Research Report

Physical and Chemical Properties of Biobased Plastic Resins Containing Chicken Feather Fibers

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Abstract. This study was conducted to (a) characterize bio-plastic pellets containing feather fibers by low temperature-scanning electron microscopy (LT-SEM) and X-Ray diffraction analysis, (b) evaluate growth and flowering of *Begonia boliviensis* A. DC. 'Bonfire' when grown in a medium amended with pellets, and (c) analyze macro- and micro-elements in the medium and plant tissues of begonia. Based on physical and chemical analyses of six types of pellets mixed in the medium, pellets 32 (P 32), 37 (P 37), 40 (P 40), and P NaS (P NaS), P NaS containing sodium sulfite and > 30% glycerol were not suitable and not recommended for future evaluations. Pellets containing glycerol at 10% were not suitable for the use as an ingredient for commercial growing media and P 45 is considered suitable pellets to prepare pots.

Additional key words: growth and flowering, low temperature-scanning electron microscopy (LT-SEM), poultry feather, soil and foliar analysis, X-ray diffraction analysis

Introduction

Approximately four to six billion pounds of waste poultry feathers are produced each year in the United States and are processed into cheap animal feed, buried, or incinerated (Kornillowicz-Kowalska and Bohacz, 2011). Feathers, which are approximately 91% keratin, are an important source of keratin protein with a unique feature of a cross-linked semi-crystalline structure at the molecular level (Schmidt and Jayasundera, 2004). The use of feathers with polymers or plastics derived from renewable resources will allow many environmental issues to be resolved.

Pellets are prepared from various formulations containing varying amounts of poultry feather fibers, and other additives such as petrochemicals and biodegradable polymers need to be mixed with the poultry fibers. Development of a nursery growing container with keratin would be desirable. Pots or containers are produced by preparing bioplastic pellets (pellets) composed of keratin fibers mixed into high-density polyethylene and injecting this mixture at 20% by weight into

molds (Barone, 2005; Barone et al., 2005).

However, no information is currently available to evaluate the impact of pellets containing feather fibers, and eventually pots, on growth and development of crops. Growth and flowering of floral crops depends on many factors including the medium with acceptable pH and other physical and chemical properties. Medium with 30% feather fibers did not affect growth of floral crops that include 'Cooler Blush' Vinca (*Catharantus roseus* G. Don) (Evans, 2004).

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White feathers of broiler chickens following steam hydrolysis released the greatest amount of nitrogen during the first 5 weeks, but did not add to the 12-week profiles (Choi and Nelson, 1996a) and the microbial hydrolysis with *Bacillus licheniformis* was observed during weeks 8 through 11, which suggested that nitrogen would be slowly released following treatment with *B. licheniformis* (Choi and Nelson, 1996b). Roots readily penetrated the walls of both feather and peat pots (Evans and Hensley, 2004). The nitrogen concentration was higher in 'Better Boy' tomato plants when grown in feather containers which may release additional



nitrogen, resulting in plants with higher dry weight as compared to those grown in plastic or peat pots (Evans, 2004).

At present, there is no report on how pellets may affect the chemical properties of the medium and plant growth and development. We selected *Begonia boliviensis* A. DC. 'Bonfire' to evaluate the growth and flowering in 30- to 90-day projects. The objectives of this study were to (a) examine and characterize several plastic pellets (pellets) containing feather fibers using low temperature scanning electron microscopy (LT-SEM) and X-Ray diffraction analysis, (b) evaluate growth and flowering of *B. boliviensis* A. DC. 'Bonfire' with pellets mixed in the medium, and (c) analyze the concentrations of macro- and micro- elements in pellets, growing media amended with pellets, and plant tissues.

