# Partial Characterization of Allelopathic Substances in Sorghum Stem by Different Organic Solvents and pH

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# 수수 줄기에 含有된 他感物質의 溶媒와 pH에 따른 特性究明

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## ABSTRACT

The toxic compounds of sorghum stem extracts were isolated by different organic solvents and pH, and characterized and quantified in terms of their inhibition of seed germination and seedling growth in *Echinochloa colona*(L.) Link and radish(*Raphanus sativus* L.).

Sequential partitioning of stem extract with various organic solvents with increasing polarity showed that all fractions of hexane, ethyl ether, methylene chloride, ethyl acetate, and the aqueous remainder inhibited germination and seedling growth in *E. colona*. Of the five fractions, the ethyl ether fraction had the greatest inhibitory effect on *E. colona*.

Further separation of the ethyl ether fraction at different pH(pH 2-11) showed that phytotoxic compounds were acidic. The result indicates that the phytotoxin present in the stem extract may be nonpolar and acidic.

Key words : allelochemical, sorghum, solvent, pH

## INTRODUCTION

Previous experiments showed that stem, root and leaf of the sorghum exhibited reduced germination and seedling growth of *E. colona* and radish[9]. Of the three parts, stem extract was most inhibitory to plant growth.

In order to confirm the allelopathic activity and to determine the characteristics of the inhibitor, chemical isolation and identification works are necessary. Several researchers have used polar to nonpolar solvents for the extraction of allelochemicals from live or dried plant in order to find the best solvent for the isolation of the most toxic compound[2,3,7,8,10,12]. They indicat-

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ed that different solvents needed depending on the nature of the allelochemicals. In spite of the extensive works on the isolation and characterization of the allelochemicals in other plants few reports are avaiable, however, on the effect of solvents and pH on the extraction of allelopathic compounds in the sorghum stem, especially on the aqueous extracts.

Therefore, the objective of this investigation was to characterize the allelochemical in sorghum stems extracted with different solvents and pH.

## MATERIALS AND METHODS

### Partitioning of Stem Extracts with Various Solvents

Since the sorghum tissue extract which gave the greatest inhibitory effect was the stem extract[9], ground stem tissue (100g) was extracted for 72 hr with 3 liters of distilled water. The mixture was filtered and centrifuged at 16000g for 30 min to remove particulate material. Nine liters of acetone were added to the aqueous extract. The mixture was kept for 24 hr in a 4℃ cold room. Proteinaceous material precipitated in the solution was removed by vacuum filtration through Whatman No. 1 and 42 filter paper, and discarded. Acetone was removed from the extract by rotary evaporation at 40℃. The clear aqueous extract was sequentially partitioned with the following solvent series ranging from least polar to most polar : hexane, ethyl ether, methylene chloride, and ethyl acetate. Each liter of the aqueous stem extract was sequentially partitioned six times with 250ml of each of the four solvents. Solvent was evaporated to dryness under vacuum at 40℃. The entire process of extraction and partitioning was repeated five times to gather enough material for experimentation.

Each fraction from the liquid-liquid extraction was redissolved in methanol to from solutions at a concentration of 4, 8, and 16mg/ml. One milliliter of each fraction was placed in a plastic petri dish (60× 15mm) lined with filter paper (Whatman No. 1). Methanol was allowed to evaporate from each dish inside the hood at room temperature, and 2ml distilled water was added. Twenty seeds of *E. colona* were placed in petri dish at 28℃ temperature-controlled incubator. Germination percentage, shoot and root length, and percent growth inhibition were measured at 4 days after treatment.

### Ethyl ether Partitioning at Different pH

Based on the bioassay of the previous experiment, further experiments involving different pH were carried out using the solvent fractions with maximum inhibitory activity. The ethyl ether extract was evaporated to dryness and the residue was dissolved in 100ml of distilled water. A 50-ml portion of this fraction was extracted at pH 2, 7, and 11. Acidic and basic fractions were dried over anhydrous $(NH_4)_2SO_4$. One, 2, and 3ml of each fraction was placed on a plastic petri dish (60× 15mm) lined with a filter paper (Whatman No. 1). The inhibitory activities of each fraction were tested with *E. colona* and radish as described earlier[9].

### Statistical Design and Analysis of Data

Experiments were laid out in a compeletly randomized desingn with four replications. Data were analyzed using single or two-factor analysis. Treatments were compared using Least Significant Differences (LSD).

