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Assessing Biological Activity of Agricultural Biostimulants: Bioassays for Plant Growth Regulators in Three Soil Additives

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ABSTRACT

Laboratory bioassays were used to investigate plant growth-regulating effects of three different experimental soil additives, designated *EXP95*, *W91*, and *Z96*. A yeast growth test was used as a general assay of bioactivity, responses to soil additives were compared to those of known plant growth regulators [indoleacetic acid (IAA), gibberellic acid (GA_3), and kinetin]. A corn coleoptile elongation test was used to assay for auxin-like activity and a dwarf pea bioassay was used for gibberellin-like activity. The three soil additives were tested at five solution concentrations ranging from 1 to 10,000 ppm (by volume). All three soil additives stimulated yeast growth, depending on the concentration of the test solutions. However, all three soil additives inhibited plant growth in the two plant bioassays. Although this study clearly demonstrated that the three soil additives had significant biological activity at very low concentrations, there was little evidence for auxin-like or gibberellin-like activity.

INTRODUCTION

Non-conventional soil additives, such as soil biostimulants, although rarely recommended by State University extension programs, are nevertheless widely used in both horticultural and field crop production. There are numerous products claimed to enhance crop growth and yield through widely varying mechanisms, such as inoculation of soil with microorganisms, activation of soil microbial activity, promotion or augmentation of the activities of critical soil enzymes or plant growth hormones, or supplementation of micronutrients. However, there is very little scientific evidence to support or refute the claims of most of these products.

Despite the general lack of information on the effects of biostimulants on soil-plant systems, the modes of action of some soil treatments have been investigated in some detail (Russo and Berlyn, 1990, 1992; Subler et al., 1995a, 1995b, 1998). These include two experimental soil treatments, designated *Z93* and *W91* (Ag Spectrum Co., DeWitt, IA), that are comprised of solutions of fermentation products and trace minerals. There is good evidence that these treatments, when applied to the soil at remarkably low rates (typically 3 to 15 ounces per acre) can alter soil microbial communities and activities, stimulate the decomposition and mineralization of organic materials in the soil, increase nutrient availability, and enhance plant growth (Subler et al., 1995a). Yet, the precise mechanisms by which these additives influence soil and plant processes are not well understood. Although there is an apparent link between increased nutrient mineralization and availability and nutrient uptake and growth of plants in soils treated with these materials, other alternative mechanisms cannot yet be ruled out.

One such possible mechanism is the direct chemical action of the soil additives on plant development and growth. Plant growth regulators (PGRs), organic compounds that modify plant physiological processes, have been used widely in both horticultural and field crop production (Harms and Oplinger, 1988). It is well established that soil microorganisms can produce specific PGRs such as auxins, gibberellins, and cytokinins (Frankenberger and Arshad, 1995). Microorganisms can also influence the production of humic acids which can influence plant growth directly (Chen and Aviad, 1990).

We wanted to determine if soil additives, such as *Z93* and *W91*, could influence plant growth directly. In this study, we used a series of bioassays to evaluate the potential influences of three soil additives on plant growth.

METHODS

Three bioassays, a yeast growth test, a corn coleoptile elongation test, and a dwarf pea growth test (Hill, 1980) were used to investigate the influence of the three soil additives on various aspects of plant growth and to compare the growth

responses of the soil additives with those of specific plant growth compounds (auxins, cytokinins, and gibberellins).

Yeast Growth Test

A culture of *Saccharomyces cerevisiae* was obtained from Carolina Biological Supply Company. An active and standardized inoculum of *S. cerevisiae* for bioassay was obtained after incubating 50-mL portions of inoculated NG liquid medium in 250-mL Erlenmeyer flasks on a rotary shaker at 25°C for 24 h. Gibberellic acid, kinetin (6-furfuryl-aminopurine), and indolyl-3-acetic acid were obtained from the Sigma Chemical Co., St. Louis, MO.