Materials and Methods

Poultry Feather Fiber Based Biobased Plastic Resin Pellets

Poultry feather fibers as previously described (Barone et al., 2005) was used in this study. Glycerol (CQ Concepts Inc., Ringwood, IL, USA) was used as a low molecular weight plasticizer. Polypropylene (Pro-Fax PH920S), a thermoplastic polymer matrix (Basell Polyolefins, Elkton, MD, USA), ethylene acrylate (C₈H₁₀O₄) copolymer, Biomax[®] Strong 100, an additive for the biodegradable polymer (DuPont, Wilmington, DE, USA) were used. Epolene® E-43 (MAGP), to improve the performance of the biodegradable materials (Westlake Chemical Co., Houston, TX, USA) as coupling agent, polylactide (PLA 3001D)), a corn-based polymer (NatureWorks LLC, Blair, NE, USA), and Talc as a nucleating agent (Rio Tinto Minerals, Greenwood Village, CO) were used to prepare pellets. Sodium sulfite (Na₂SO₃, NaS) was dissolved in de-ionized water at a concentration of 2% by weight of total feather keratin content. The poultry feather was placed into a PBB2 Professional Bar Blender (Waring Consumer Products, East Windsor, NJ, USA) and the aqueous solution of sodium sulfite was added gradually.

Processing of Biobased Plastic Resin Materials and Fabrication of Resin

White poultry feathers were dried at 80°C under a vacuum for 18 h to reduce moisture content to 2-3%. The poultry feathers with other ingredients (Table 1) was mixed (Brabender Plasti-corder, Brabender GmbH & Co., Duisburg, Germany) and then melt blended in a Brabender PL2000 (Brabender Instruments, Inc., South Hackensack, NJ, USA) twin-screw extruder. The extrusion was conducted at a speed of 80 rpm and at a barrel temperature of 175, 179, and 185°C from the feeding zone to the die zone. A pelletizer (Bay Plastics Machinery, Bay City, MI) was used to chop the extruded strands into pellets.

Low Temperature Scanning Electron Microscopy (LT-SEM)

Low temperature scanning electron microscopy (LT-SEM) observations of the cut surface of pellets were performed using the techniques previously described by Ochoa et al. (2011). An accelerating voltage of 5 kV was used to view the specimens. Images were obtained using 4pi Analysis System (Durham, NC) graphic software integrated to the SEM. Images were sized and placed together to produce the figures using Adobe[®] Photoshop 7.0. graphics editing program.

X-ray Diffraction Analysis

A S-3700 Variable Pressure SEM (Hitachi High Technologies America, Inc., Pleasanton, CA) with an Oxford Instruments using INCA® X-Ray Diffraction System (Bucks, UK) attached to the SEM was used. Images of the pellets were obtained at 300X and a minimum of 50,000 counts were obtained per sample. INCA® using software was utilized to analyze the chemical composition of each sample. Six different pellets, chicken feather fibers, perlite and peat moss from ProMix BM (Premier Horticulture, Quakertown, PA, USA) were analyzed.

Table 1. Bio-based plastic resin (pellet; P) material formulations (weight percenta	Table	1. Bio-based	plastic resin	(pellet; P)	material	formulations	(weight	percentage
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Pellets	Feather keratin	Glycerol	Biomax strong additive	Poly- propylene	Epolene (MAGP)	Water	Sodium sulfite	Polylactide (PLA)	Talc
P 29	40	10	0	50	0	0	0	0	0
P 32	50	20	10	0	0	0	0	10	10
P 37	50	20	20	0	0	0	0	10	10
P 40	50	20	0	0	0	0	0	30	0
P 45	30	0	0	60	10	0	0	0	0
P NaS ^z	60	30	0	0	0	8	2	0	0

^zSodium sulfite (Na₂SO₃; NaS).



The Effect of Mixing Different Pellets in the Medium on Growth, Flowering, and Substrate and Foliar Analyses

Preparation of Stock Plants and Propagules

Begonia boliviensis 'Bonfire' (Gro-N-Sell Inc., Chalfont, PA) stock plants were grown in an air-conditioned greenhouse maintained at 21/18.3°C (day/night) (Lee and Roh, 2001). Stem tip cuttings, 7-8 cm long with 4 to 5 leaves and lateral shoots formed at the two-lower nodes of the cuttings, were rooted in a rooting medium composed of coarse grade vermiculite: ProMix BX (1:1, by volume) under a mist bench for 10 days in the greenhouse maintained at 23-27/21-23°C.

