Plant Growth Regulatory Effects of Chicken Litter Extract

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Joe M. Bradford

ABSTRACT. Chicken litter is often used in organic farming as a source of plant food, and to improve soil organic matter and microbial populations. Both positive and negative effects of such an amendment have been reported. Because of the complex interactions involving soil, plant, and microbial populations in the most common test systems, it is difficult to attribute the observed plant responses to any one component of the test system affected by chicken litter. We have therefore conducted studies on the chicken litter extracts in a soil-less system to ascertain whether chicken litter affects plants simply by providing plant nutrients or if it also affects plants through plant growth regulatory substances. Since chicken litter is generally rich in available ammonium nitrogen, we studied the effects of chicken litter extract on ammonium induced rise in putrescine levels in oat leaves. Our results showed increases in putrescine levels in oat leaves floating on ammonium sulfate solution (0.033%) but

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Journal of Sustainable Agriculture, Vol. 30(2) 2007
Available online at http://jsa.haworthpress.com
doi:10.1300/J064v30n02_03

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not in leaves floating on chicken litter extract containing equivalent amount of ammonium nitrogen. Mixing chicken litter extract with the ammonium sulfate inhibited the ammonium induced rise in putrescine levels thus supporting the hypothesis that regulatory substances in chicken litter extract affect plant growth. In addition, our experiments showed that chicken litter extracts inhibited root growth in cowpeas that could not be attributed to the levels of ammonium nitrogen present in the extract. In fact, emerging roots of young cowpea seedlings exhibited an anti-geotropic response more akin to hormonal effects than a nutritional phenomenon.

doi:10.1300/J064v30n02_03 [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-HAWORTH. E-mail address: <docdelivery@haworthpress.com> Website: <http://www.HaworthPress.com>.

KEYWORDS. Ammonium sulfate, chicken litter, growth regulatory, organic farming, polyamine, putrescine

INTRODUCTION

Chicken litter has long been used as a soil amendment to provide nutrients for plant growth (Bush et al., 1999; Qafoku et al., 2001), increase organic matter in the soil (Mello and Vitti, 2002), improve microbial populations in the soil (Riegel et al., 1996; Doubs et al., 1997; Riegel and Noe, 2000), and to control plant pests such as nematodes (Rodriguez et al., 1995) and weeds (Craft and Nelson, 1996). In addition, chicken litter has been included in the diet of cattle to provide nutrients (e.g. proteins) (Devendra, 1983). In either case, both positive effects, in the form of improved plant growth (Snyder et al., 1993) or better animal health and production (Holzer et al., 1980; Ayangbile et al., 1993), and negative effects, in the form of phytotoxicity (Asmus et al., 2002) or adverse effects on animal health (Popoff, 1989) have been reported. We observed that chicken litter applied as a soil amendment, according to suppliers recommended rates, produced severe phytotoxic effects on grape vines and on three month old olive rooted cuttings, but had no negative effect when sprayed on foliage; in fact, foliage looked greener after the sprays (Malik and Bradford, unpublished results).

Most studies on organic amendments are conducted in complex systems involving interactions between plant, soil, and micro flora. Therefore, it is usually difficult to assess whether a particular plant response to an organic amendment is due to a direct effect of the amendment mediated by nutrients or some other substances such as growth regulatory compounds, or is an indirect manifestation of changes in soil physical or
biological conditions. This lack of knowledge about the nature of organic amendments and their interactions in different environments may be partly responsible for some of the contradictory results obtained by different workers. It would be valuable to have an understanding of individual effects of this complex system, and hence, to make appropriate management decisions in an organic farming system. In this study, regulatory effects of chicken litter extract on plants in a soil less system were studied, thus eliminating the variable of soil physics or microflora interactions. Since ammonium nitrogen is a major component of chicken litter extract, and it is well-known that ammonium nitrogen increases putrescine levels in leaves (Smith, 1975), we decided to study putrescine levels in oat leaf segments floating on chicken litter extract and on ammonium sulfate solutions. Upto this date we are not aware of any reports that specifically describe plant growth regulatory responses to chicken litter sold as plant food.

**MATERIALS AND METHODS**

**Plant Growth and Test System**

*Studies on Leaf Metabolism.* The effect of chicken litter extract and ammonium sulfate (the reason for choosing ammonium sulfate has been described in the previous section) on leaf metabolism was determined using oat leaf segments placed under light. This system has been used to study leaf metabolism in response to various stimuli in several of our previous studies (Malik, 1987 and references therein) also in various other laboratories (Thimann, 1978 and references therein). Procedure for growing oat seedlings and testing the effect of various agents on metabolism of sub-apical oat-leaf segments has been described previously (Malik and Thimann, 1980; Malik, 1987). Briefly, oat seeds (*Avena sativa* L. cv Jerry) were sown 1.5 cm deep in rows in plastic trays (4 cm deep) filled with water-soaked vermiculite. The trays were placed under a bank of fluorescent light giving 30 μmol m−2 s−1 at plant level at 23 ± 1°C. After seven days, 3 cm sub-apical segments were taken from the first fully elongated leaves of oat seedlings. Seven segments were placed abaxial side down in Petri dishes containing 10 ml of test solution and were incubated under light for five days. At the end of the incubation period, the leaf segments were blotted dry and frozen at −80°C until needed for analyses.