## RESULTS AND DISCUSSION

### Partitioning of Water Extracts with Various Solvents

**Germination**. Repeated partitioning with various solvent series was successful in removing considerable toxicity from the crude aqueous

**Table 1**. Quantity extracted from 100g sorghum stem material and its activity on root elongation of *E. colona*.

| Fraction | Quantity extracted (mg) | Percentage of recovered material | $I_{50}$[a] (mg/ml) |
|---|---|---|---|
| Acetone ppt | 1263.0 | 9.10 | |
| Hexane | 3.3 | 0.02 | 14.29 |
| Ethyl ether | 75.6 | 0.05 | 2.54 |
| Methylene chloride | 24.9 | 0.20 | 6.77 |
| Ethy acetate | 95.5 | 0.70 | 5.73 |
| Aqueous remainder | 12406.5 | 89.50 | 7.54 |

[a] Based on weight(mg) of fractions recoverd(per 3000ml crude aqueous extract) per weight(mg) of fraction necessary for 50% inhibition of root elongation(mm).

extract of sorghum stem. The greatest amount of material was removed from the aqueous stem extract through partitioning with ethyl acetate (Table 1). This fraction accounted for 0.7% of the removed material. After initial separations, 98.6% was associated with the protein precipitate and final aqueous extracts. Their specific activity was considerably less than that of the organic extracts. The organic fractions had only 1.42% of the recovered material. Among the organic fractions, the ethyl ether fraction had the greatest specific activity with and $I_{50}$ of 2.54mg/ml. The second most active was the ethyl acetate fraction, followed by the methylene chloride and aqueous fractions. However, most of the inhibitory activity occurred in the ethyl ether, methylene chloride, and ethyl acetate fractions, as measured by the inhibition of seed germination and seedling growth of *E. colona*.

Seed germination was reduced by the five fractions(Table 2). However, the response depended on the fraction source and concentration. Germination in *E. colona* was inhibited with increase in concentration of all five extracts. At the lowest concentration(4mg/ml), only the ethyl ether fraction inhibited *E. colona* germina-

**Table 2**. Effect on seedling growth of *E. colona* of different solvents fractionated from the stem extract.

| Fraction | Concentration (mg/ml) | Germination (%) | Mean length (mm) | |
|---|---|---|---|---|
| | | | Shoot | Root |
| Control | | 78.8 | 13.1 | 19.9 |
| Hexane | 4 | 82.5 | 12.9 | 15.3 |
| | 8 | 71.3 | 10.3 | 13.9 |
| | 16 | 58.6 | 8.1 | 9.0 |
| Ethyl ether | 4 | 21.3 | 2.5 | 1.7 |
| | 8 | 0 | 0.0 | 0.0 |
| | 16 | 0 | 0.0 | 0.0 |
| Methylene chloride | 4 | 80.0 | 9.8 | 16.1 |
| | 8 | 43.8 | 7.3 | 2.0 |
| | 16 | 15.0 | 4.6 | 0.2 |
| Ethy acetate | 4 | 80.0 | 9.9 | 12.6 |
| | 8 | 55.0 | 7.5 | 0.4 |
| | 16 | 28.8 | 5.9 | 0.1 |
| Aqueous remainder | 4 | 71.3 | 11.6 | 11.7 |
| | 8 | 68.8 | 9.2 | 5.6 |
| | 16 | 55.0 | 5.6 | 4.2 |
| LSD 0.05 | | 14.5 | 2.6 | 4.0 |

tion. However, as the solvent concentration increased, germination of *E. colona* was gradually reduced by all solvents. Inhibition increased with an methylene chloride fraction reduced germination significantly at 8mg/ml, but hexane and the aqueous fractions reduced it slightly. At a concentration of 8mg/ml, but hexane and the aqueous fractions reduced it slightly. At a concentration of 8mg/ml, ethyl ether inhibited germination 100%.

At the high concentration (16mg/ml), all solvents inhibited germination. However, the most inhibitory activity occurred with the ethyl ether fraction. Even at 4mg/ml, ethyl ether fraction inhibited germination by 73% whereas the other solvents did not. These experiments indicate that ethyl ether extract of sorghum stem at a relatively low rate inhibits radicle growth in *E. colona*. Therefore, the most inhibitory phytotoxins present in sorghum stem appear to be primarily nonpolar. The existence of less polar, allelopathically active substances in sorghum extract has previously been reported. Sequential extraction with hexane and toluene of the acidic residue partitioned from the cation exchange column effluent resulted in two fractions of enriched inhibitory potential[10]. Residues recovered from the hexane-soluble fraction were the most inhibitory and had a specific inhibitory activity about 16 times that present in the acid residues. The ethyl ether extract of quackgrass shoots also caused a significant decrease in radicle length in snapbeans (*Phaseolus vulgaris* L)[12]. Radicle length was a more sensitive indicator of inhibition than and seed germination or shoot length. Chou and Waller[5] also found that the ethyl ether fraction of an aqueous extract of coffee leaves significantly inhibited radicle growth in rye grass.

