

**Evaluation and Use of Neem (*Azadirachta indica* A. Juss)  
as an Organic Substrate Component**

by

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## Abstract

With “green-mindedness” increasing in popularity, the use of organic substrates has become even more important in the retail horticultural market. Multiple experiments were conducted to evaluate commercially available bagged substrates. The effects of eleven commercially available substrates on plant growth were evaluated using four species of plants. In experiment one, Jungle Growth<sup>®</sup> organic substrates outperformed other commercial potting substrates and were only slightly inferior in performance to the best-ranking substrate, Miracle-Gro<sup>®</sup> Moisture Control<sup>®</sup>. The second experiment revealed that, where different, the Jungle Growth<sup>®</sup> substrates outperformed all other substrates tested (though not statistically greater than Sta-Green<sup>®</sup> Flower and Vegetable mix when used on tomatoes and petunias). The search for beneficial amendments for horticultural soilless media is a constant and ongoing process. Neem (*Azadirachta indica* A. Juss) cake powder is currently being used as a substrate component, but its full effects on the rhizosphere and subsequent plant biomass have yet to be explored. Therefore, a third study was conducted to evaluate neem at varying percentages ranging from 0-5% in two stock substrates – one containing poultry protein compost and the other containing peat in place of the compost and receiving nutrients via fertigation. Results show the benefit of the addition of neem, especially at 5%, in the compost-containing treatments. The same cannot be said of the non-compost treatment. Plants grown in 0-1% neem

treatments within the non-compost mixes had results that outperformed any of the compost treatments and often outperformed other non-compost treatments. Therefore, neem appears to be beneficial when amended into poultry protein compost-containing substrates, but antagonistic when added to standard mixes that will be fertigated. Nursery and greenhouse growers continue to seek materials to decrease costs of plant production while maintaining environmental stewardship. Incorporation of neem cake as a substrate component could potentially impact nitrogen release as a result of altering substrate bacterial activity. A preliminary study investigated the impact of neem on substrate gas release and provides a starting point for further investigation regarding neem use as a substrate component. With three substrate groups being tested with varying percentages of neem, this study reports on both across-group results as well as within-group results. Across all three groups, 3% neem within the pine bark + poultry protein compost + neem group was significantly greater in CO<sub>2</sub> production than all treatments within the pine bark + neem group as well as zero percent neem within its own group and the pine bark + poultry protein compost + 19-6-12 + neem group. Nitrous oxide emission was significantly greater in the pine bark + poultry protein compost + 19-6-12 + neem group than all other treatments. Within-group comparisons reveal that three percent neem had greater CO<sub>2</sub> emission than zero percent neem for both the pine bark + neem and pine bark + poultry protein compost + neem groups. Three percent neem also produced significantly greater CH<sub>4</sub> than zero percent neem in the pine bark + poultry protein compost + neem group – and within the same substrate group, two percent neem had significantly greater N<sub>2</sub>O emission than zero percent neem. There were no significant

differences among treatments within the pine bark + poultry protein compost + 19-6-12 + neem group for all three gases analyzed.

## Acknowledgements

The author, first, foremost and most importantly, would like to thank God, “Who delivers me through Jesus Christ our Lord!” (Romans 7:25 NIV). It is only by His grace that the author has drawn breath and strength to accomplish the tasks set before him. The author’s life was bought by the spotless blood of the Lamb and the author is forever indebted to this supreme act of grace and love – his life and soul are Yours, Lord!

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And Jess, what can the author say? The Lord has been so faithful in bringing you in his life and in giving you the patience to put up with him. You have been invaluable, not only in your help with research, but also as his best friend. As the author writes this, the countdown to marriage begins, but as you read it, the lifelong journey of serving God together will have already begun. The author loves you and thanks you for everything! Onward you, Otto and the author go together as a family!

There is only one thing left to say:

**Praise God from Whom all blessings flow!!**

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## List of Abbreviations

EC	Electrical Conductivity
GI	Growth Index
PB	Pine Bark
PPC	Poultry Protein Compost
JG	Jungle Growth <sup>®</sup>
MG	Miracle-Gro <sup>®</sup>

## **Chapter I**

### **Literature Review**

#### **Soiless Media:**

Beginning in the 1970's, pine bark quickly became an invaluable resource to the horticulture industry. Present-day demands for this commodity, however, have also increased and, with the growth of the horticulture sector, our industry is feeling the pressure to find some alternatives to exclusive pine bark usage (Avent, 2003). While greenhouse plants are almost exclusively produced in peat-substrates, 75-100%, by volume, of container substrates in the eastern US are comprised of pine bark (Lu et al., 2006). Future projections project the rising cost of pine bark combined with less availability to the horticulture industry (Lu et al., 2006).

