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EFFECTS OF JUGLONE (5'-HYDROXY-1,4-NAPHTHOQUINONE) ON THE ALGAE ANABAENA FLOS-AQUAE, NOSTOC COMMUNE AND SCENEDESMUS ACUMINATUS

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ABSTRACT

Three species of algae, Anabaena flos-aquae, Nostoc commune and Scenedesmus acuminatus were selected for their sensitivity to juglone and studied for the effects of juglone concentrations of 10, 1, 0.5, 0.1 and 0.01 µg/ml upon their growth. A. flos-aquae was most sensitive, with significant inhibition by the 0.5 µg/ml concentration. N. commune was inhibited least, with significant inhibition only in the 10 µg/ml concentration. S. acuminatus was found to be moderately inhibited at the 0.5 µg/ml concentration. All species were found to be non-viable after 14 days exposure to 10 µg/ml juglone.

Tests with 7 and 14 day old cells of S. acuminatus showed significant differences in growth. Seven day old cells used as inoculum were inhibited by all concentrations while 14 day old cells showed growth in excess of controls in three concentrations (0.5, 0.1 and 0.01 µg/ml). All studies with 14 day old cells showed slight, but not significant, increases in growth in the 0.01 µg/ml concentration. These results suggest that juglone may enhance growth of some soil micro-organisms.

INTRODUCTION

The deleterious effect of the black walnut, Juglans nigra, upon higher plants has been known since ancient times. Many studies have shown that juglone, 5-hydroxy-1,4-naphthoquinone, is the compound responsible for inhibiting the growth of higher plants around the walnut tree (Reitveld, 1983; Funk et al., 1979). Juglone has also been studied as a fish toxicant (Marking, 1970), and has been found to be a depressant in other animals (Westfall et al., 1961). Studies by Koepp (1972), Harmon and Crane (1974, 1976), Cobley et al. (1973), and Grossman et al. (1974) have established a mode of action for juglone as a respiratory inhibitor, specifically of NADH dehydrogenase. Studies by Van Duuren et al. (1978) demonstrated the substance to have potentiating properties. Investigations of micro-organisms have indicated that juglone may serve as a resistance factor to plant pathogens such as Pseudomonas effusa (Hedin et al., 1979), Solkov et al. (1972) presented evidence of juglone inhibition for a variety of plant pathogens and other microbes. Krajci and Lynch (1977) used the antibiotic disc assay method to determine inhibition by crude walnut hull extracts and pure juglone against a broad spectrum of microbes including bacteria, algae and fungi. More recently, Dawson and others have demonstrated inhibitory effects on the symbiotic nitrogen-fixing micro-organisms Rhizobium japonicum strain 71 and Frankia Ar 13 (Dawson et al., 1981; Dawson and Seymour, 1983).

Although some work has been done with members of both Cyanophyceae and Chlorophyceae, those studies have neglected to assess either extremely low-level exposure or the possibility of growth enhancement at non-inhibitory concentrations. In addition, the low solubility of juglone in water has led to the use of organic solvents to provide a carrier for the substance. The purpose of this study was to examine the effects of juglone on two free-living soil algae (Cyanophyceae), and on different aged cultures of a sensitive green alga (Chlorophyceae). Water was used as the solvent for the juglone since it is the natural carrier of the substance.

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MATERIALS AND METHODS

Algae were obtained from Richard C. Starr's collection at the University of Texas. Those selected were Anabaena flos-aquae (Lyng.) Breb. UTEX 1444; Nostoc commune Vaucher UTEX 584; Scenedesmus acuminatus (Lagerh.) Chodat UTEX 415; and Chlorella pyrenoidosa Chick UTEX 252. Preliminary studies showed C. pyrenoidosa to be unaffected by juglone at the concentrations tested so it was abandoned. Juglone (99% pure) was obtained from Sigma Chemical Corporation. A saturated stock solution was made by dissolving juglone in approximately 1L of distilled water with constant stirring for 24 hours. Suspended solids were removed by filtration through a 0.45 µm filter (Millipore). Concentration was calculated by deducting the weight of undissolved solid from the original amount of juglone, correcting for purity and adjusting the volume to 1L. The saturated solution was found to contain 0.1032 g/1 juglone.

Test organisms were cultured in 250 ml flasks containing 100 ml of Bristol's solution in a Lab-Line Environ-Shaker at 20°C, 100 rpm on a 12 hour day/night cycle, for 14 days prior to introduction to test conditions. In studies of S. acuminatus, the inoculum for one series was

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Scenedesmus</th>
<th>Anabaena</th>
<th>Nostoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.0</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0.01</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) observable growth
(-) no observable growth

Table 1. Viability study (1 ml test culture removed from flasks at end of two week exposure to juglone, cultured 7 days on Bristol's medium solidified with 15 g/1 agar. Microscopic examination used to verify negative results.)
allowed to grow for only 7 days. Ten ml aliquots of inoculum were transferred aseptically to flasks containing 80 ml Bristol’s solution and 10, 1, 0.5, 0.1 or 0.01 ml of filter sterilized juglone. Volumes were adjusted to 100 ml for all concentrations. Controls contained 10 ml sterile distilled water. After 14 days incubation the cells were harvested by centrifugation in tared centrifuge tubes at 15,000 rpm for 30 min. Dry weights were obtained by drying at 65°C to constant weight (approximately 7 days).

