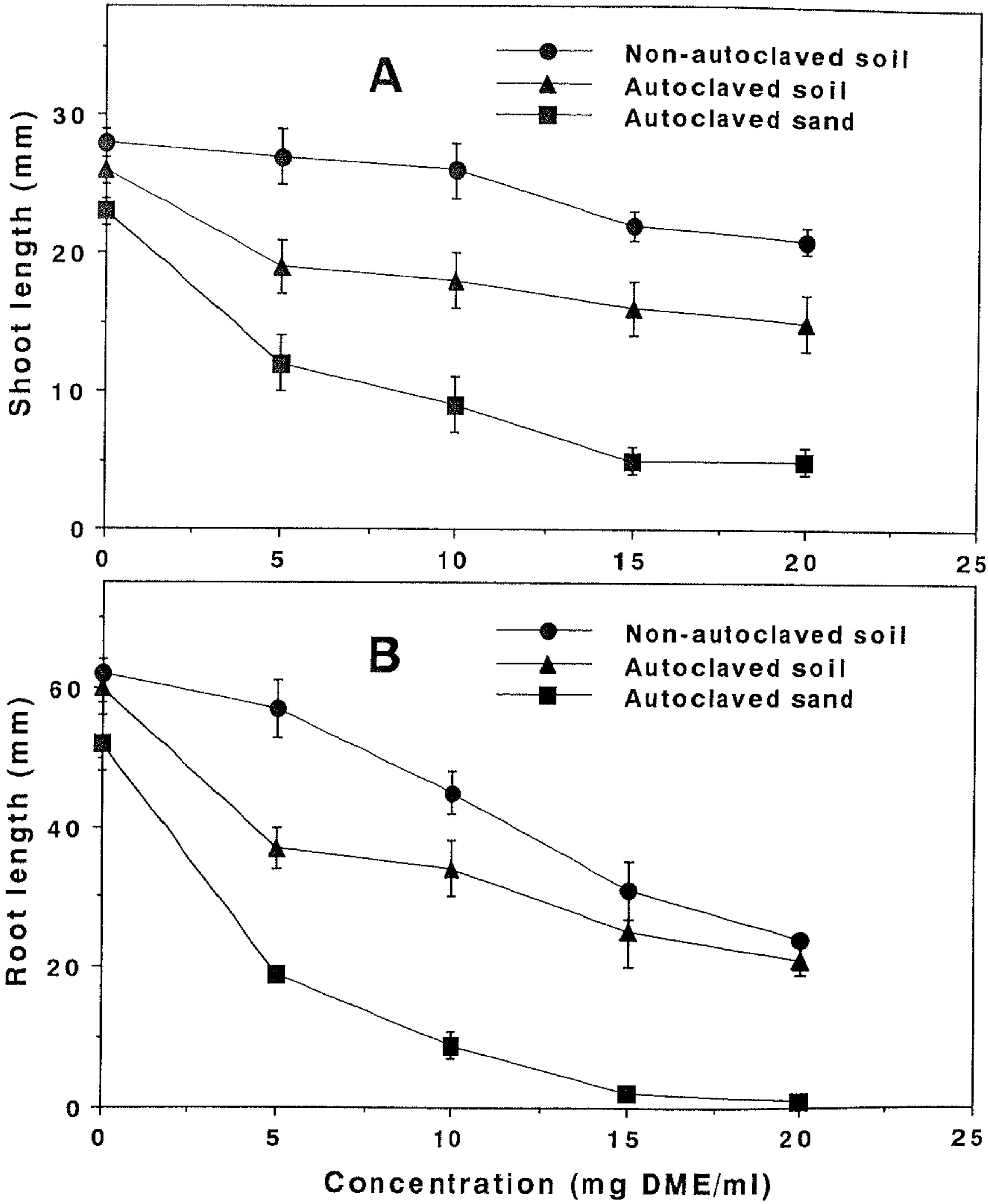

Fig. 3.3 Shoot growth (A) and root growth (B) of rice seedlings in non- autoclaved and autoclaved soil and in autoclaved sand treated with water extract from Mexican sunflower leaves.