Numerous organic and inorganic substances are being used to develop varying substrates. Clean chip residual (the material left on the forest floor following timber harvest) can be a realistic alternative to pine bark-exclusive substrates for ornamental plant production (Boyer et al., 2008). Fain et al. (2008) successfully used WholeTree (whole pine tree shoots) in varying percentages as suitable greenhouse media for growing marigolds and petunias. With additional fertilizer, Jackson and Wright (2007) were able to grow plants in 100% pulverized pine wood. Cotton gin compost was used as a viable substrate component by Cole et al. (2002). Spent tea grinds can be successfully used in

horticultural substrates (Wells, 2008). Poultry litter was a suitable amendment for substrates comprised primarily of either pine bark, WholeTree (created by chipping, then further milling entire pine trees – log, limbs, needles and bark), or clean chip residual for growing plants in containers with favorable results and is a readily available source of nitrogen (Fain et al., 2008; Marble et al., 2010; Mitchell and Donald, 1995).

Parboiled fresh rice hulls can be used successfully as a substrate component comparable to perlite (Evans and Gachukia, 2007). Using substrates from peanut hulls, pine bark, peatmoss and combination thereof, Bilderback et al. (1982) reported success in growing azaleas. In searching for an ecologically-friendly alternative to rockwool, Urrestarazu et al. (2005) concluded that almond shells can be an effective and beneficial soilless media for plant production. Coconut coir can be a viable substrate for plant production, however its success depends on particle size (Noguera et al., 2003). Peat-vermiculite substrates served as viable substrates for growing chrysanthemums (Paul and Lee, 1976). Mineral substrates have the potential to perform similarly to peat-based mixes (Smith et al., 1995). Smith and Hall (1994) determined that a perlite-based substrate can be comparable in management and productivity to peat-based potting mixes.

While potential alternative substrates seem to abound, there are requirements that must be met of the material in order for it to be usable and effective. Chang and Lin (2007) report that basic requirements for a successful and beneficial plant-growing medium include: excellent chemical resistance properties, light weight, inexpensiveness, absence of pests and diseases and availability. Nkongolo and Caron (2006) noted that particle size, specifically in peat and pine bark-based substrates, influences plant

response. Handreck (1983) reports the importance of particle size and advises that substrate formulators heed the “fines” fraction of a substrate, especially particle size smaller than 0.5mm, as this size controls the physical properties of pine bark-based substrates. Shrinkage is a physical property of organic soils (such as peat) that must be managed (Schwärzel et al., 2002). Nemati et al. (2002) noted that insufficient aeration of artificial growing media is a common problem in greenhouse production.

**Neem:**

The soilless media amendment in question is a product of *Azadirachta indica* A. Juss, or the neem tree. The neem tree is known as the “village dispensary” in India and Southeast Asia, where it is native (Biswas et al., 2002). This is due to the results of both science and tradition about the medicinal and agricultural uses that neem provides. The neem tree is an evergreen tree belonging to the mahogany family, Meliaceae, of which *Melia azedarach* Chinaberry is also a member (Biswas et al., 2002). Steeped in Indian tradition and lore, the neem tree continues to play roles in their traditions, medicine and agriculture and now the rest of the world is beginning to pay more attention to neem, which has been somewhat controversial to the traditionalists in Asia (Marden, 1999).

Almost every part of the neem tree (bark, leaves, seeds, fruit, flowers and roots) is used in various ways, with more than 140 chemical compounds having been isolated by scientists (Brahmachari, 2004). These compounds have proven effective against and to combat intestinal worms, treat asthma and rheumatism, as well as being useful as analgesic, anti-malarial, anti-fungal, insecticidal as well as a “general health promoter” (Brahmachari, 2004). All of these uses also seem to come with little to no ill effect to humans (Marden, 1999).

The chemicals isolated from neem can be categorized into two groups: isoprenoids and non-isoprenoids (Brahmachari, 2004). Non-isoprenoids are amino acids, carbohydrates, flavonoids and others, while isoprenoids contain compounds such as azadirachtin (Brahmachari, 2004). This last compound, azadirachtin is used today in many insecticides in the United States. In the early 1990's a company in Florida patented a stabilized version of azadirachtin to increase its usefulness and was then approved for use on food crops by the Environmental Protection Agency in 1994 – the first neem-based product marketed in the U.S. (Marden, 1999). Many studies have followed in search for the insecticidal and medicinal applications for compounds including neem, but other uses for neem have not been researched in the United States.

Neem has been used as a soil amendment in many studies in India. Neem seed residue provided a nitrogen value, after oil extraction, of 7% and at a release rate fast enough to satisfy maize nutrition (Agbenin et al., 1999). Neem oil also enhanced plant growth when incorporated into soil at 2.5, 5.0 and 20 ppm, though 10 ppm decreased plant growth (Bhaskar and Charyulu, 2005). Likewise, Agyarko et al. (2006) reported that soil nutritional levels increased with poultry manure and increasing levels of neem leaves. Bhalla and Devi Prasad (2008) reported higher vegetative growth in plants than reproductive growth (both showing higher growth than the control) when neem cake was incorporated into the soil.