*Studies on Root Growth.* For these studies, cowpeas (*Vigna unguiculata* L. cv. clay) were grown in plastic growth pouches as used in our earlier
studies on soybean (Malik et al., 1987). Briefly, the seeds were surface sterilized by soaking for 7 minutes in 5.25% sodium hypochlorite with one drop of Tween 20 as a surfactant. The seeds were then washed seven times with sterilized water, soaked for 1 h in water or in a test solution, and then spread over filter paper and placed in Petri dishes containing 10 ml of test solution. The seeds were allowed to germinate in dark at 26°C for 48 h. Two seedlings were transferred to plastic growth pouches (Northrup King Seed Co. Minneapolis, MN) containing 10 ml of the appropriate test solution. The pouches were then placed in a growth chamber (Revco, series 1500) maintained at 29 ± 1°C during day (12 h photoperiod) and 26 ± 1°C in the night. The light intensity at plant height was 300 μmol m⁻² s⁻¹. The pouches were kept in the dark by covering them with aluminum foil during first 24 h after which the foil was removed. The root length was measured after two days. At least 20 replicate plants were used in each treatment.

Cowpea seeds were also sown in 4 cm deep plastic trays filled with a 50:50 mix of vermiculite: Perlite soaked with either inorganic nutrient solution or 1% chicken litter extract. The temperature in the greenhouse was maintained at 29 ± 2°C during day and at 26 ± 2°C during night in the winter. Light intensity in the greenhouse at mid-day varied between 500-800 μmol m⁻² s⁻¹.

Preparation of Chicken Litter Extract. Commercial chicken litter preparation sold as organic amendment was purchased from Ag Org Inc., Houston, Texas. One gram of this material was stirred in 100 ml of deionized water with a magnetic stirrer at high speed for 1 h at room temperature and then centrifuged at 39,000 g for 30 min; at this centrifugal force bacteria and spores settled in the pellet and hence are removed from the extract. The supernatant liquid (1× extract) was adjusted to neutral pH and used for tests with cowpea roots or oat leaf segments. Fresh extract was prepared for each application. The amounts of ammonium nitrogen in samples of chicken litter and 1% chicken litter extract were determined by Midwest Laboratories, Omaha, Nebraska.

Chemical Analyses

Extraction and Derivatization of Polyamines. Polyamines, primarily putrescine, were extracted from plant tissue by a modified procedure described by Flores and Galston (1982) and Reddy et al. (1993). Plant tissue was pulverized into fine powder in liquid nitrogen with pestle and mortar. A 0.25 g sample of frozen oat leaf powder was mixed with 1.5 ml of 5% (v/v) perchloric acid and vortexed overnight at 4°C. The
mixture was centrifuged at 13,000 g for 20 min. A 0.5 ml aliquot of the supernatant liquid was mixed with 1 ml of 2 N KOH, briefly vortexed, and then mixed well with 10 μl of benzoyl chloride and allowed to incubate at room temperature for 15 min. After incubation, the mixture was centrifuged at 13,000 g for 15 min. The supernatant liquid (containing derivatized free polyamine from plant tissue) was aspirated and placed in a 15 ml propylene tube and partitioned three times with ethyl ether. Ether fractions were pooled and evaporated under nitrogen at 40°C, and the residues were reconstituted with 200 μl of HPLC grade methanol. Putrescine standard was also similarly passed through the same derivatization and partitioning procedure.

Separation and Measurements of Putrescine by HPLC. A slightly modified method of Flores and Galston (1982) was used to analyze putrescine in different extracts. The method allows clear separation of putrescine from other polyamines for quantitative measurements. HPLC analyses were performed using Waters (Milford MA) Alliance HPLC system (model 2695) equipped with photodiode array detector (model 2996). Polyamines were separated by reverse phase HPLC using Waters Symmetry C$_{18}$ (5 μm particle size) column (3.9 × 150 mm) maintained at 35°C during chromatographic runs. The solvent system consisted of acetonitrile: 0.02% trifluoroacetic acid in water (46:54) ran isocratically at flow rate of 1 ml/min. A 10 μl aliquot of extract or standard solution was injected for each run and bezoylpolyamines were detected at 254 nm. Putrescine in the extracts was identified by comparing retention times and UV spectra with the standard peak.

RESULTS AND DISCUSSION

The Effect of Chicken Litter Extract on Leaf Metabolism

Putrescine levels in oat leaf segments floating on ammonium sulfate solution (0.033%, 1×) under light for five days were approximately 250% higher compared with leaf segments floated on water (i.e., 349.67% of water controls) for the same period of time (Table 1). These results confirm previous reports that ammonium nitrogen increases putrescine levels in plant tissue (Smith, 1975). Putrescine levels did not increase in leaf segments kept on 1× (1% in water) chicken litter extract (about 17% less than water control) that contained the same amount of ammonium nitrogen as was in 1× ammonium sulfate solution (Table 1). In fact, leaf segments placed on chicken litter extract (i.e., containing
TABLE 1. The effect of chicken litter extract and ammonium sulfate on ammonium induced rise in putrescine levels in oat leaf segment kept under light for 5 days.

