Allelopathic activity and movement of water leachate from Mexican sunflower [Tithonia diversifolia (Hemsl.) A. Gray] leaves in soil

Suthep Tongma*, Katsuichiro Kobayashi* and Kenji Usui*

Abstract: Laboratory experiments were conducted to determine the phytotoxic activity of water leachates from Mexican sunflower [Tithonia diversifolia (Hemsl.) A. Gray] leaves and the effect of water movement on efficacy of the leachate in soil. The water leachates from both the green and senescent leaves applied onto soil inhibited root growth of rice seedlings. The phytotoxicity of leaf leachate depended on the concentration of phytotoxic substances which was determined by the amount of leaves extracted. Mobility of phytotoxic substances from Mexican sunflower leaves in soil was dependent on water movement and soil moisture conditions. More downward movement was observed in dry soil than in wet soil. The inhibitory activity of water leachate in soil was well correlated with the concentration in soil-water. The allelopathic potential of Mexican sunflower leaves in natural field was discussed.

Key words: Tithonia diversifolia, allelopathy, water leachate, leaching

Introduction

Mexican sunflower is a perennial plant of the family Asteraceae, native to Mexico and Central America6), and introduced to many countries in Asia, Africa, North America and Australia to serve as green manure and to prevent soil erosion8,11,14). In the northern part of Thailand, Mexican sunflower is widespread in the mountainous areas where it has become a dominant plant19). During the rainy season, this plant grows rapidly and usually forms an almost pure stand with few other plant species. The plant is reported to contain sesquiterpene lactones, tagitinin A and C, and a flavonoid, hispidulin, each of which has phytotoxic activity3,4). This suggests its strong competitive ability and possible allelopathic activity on growth of other plants under natural conditions.

Our previous study14) demonstrated that water extracts from leaves, stems, and roots of Mexican sunflower inhibit seed germination and seedling growth of some plants tested. Soil applied with leaf water extract, soil incorporated with leaf residue and soil previously planted with Mexican sunflower have a phytotoxic effect on growth of other plants14,15). The phytotoxic activity was also influenced by soil factors such as soil adsorption and biodegradation15). These studies14,15) suggested that Mexican sunflower has allelopathic potential. Under natural conditions, however, most allelopathic substances are released into the soil through water leaching directly by rain and dissolving the plant residues in soil19). Water leaching and soil conditions have been reported to affect viability and activity of phytotoxins in soil17).

The present study was conducted to deter-
mine the phytotoxic activity of water leachates from Mexican sunflower leaves by water spray and to investigate the effect of downward movement of water in soil on the phytotoxicity in order to obtain basic information about the allelopathic activity of Mexican sunflower under natural conditions.

Materials and Methods

Phytotoxic activity of leaf leachates from Mexican sunflower

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 rate of 50, 100, 150, 200, 250 and 300 g/m². 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 [light colored andosol, total carbon 3.29%, total nitrogen 0.29%, cation exchange capacity 19.0 me/100 g, clay content 22.3%, maximum water holding capacity (MWHC) 69.8% of dry soil]. The leaves were gently sprayed with 200 ml of distilled water, equivalent to a 3 mm rainfall, by a hand sprayer. 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 MWHC with distilled water by sub-irrigation. Thirty uniformly germinated rice seeds (Oryza sativa L. cv. Nipponbare) having 1 mm coleoptile were planted in each box and allowed to grow in a growth chamber at 25/20°C, 12/12 hr, day/night period for 4 days. Shoot and root lengths of rice seedlings were measured and compared to the seedlings grown in the control soil.

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

To determine the effect of a portion of leaf water leachate in water spray on the phytotoxic activity, 6.67 g of green or senescent leaves was sprayed 7 times with 50 ml of distilled water each time. The leachate passing through the screen each time was separately collected and bioassayed with germinated rice seeds in soil. Ten grams of air-dried Kannondai soil was put into small glass bottles (4 cm in diameter and 8 cm in height) 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, then shoot and root lengths of rice seedlings were measured.