Rooted cuttings of *B. boliviensis* were planted on June 3, 2010 in ProMix BM media amended with either 8 grams of P 29, P 32, P 37, P 40, P 45, or P NaS into 95 grams of medium (Table 1). At planting, 0.8 grams of controlled release fertilizer (14N-6P-11.3K) was added to the second control group that contained no pellets. Shoots were pinched leaving two lateral shoots. During culture, 200 mg·L⁻¹ N from water soluble fertilizer (20N-8.6P-11.7K) was applied once a week. Plants were in a completely randomized design with 8 single plant replications. When the main shoot of control plants that were grown in the second control group had formed 3 to 5 flowers, data were collected with the following criteria based on the length stage of flowers of primary shoot and the presence of the secondary and tertiary shoots and the development of flower buds on these shoots 34 days after transplanting (DAP) (Table 2). Soil and leaf samples were collected in duplicate. Newly developed and expanded leaves following transplanting were collected.

Analysis of Pellets, Medium, and Foliar Samples

Dry P 29, P 32, P 37, P 40, P 45, and P NaS, grounded feather fibers (treating them as a medium), and leaf samples in duplicate were analyzed (JR Peters Laboratory, Allentown, PA). In brief, media extracts and tissue acid digestion extracts were analyzed on the inductively coupled atomic emission spectrometer (ICP)-IRIS Plasma Spectrometers (Thermo Jarrell Ash, Corp, Franklin, MA) for phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), boron (B), copper (Cu), zinc (Zn), molybdenum (Mo), sulfur (S), and other elements. Plant tissue analysis for total N was performed following combustion. Ammonia and nitrate nitrogen was analyzed on the SAN++ Segmented Flow Analyzer (Skalar Inc., Buford, GA, USA). Soil and foliar analysis was performed in triplicate.

Results

Chemical Analysis of Dry Pellets

The pH of the dry pellets ranged from 6.0 in pellet 37 (P 37) to 6.5 in P 45 and was the highest (7.6) in P NaS (Table 3). Soluble salts level was the highest in P NaS (3.40 dS·m⁻¹). The concentration of ammoniacal nitrogen (NH₄-N) was the highest in P 37 (118 mg·L⁻¹) followed by in P 32 (84 mg·L⁻¹) and P 40 (55 mg·L⁻¹) which is higher than the suggested ranges (< 20 mg·L⁻¹) (JR Peters Laboratory, Allentown, PA, USA). The concentration of NH₄-N was the lowest in P 45 (2 mg·L⁻¹). The concentration of nitrate nitrogen (NO₃-N) and soluble salts in all pellets and feather fibers were in the suggested ranges, i. e., 35-180 mg·L⁻¹ and 0.75-3.5 dS·m⁻¹, respectively. The level of phosphorus in P 32 (81.7 mg·kg⁻¹)

Table 2. Grading scales and criteria to assess plant growth and development (plant appearance) by mixing pellets (P) with a growth medium.

Cradina	No. of	Primary shoot		Presence	and development of	Dignt appearance and pollete	
Grading scales ^z	No. of new shoots	Length (cm)	Flowering of	Secondary shoots	Tertiary shoots	Plant appearance and pellets (No. of plants-pellet)	
1	1	< 5	bud stage	Not formed	Not formed	5-P NaS ^y	
2	2	5	flowered	Not formed	Not formed	2-P 32; P 37; 7-P 37, 7-P 40	
3	2-3	10	flowered	Formed with flower buds	Not formed	4-P 32; P. 37; P 40	
4	2-3	10-15	flowered	Formed with flower buds	Not formed	1-P 45; 3-P 29	
5	3-5	10-15	flowered	Formed with flower buds	Formed with flower buds before anthesis	6-P 45	
6	3-5	15-20	flowered	Formed with flower buds	1 to 2 tertiary shoots and flowered	8-P control	
7	3-5	15-20	flowered	Formed with flower buds	> 3 tertiary shoots and flowered	9-P control	

^zRefer to Fig. 3 for grading 1 (poor) through 7 (acceptable), which are noted at the upper left corner at the frames.

YRefer to Fig. 3 for plant appearances grown in growth medium mixed with pellets. 5-P NaS means that 5 plants grown in pellet NaS (P NaS) did not reach anthesis (bud stage) and produced no secondary and tertiary shoots.



Table 3. pH, total soluble salts (SS, dS·m⁻¹), and macro- and micro-elements of dry pellets treated a growing medium and feather fibers treated as a foliar sample.

