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Allelopathic Effects of Volatile Substances Emitted by *Lycopersicon esculentum*

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토마토에서 방산된 휘발성 물질의 알레로파시 효과

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ABSTRACT

The phytotoxic effects of volatile substances emitted from the tomato plant (*Lycopersicon esculentum*) on receptor plants such as *Bidens bipinnata, Plantago asiatica, Lactuca sativa, Eragrostis ferruginea and Achyranthes japonica* were investigated. Volatile substances from the leaves inhibited seedling growth of receptor plants in the laboratory tests. The inhibition response varied with the concentration of compounds.

To identify the phytotoxic compounds from tomato plant, a GC/MS method was employed. Forty compounds, such as trans-2-hexenal, linalool, phenylacetaldehyde, methylsalicylic acid and tetradecanaic acid were identified from the essential oil of tomato plants.

The findings suggest that the tomato plant may have allelopathic potential.

Key words: *Lycopersicon esculentum*, Allelopathy, Essential oil, Germination, Seedling growth

INTRODUCTION

Many researchers have found that the inhibitory substances involved in allelochemic interactions were phenolic compounds and volatile substances from leaves, roots, fallen leaves and under the soil of growing plants. These inhibitory substances were identified by paper chromatography, thin layer chromatography, gas chromatography and mass spectrophotometry(Grummer, 1961: Lodhi, 1976; Chou and Chen, 1976; Jackson and Willemsen, 1976). Few phenols have been reported to be present in tomato plants(Karrer 1958; Bate-Smith 1972).

Volatile substances, probably terpenes, exuded from *Artemisia cana, A. absinthium, A. frigida, A. dracundus* and *A. tridentata* var. *vaseyana* inhibit germination of tested species(Hoffman and Hazlett, 1977; Weaver and Klarich, 1977; Groves and Anderson, 1981).

Bioassays of *Calamintha ashei* monoterpenes demonstrated effects on germination in concentrations as low as 0.05mM for (t)-evodone(Weidenhamer, *et al.*, 1994)

The role of the terpenoid mixture, either constitutive or induced, in its intraspecific qualitative and quantitative compositional variations, and its dosage dependent effects(Langenheim, 1994) are important in terms of allelopathic research.

Allelochemicals are released from plant tissue in a variety of ways including exudation of volatile chemicals from living plant parts, exudation of water soluble toxins from ground parts, or leaching of water soluble chemicals from above ground parts in response to the action of rain, fog or dew. Tomato plants are known to exhibit allelopathic effects or to produce a number of terpenoid allelochemicals.

The purpose of the present study is to gather evidences of the allelopathic characteristics of tomato plants: (1)to verify the presence of growth inhibitors in the tomato plant, (2) to identify the chemical substances responsible.

MATERIALS AND METHODS

Donor and receptor plants

As strong smell spread from tomato plants, so the study on the allelopathic potential of tomato plants (*Lycopersicon esculentum*) was chosen as the donor plant.

The receptor species were chosen because *Lactuca sativa* for seedling growth, *Bidens bipinnata, Plantago asiatica, Lactuca sativa* and *Eragrostis ferruginea* for relative seedling elongation test grown with growth chamber, respectively.

Growth test in Growth Chamber

Petri-dishes(diameter 12 cm) with one layer of filter paper were used. Fifty seeds of *L. sativa* were evenly disposed in each dish. Control was treated with distilled water.

The Petri-dishes were put into a growth chamber giving alternating temperatures (20℃ for 16 hours in light and 15 for 8 hours in dark). The experiment extended over a period of 10 days to allow maximum seed germination and seedling growth.

The results were measured by measuring the length of seedlings in millimeters and calculated as relative seedling elongation for quantitative evaluation of the effect of phytotoxic substances on the experimental species. All data obtained from three or more treatments and bioassay results were analyzed by Duncan's multiple-range test.

Volatile substances test

To study the effects of volatile substances from the tomato plant, a 1.8-liter glass chamber(Baker, 1966) were provided for the germination test(Yun, *et al.*, 1993).