<table>
<thead>
<tr>
<th></th>
<th>Chicken litter extract 1×</th>
<th>Ammonium sulphate 1×</th>
<th>Ammonium sulphate 1× + Chicken litter extract 1×</th>
<th>Ammonium sulphate 0.5×</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine levels</td>
<td>83.33</td>
<td>349.67²</td>
<td>122.80³</td>
<td>237.67</td>
</tr>
<tr>
<td>as % water controls</td>
<td>±10.48⁴</td>
<td>±19.65</td>
<td>±30.06</td>
<td>±52.07</td>
</tr>
<tr>
<td>Standard error of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹1% water extract of chicken litter containing equivalent amount of ammonium nitrogen as in 1× ammonium sulfate solution (0.033% (NH₄)₂SO₄).
²The putrescine levels, as percent water controls, in leaves floating on 2× ammonium sulfate were 596.67 ± 92.59.
³The putrescine levels, as percent water controls, in leaves floating on 50:50 mixture of 1× chicken litter extract and 2× ammonium sulfate were 239.00 ± 38.37.
⁴± is standard error of mean. The means differ significantly at P < 0.05.

are known to play important role in regulating various developmental phenomenon (Peeters et al., 1993; Rugini and Mencuccini, 1985; Smith, 1985). Any agent such as chicken litter extract that modulate putrescine levels may affect plant growth regulation. The fact that chicken litter extract inhibited the rise in ammonium induced putrescine may not always have negative effects on
plant growth and productivity because high levels of putrescine in leaves could produce deleterious effects on leaves in light (Kauer-Sawhney and Galston, 1979). Thus, regulating the rise of putrescine in leaves under high ammonium levels might be beneficial in some cases.

**The Effect of Chicken Litter Extract on Root Growth**

Table 2 shows that 1× chicken litter extract (1%) applied to cowpea roots in a soil less system produced approximately 50% inhibition in root length. This inhibition does not seem to be due to the presence of high ammonium nitrogen because ammonium sulfate providing equal amount of ammonium nitrogen as in chicken litter extract did not produce similar inhibitory effects (Table 2). Since the extract was centrifuged at speeds sufficient to remove microbes and spores in the supernatant, it is unlikely that the observed effects here could be due to the indirect effects of microbes. Similarly, it is unlikely that the root growth inhibition in cowpeas could be due to any interactions of materials in chicken litter and components of soil system as the experiment was conducted in the soil-less system. Thus, it appears that chicken litter extract contain substances that regulate the inhibition of root growth in cowpeas. These results can help explain part of our earlier findings that application of chicken litter at recommended applications rate (much higher concentration than 1% extract) produced strong toxic effects on two-month-old rooted olive cuttings and the rooted grapevine cuttings (unpublished results of Malik and Bradford).

When cowpeas were sown in vermiculite trays supplied with 1% chicken litter extract, a majority of seedlings had roots coming out on

**TABLE 2. The effect of chicken litter extract and ammonium sulfate on root elongation in cowpeas.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root length (cm)</th>
<th>Percent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water control</td>
<td>8.0 ± 0.82</td>
<td>0</td>
</tr>
<tr>
<td>Chicken litter extract</td>
<td>4.2 ± 0.36</td>
<td>48</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>7.5 ± 1.0</td>
<td>6</td>
</tr>
</tbody>
</table>

1 ± is standard error of mean. The means marked with letter ‘a’ are significantly different from the mean marked with letter ‘b’ at P < 0.05.

2 1% extract was used in this experiment.

3 Average inhibition of three repeat experiments was 55%.

4 0.033% ammonium sulfate (providing equivalent amount of ammonia nitrogen as determined in 1% chicken litter extract) was used in this experiment.
the surface as opposed to normal pattern of root growth down towards gravity through the vermiculite (Figure 1). Even when the roots were covered with additional vermiculite, large number of seedlings could not stand erect for 4-5 days after emergence. This anti-geotropic response of cowpea seedling roots also indicate that chicken litter extracts could also affect plant growth through the presence of regulatory substance(s).

FIGURE 1. Cow pea seedlings growing in 1% chicken litter extract (A) show anti-geotropic response, while seedling growing in inorganic nutrient solution (B) show normal development with roots going down and cotyledons standing up.
CONCLUSIONS

The results of the present studies show that organic amendments such as chicken litter could affect plant growth and productivity in more ways than by simply supplying plant nutrients. Thus, it would be prudent to conduct initial studies under controlled conditions to develop effective management practices for large scale organic farming under different conditions for different crops. For example, application of high doses of chicken litter directly at the root zone during early seedling development may cause serious damage to crop while various concentrations of chicken litter extract (depending on crop and stage of development) alone, or in combination with other amendments, may prove beneficial for crops if applied as foliar sprays. The present study was done under laboratory conditions, and therefore, careful field studies at limited scale are needed before applying these results for large scale field management of crops.

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