The test solutions, designated *EXP95*, *W91*, and *Z96* (equivalent to *Z93*) were obtained from Ag Spectrum Co. (DeWitt, IA). They were described as aqueous solutions of complex organic compounds derived from fermentation processes and supplemented with trace minerals. Different concentrations of these solutions (0.0001%, 0.001%, 0.01%, 0.1%, and 1% by volume) were added in 5 mL amounts to flasks containing 50 mL of NG agar containing 0.1% magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.2% potassium dihydrogen phosphate (KH_2PO_4), 0.4% ammonium sulfate [$(NH_4)_2SO_4$], 0.3%; 0.4% yeast extract (Oxoid), 0.5% peptone (Orthane; Analema Chemical), and 1.0% glucose by weight. Each flask was inoculated with 0.1 mL of standardized inoculum of *S. cerevisiae* and then incubated on a rotary shaker at 25°C for 24 h. Optical density measurements at 650 nm were made on each sample. Deionized water was used as a control. Solutions of GA_3, kinetin, and IAA (each 5 ppm) were used as positive controls. There were four replicates for each treatment.

Corn Coleoptile Elongation Test

In this test, corn seedlings were grown in the dark until coleoptiles were 2-3 cm long. The apical 3-4 mm of the coleoptiles were removed and a segment of standard length cut from the remaining part and floated on the test solutions. The increase in length of this segment after 24 h in the dark was taken as a measure of auxin activity. Deionized water and IAA (10^{-5}M) were used as controls. Five replicates were used for each treatment.

Dwarf Pea Bioassay

Gibberellins are physiologically defined by their ability to induce shoot elongation in certain dwarf plants and they have been demonstrated in various plant extracts using maize plants and dwarf peas as assay material. Seeds of dwarf pea and normal pea were grown in the greenhouse at the rate of three seeds per pot. Four days after germination, a drop of the test solutions containing 0.05% Tween 20 surfactant solution were applied to the shoot of the plants. The application of the solution was repeated after 48 and 96 hours. Stem length increase of dwarf

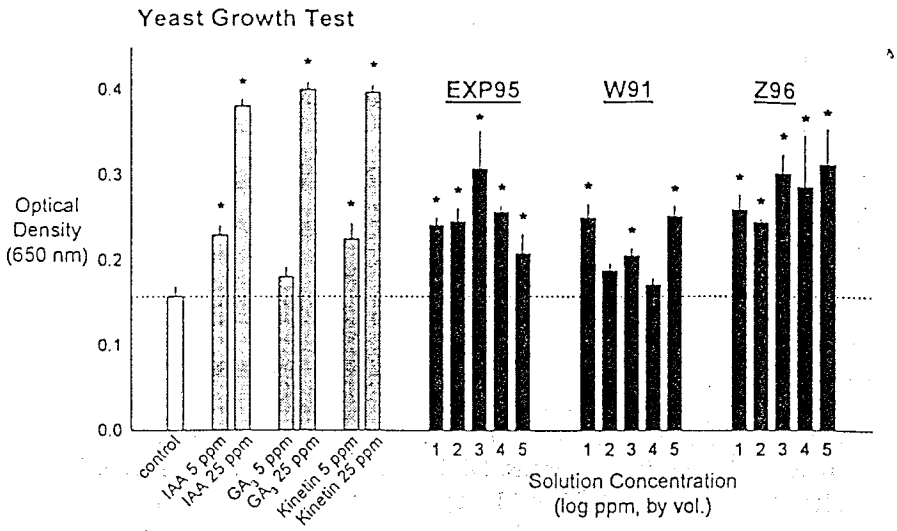

FIGURE 1. Yeast cell proliferation (measured as optical density) in solutions with different concentrations of the three soil additives. Known plant growth regulators, indole acetic acid (IAA), gibberellic acid (GA_3), and kinetin were used as positive controls. Mean±SE (n=5). Dashed line indicates mean control response. Asterisks indicate significant differences ($P<0.05$) between treatment and control means.

pea plants compared to normal pea plants indicate the effect of gibberellin-like compounds. Deionized water and GA_3 (100 ppm) were the blanks. Five replicates were used for each treatment.