In a glass chamber(d 14cm, height 10.5cm), fifty seeds of the receptor plants such as A. *japonica, L. sativa* and *P. asiatica* were placed on the filter paper which was layered on absorbent cotton with sufficient moisture. Grown leaves of the tomato plant were picked for the test. Fresh leaves in different quantities (5, 10, 15, 20, 25, 30g) were placed in glass beakers embraced within every glass chamber. The same type chamber, but without sliced leaves, was used as the control.

The chamber was covered with vinyl wrap and placed at room temperature(Ca. 25°C). After 2 to 4 days, the radicle elongation were measured.

All the results of the experiments were means of the four replicates.

Essential oils extraction and chemical analysis

Essential oils from the tomato plant were obtained using Karlsruker's apparatus (Stahl, 1973). Chromatography materials and methods were that of Kil and Yim(1983). Gaschromatography (Hewlett Packard 5890A capillary) was employed for analysis of the volatile substances from the tomato plants. Authentic compounds purchased from Sigma and Aldrich company.

Oven temperature was set to 100℃, then programmed to increase 4℃/min and then maintained at 300℃. Split ratio was 1 : 50. A one-microliter sample injection was made with a 5 ml SGE syringe employing the solvent flush technique.

RESULTS

Relative elongation of receptor plants treated with volatile substances of tomato plant was reduced as two types: seedling elongation of *Plantago asiatica* and *Bidens bipinnata* showed that the more the volatile substances of the tomato leaf, the worse the growth was showed and that *Eragrostis ferruginea* and *Lactuca sativa* did not much respond to increasing chemical concentration of tomato plant. Thease latter grew over 75% of relative seedling elongation at all tested plots compared to control(Fig. 1).

In addition to the test growth of three selected species the relative elongation of *Plantago asiatica* was heavily reduced, but that of Lactuca sativa was comparatively high. While *Achyranthes japonica* did not appear to be much influenced by the volatile substances of the tomato plant(Fig. 2).

Figure 3 shows the gas chromatogram of essential oil extracted from tomato plant leaf.

The identification of each component was made by comparison of the retention time and mass spectra with those of commercial authentic samples.

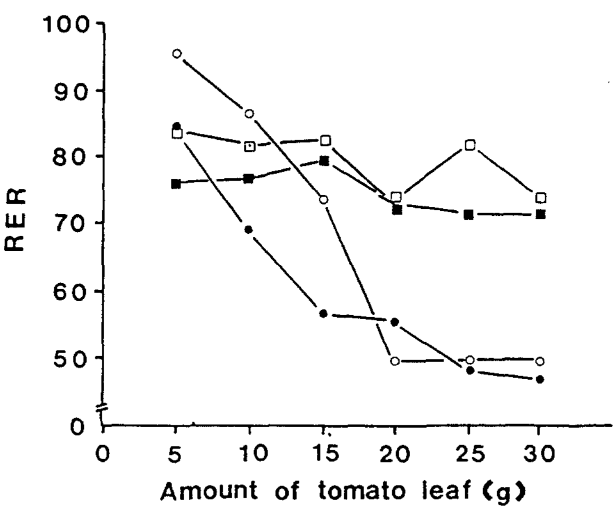

Fig. 1. Relative seedling elongation of receptor plants grown in growth chamber with different quantities of tomato leaves. Keys to species : ● ; *Bidens bipinnata*, ○ ; *Plantago asiatica*, ■ ; *Lactuca sativa*, □ ; *Eragrostis ferruginea*.

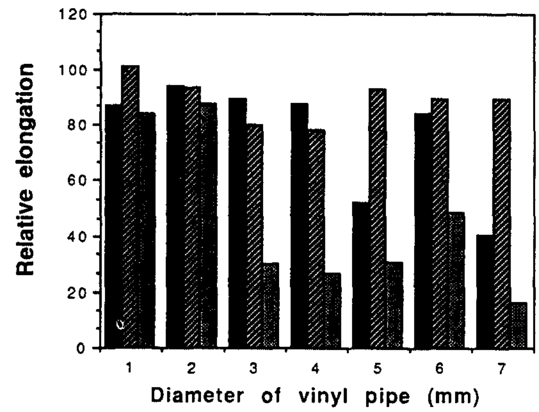

Fig. 2. Relative seedling elongation of receptor plants grown in growth chamber at different size of vinyl pipe. Keys to species ; ■, *Achyranthes japonica;* ▨, *Lactuca sativa* ; ▦, *Plantago asiatica.* Values are based on the mean of three determinations.