At time of harvest, 1 ml aliquots of test cultures were pipetted onto Bristol’s medium solidified with agar (15 g/l) in order to test for viability (Table 1). All cultures were tested for purity by streaking on nutrient agar plates at the time of inoculation.

Data for S. acuminatus and N. commune represent the means of three series of three repetitions each. Data for A. flos-aquae represent the means of four series of tests. Significance was determined by both linear regression and Fisher’s Least Squares Determination of analysis of variance. All tests were done at the 0.05 level of significance.

Figure 1. Effects of juglone on the growth (dry weight) of Anabaena flos-aquae shown as a percent of control (vertical line = standard deviation). Correlation = -0.8541; Significance = 0.0001.

Figure 2. Effects of juglone on the growth (dry weight) of Scenedesmus acuminatus shown as a percent of control (vertical line = standard deviation). Correlation = -0.5497; Significance = 0.0003.

Figure 3. Effects of juglone on the growth (dry weight) of Anabaena flos-aquae and Scenedesmus acuminatus shown as a percent of control (vertical line = standard deviation). Comparison of 1 and 2 week old inocula for initial difference in inoculum dry weight. Correlation = -0.8585; Significance = 0.0001 for one week inoculum. Correlation = -0.7314; Significance = 0.0001 for two week inoculum.

DISCUSSION

Krajci and Lynch (1977) demonstrated the inhibition of several different bacteria, algae and fungi by juglone. The algae tested were Calothrix flaccumbicens, Anacystis sp., Bracteacoccus cinnabarinus, Coelastrium microsporum, and Anabaena variabilis. Their results showed A. variabilis to be inhibited by juglone concentrations of 0.0625 mg/ml. Corresponding concentrations of 10 and 1 ug/ml used in this study significantly inhibited A. flos-aquae. Apparently, the two Anabaena species exhibited a similar sensitivity to juglone.

Although this is the first report of algal growth stimulation by juglone, others have reported similar results for higher plants. Funk et al. (1979) reported the stimulation of some coniferous seedlings at concentrations of juglone as low as 10^-4 and 10^-5 M. Reitveld (1983) also reported some increased growth in 10^-3 and 10^-4 M concentrations of juglone, but the increases were not significant. Data presented here indicate...
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Juglone was the subject of a study to determine its effects on blue-green algae. The study was conducted by BARRY, A. L. (1964) on the routine antibiotic disc-plate sensitivity test II. The study was published in the American Journal of Medical Technology, Volume 30, Issue 333-342.

COBLEY, J. G., S. GROSSMAN, H. BEINERT, and T. P. SINGER (1983) conducted a study on the catalytic activity and EPR signals of DPNH dehydrogenase and its relationship to the acquisition and loss of pteridin sensitivity and the coupling site 1. This study was published in Biochim Biophys Acta, Volume 73, Issue 1273-1281.


GROSSMAN, S., J. G. COBLEY, and T. P. SINGER (1974) conducted studies on the inhibition of various microorganisms with juglone. These studies were published in the Journal of Bacteriology, Volume 122, Issue 381-3826.

HARMON, H. J., and F. L. CRANE (1974) studied the topographical distribution of new sites on the mitochondrial electron transport chain. This study was published in the Journal of Biological Chemistry, Volume 250, Issue 326-333.


KOEPPE, D. E. (1972) studied the reactions of isolated corn mitochondria influenced by juglone. This study was published in the Journal of Biological Chemistry, Volume 240, Issue 89-94.

KRAJC, W. M., and D. L. LYNNCH (1977) conducted a study on the inhibition of various microorganisms by crude walnut hull extracts and juglone. This study was published in Microbiol Lett., Volume 4, Issue 175-181.

LANGHANS, V. E., P. A. HEDIN, and C. H. GRAVES (1976) studied fungitoxic substances found in pecan (Carpa illinoensis K. Koch). This study was published in the Journal of Agricultural Food Chemistry, Volume 28, Issue 89-94.

MARKING, L. L. (1970) studied the growth (5-hydroxy-1,4-naphthoquinone) as a fish toxicant. This study was published in the Journal of the American Chemical Society, Volume 99, Issue 510-514.

REITVELD, W. J. (1983) conducted a study on allelopathic effects of juglone on germination and growth of several woody species. This study was published in the Journal of the American Chemical Society, Volume 92, Issue 295-308.

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