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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 (Reitvedt, 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 potent tumor promoting properties. Investigations of micro-organisms have indicated that juglone may serve as a resistance factor to plant pathogens such as Pseudomonas effusum (Hedin et al., 1979), Solkow 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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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.

![Graph 1](image1.png)

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.

**DATA AND RESULTS**

The results of viability checks for all tests showed no growth in the 10 ug/ml concentration. A. flos-aquae showed no growth in the 1 ug/ml concentration. Analysis of dry weights showed a significant change between the 0.1 and 0.5 ug/ml concentrations, resulting in a biomodal distribution (Figure 1). The lower concentrations were grouped with the controls and the 10, 1 and 0.5 ug/ml tests were related. It is of interest to note the slight increase in growth associated with the 0.01 ug/ml concentration. While not significant, it nonetheless was seen in all experiments with the exception of S. acuminatus cultures initiated with 7 day old inocula. N. commune was significantly affected by only the 10 ug/ml concentration. All other data were within one standard deviation of the control. Once again there was a slight elevation in growth in the lowest concentrations of juglone (Figure 2).

The study of different inoculum ages of S. acuminatus displayed no significant difference between the two highest concentrations of juglone when adjusted for difference in initial inoculum weights (Figure 3). The 7 day old inoculum was inhibited in all concentrations of juglone. Significant inhibition occurred only in the 10, 1 and 0.5 ug/ml concentrations, with the 69% weight at 0.5 ug/ml being the only example of significant inhibition which differs from both the controls and the nonviable cultures. The 14 day inoculum showed growth above the level of controls in all but the 10 and 1 ug/ml concentrations. There was again an increase in overall growth at 0.01 ug/ml juglone solution for the 14 day old inoculum, although the 7 day inoculum was significantly inhibited at this concentration. Results of viability checks indicated that only the 10 mg/ml solution was lethal.

![Graph 2](image2.png)

Figure 3. Effects of juglone on the growth (dry weight) of Scenedesmus acuminatus shown as a percent of control (vertical line = standard deviation). Comparison of 1 and 2 week old inocula adjusted 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 flaccumbiens, Anacystis sp., Bracteacoccus cinnabarinus, Coelastrum 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 canisterous seedlings at concentrations of juglone as low as 10⁻⁴ and 10⁻⁶ 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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