Dellet (D)		NO ₃ -N	NH ₄ -N	Р	K	Ca	Mg	S	В	Fe	Mn	Cu	Zn
Pellet (P)	pН					(mg·L ⁻¹)							
P 29	6.2	5.4	11	8.5	10	8.1	2.93	18	0.00	0.02	0.01	0.07	0.09
P 32	6.4	8.2	84 ^z	43.5	48	14.1	6.04	62	0.00	0.03	0.02	0.11	0.13
P 37	6.0	13.0	118	81.7	65	71.2	19.41	201	0.02	0.15	0.09	0.12	0.27
P 40	6.3	12.1	55	34.6	38	17.9	7.36	53	0.00	0.04	0.03	0.09	0.10
P 45	6.5	14.7	2	0.5	1.0	1.2	0.23	1	0.00	0.01	0.00	0.03	0.18
P NaS	7.6	0	10	231	1,238	694	124	1,940	1.67	3.07	9.28	8.89	3.54
Fibers ^y	5.7	0	0	137	50.1	50.1	22.0	38.3	0.04	0.24	0.47	0.16	0.67
Suggested range ^x	5.2-6.3	35-180	0-20	5-50	35- 300	40-200	20-100	0-250	0.05-0.5	0.3-3.0	0.02-3.0	0.001-0.5	0.30-3.0
Foliar sample	e			(%)						(mg·kg	·1)		
Fibers	_	13.2	(N^w)	0.10	0.15	0.14	0.03	-	1.33	464	10.7	9.64	49.0
Suggested range ^v		3.5-5.	5 (N)	0.35-1.0	2.0-8.8	0.8-3.0	0.2-1.5	-	30-150	60-200	50-200	5-25	30-150

²Numbers in bold face suggest that the concentration is higher than the upper limit of the suggested range for general horticultural crops.

^vGeneral suggested ranges by JR Peters Laboratory. Soluble salts (dS·m⁻¹) that ranges from 0 (P45) to 3.40 (PNaS) and molybdenum was not presented, since they were within the suggested ranges and no significant differences were observed.

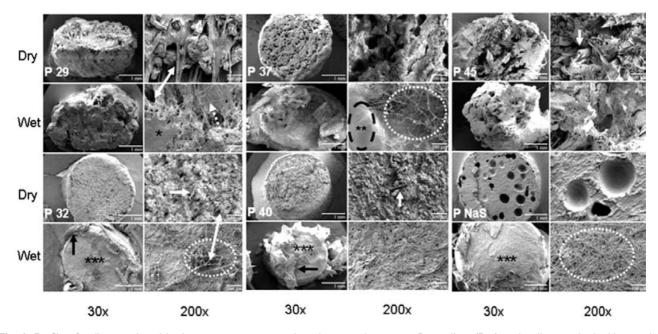


Fig. 1. Profile of pellets analyzed by low temperature scanning electron microscopy. Dry pellets (Dry) and pellets packed with a moist medium (Wet) for 96 h at 30 and 200x magnification. Feather fibers are indicated by arrow (→) in P 32, P 40, and P 45. Massive fungal mycelia with broken circle in wet P 32, P 37, and P NaS of hyphae as indicated with dotted arrow (--->, P 29 dry at 200x for example), waters covering the cut surface of in P 29 (*), P 32 (***) P 37 (**) and P 40 (****) and P Na S (****) were evident. The number of asterisks (*) indicates the relative surface areas which were covered by water.

was higher than the suggested upper limit. The level of Ca except in P 37, S, B, Fe, and Mo in all dry pellets were either close to or lower than the lower range of suggested limit. The level of all elements in P NaS, except soluble salts, NH₄-N, and NO₃-N, was higher than the upper limit of suggested ranges. Feather fibers, when analyzed as if

they are a foliar sample, had 13.2% nitrogen (N) and 464 mg \cdot kg⁻¹ Na.

Characteristics of Polymers Analyzed by Low Temperature-SEM

Color and surface appearance of P 32, P 37, and P 40



^yFeather fibers treated either as a growth medium or foliar sample.

^xGeneral suggested range set by JR Peters Laboratory (Allentown, PA, USA).