Neem's impact within a substrate has been attributed to its potential urease retardation activity (B. Hurst, personal communication). Urease is the naturally occurring enzyme in soil responsible for the hydrolysis of urea into carbon dioxide and ammonia (Manunza et al., 1997). Ammonium and ammonia are the nitrogen sources

within fertilizers (be it synthetic fertilizer or organic), but the ammonia can undergo volatilization, which may decrease nitrogen available to the plant. Therefore, it is often desirable to have certain levels of urease inhibition in order to constrain ammonium production, resulting in a more readily available supply of nitrogen over a longer period of time. The amassing of ammonium can lead to problems other than ammonia volatilization, such as nitrite toxicity and damage to young plants (Bremner and Douglas, 1971).

Urease prevalence in soil is closely linked with organic matter content within a soil or substrate (Burns et al., 1972). Fishbein et al. (1973) have shown that urease enzyme purified from jack bean meal may actually have more than a dozen molecular forms. Each dimer of urease is composed of two half-units, which means that the enzyme itself can be dissociated into constituents (Fishbein et al., 1973), though it is not stated whether these subunits themselves are functional. However, if neem breaks down the enzyme dimers, it is currently unknown if the urease then becomes non-functional.

There have been some directed studies aimed at urease and/or nitrification inhibition. Burns et al. (1972) showed that pronase, a proteolytic enzyme, degrades jack bean urease, but has no effect on urease within the soil matrix. Bremner and Douglas (1971) found that metallic salts containing silver, mercury, gold or copper can have an effect on urease activity (silver sulfate had a 48% inhibitory effect). Organic compounds such as phenylmercuric acetate and catechol inhibited urease by 67% and 74%, respectively. Other inhibitors of urease include phosphorodiamides, phosphotriamides and hydroxamic acid (Manunza et al., 1997). In corn production, Schlegel et al. (1986) noted the following urease inhibitors: hydroxamates, heterocyclic sulfur compounds,

xanthates, organophosphorus insecticides, quinones and phosphoroamides, noting phenylphosphorodiamide as the most effective inhibitor. Schlegel et al. (1986) showed that urease inhibitors did not increase yield of corn but inhibitors had the greatest potential effect when added to surface applied urea that was not watered in. Effects of pesticides on urease was the focus of Ingram et al. (2005) who reported that pesticides can have a noticeable effect on bacterial urease, but no significant effect on free urease in the soil. Treatment of soils with toluene resulted in increases in soil urease activity, as did chloroform fumigation (Klose and Tabatabai, 1999). Also, the addition of glucose, or any organic matter that is hospitable to microbial activity, was shown to increase urease function in soils (Zantua and Bremner, 1976).

Mohanty et al., (2007) reported on the potential inhibitory effects of neem seed kernel powder on urease in three mineral soils native to India, showing slight suppression of urease activity when applied to acidic soils. Méndez-Bautista et al., (2009) studied the effects of neem leaf extracts on greenhouse gas emissions and inorganic nitrogen in urea-amended soil and reported that the leaf extract had no significant effect on urease, but may limit nitrification. Majumdar et al., (2000) coated urea with neem seed powder before adding to rice fields in North India, resulting in slight nitrification inhibition. Kumar et al., (2007) used neem oils to coat urea and added it to sandy-loam soils resulting in some nitrification inhibition as well. Bhalla and Devi Prasad (2008) showed, in one of their studies, that addition of neem cake into a mineral soil is an economical and effective method for reducing fertilizer application by prolonging fertilizer available to the plant.

The question now is whether neem will have any effect on urease activity within soilless potting media, especially a pine bark-based substrate. Secondly, would neem cake use be cost-effective? Patra and Chand (2009) noted that though neem is being proved as an effective soil amendment, it is not being used wide-scale because of, in their case, the coating process for the urea is cumbersome and the materials are not readily available. Therefore, one area of research to be addressed in this thesis is to evaluate neem cake's effectiveness in soilless media and, if effective, can neem be used in cost-effective amounts incorporated in the substrates with beneficial results to the plant.

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## Chapter II

### Evaluation of Neem Cake Powder Percentages as an Organic Substrate Component

#### Significance to Industry:

The search for beneficial amendments for horticultural soilless media is a constant process. Neem (*Azadirachta indica* A. Juss) is currently being used as a substrate component, but its full effects on the rhizosphere and subsequent plant biomass have yet to be explored. Therefore, this study was conducted to evaluate neem cake powder (referred to as neem) at varying percentages ranging from zero-to-five percent in two stock substrates – one containing poultry protein compost and the other containing peat in place of the compost and receiving nutrients via fertigation. Results show the benefit of the addition of neem, especially at five percent, in the compost-containing treatments. The same cannot be said of the non-compost treatment. Plants grown in zero-to-one percent neem treatments within the non-compost mixes had results that outperformed any of the compost treatments and often outperformed other non-compost treatments. Therefore, neem appears to be beneficial when amended into poultry protein compost-containing substrates, but antagonistic when added to standard mixes that will be fertigated.

