Chapter 4

Phytotoxic activity of water leachate from Mexican sunflower leaves in soil

Introduction

Allelochemicals may be released from plant to the environment in many ways including volatilization, root exudation, water leaching and decomposition of plant residues (Putnam, 1985; Rice, 1984, 1987; Chung and Miller, 1995c). However, it is believed that most allelopathic substances are released into soil through water leaching directly by rain and by dissolution of the plant residues in soil (Nelson, 1996). The results in chapter 1 and chapter 2 demonstrated that water extract from both the green and the senescent leaf powders of Mexican sunflower could inhibit plant seedling growth. Soil applied with water extract from Mexican sunflower leaf powder and soil incorporated with Mexican sunflower leaf powder had a phytotoxic effect on the growth of the plants tested. These results suggested that Mexican sunflower leaves contain plant growth inhibitor(s) and these compounds could be released from the leaf residue to the soil. Under natural conditions, Mexican sunflower has the capacity to produce a lot of living and senescent leaves due to its fast growing character and its perennial life cycle

(Ayeni *et al.*, 1997a, 1997b). The phytotoxic substance(s) contained in the leaves might possibly be washed out by rain water. The objective of this study was to investigate the phytotoxic activity of water leachates from Mexican sunflower leaves by water spray in order to obtain basic information about the release of phytotoxic substance(s) from Mexican sunflower leaves under natural conditions.

Materials and Methods

Phytotoxic activity of Mexican sunflower leaf leachates

The green and senescent leaves of six-month old Mexican sunflower grown in a greenhouse were harvested and air-dried at room temperature. The green and senescent leaves were separately spread on a stainless screen (215 mm x 150 mm) at the rates of 50, 100, 150, 200, 250 and 300 g/m^2 . The screen was placed over a collecting plastic box (215 mm x 150 mm x 50 mm) containing 330 g of air-dried sieved Kannondai soil. The leaves were gently hand-sprayed with 200 ml of distilled water, being equivalent to a 6 mm rainfall. The leached water passing through the screen was allowed to gravitationally drip onto the soil in the box for 30 min. Soil moisture content in each box was adjusted to maximum water holding capacity (MWHC) with distilled water by sub-irrigation. Thirty uniformly germinated rice seeds, having 1 mm coleoptile were planted in each box and allowed to grow in a

growth chamber at 25/20℃, 12/12 hr, day/night period for 4 days. Shoot and root length of the rice seedlings were measured and compared to the seedlings grown in the control soil.

To determine the effect of the amount of rainfall on the phytotoxicity of Mexican sunflower leaf leachate, 6.67 grams of air-dried green or senescent leaves placed on the screen was sprayed with 50,100,150, 200 and 250 ml of distilled water, the amounts being equivalent to 1.5, 3.0, 4.5, 6.0 and 7.5 mm of rainfall, respectively. After the leachates had completely dripped into the soil boxes, soil moisture content in each box was adjusted to MWHC. The treated soils were bioassayed with germinated rice seeds as described above. Shoot and root length of the rice seedlings were measured and compared to the seedlings in the control soil.

To determine the effect of the portion of leaf water leachate in water spray on the phytotoxic activity of Mexican sunflower leaf leachate, 6.67 grams of green or senescent leaves was sprayed with 50 ml each of distilled water for 7 times. Each leachate passing through the screen was separately collected and bioassayed with germinated rice seeds in soil. Ten grams of air-dried Kannondai soil was put into small glass bottles (8 cm in height and 4 cm in diameter) and filled with 7 ml of the leachate. Five germinated rice seeds were planted in each bottle and allowed to grow in a growth chamber for 4 days. Shoot and root lengths of the rice seedlings were measured. All experiments

were carried out twice with three replications each time.

Results and Discussion

Phytotoxic activity of Mexican sunflower leaf leachates

Shoot and root growth of the rice seedlings in soil applied with water leachates from green and senescent leaves of Mexican sunflower at different spray volumes is shown in Fig. 4.1. Both green and senescent leaf leachates inhibited rice root growth. The green leaf leachate showed a slightly higher inhibitory effect than the senescent leaf leachate. Rice root growth decreased with increasing amounts of leaves. Shoot growth of the rice seedlings was also inhibited but the degree of inhibition on shoot growth was less than on root growth. This result indicated that the phytotoxic compound(s) contained in both green and senescent leaves of Mexican sunflower could be eluted by water spraying.