[&]quot;Total nitrogen.

which contained 50% of feather keratins or 20% glycerol (Table 1) is darker and smoother than those of P 29 (10%) and P 45 (0%) (images not presented). When the cut surfaces of dry and wet pellets were analyzed by LT-SEM (Fig. 1), feather fibers were observed in P 32, P 40, and P 45. In dry pellets, very similar surface image profiles were observed in P 32, P 37, and P 40 which is different from those in P 29 and P 45; the former group of pellets showing small pores/ cavities and the later group have coarse cavities with stretched structures. The least changes of image profiles were observed in moist P 29 and P 45 as compared to dry pellets. Changes in image profiles between dry and moist pellets were most significant in P NaS. The size of pores/cavities was reduced in moist pellets which was due to the degree of surface area which was covered by water covering the cut surface. In fact the entire surface of moist P NaS was covered with water.

The surface of all moist pellets had fungal mycelia forming an extensive network (Fig. 1). The massive formation of hyphae was evident covering pores/cavities in P 32, P 37, and especially in P NaS. The entire surface of P 32, P 40, and P NaS was covered with an enormous body of mycelium and water molecules. Mycelia formation was less obvious in P 29 and P 45.

Chemical Analysis of Pellets Using X-ray Diffraction and Medium Analysis

Sulfur (S), a main component of chicken feathers, was detected in all dry or moist pellets and fibers except in dry

or moist P 29, moist P NaS, and dry or moist perlite (Fig. 2) or peatmoss (data not shown). Magnesium (Mg) and silica (Si) were detected only in dry P 37; however, they were not detected in moist P 37 (data not shown). Sodium (Na) and S were detected in dry P NaS (P NaS dry), but not in P NaS moist. Calcium (Ca) was detected in both dry and moist P NaS. In moist perlite, K, Na, Si, and aluminum (Al) was detected.

Growth, Flowering, and Analysis of Soils and Leaves

When 4 grams of P 29, P 32, P 37, P 40, P 45 or P NaS were mixed in the medium, growth and flowering responses were vigorous to poor in the following order: P 45 plant (vigorous, pale in leaf color), P 29 plant (vigorous, pale in leaf color, branching) > P 32 plant, P 37 plant > P 40 plant > P NaS plant (less vigorous, dark in leaf color, weak lateral shoots, and inhibited or delayed flowering) (data not presented). In a subsequent experiment, shoot growth and flowering 34 DAP were rated 1 (poor in growth and flowering in P NaS plant) (Fig. 3E) to 7 (acceptable in control) (Figs. 3H and 3I) (Table 2) with 8 grams of pellets mixed into the medium. Six P 45 plants were rated 5 (Fig. 3F) which formed tertiary shoots with flower buds developed. However, foliar color was lighter in green than those in the controls (rating 6 and 7, Figs. 3H and 4I, respectively).

Plants with weak shoots were observed in P 32 plant (Figs. 3B and 3E), P 37 plant (Fig. 3D), P 40 plant (Fig.

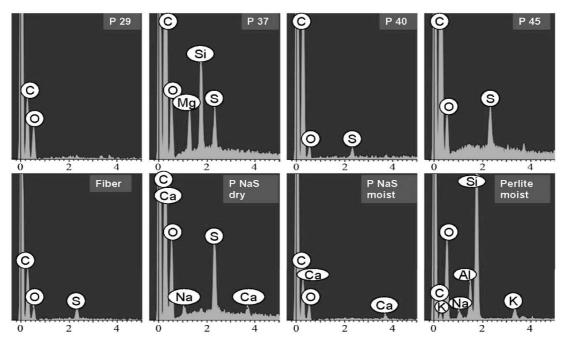


Fig. 2. X-ray diffraction analysis profiles of dry pellets or moist pellets and perlite in a soilless medium. Cabon (C), oxygen (O), magnesium (Mg), silica (Si), sulfer (S), sodium (Na), calcium (Ca), aluminum (Al), and potassium (K) was detected. Analysis profiles are similar in both dry and wet pellets, if not indicated.