Forty components were identified in leaf essential oil of tomato plant. These chemicals are able to classify into several substances such as alkane, alcohol, aldehyde, ester. carboxylic acid, phenol, terpenoid, ketone, epoxide and tricosine. The major constituents of leaf essential oil were trans-2-hexenal, cis-hexen-1-ol, eugenol and hexadecanoic acid.

Among identified 40 components -terpineol, linalool, eugenol and tetradecanoic acid were provided and bioassayed of *Amaranthus mangostanus.* Seed germination of *A. mangostanus* were reduced of 60% at all tested concentration of -terpinene and linalool solution, while those of A. mangostanus were inhibitory proportionally by increasing concentration of three chemical compounds except -terpineol solution (Fig. 4).

This result suggests that natural chemicals contained in tomato plants affect to inhibit seed germination of selected species, but may not affect to that of another species.

When egg plant planted near tomato plants the growth of egg plant was reduced severely(Kim and Kil, 1987). Therefore allelopathic effects of tomato plant to selected species may be assumed both in field and crop plantation.

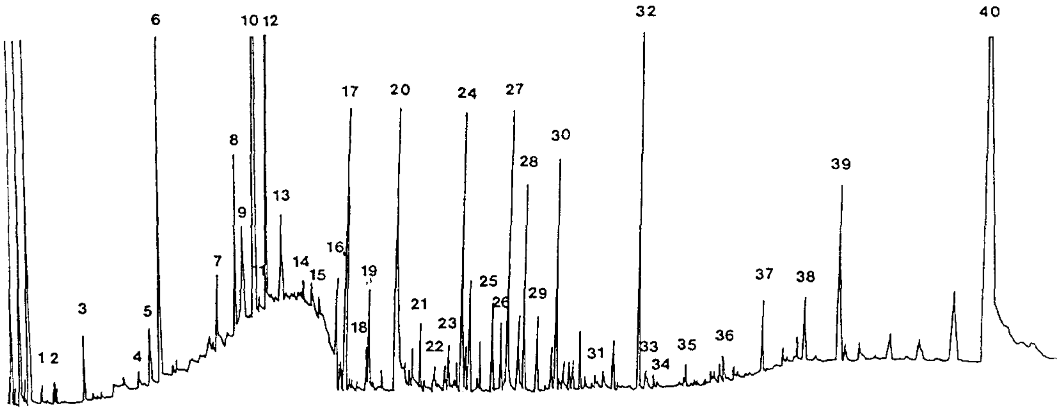

Fig. 3. Gas chromatograms of essential oil of tomato leaf. Keys to numbers; 1, Methylbenzene; 2, Decane; 3, Hexanal; 4, Dodecane; 5, iso-Amylalcohol; 6, *trans*-2-Hexenal; 7, 1-Hexen-3-ol; 8, n-Hexanol; 9, *cis*-3-Hexenyl acetate; 10, *cis*-Hexen-1-ol; 11, Tetradecane; 12, *trans*-2-Hexen-1-ol; 13, Linalooloxide; 14, Acetic acid; 15, Pentadecane; 16, Benzaldehyde; 17, Linalool; 18, Hexadecane; 19, β-Caryophyllene; 20, Phenylacetaldehyde; 21, α-Terpineol; 22, Methylbenzoate; 23, Ethyl phenylacetate; 24, Methyl salicylate; 25, Geraniol; 26,Guaiacol; 27, Benzyl alcohol; 28, 2-Phenylethyl alcohol; 29, β-Caryophyllene epoxide; 31, Heneicosane; 32, Eugenol; 33, Thymol 34, Docosane; 35, Tricosine; 36, Tetracosane; 37,Pentacosane; 38, Hexacosane; 39, Tetradecanoic acid; 40, Hexadecanoic acid. Components are listed only if their presence was confirmed by mass spectral analysis.