The response of rice seedlings to water leachate obtained from various spray volumes differed (Fig. 4.2). Spraying water at the rate of 50 ml onto 6.67 grams of green Mexican sunflower leaves showed a slight inhibitory effect on shoot and root growth. The highest phytotoxicity of leachate was found at the spray volume of 150 ml. The inhibitory activity on rice seedling growth declined in larger spray volumes. The similar dynamic pattern of rice response was found in the senescent leaf leachate treatments. This demonstrated that plant growth inhibitory substance(s) in

Mexican sunflower leaves could easily be washed out by water spray, and that they require a sufficient amount of water to dissolve and be released into the environment. However, too much leaching water may lead to dilution of the phytotoxic compound(s) after the washing out .

The phytotoxic effect of leachates by intermittent spraying of 50 ml water on the growth of rice seedlings is shown in Fig. 4.3. All leachates from both green and senescent leaves inhibited rice root growth but the degree of inhibition varied among the leachates. The second leachate from green leaves showed the greatest inhibition. The phytotoxic activity declined in the following leachates. From the senescent leaves, the second and third leachates inhibited rice seedling growth more remarkably than the first leachate and the phytotoxic activity decreased in the following water spray. Similar patterns of rice shoot growth in response to each water leachate were also observed but the degree of inhibition was less than that which occurred on the root growth. This was well correlated with the results that the spray volume of 150-200 ml provided the highest phytotoxic leachate (Fig. 4.2). In this study, the phytotoxic activity of senescent leaf leachate seemed to be lower than that of the green leaf leachate. This is probably due to the difference in water absorption and/or content of the water-extractable phytotoxic substances(s) between the senescent and the green leaves. At the same amounts of leaves and water spray, the senescent leaves absorbed

more volume of sprayed water compared to the green leaves, therefore, the final volume of leachate, which gravitationally dripped into the collecting box, from the senescent leaves was slightly less than that from the green leaves. The lesser volume of the senescent leaf leachate may be responsible for the lower phytotoxic activity observed in this bioassay.

The living and senescent leaves are the major parts of Mexican sunflower plant which contain and release the phytotoxic compound(s) into the soil compared to the root and stem as described in chapter 1. In the preliminary study, it was found that water leachate from both fresh and air-dried green leaves of the plant at the same dry weight inhibited rice seedling growth to a similar extent. In this study, the air-dried green mature and senescent leaves were used as the material for study in order to compare the phytotoxic activity of the leachates from mature and senescent leaves by water spray. In chapter 1, the water extract from dry leaf powder at the concentrations of 10 and 20 mg DME/ml applied into Kannondai soil reduced rice root growth to 60 % and 39 % of the control, respectively. In the present study, water leachate in the 300 g/m^2 treatment, a concentration being equivalent to 100 mg DME/ml of spray water, inhibited rice root growth to about 60 % of the control. It could be assumed that phytotoxic substances in Mexican sunflower leaves might be eluted by water spraying at one-tenth the concentration of the water extract from the dry leaf powder by the conventional

shaking method. The extracting rate of the phytotoxic compounds by water spray may depend on the differences in their water solubility.

Water sprayed at the rates of 50, 100, 150, 200, and 250 ml on the 215 mm x 150 mm screen are equivalent to 1.5, 3.0, 4.5, 6.0 and 7.5 mm of rainfall, respectively. The water leachates obtained from these spray volumes showed phytotoxic activity on seedling growth, suggesting that phytotoxic substance(s) in both green and senescent leaves could easily be washed out by rain water. The spray volume of 150-200 ml, being equivalent to 4.5-6.0 mm rainfall, could extract some phytotoxic compound(s) from Mexican sunflower leaves in sufficient amounts to reduce the root growth of plant grown in the soil. The spray volumes over 250 ml (more than 7.5 mm rainfall) might result in the dilution of phytotoxin(s) in the leachate which may lead to reduced phytotoxicity. This was supported by the observations that the second and third leachates from intermittent spraying of 50 ml water had the highest inhibitory activity and the phytotoxicity began to reduce in the following leachates (Fig. 4.3).

Under natural field conditions, during 5 months of rainy season in Mexican sunflower infested areas in the northern part of Thailand, it was found that this plant is able to produce green and senescent leaves that are about 80-300 and 25-150 grams in dry weight per m^2, respectively. The range depended on age, plant density and soil fertility. The average rainfall was 950 mm

with 32 rainy days in the season. Very few other plant species were observed under the Mexican sunflower canopy, except for *Eupatorium odoratum*, *Mimosa invisa* and *Imperata cylindrica*. It suggested that leaf leachate obtained by these amounts of rain from Mexican sunflower leaves seemed to be sufficient to suppress seed germination and seedling growth of other plants under natural conditions.