**Introduction:**

Beginning in the 1970's, pine bark quickly became an invaluable resource to the horticulture industry. Present-day demands for this commodity, however, have also increased and, with the growth of the horticulture sector, our industry is feeling the pressure to find some alternatives to exclusive pine bark usage (Avent, 2003). While greenhouse plants are almost exclusively produced in peat-substrates, 75-100%, by volume, of container substrates in the eastern US are comprised of pine bark (Lu et al., 2006). Future projections project the rising cost of pine bark combined with less availability to the horticulture industry (Lu et al., 2006).

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managed (Schwärzel et al., 2002). Nemati et al. (2002) noted that insufficient aeration of artificial growing media is a common problem in greenhouse production.

The soilless media amendment in question is a product of *Azadirachta indica* A. Juss, or the neem tree. The neem tree is known as the “village dispensary” in India and Southeast Asia, where it is native (Biswas et al., 2002). This is due to the results of both science and tradition about the medicinal and agricultural uses that neem provides. The neem tree is an evergreen tree belonging to the mahogany family, Meliaceae, of which *Melia azedarach* Chinaberry is also a member (Biswas et al., 2002). Steeped in Indian tradition and lore, the neem tree continues to play roles in their traditions, medicine and agriculture and now the rest of the world is beginning to pay more attention to neem, which has been somewhat controversial to the traditionalists in Asia (Marden, 1999).

Neem has been used as a soil amendment in many studies in India. Neem seed residue provided a nitrogen value, after oil extraction, of 7% and at a release rate fast enough to satisfy maize nutrition (Agbenin et al., 1999). Neem oil also enhanced plant growth when incorporated into soil at 2.5, 5.0 and 20 ppm, though 10 ppm decreased plant growth (Bhaskar and Charyulu, 2005). Likewise, Agyarko et al. (2006) reported that soil nutritional levels increased with poultry manure and increasing levels of neem leaves. Bhalla and Devi Prasad (2008) reported higher vegetative growth in plants than reproductive growth (both showing higher growth than the control) when neem cake was incorporated into the soil.

Neem’s impact within a substrate has been attributed to its potential urease retardation activity (B. Hurst, personal communication). Urease is the naturally occurring enzyme in soil responsible for the hydrolysis of urea into carbon dioxide and

ammonia (Manunza et al., 1997). Ammonium and ammonia are the nitrogen sources within fertilizers (be it synthetic fertilizer or organic), but the ammonia can undergo volatilization, which may decrease nitrogen available to the plant. Therefore, it is often desirable to have certain levels of urease inhibition in order to constrain ammonium production, resulting in a more readily available supply of nitrogen over a longer period of time. The amassing of ammonium can lead to problems other than ammonia volatilization, such as nitrite toxicity and damage to young plants (Bremner and Douglas, 1971).

Mohanty et al., (2007) reported on the potential inhibitory effects of neem seed kernel powder on urease in three mineral soils native to India, showing slight suppression of urease activity when applied to acidic soils. Méndez-Bautista et al., (2009) studied the effects of neem leaf extracts on greenhouse gas emissions and inorganic nitrogen in urea-amended soil and reported that the leaf extract had no significant effect on urease, but may limit nitrification. Majumdar et al., (2000) coated urea with neem seed powder before adding to rice fields in North India, resulting in slight nitrification inhibition. Kumar et al., (2007) used neem oils to coat urea and added it to sandy-loam soils resulting in some nitrification inhibition as well. Bhalla and Devi Prasad (2008) showed, in one of their studies, that addition of neem cake into a mineral soil is an economical and effective method for reducing fertilizer application by prolonging fertilizer available to the plant.

The objective of this experiment was to determine the impact of neem cake powder as a substrate component on plant growth or quality.

## **Materials and Methods:**

Two stock substrates were used for this study: one with poultry protein compost (PPC) as the fertilizer source and the other with a starter charge fertilizer (a custom 7-2-10 blend) incorporated into the mixture. The compost stock mixture consisted of pinebark, poultry protein compost, perlite and vermiculite in a 50:17:10:5 ratio. To account for the volume of the PPC in the first mixture, peat was used as a substitute.

With the two stock substrates on hand, neem cake powder (a residual of the neem tree seeds or kernels after being cold-pressed for oil) was incorporated at 1, 2, 3, 4 and 5 percent by volume while also keeping a “control” treatment with no neem added. Due to neem cake’s small particle size and the relatively negligible amounts that were used, it was not necessary to compensate for its addition by the subtraction of any of the other amendments. The neem was thoroughly incorporated into the mixtures as the starter charge was added to the non-compost stock substrate.