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

To determine the downward mobility of Mexican sunflower leachates in the dry condition, Mexican sunflower leaf extract from the air-dried green leaf powder at the concentration of 20 mg/ml was applied into soil column. Seven hundred and fifty grams of air-dried Kannondai soil was put into a column consisting of 10 pieces of plastic ring (10.5 cm in diameter and 1 cm in height). Five hundred ml of water extract was poured onto the top of soil column to the MWHC. After 3 hr, the treated soil columns were divided into 10 layers, 17 grams of soil from each layer was put into small glass bottles and bioassayed with germinated rice seeds as
described above. Soil-water from each soil layer was separated by centrifugation with double tubes and bioassayed with the rice seeds in sea sand. To investigate the downward movement of Mexican sunflower leaf leachate in wet soil, a similar experiment was conducted using the soil columns which had been pre-moistened with distilled water by sub-irrigation to be the MWHC. The effect of rain on mobility of phytotoxic substances from Mexican sunflower leachate previously existing in the top soil was determined by placing 250 g of soil previously applied with 175 ml of Mexican sunflower leaf extract at the concentration of 40 mg/ml on top of the wet soil column. Into this column was poured 600 ml of distilled water 24 hr after the treated soil had been placed on top, and the applied water was allowed to completely move to the bottom over a 3 hr period. The treated soil was removed and the soil and its soil-water in each layer were bioassayed by the above procedures. All experiments were conducted twice using a completely randomized design with three replications. Data were initially analyzed to test for homogeneity. Because there was no significant difference between duplicate experiments, the data were combined and are presented as pooled mean values. Duncan’s Multiple Range Test (DMRT) was used to determine the difference among means.

Results

Phytotoxic activity of leaf leachates from Mexican sunflower

Root growth of rice seedlings in soil applied with water leachates from green and senescent leaves of Mexican sunflower at different spray volumes is shown in Fig.1. Both green and senescent leaf leachates inhibited rice root growth, with the green leaf leachate showing a slightly more inhibitory effect. Rice root growth decreased with increase in the amount of leaves. Shoot growth of rice seedlings was also inhibited but the degree of inhibition was less than on root growth (data not shown). The result indicated that the phytotoxic compounds contained in both green and senescent leaves of Mexican sunflower could be easily washed out by water spraying.

The response of rice seedlings to water leachate obtained from various spray volumes differed (Fig. 2). Spraying water at the rate of 50 ml onto 6.67 g of green Mexican sunflower leaves had a slight inhibitory effect on rice root growth. The highest phytotoxicity of leachate was found at the spray volume of 150 ml. The inhibitory activity on rice seedling growth declined with the larger spray volumes. A similar dynamic pattern of rice response was found in the senescent leaf leachate treatments. This demonstrated that plant growth inhibitors in Mexican sunflower leaves could be easily washed out by water spray and that they require a sufficient
amount of water to dissolve and release into the environment, although too much leaching water led to the dilution of phytotoxins.

The phytotoxic effect of leachates on rice seedling growth by the 50 ml water spraying is shown in Fig. 3. All leachates from both green and senescent leaves inhibited root growth but the degree of inhibition varied among the leachates. The second leachate from green leaves showed the greatest inhibition, and the phytotoxic activity declined in subsequent 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. A similar pattern of rice shoot growth in response to each water leachate was also observed but the degree of inhibition was less than that occurring on root growth (data not shown). This was well correlated with the results that the spray volume of 150-200 ml provided the highest phytotoxic leachate (Fig. 2).

Effect of water movement on phytotoxic activity of Mexican sunflower leachate in soil

The phytotoxic activity of water extract from Mexican sunflower leaves applied in dry and wet soil conditions on rice root growth is shown in Fig. 4A and 4B. The phytotoxic activity of each soil layer and its soil-water on root elongation was also compared. The effect of downward movement of water on the phytotoxic activity of phytotoxic substances previously adsorbed on the top soil was simulated by placing the soil previously treated with the water extract from Mexican sunflower leaves onto the top of wet soil column (Fig. 4C).

When leaf extract was applied onto dry soil columns, root growth of rice in the upper layers was inhibited more than those in the deeper layers (Fig. 4A), whereas in wet soil column, root elongation of rice in the 5-6 cm layer was inhibited more than the seedlings in the upper or lower layers (Fig. 4B). The results indicate that phytotoxic activity of Mexican sunflower extract applied in soil
differed among the soil layers and was affected by the moisture condition of soil before the application. Figure 4C shows that the most remarkable inhibitory effect on the growth of rice was found in the 4-6 cm layer. The inhibitory activity in the treated soil placed on the top of soil column on root growth was clearly decreased by the application of water compared to the activity before the water application (data not shown). This indicated that phytotoxic substances previously adsorbed on soil could be leached down from the treated soil to the lower layers of the column by water movement after adsorption.

Root elongation of rice seedling in sea sand treated with soil-water from each soil layer was inhibited in the same manner as those grown directly in the soil layers (Fig. 4A, B and C). Thus, phytotoxic activity of Mexican sunflower leachate in soil was correlated well with that in soil-water.