Partitioning crude aqueous extracts using organic solvents led to fractionation of the activity. Initial partitioning with hexane resulted in inactive organic fractions. Subsequent fractionation of the aqueous fractions with ethyl ether yielded toxicity. Partitioning of the second aqueous fraction against the three solvents again resulted in activity. Ethyl ether was the most effective partitioning of the solvent tested with regard to overall growth inhibition. The fact that all fractions had inhibitory effect after sequential partitions indicates that the partitioning resulted in the division of the inhibitory activity. Sequential inhibitory potential was present in several isolated aqueous fractions after these were partitioned with nonpolar solvent extracts of sorghum herbage[10]. All previously identified inhibitors isolated from sorghum residues were phenolic acids and liphophilic in terms of solubility[1,6]. It was also interesting that, even after partitioning with various solvents, significant inhibitory activity was still present in the aqueous remainder. These results suggest that sorghum stem contains water soluble phytotoxin. This is consistent with our observation that water leachates of sorghum residues used as mulch on the soil surface were inhibitory to *E. colona* and radish seedling growth in the greenhouse experiment. The studies reveal that the ethyl ether fraction in combination with less polar compounds present in methylene chloride and ethyl acetate or more polar aqueous remainder may be responsible for inhibiting germination and early seedling growth as observed in the greenhouse experiment.

**Seedling growth**. Root and shoot growth of *E. colona* was significantly inhibited by all solvents. The ethyl ether fraction had the most inhibitory effect (Table 2). The root was more sensitive than the shoot and germination. The emerging root possibly absorbed more substance than the shoot.

Overall, the organic solvent fractions were much more active than the aqueous fractions. The ethyl ether fraction had the greatest specific

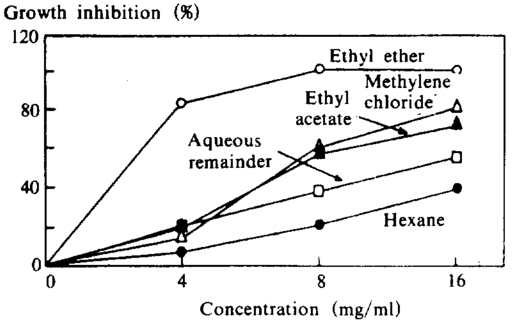


**Fig. 1.** Effect of inhibitor fractionated by different organic solvents from sorghum stem extract on the growth of *E. colona*.

activity with and $I_{50}$ of 2540ppm(w/v). The second most active was the methylene chloride fraction, followed by ethyl acetate and hexane fractions.

Since the greatest toxicity occurred in the ethyl ether fraction(Fig. 1), the crude ethyl ether extract was used in further separation and bioassay to characterize the inhibitor responsible for reducing seedling growth.

### Ethyl ether Partitioning at Different pH

***E. colona* germination**.The different ethyl ether fractions varied in their ability to affect germination and growth of *E. colona*. The degree of inhibition was dependent on the volume of the extract used. The acidic and neutral fractions were inhibitory to *E. colona* germination (Table 3). The acidic and neutral fractions were considerably more inhibited *E. colona* than was the base and aqueous extracts. The acidic extract at 1ml inhibited germination by about 60%, whereas the base extract at the same volume inhibited *E. colona* germination by only about 24%. The acidic fraction at 2ml completely inhibited germination. Germination with the neutral fraction decreased with increasing volume of the extract, but inhibition was lower than with the acid fraction. The base extract had slight impact and the aqueous extract had no inhibitory effect.

**Radish germination**.Radish germination was significantly inhibited by the acid and neutral fractions. However, the base and aqueous extracts did not have any inhibitory effects (Table 4).

When root exudates of sorghum were fractionated with chloroform at different pH, most of the inhibitory effect was found in the neutral and acetone fractions[4]. A similar high inhibition by the acid fraction was reported by Jain et al.[8] and Park[11].

**Table 3.** Seedling growth in *E. colona* as affected by ethyl ether fraction extracted at different pH.

| Fraction | Volume (ml) | Germination (%) | Mean length (mm) | |
|---|---|---|---|---|
| | | | Shoot | Root |
| Control | | 81.0 | 13.0 | 14.1 |
| Acid | 1 | 32.0 | 4.9 | 4.5 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| Neutral | 1 | 61.3 | 6.3 | 8.3 |
| | 2 | 33.8 | 5.9 | 6.9 |
| | 3 | 8.8 | 2.0 | 0.5 |
| Base | 1 | 82.5 | 9.7 | 16.2 |
| | 2 | 71.3 | 9.3 | 10.3 |
| | 3 | 75.0 | 7.5 | 5.0 |
| Aqueous remainder | 1 | 83.8 | 12.7 | 19.5 |
| | 2 | 72.5 | 13.3 | 18.9 |
| | 3 | 70.0 | 13.0 | 13.5 |
| LSD 0.05 | | 15.3 | 1.8 | 3.3 |