There were twelve treatments (six percentages of neem and two stock substrates) replicated 10 times for a total of 120 experimental units. Square pots (4”x 4”x 6”) were filled with each substrate and planted with blue salvia (*Salvia farinacea* ‘Far Rhea Blue’). Pots were arranged in a randomized complete block design and watered on an “as needed” basis. Clear water was used on all treatments for the first week and on the compost treatments for the duration of the study. Non-compost treatments, however, received fertigation three times a week and clear water every fourth watering after the initial week of installation with 20-20-20 soluble fertilizer (TotalGro™, Inc, Winnsboro, Louisiana) at 175 ppm nitrogen. Percent nitrogen sources for this fertilizer are 5.98% nitrate nitrogen, 5.58% ammoniacal nitrogen and 8.44% urea nitrogen. The study was

conducted in a double-poly greenhouse for the 8-week study with the thermostat set at min/max temperatures of 65/78°F.

Data taken 28 and 56 days after planting (DAP) included: growth indices, pH, electrical conductivity (EC), SPAD-502 (measures chlorophyll levels in the plants) readings and shoot dry weight (shoot dry weights was measured at 56 DAP; the shoot fresh cuttings were placed in a 175°F oven for four days and weighed). All data were analyzed using Waller-Duncan k-ratio t-test in SAS software.

### **Results:**

*Mid-study (28 DAP):* There were few differences among all treatments 4 weeks into the study (Table 1). Non-compost treatments without neem and with 1% neem had significantly higher growth indices than any of the PPC treatments. The 4% neem, non-compost treatment also had significantly higher growth indices than did the 0-4% neem compost treatments, but GI's were not significantly greater than the 5% neem, PPC treatment.

Measurements of pH at 28 DAP show that the one percent neem, non-compost mixture had the lowest pH and was not significantly different from the 2, 3 and 5% neem, non-compost mixtures (Table 1). It was, however, significantly lower than all other mixtures. Electrical conductivity shows that the zero percent, PPC mixture was significantly higher in EC than 4 and 5% neem, PPC mixtures as well as the 0%, non-compost mixture (Table 1). All other mixtures are not significantly different.

SPAD results show that the 0% neem and 3% neem, non-compost mixtures had significantly higher SPAD readings than did 0, 2 and 4%, compost mixtures (Table 1). The rest of the treatments had no significant difference in values.

*Termination (58 DAP):* At termination, results show that one percent neem in the non-compost mixture had significantly greater GI's than any of the PPC mixtures, as well as the 4 and 5% neem, non-compost treatments (Table 2). The 2% neem, non-compost mixture also had significantly greater GI's than the 0, 1, 2, 3 and 5% neem, PPC mixes as well as the 5% neem, non-compost treatment. Within the PPC treatments, there were no significant differences in GI.

SPAD results show that the 2 to 5% neem, non-compost mixtures had significantly higher SPAD readings than did any of the PPC treatments and the 1% neem, non-compost mix (Table 2). In addition, the 5% neem, non-compost treatment was also significantly greater than the 0% neem, non-compost treatment at 56 DAP.

Measurements for pH show that the 4% neem, non-compost mixture had a pH significantly lower than any of the PPC treatments as well as the 0% neem, non-compost mix (Table 2). The 0 and 2% neem, PPC mixes, likewise, had a significantly higher pH than all non-compost mixtures except the 0% neem, non-compost treatment. At 56 DAP, the 4 and 5% neem, non-compost mixtures had significantly greater EC measurements than the 0-2% neem, PPC mixtures and the 0% neem, non-compost treatment (Table 2). In the same way, the 0% neem, non-compost treatment had a significantly lower EC value than the 1, 3, 4 and 5% neem, non-compost treatments.

Shoot dry weights of 0 and 1% neem, PPC treatments were significantly lower than any of the non-compost treatments (Table 2). The 2% neem, PPC treatment was

also significantly less than all non-compost treatments except for the 4% neem, non-compost mix. In the same way, the 0-3% neem, non-compost treatments had significantly greater dry weights than all PPC mixes, except for the 5% neem, PPC treatment.

### **Discussion:**

Non-compost treatments, those containing peat and fertigated, outperformed the PPC treatments. As a whole, the non-compost treatments had greater growth indices, higher SPAD readings and greater fresh and dry weights. Within the non-compost treatments, the treatments with less neem (especially 0 and 1%) often outperformed the other treatments within the non-compost group. Within the PPC group, though, the opposite was true: the 5% neem with PPC treatment outperformed all other mixes within the PPC group.

It is difficult to draw one overall conclusion from this experiment, especially since the initial purpose of this study was to consider the effect of neem at different percentages. It seems, however, that the PPC used in 6 of the treatments may hinder the performance of the plants when compared to the superior results of plants in the fertigated, non-compost treatments. Examining the neem percentages seems to render a twofold conclusion: when used in conjunction with PPC, higher neem percentages (in this case 5% neem) aid the plant in growth; but, within the fertigated, non-compost treatments, little-to-no neem outperformed the higher percentages of neem (0 and 1% neem, in this case, outperformed 4 and 5% neem treatments within the non-compost group). Consequently, when using poultry protein compost, the addition of neem has a

beneficial effect on the plant, however it has an antagonistic effect with increasing amounts added as an amendment in the case of fertigated treatments.