The present study suggested that phytotoxic substance(s) in Mexican sunflower leaves could be washed out from both living and dead leaves by rain water. With an appropriate amount of leaves and rainfall, the phytotoxic compound(s) should be released to a sufficient concentration leading to inhibit the growth of other plant species under natural field conditions. It can be assumed that the phytotoxic compounds are continually accumulated in the soil, brought about by sufficient amounts of rainfall and the biomass of Mexican sunflower and by exudation from the roots.

Summary

1) The water leachates from air-dried green and senescent leaves of Mexican sunflower obtained by water spray have inhibitory activity on the growth of rice seedlings in soil.
2) The phytotoxic activity of the leaf water leachates was dependent on both the amount of Mexican sunflower leaves

and the amount of water spray.

3) These results suggested that phytotoxic substance(s) in the green and the senescent leaves of Mexican sunflower could be eluted by rain and have a potential to inhibit the growth of other plant seedlings in soil.

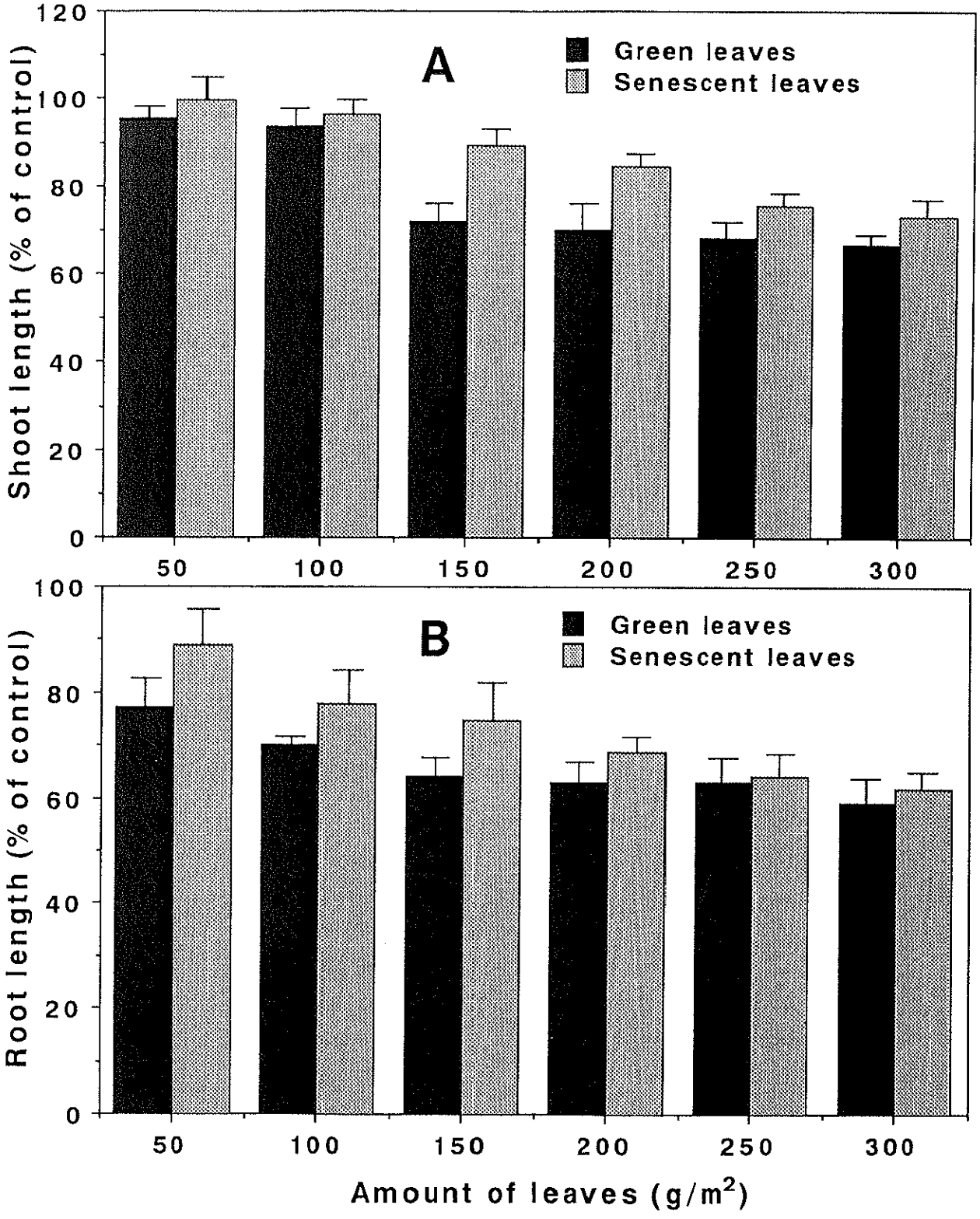

Fig. 4.1 Shoot growth (A) and root growth (B) of rice seedlings in soil applied with the leachates from various amounts of green and senescent Mexican sunflower leaves by water spray. Shoot and root length of the control were 40 ±2 mm and 79 ±4 mm, respectively.

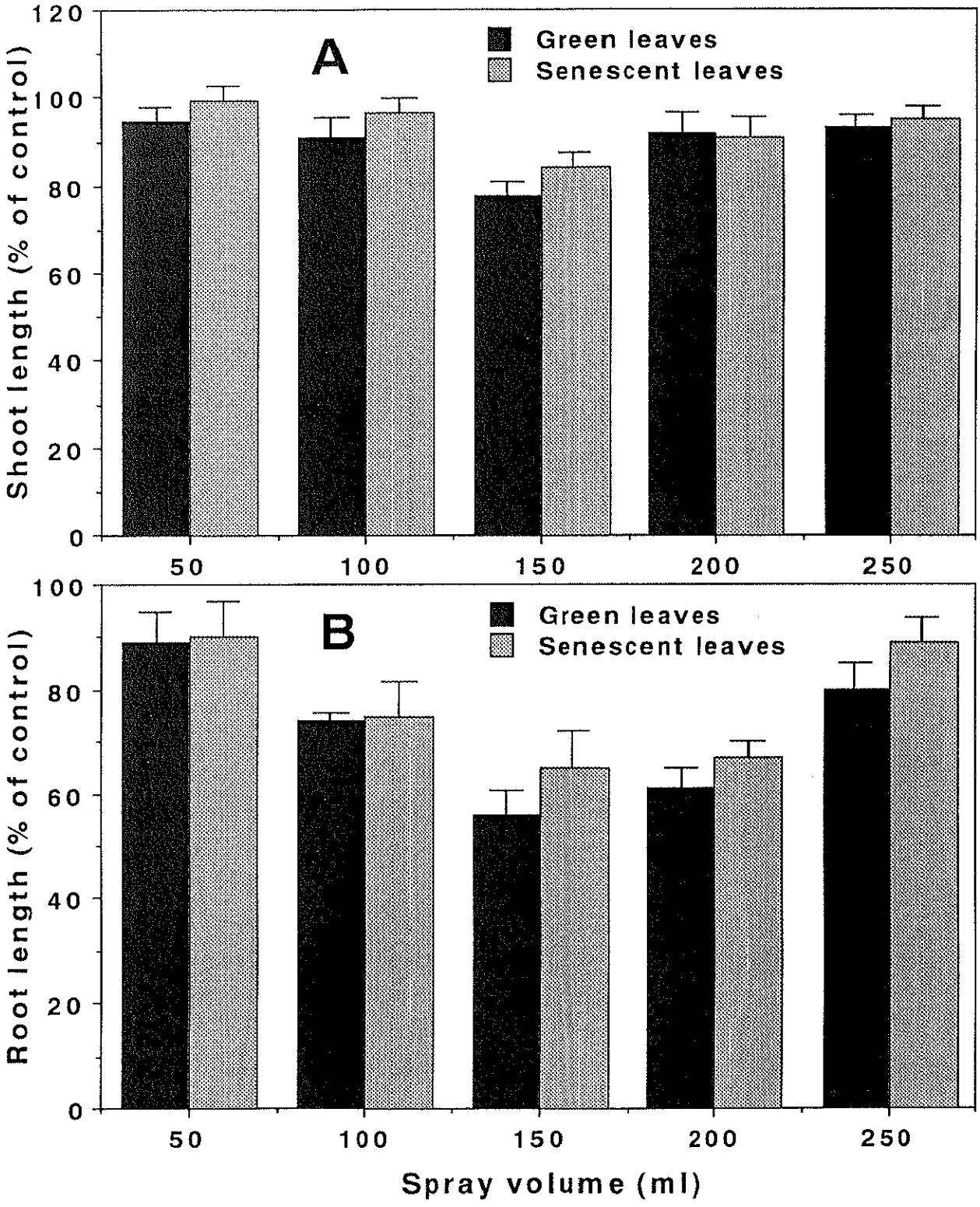

Fig. 4.2 Shoot growth (A) and root growth (B) of rice seedlings in soil applied with the leachates from green and senescent Mexican sunflower leaves by various water spray volumes. Shoot and root length of the control were 39±3 mm and 75±4 mm, respectively.