**Table 4.** Seedling growth in radish as affected by ethyl ether fraction extracted at different pH.

| Fraction | Volume (ml) | Germination (%) | Mean length (mm) | |
|---|---|---|---|---|
| | | | Shoot | Root |
| Control | | 81.3 | 8.3 | 17.0 |
| Acid | 1 | 82.5 | 4.6 | 8.8 |
| | 2 | 66.3 | 3.5 | 7.0 |
| | 3 | 38.8 | 3.0 | 3.6 |
| Neutral | 1 | 83.8 | 4.6 | 10.1 |
| | 2 | 61.3 | 2.5 | 4.6 |
| | 3 | 48.8 | 2.2 | 4.5 |
| Base | 1 | 82.5 | 8.6 | 17.0 |
| | 2 | 78.8 | 7.5 | 13.4 |
| | 3 | 81.3 | 7.2 | 16.6 |
| Aqueous remainder | 1 | 88.8 | 7.6 | 17.0 |
| | 2 | 85.8 | 7.9 | 15.8 |
| | 3 | 90.0 | 6.2 | 13.8 |
| LSD 0.05 | | 12.3 | 1.8 | 3.3 |

Germination was similarly affected in both test species. The acidic fraction of the ethyl ether fraction was most inhibitory to germination in *E. colona* and radish, followed by neutral fraction. Inhibition increased with increasing extraction volume. There was little inhibition with the basic and no inhibition with the aqueous fractions.

***E. colona* seedling growth.** The acidic, neutral, and basic fractions were inhibitory to seedling growth of *E. colona*, but the aqueous fraction had no effect(Table 3). Stem and root lengths were significantly reduced by the acidic and neutral fractions, and inhibition increased with increasing volume of the extracts. The basic fraction had little effect. The acidic fraction was more inhibitory than the neutral fraction(Fig. 2).

**Radish seedling growth.** Regardless of concentration, radish shoot and root growth were greatly affected by the acid and neutral fractions of ether extracts(Table 4). However, the two fractions did no markedly differ in inhibiting shoot and root length. Unlike in *E. colona*, inhibition percentage based on germination, and shoot and root length were similar in the acid and the neutral fractions(Fig. 2).

Regardless of the test species, germination, shoot length, and root length were adversely affected by the different extraction volume of the acidic and neutral fracions of the ethyl ether extract fractionated from the aqueous extract of the sorghum stem. The acidic fraction had greater inhibitory activity on the growth parameters of the test species than the other fractions.

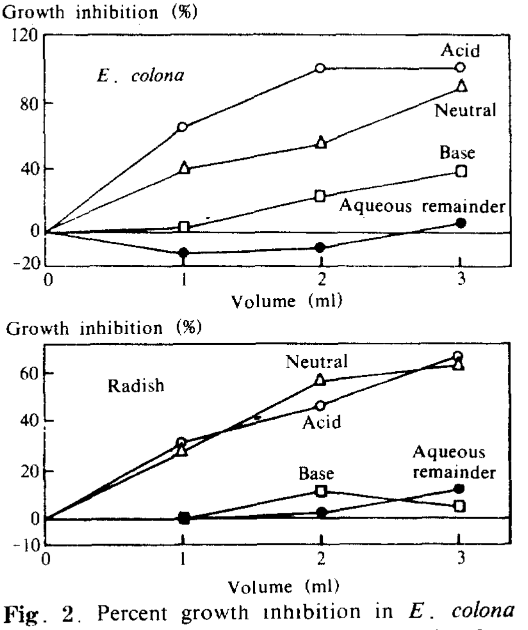


**Fig. 2.** Percent growth inhibition in *E. colona* and radish as affected by ethyl ether fraction extracted at different pH.

## 摘 要

수수 줄기에 함유된 타감물질의 특성을 구명하기 위해서 극성, 비극성 용매로 분획한 결과 사용한 용매에 따라 억제 효과가 달리 나타났는데 ethyl ehter 분획에서 억제작용이 가장 크게 나타났으며 또 methylene chloride와 ethyl acetate 분획에서도 8mg/ml과 16mg/ml 농도에서 상당한 억제 효과가 나타났으나 hexane과 남은 수용층 분획에서는 거의 억제 효과가 나타나지 않았다.

Ethyl ether 분획을 pH에 따라 알칼리성, 중성 및 산성으로 다시 분획한 결과 산성 분획에서 억제 효과가 가장 크게 나타났다.

이상의 결과에서 수수 줄기와 함유하고 있는 타감물질은 비극성이며 산성 특성을 가진 물질이라 추측된다.

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