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**Table 1. Growth Indices, pH, EC and SPAD 28 DAP<sup>Z</sup> for salvia from neem percentage study 2010.<sup>Y</sup>**

Percent Neem Cake <sup>X</sup>	Primary Substrate Treatment <sup>W</sup>	Growth Indices <sup>V</sup>	pH <sup>U</sup>	EC <sup>T</sup>	SPAD <sup>S</sup>
0%	neem + PB + PC	14.8 d <sup>R</sup>	7.03 a	0.56 a	34.3 c
1%	neem + PB + PC	14.1 d	6.90 ab	0.47 ab	38.3 ab
2%	neem + PB + PC	15.6 d	6.91 a	0.46 ab	35.1 bc
3%	neem + PB + PC	15.1 d	6.84 ab	0.46 ab	37.4 abc
4%	neem + PB + PC	15.0 d	6.78 ab	0.35 b	34.8 c
5%	neem + PB + PC	16.2 cd	7.02 a	0.32 b	36.4 abc
0%	neem + PB + peat	24.0 a	6.82 ab	0.30 b	38.8 a
1%	neem + PB + peat	21.6 ab	6.26 c	0.50 ab	37.6 abc
2%	neem + PB + peat	14.2 d	6.49 bc	0.46 ab	39.5 a
3%	neem + PB + peat	15.2 d	6.66 abc	0.44 ab	37.7 abc
4%	neem + PB + peat	19.4 bc	6.77 ab	0.45 ab	38.0 abc
5%	neem + PB + peat	14.2 d	6.65 abc	0.45 ab	36.2 abc

<sup>Z</sup>Days after planting.

<sup>Y</sup>Data presented from neem percentage substrate study 2010.

<sup>X</sup>Neem cake added as percentage by volume.

<sup>W</sup>Stock substrate based on a 50:17:17:10:5 ratio, by volume, of pine bark (PB):compost (PC) or peat, dependent upon treatment listed:perlite:vermiculite.

<sup>V</sup>Measurements taken as [(height + width<sub>1</sub> + width<sub>2</sub>) / 3] in centimeters, at respective days after installation.

<sup>U</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>T</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>S</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter, at respective days after installation.

<sup>R</sup>Means within column followed by the same letter are not significantly different (Waller-Duncan K-ratio t test  $p \leq 0.05$ ,  $n=10$ ).

**Table 2. Growth Indices, pH, EC and SPAD 56 DAP<sup>Z</sup> for salvia from neem percentage study 2010.<sup>Y</sup>**

Percent Neem Cake <sup>X</sup>	Primary Substrate Treatment <sup>W</sup>	Growth Indices <sup>V</sup>	pH <sup>U</sup>	EC <sup>T</sup>	SPAD <sup>S</sup>	Dry Weight <sup>R</sup>
0%	neem + PB + PC	20.2 e <sup>Q</sup>	6.80 a	0.24 bcd	24.6 f	8.80 g
1%	neem + PB + PC	21.0 e	6.66 ab	0.23 cd	26.8 f	9.30 fg
2%	neem + PB + PC	21.5 cde	6.84 a	0.24 bcd	30.2 e	10.03 efg
3%	neem + PB + PC	20.9 e	6.51 abcd	0.31 abcd	31.7 de	10.68 de
4%	neem + PB + PC	23.5 bcde	6.62 abc	0.31 abcd	34.3 d	10.20 ef
5%	neem + PB + PC	21.2 de	6.67 ab	0.35 abc	34.1 d	12.25 abc
0%	neem + PB + peat	24.9 abc	6.65 ab	0.18 d	39.9 bc	12.25 abc
1%	neem + PB + peat	27.4 a	6.27 de	0.34 abc	37.5 c	12.18 abc
2%	neem + PB + peat	25.1 ab	6.22 de	0.30 abcd	40.2 ab	13.25 a
3%	neem + PB + peat	24.7 abcd	6.37 bcde	0.37 ab	41.1 ab	12.53 ab
4%	neem + PB + peat	22.5 bcde	6.13 e	0.43 a	42.3 ab	10.95 cde
5%	neem + PB + peat	21.5 cde	6.31 cde	0.41 a	42.8 a	11.88 bcd

<sup>Z</sup>Days after planting.

<sup>Y</sup>Data presented from neem percentage substrate study 2010.

<sup>X</sup>Neem cake added as percentage by volume.

<sup>W</sup>Stock substrate based on a 50:17:17:10:5 ratio, by volume, of pine bark (PB):compost (PC) or peat, dependent upon treatment listed:perlite:vermiculite.

<sup>V</sup>Measurements taken as [(height + width<sub>1</sub> + width<sub>2</sub>) / 3] in centimeters, at respective days after installation.

<sup>U</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>T</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>S</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter, at respective days after installation.

<sup>R</sup>Shoot biomass taken at 56 DAP was dried in a 175°F drying oven for four days and weighed again in grams.