Statistical Analysis

Analysis of variance and regression procedures were used to evaluate the significance of treatment effects. For each bioassay, Dunnett's two-tailed t-test (Dunnett, 1955) was used to determine if the treatments were significantly different ($P<0.05$) from the untreated controls; this test holds the experiment-wise error rate to a specified alpha level. The SAS software was used for statistical analyses (SAS, 1990).

RESULTS AND DISCUSSION

Yeast Growth Test

The yeast growth test determines the direct biological activity of chemical treatments on yeast cell proliferation. It has been found to respond well to the presence of various plant growth hormones and has been suggested as a simple, yet sensitive, general bioassay for plant growth regulating substances (Barea et

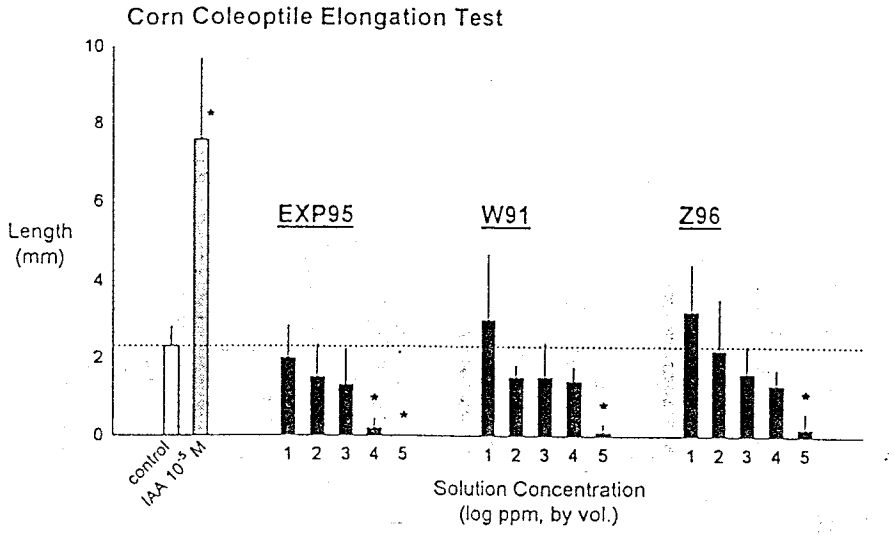

FIGURE 2. Corn coleoptile elongation in solutions with different concentrations of the three soil additives. Indoleacetic acid (IAA) was used as a positive control. Mean±SE (n=5). Dashed line indicates mean control response. Asterisks indicate significant differences ($P<0.05$) between treatment and control means.

al., 1974). In this study, the three plant growth hormones used as positive controls showed significant yeast growth responses, especially at 25 ppm (Figure 1).

All three soil additives significantly increased yeast growth, depending upon treatment concentration (Figure 1). *EXP95* increased yeast growth compared to the control at all concentrations tested; growth was highest at 100 ppm (0.01%). *W91* increased yeast growth significantly at the low and high concentrations, but had less response at intermediate concentrations. Of the three soil additives, *Z96* had the greatest influence on yeast growth, exhibiting a log-linear increase in growth with increasing concentration.

Figure 1 clearly demonstrates that all three of the soil additives tested had significant direct biological activity at relatively low solution concentrations. Since the test solution contained a complete complement of macro- and micronutrients adequate for short-term growth, this suggests that the biostimulant effect of the soil additives was due to some mechanism other than simple nutrient amendment. The nonlinear response with concentration for two of the three additives also indicates a non-nutrient mediated response. Although it is not possible to determine if the observed growth responses to the treatments resulted from the activity of specific plant growth hormones, the responses fell within the range of those elicited by the three positive controls (IAA, GA_3, and kinetin). Nevertheless, this provides