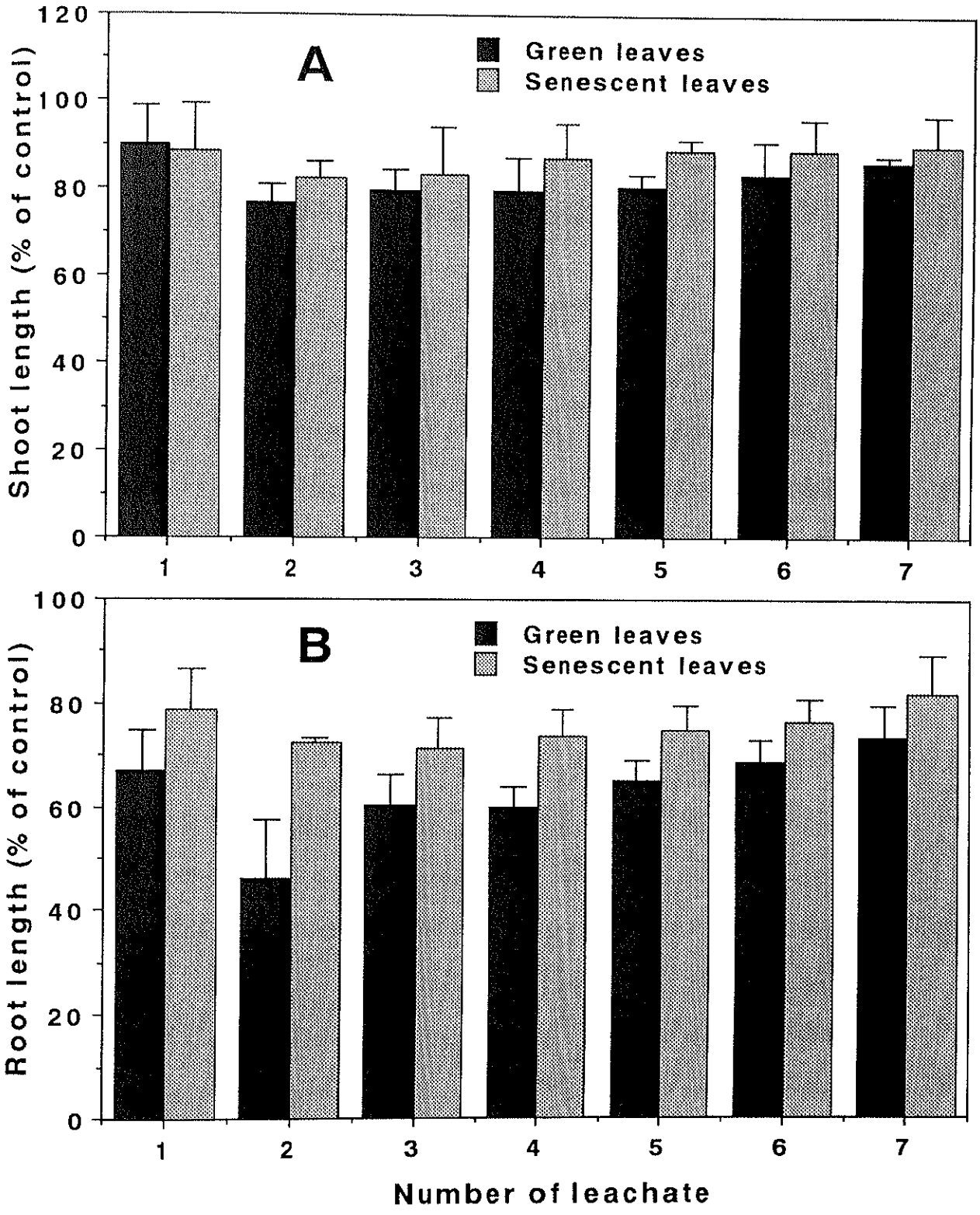

Fig. 4.3 Shoot growth (A) and root growth (B) of rice seedlings in soil applied with the leachates from green and senescent Mexican sunflower collected at 50 ml intervals of water spray. Shoot and root length of the control were 39 ±2 mm and 73 ±2 mm, respectively.