<sup>Q</sup>Means within column followed by the same letter are not significantly different (Waller-Duncan K-ratio t test p≤0.05, n=10).

## Chapter III

### Gas Production from Soilless Media Amended with Neem Cake Powder

#### Significance to Industry:

Nursery and greenhouse growers continue to seek materials to decrease costs of plant production while maintaining environmental stewardship. Incorporation of neem cake as a substrate component could potentially impact nitrogen release as a result of altering substrate bacterial activity. This preliminary study investigates the impact of neem on substrate gas release and provides a starting point to further investigation regarding neem use as a substrate component. In this study, three substrate groups were tested with varying percentages of neem, and this paper reports on both across-group results as well as within-group results. Across all three substrates, three percent neem within the pine bark + poultry protein compost + neem group was significantly greater in CO<sub>2</sub> production than all treatments within the pine bark + neem group as well as zero percent neem within its own group and the pine bark + poultry protein compost + 19-6-12 + neem group. Nitrous oxide emission was significantly greater in the pine bark + poultry protein compost + 19-6-12 + neem group than all other treatments. Within-group comparisons reveal that three percent neem had greater CO<sub>2</sub> emission than zero percent neem for both the pine bark + neem and pine bark + poultry protein compost + neem groups. Three percent neem also produced significantly greater CH<sub>4</sub> than zero percent neem in the pine bark + poultry protein compost + neem group – and within the same

substrate group, two percent neem had significantly greater N<sub>2</sub>O emission than zero percent neem. Comparing within-group, there were no significant differences among treatments in the pine bark + poultry protein compost + 19-6-12 + neem group for all three gases analyzed.

### **Introduction:**

Fertilizer is an expensive part of any plant production program and environmental safety is becoming an increasingly important subject. Therefore, any cost-effective method to reduce fertilizer use in an environmentally-friendly manner is a needed and valuable product. Nitrogen is often viewed as a limiting factor in plant nutrition, and while there are many forms or sources of nitrogen, our study focused specifically on urea. Urea breaks down into ammonium with the aid of an enzyme known as urease. Ammonium then further breaks down into ammonia, which then undergoes volatilization. Therefore, slowing down this catalysis of urea could, in theory, prolong substrate nitrogen supplies. Since urease in soil is a byproduct of bacteria, limiting urease production by affecting the enzyme itself or its bacterial producers could inhibit the breakdown of urea.

Beginning in the 1970's, pine bark quickly became an invaluable resource to the horticulture industry. Present-day demands for this commodity, however, have also increased and, with the growth of the horticulture sector, our industry is feeling the pressure to find some alternatives to exclusive pine bark usage (Avent, 2003). While greenhouse plants are almost exclusively produced in peat-substrates, 75-100%, by volume, of container substrates in the eastern US are comprised of pine bark (Lu et al.,

2006). Future projections project the rising cost of pine bark combined with less availability to the horticulture industry (Lu et al., 2006).

Numerous organic and inorganic substances are being used to develop varying substrates. Clean chip residual (the material left on the forest floor following timber harvest) can be a realistic alternative to pine bark-exclusive substrates for ornamental plant production (Boyer et al., 2008). Fain et al. (2008) successfully used WholeTree (whole pine tree shoots) in varying percentages as suitable greenhouse media for growing marigolds and petunias. With additional fertilizer, Jackson and Wright (2007) were able to grow plants in 100% pulverized pine wood. Cotton gin compost was used as a viable substrate component by Cole et al. (2002). Spent tea grinds can be successfully used in horticultural substrates (Wells, 2008). Poultry litter was a suitable amendment for substrates comprised primarily of either pine bark, WholeTree (created by chipping, then further milling entire pine trees – log, limbs, needles and bark), or clean chip residual for growing plants in containers with favorable results and is a readily available source of nitrogen (Fain et al., 2008; Marble et al., 2010; Mitchell and Donald, 1995).

Parboiled fresh rice hulls can be used successfully as a substrate component comparable to perlite (Evans and Gachukia, 2007). Using substrates from peanut hulls, pine bark, peatmoss and combination thereof, Bilderback et al. (1982) reported success in growing azaleas. In searching for an ecologically-friendly alternative to rockwool, Urrestarazu et al. (2005) concluded that almond shells can be an effective and beneficial soilless media for plant production. Coconut coir can be a viable substrate for plant production, however its success depends on particle size (Noguera et al., 2003). Peat-vermiculite substrates served as viable substrates for growing chrysanthemums (Paul and

Lee, 1976). Mineral substrates have the potential to perform similarly to peat-based mixes (Smith et al., 1995). Smith and Hall (1994) determined that a perlite-based substrate can be comparable in management and productivity to peat-based potting mixes.