Discussion

Living and senescent leaves are major parts of the Mexican sunflower plant which contain and release more phytotoxic compounds into the soil than do the root or stem. In a 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 to compare the phytotoxic activity of the leachates from mature and senescent leaves by water spray. The water extract from dry leaf powder at the concentrations of 10 and 20 mg/ml applied to Kannondai soil reduced rice root growth to 60% and 39% of the control, respectively. In the present study, water leachate in the 300 g/m² treatment, equivalent to 100 mg/ml of spray water, inhibited rice root growth to about 60% of the control. It was assumed that phytotoxic substances in Mexican sunflower leaves can be
eluted by water spraying at a one tenth concentration of the water extract from the dry leaf powder by the conventional shaking method\(^\text{14,15}\).

Water sprayed at the rate of 50, 100, 150, 200, and 250 ml on the 215 mm \(\times\) 150 mm screen is 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 root growth, suggesting that phytotoxic substances in both green and senescent leaves could be easily washed out by rainwater. The spray volume of 150–200 ml, equivalent to 4.5–6.0 mm rainfall, extracted phytotoxic compounds from Mexican sunflower leaves in a sufficient amount to reduce the root growth of plants grown in the soil. A spray volume of over 250 ml (more than 7.5 mm rainfall) might result in dilution of phytotoxins in the leachate, thus reducing phytotoxicity. This was supported by the second and third leachates from 50 ml of water spray showing the highest inhibitory activity and the phytotoxicity being reduced in subsequent leachates (Fig. 3).

During 5 months of the rainy season in Mexican sunflower infested areas in the northern part of Thailand, this plant was found to produce green and senescent leaves of about 80–300 and 25–150 g in dry weight per m\(^2\), respectively, depending on age, plant density and soil fertility\(^*\). The average rainfall was 950 mm with 32 days of rain during in the rainy season\(^*\). Very few other plant species were observed under the Mexican sunflower canopy\(^\text{18}\). Leaf water leachate by rain thus seems to be sufficient to suppress the seed germination and growth of other plants.

The application of Mexican sunflower extract to dry and wet soil columns simulates the movement of phytotoxic substances released from the plant in the natural field after the first and subsequent rainfall events of the rainy season. The first rainfall begins when the top soil layer is dry, and the soil profile thereafter retains various levels of moisture up to its field water holding capacity by the following rains. Therefore, it is suggested that the phytotoxic substances in Mexican sunflower leaves could be extracted by rain, reached to the soil surface and moved down with water movement to the lower soil layer, at least to the top 5 cm where most of the weed seeds commonly locate, and that phytotoxic compounds, which have been previously released from the leaves onto the soil surface by the first rainfall, could be further mobilized into the lower soil layers by the following rains. The reason for the different effect of water movement on the phytotoxic activity between dry and wet soil remains to be investigated.

Similar response pattern of root growth to the soil which received the leachate and to its soil-water suggested that phytotoxic activity of the water leachate was actually induced by the concentration of phytotoxic substances in the soil-water.

Little is known about water leaching or mobility of plant allelochemicals in soil. However, it is believed that environmental factors affecting mobility of plant phytotoxins in soil are similar to the factors influencing herbicide movement\(^\text{5,7,12}\). Precipitation is the main factor involved in the downward movement of chemical substances after application. Weidenhamer\(^\text{16}\) noted that, like herbicides, allelochemicals can be bound and made unavailable by soil organic matter and clays and that, unlike herbicides which are applied at a given rate and subsequently decrease in concentration with time, allelo-

chemicals are continually being added to and removed from the soil associated with the downward movement of water. Thus, it is likely that phytotoxicity of allelochemicals is a function of both static availability and dynamic availability based on the total amount of chemicals moving in and out the system over a period of time\(^{17}\).

Finding in the present study suggested that phytotoxic substances in Mexican sunflower leaves could be washed out from both living and dead leaves by rainwater. With an appropriate amount of leaves and rainfall, the phytotoxic compounds are released in a concentration sufficient to lead to inhibition of growth of other plant species. It seems that the phytotoxicity and the mobility of substances released from Mexican sunflower in soil are affected by water movement and soil moisture conditions as well as other soil factors. We reported earlier that the phytotoxic effect was influenced by soil adsorption and microbial degradation of Mexican sunflower extract and residue in soil\(^{19}\). It is assumed that the phytotoxic compounds are continually accumulating in soil due to the sufficient amount of rainfall and the perennial life cycle of Mexican sunflower and result in inducing allelopathy under natural conditions. Further studies are needed on the performance of the isolated chemicals\(^{4,11,13}\) in Mexican sunflower leaves in soil after their elution by water leaching and the mechanism of their phytotoxic activities in soil in terms of their allelopathy.

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References

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