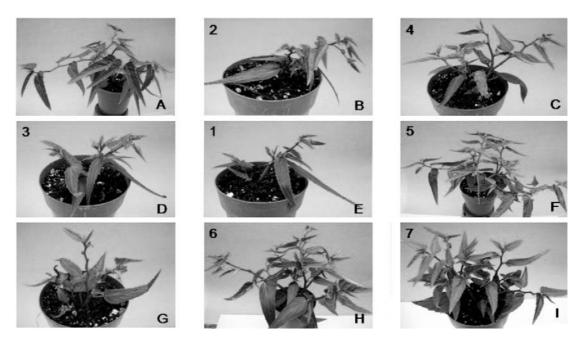


Fig. 3. Short-term evaluation on growth and flowering of *Begonia* 'Bonfire'. Refer to Table 2 for grading scales from 1 through 7 shown at the upper left corner of frames. Plant was grown in P 29 medium (29 plant, C), P 32 plant (B), P 37 plant (D), P 40 plant (G), P 45 plant (F), P NaS plant (E), P control 1 plant without slow release fertilizer (A), P control 2 plant with slow release fertilizer (H and I). Plant shown in H (scale 6) and I (scale 7) is acceptable.

Table 4. Mean comparisons for soil analysis data for pH, soluble salts (SS; mmhos·cm⁻¹), macro- and micro-elements in growth medium mixed with different pellets with *Begonia* 'Bonfire'.

		pH, tot	al soluble sa	lts (SS; dS·r	m ⁻¹), and ma	acro- and mic	ro-elements ((mg·L ⁻¹)	
Pellets	pН	SS	NO ₃ -N	NH ₄ -N	Р	K	Ca	Mg	S
						(mg·L ⁻¹)			
P control	6.8	0.79	45	6.5	26.4	161	27	4.9	28.5
P 29	5.8	2.18	215 ^z	25.2	20.8	252	162	32.9	52.3
P 32	5.3	5.47	442	139.5	41.4	332	369	70.6	214.0
P 37	5.1	3.02	330	8.2	26.7	239	339	61.9	70.4
P 40	6.0	2.73	241	88.3	26.1	234	168	35.6	100.0
P 45	6.9	0.74	34	6.4	25.9	143	30	5.3	34.7
P NaS	5.0	3.35	378	12.1	30.9	268	378	67.9	77.8
Level of signific	cance ^y								
Pellets	***	***	***	***	**	**	***	***	***
HSD at 0.01	0.29	0.34	24	8.76	8.34	17.9	20.7	8.37	11.9
Suggested ranges ^x	5.2-6.3	0.75-3.5	35-180	0-20	5-50	35-300	40-200	20-100	50-250

^zNumbers in bold face suggest that the concentration is higher than the upper limit of the suggested range for general horticultural crops.

3G), and P NaS plant (Fig. 3E) as compared to P 29 plant (Fig. 3C), P 45 plant (Fig. 3F), and P control (P con) plant (Figs. 3H and 4I). Leaf color of P 45 plant (Fig. 3F) was light in green as compared to other plants, particularly of P 32 plant, P 40 plant, and especially in P NaS plant.

The pH of the P 45 medium (6.9) was higher, and the pH of P NaS medium (5.0) was lower than the suggested range (5.2-6.3). The pH of all other pellet media fell within the suggested ranges. The concentration of NO₃-N in P 29, P 32, P 38, P 40, and P NaS medium was higher (> 215 mg·L⁻¹



^ySignificant difference at 5% (*), 1% (**), and 0,1% *(***), F-test.

^xGeneral suggested ranges by JR Peters Laboratory. Data on B. Fe, Mn, Cu, and Zn are not presented since all were within the suggested ranges.

in P 29) than the concentration in P control (45 mg·L⁻¹) and P 45 (34 mg·L⁻¹) medium. The concentration of NO₄-N, K, and Ca in P 32 medium was higher as compared to other pellets and above the upper limit of suggested ranges. Other elements that include Mg and copper Cu and other minor elements, S, B in other pellet mixed media, for example, were within the suggested ranges. Sulfur concentration in P NaS medium was 77.8 mg·L⁻¹, which is within the suggested ranges (50-250 mg·L⁻¹). Most of the elements in P control medium and in P 45 medium were not significantly different from each other (Table 4). For example, the concentration of the total soluble salts was 0.79 and 0.74 dS·m⁻¹ and of Ca was 27 and 30 mg·L⁻¹, and iron (Fe) was 0.07 and 0.07 mg·L⁻¹, respectively.