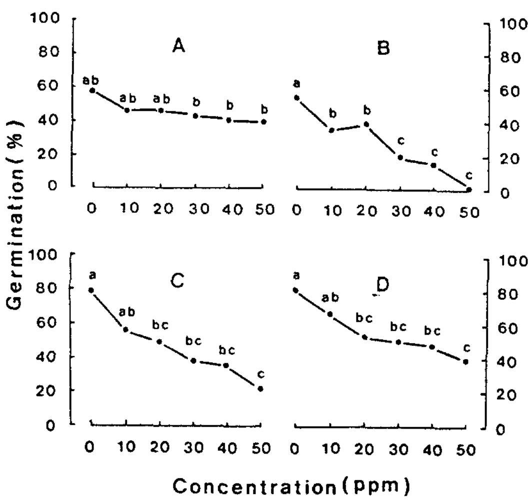

Fig. 4. Effect of α-terpineol(A) and linalool(B), eugenal(C) and tetradecanoic acid(D) on germination of *Amaranthus mangostanus*. Values not followed by the same letters are significantly different($P<0.05$).

DISCUSSION

Volatile substances emitted from tomato plant leaves inhibited radicle growth of receptor plants. Essential oil of tomato plants also inhibited strongly elongation of the test species owing to the presence of chemical substances. These results clearly show that the tomato plant leaf released volatile substances that acted as inhibitions to growth. Furthermore, the degree of toxicity of released volatile substances was dependent on the receptor plant. This finding was in agreement with Halligen(1976), Heisey and Delwiche (1983) and Oleszek(1987).

Yun(1990) reported that the germination and elongation of receptor plants was inhibited by volatile substances emitted from wormwood leaf and was concentration dependent.

Essential oil of the plant extracted by Karlsruker's apparatus suppressed germination and elongation of the receptor plants, and their threshold concentration for the inhibition of elongation of receptor plants was 4.8 ul/100ml in this study.

Yun(1990) reported that volatile substances emitted from wormwood leaf inhibited seed germination and radicle growth of receptor plants. There are many reports on active chemicals isolated from plants. Although such naturally occurring substances have been identified in part from several kind of plant species, these findings was contributed and have played a bridge role basically. For example, twenty six components were identified in wormwood leaf essential oil, 28 in flower oil and 23 in root oil(Yun, 1990). Phenolic compounds are a main inhibitor of germination and seedling growth(Olmsted and Rice, 1970; Whittaker and Feeny, 1971; Tinnin and Muller, 1972; Horsely,1977; Carballeire, 1980). Forty components for the first time were identified in tomato leaf essential oil. Among them the major constituents of the essential oil of tomato leaf were trans-2-hexanal, cis-hexen-1-0l, trans-2-hexen-1-0l, eugenol, and hexadecanoic acid.

Besides, Vostrowsky *et al.*(1984) presented that the essential oil from leaves of *A. abrotanum* was identified to contain about 40 different chemicals including 1,8-cineole by GC/MS.

Five phenolic acids, tannic acid, hydroquinone, p-hydroxybenzoic acid, vanillic acid, and ferulic acid were identified from tomato plants leaf by HPLC (Kim and Kil, 1989).

Muller(1965) pointed out that terpenes were first noticed to cause cucumber radicles and hypocotyls to be short and thick, due to reduced cell elongation and cell division.

Also the cells of inhibited roots frequently contain abnormal nuclei and globules of fats or oils which presumably accummulated because new cells were not able to form owing to phytotoxic chemicals originated from tomato.

Therefore, inhibition effect of seedling growth of receptor species, abnormal morphology of root hair development and root tip tissue, etc would have allelopathic activitiy by tomato volatiles.

적 요

토마토식물로부터 방산되는 휘발성 물질이 도깨비바늘, 질경이. 상치, 그령, 쇠무릎에 미치는 식물독성효과를 조사하였다. 실험실실험 결과 토마토 잎의 휘발성 화학물질은 실험에 사용된 식물들의 유식물 생장을 억제했고 억제의 정도는 화학물질의 농도에 따라 차이가 있었다.

그래서 토마토식물 정유속에 함유되어 있는 식물독성물질은 GC/MS로 분석하여 본 결과trans-2-hexanal, linalool, phenylacetaldehyde, methylsalicylic acid, tetradecanaic acid 등 40종류 성분이라고 최초로 분리 확인해 냈다.

이러한 실험결과로 보아 토마토 식물의 휘발성 물질은 알레로파시 효과가 있다는 사실을 밝혀냈다.

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