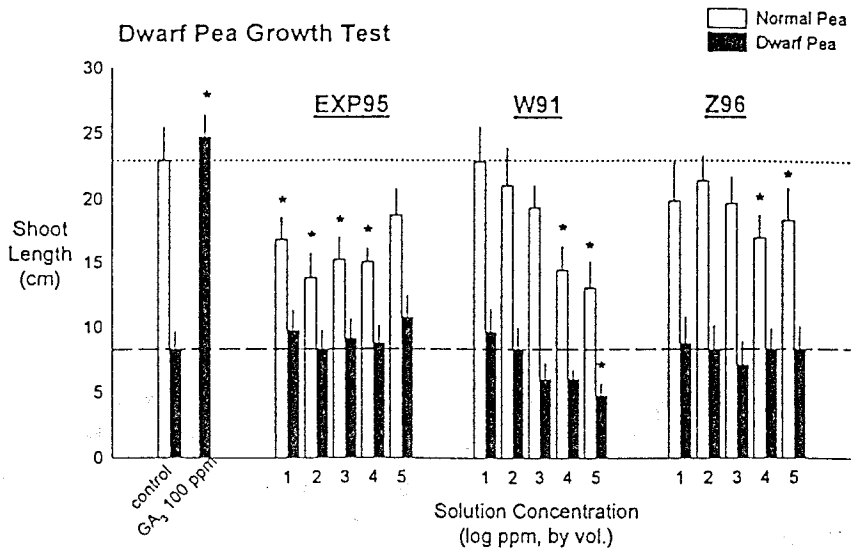

FIGURE 3. Shoot length of normal and dwarf pea plants treated with in solutions with different concentrations of the three soil additives. Gibberellic acid (GA_3) was used as a positive control. Mean±SE (n=5). Dashed lines indicate mean control responses. Asterisks indicate significant differences ($P<0.05$) between treatment and respective control means.

strong evidence for significant biostimulant activity of the three soil additives, at least on specific microorganisms, and suggests possible plant growth-regulating effects as well.

Corn Coleoptile Elongation Test

The corn coleoptile elongation test is used to assay for activity of auxin-like compounds. In this study, the IAA control led to a dramatic increase in coleoptile elongation (Figure 2). However, all three soil additives led to significant inhibition of coleoptile elongation, generally increasing with concentration. There was a tendency for both *W91* and *Z96* to have slightly greater mean coleoptile elongation at the lowest concentrations than the untreated control, but these differences were not statistically significant. It would be interesting to determine if concentrations lower than those used in this study would cause significant positive responses.

All three soil additives tested inhibited corn coleoptile elongation at most concentrations. This suggests that, even though the soil treatments had significant direct biological activity on plant growth, there is little evidence for the activity of auxin-like plant growth regulators.

Dwarf Pea Bioassay

The dwarf pea growth test is used to assay for activity of gibberellin-like compounds. Treatment of dwarf plants with gibberellic acid increased their shoot length to levels comparable to normal pea plants (Figure 3). All three soil additives significantly inhibited shoot elongation in the normal pea plants. *W91* had the widest range of response, increasing from no response relative to the normal pea control at the lowest concentration, to nearly 50% reduction in length at the highest concentration. Neither *EXP95* nor *Z96* affected the shoot length of the dwarf pea plants. However, *W91* significantly inhibited dwarf pea shoot elongation with increasing concentration.

All three of the soil additives inhibited normal plant growth in the dwarf pea growth test, providing additional evidence for the direct influence of each treatment on plant growth. However, due to the lack of stimulation of shoot elongation in the dwarf pea plants, there is no evidence of gibberellin-like activity.

CONCLUSIONS

The bioassays used in this study provide strong evidence for the direct biological activity of the three soil additives (*EXP95*, *W91*, and *Z96*) at very low with a concentrations. All three additives exhibited biostimulant activity in the bioassay microorganism (yeast), but had inhibitory effects on various plant growth parameters. Despite their significant effects on plant growth, there was little evidence for the activity of either auxin-like or gibberellin-like compounds in the soil additives. Although our results suggest non-nutrient mediated effects, further research would be necessary to elucidate the mechanisms by which these soil additives directly influence microbial and plant growth.

ACKNOWLEDGMENTS

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