Foliar analysis indicated that N concentration of plants grown in P control medium (P control plant) without and with an addition of controlled release fertilizer and 200 mg·L⁻¹ liquid N application was 4.2% and 5.5%, respectively, which fell in the suggested ranges. In P 32, P 37, P 45, and P NaS plant were higher than 6.3%, which is above the upper limit of the suggested range (data not presented). Except for N and Cu, the concentration of all other elements fell within the suggested ranges when plants were grown in medium mixed with different pellets.

Discussion

Most large scale greenhouse floral and nursery crops are grown in petroleum based plastic pots. Recently, physical properties of several types of biodegradable pots such as rice-hull based pots and containers have been evaluated (Evans et al., 2010). These reports indicated that plants were grown successfully in the media containing up to 30% feather fiber (Evans, 2004; Evans and Hensley, 2004). Tomato plants grown in pots with ground feathers (Tyson, Inc., Inc., Springdale, AR) had higher N concentration in the tissue and higher dry weight (Evans, 2004). However, production of the prototype models has been discontinued. (Personal communication, Hickman, 2011).

Analysis of Dry Pellets as a Soil Medium

Incorporation of variable amounts of glycerol, coupling agents into the biobased plastic resin materials will produce pellets with different chemical, physical, and biological characters. Except for P NaS (pH 7.6), all dry pellets, when treated as if they are a medium, have an acceptable pH range to grow floral crops ranging from 6.0 (P 37) to 6.5 (P 45) which is slightly higher than 5.6-6.2 for soil-less media (Bailey, 1998). The high pH of P NaS could have originated from the presence of sodium sulfite (Na₂SO₃; NaS) which has pH of > 9 in a saturated aqueous solution (http://en.

wikipedia.org/wiki/Sodium_sulfite; accessed on July 12, 2011). In addition to the alkaline pH of P NaS, and high concentration of phosphorus (231 mg·L⁻¹), K (1,238 mg·L⁻¹), Ca (694 mg·L⁻¹), and particularly S (1,949 mg·L⁻¹) in P NaS medium should be viewed as detrimental for plant growth. Since pH, soluble salts, and concentration of all macro- and micro-elements in P 29 and P 45 do not exceed the upper limit of suggested ranges, it is suggested that pots made of these pellets would likely not cause problems to plants grown in commercial production.

The growth inhibition of plants in P 32, P 37, and P 40 medium containing 20% glycerol suggested that glycerol which is miscible with water and may be released from pellets affect plant growth. Plants grown in P 29 medium at 8 grams (P 29-8g plant), but not at 4 g, showed poor growth and, therefore, the amount of pellets mixed in the medium, such as P 29 containing 10% glycerol requires further evaluation.

The addition of the compostable additive Biomax as a strengthening additive to reduce the brittleness of polylactic acid (PLA), a biodegradable polymer, in P 32, P 37, and P 40 may be considered an important ingredient, since the growth and flowering of begonia was not acceptable as compared to pellets that do not contain PLA in P 29 and P 45. However, chemical changes of PLA during polymer extrusion may create low biodegradability based on the laboratory test measuring CO₂ production at 58°C (Ahn et al., 2011). Also, the addition of 50-60% of polypropylene (PP), a thermoplastic polymer matrix, is considered acceptable since pellets containing PP in P 29 and P 45 do not affect growth of plants adversely. The addition of Biomax or talc at 10% is not considered responsible for poor plant growth.

Characteristics of Polymers Analyzed by LT-SEM

Very similar image profiles were observed for the dry pellets P 32, P 37, P 40, and P NaS which all had small pores/holes. However, P 29 and P 45 were different as they had coarse cavities with stretched structures derived from additives. In moist P 32, P 37, and P 40 pores/holes were not evident as compared to dried pellets. Presence of water or a moisture layer on the surface of moist P 32, P 37, P 40, and P NaS may cause swelling of the pellets following absorption of moisture, thus reducing the size of pores or cavities. The least differences in the image profiles of cut surface pellets was noticed between the dry and moist P 29 and P 45 which may suggest that P 29 and P 45 may degrade slowly when they are exposed to frequent watering.

Various amounts of water covering the cut surface of the pellets was evident in P 29, P 37, P 40, and P Na S, most significantly in P NaS. Swelling and a significant network of massive fungal mycelia of thread-like hyphae in P 29, P



32, P 37, and P 40 in 3-4 days may suggest that these pellets may be degraded faster than P 29 and P 45. The origin of mycelia forming fungus in this study has not been identified. However, it could be from endomycorrhizal fungus, *Glomus infrardices* N. C. Schenck & G. S. Sm. inoculated in the ProMix 'BX' (Anonymous, 2011) which benefits plant growth.

Chemical Analysis of Pellets Using X-ray Diffraction and Medium Analysis

In addition to carbon (C) and oxygen (O) detected by X-ray diffraction analysis, sulfur (S) was detected in all pellets and fibers except in P 29 which contained 40% feather fibers, but not in P 45 which contains 30% feather fibers, indicating that feather fibers when mixed at > 40% may contribute to a detectable level of S on the surface of the pellets. Sodium (Na) and S detected in dry P NaS, but not in moist pellets suggests that these two elements could become soluble and leached when pellets are mixed with a medium and watered which may affect plant growth. Calcium (Ca) was detected only in dry but not in moist peat moss (data not presented). If S leaches readily (Berry et al., 1977), the high concentration of Na, S, and N present in P NaS may not be responsible for the poor growth and development upon transplanting begonia propagules.

The formulation of P NaS has a sulfur concentration that is very high as expected since it contains 60% feather fibers and is high in S, and the use of sodium sulfite to break the S-S cystine bonds during extrusion should be considered a possible source (Barone, 2005). Source of these elements in excess concentration, high concentration of NH₄-N, P, K, Ca, and particularly S, could be from feather fiber which is high in total N and with protein high in the sulfur containing amino acid cystine (Stilborn et al., 1997).

Growth, Flowering, and Analysis of Soils and Leaves

No information is available concerning how various pellets amended into a medium affect plant growth and flowering, let alone pots made of these pellets. It is desirable to find whether or not macro- and micro-elements may be released from pellets during the course of culture. Therefore, chemical properties of 6 different pellets, P 29, P 32, P 37, P 40, P 45, and P NaS and of medium amended with above pellets were evaluated by observing the growth, flowering, and analyzing foliar analysis of begonia. Mixing pellets at the rate of 4 g did not significantly affect growth and flowering of begonia (data not presented) and the maximum rate would be less than 8 grams for all pellets types except for pellet P 45.

Considering a high concentration of NH₄-N in P 32, P 37, and P 40 containing > 20% glycerol and also high P and K level in P37, P 29 and P 45 containing < 10% glycerol can

be considered suitable for plant growth when mixed in medium at the rate of 8 grams of pellets per 95 grams of medium. Glycerol, due to its hydroxyl groups, is miscible with water (http://www.dow.com/glycerine/resources/solubility. htm; accessed on July 5, 2011) and glycerol released from P 32, P 37, and P 40 may be the cause of poor growth, particularly at an early growth and development stage of begonia, if glycerol is rapidly released to a medium. The growth of wheat grass in soil/composed mixture containing 60 grams of 10% spiked glycerine is, however, better than that without glycerine (Wee and Obbard, 2011). The addition of 10⁻¹ M glycerol with auxin promotes shoot formation per cell mass of *Grateloupia doryphora* in vitro (Garcia-Jimenez et al., 1998). Therefore, poor growth of begonia may not be attributed only to the level of glycerol in the pellets. The inhibitory effect in P 37 medium on plant growth could also be related to the addition of Biomax as a strengthening additive which improves the performance of composable polylactic acid (PLA) (http://www2.dupont.com/Biomax/en US/ assets/downloads/Biomax%20Strong.pdf).

In conclusion, based on the physical and chemical analysis of six pellets, P 32, P 37, and P 40 which contain 20% of glycerol and P NaS containing sodium sulfite and 30% glycerol is not recommended for future evaluation and should be excluded to utilize pellets to prepare pots. Poor growth and development of *Begonia* 'Bonefire' grown in P NaS medium is not solely considered responsible for high pH and particularly K and S concentration among other macro-micro elements. Mixing P 45 which does not contain glycerol with 30% feather fibers with a medium is recommended for further evaluation. Based on this study, glycerol even at 10% is considered not suitable to use as an ingredient to process pellets and the use of P 45 is the best source of pellets. Pellets prepared following mixing > 30 feather fibers without using glycerol should be further tested.

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