While potential alternative substrates seem to abound, there are requirements that must be met of the material in order for it to be usable and effective. Chang and Lin (2007) report that basic requirements for a successful and beneficial plant-growing medium include: excellent chemical resistance properties, light weight, inexpensiveness, absence of pests and diseases and availability. Nkongolo and Caron (2006) noted that particle size, specifically in peat and pine bark-based substrates, influences plant response. Handreck (1983) reports the importance of particle size and advises that substrate formulators heed the “fines” fraction of a substrate, especially particle size smaller than 0.5mm, as this size controls the physical properties of pine bark-based substrates. Shrinkage is a physical property of organic soils (such as peat) that must be managed (Schwärzel et al., 2002). Nemati et al. (2002) noted that insufficient aeration of artificial growing media is a common problem in greenhouse production.

The soilless media amendment in question is a product of *Azadirachta indica* A. Juss, or the neem tree. The neem tree is known as the “village dispensary” in India and Southeast Asia, where it is native (Biswas et al., 2002). This is due to the results of both science and tradition about the medicinal and agricultural uses that neem provides. The neem tree is an evergreen tree belonging to the mahogany family, Meliaceae, of which *Melia azedarach* Chinaberry is also a member (Biswas et al., 2002). Steeped in Indian tradition and lore, the neem tree continues to play roles in their traditions, medicine and

agriculture and now the rest of the world is beginning to pay more attention to neem, which has been somewhat controversial to the traditionalists in Asia (Marden, 1999).

Neem has been used as a soil amendment in many studies in India. Neem seed residue provided a nitrogen value, after oil extraction, of 7% and at a release rate fast enough to satisfy maize nutrition (Agbenin et al., 1999). Neem oil also enhanced plant growth when incorporated into soil at 2.5, 5.0 and 20 ppm, though 10 ppm decreased plant growth (Bhaskar and Charyulu, 2005). Likewise, Agyarko et al. (2006) reported that soil nutritional levels increased with poultry manure and increasing levels of neem leaves. Bhalla and Devi Prasad (2008) reported higher vegetative growth in plants than reproductive growth (both showing higher growth than the control) when neem cake was incorporated into the soil.

Neem's impact within a substrate has been attributed to its potential urease retardation activity (B. Hurst, personal communication). Urease is the naturally occurring enzyme in soil responsible for the hydrolysis of urea into carbon dioxide and ammonia (Manunza et al., 1997). Ammonium and ammonia are the nitrogen sources within fertilizers (be it synthetic fertilizer or organic), but the ammonia can undergo volatilization, which may decrease nitrogen available to the plant. Therefore, it is often desirable to have certain levels of urease inhibition in order to constrain ammonium production, resulting in a more readily available supply of nitrogen over a longer period of time. The amassing of ammonium can lead to problems other than ammonia volatilization, such as nitrite toxicity and damage to young plants (Bremner and Douglas, 1971).

Mohanty et al., (2007) reported on the potential inhibitory effects of neem seed kernel powder on urease in three mineral soils native to India, showing slight suppression of urease activity when applied to acidic soils. Méndez-Bautista et al., (2009) studied the effects of neem leaf extracts on greenhouse gas emissions and inorganic nitrogen in urea-amended soil and reported that the leaf extract had no significant effect on urease, but may limit nitrification. Majumdar et al., (2000) coated urea with neem seed powder before adding to rice fields in North India, resulting in slight nitrification inhibition. Kumar et al., (2007) used neem oils to coat urea and added it to sandy-loam soils resulting in some nitrification inhibition as well. Bhalla and Devi Prasad (2008) showed, in one of their studies, that addition of neem cake into a mineral soil is an economical and effective method for reducing fertilizer application by prolonging fertilizer available to the plant.

The objectives of this study were to determine neem cake powder's effect on gas production and emission from soilless media. This was done in hope to extrapolate the results into the potential prolongation of nitrogen sources within the substrates.

### **Materials and Methods:**

The study consisted of three groups of treatments: 100% pine bark (PB) + neem; 80% PB + 20% poultry protein compost (PPC) + neem; and 80% PB + 20% PPC + urea + neem. Within each of these substrates were varying concentrations of neem. Within the PB + neem substrate were four treatments with 0, 1, 2 and 3% neem. The PB + PPC + neem substrate had the same percentages of neem added, but also included 20 percent PPC with 80 percent PB. The third substrate contained the same percentages of PB

(80%) and PPC (20%) as the second substrate, and with the varying neem concentrations, but with the addition of Scott's Osmocote Classic 19-6-12 at 9 lbs/yd<sup>3</sup> (1.7 lbs N/yd<sup>3</sup>).

Each of the 12 treatments contained 4 replicates for a total of 48 experimental units. Substrates were placed in trade-gallon containers without plants and placed in a glass greenhouse at the USDA Soil Dynamics Laboratory, Auburn University, Alabama. Substrates were watered as needed, but with minimal leaching. Moist conditions were necessary to mimic rhizosphere microenvironments in order to facilitate microbial growth. Data were taken at regular intervals beginning in May 2010 and ended in August 2010. Data was collected 3 days weekly for the first 2 weeks and then once weekly for the next 7 weeks. After that, data was collected biweekly. In order to determine substrate microbial activity, gas emissions were collected from an airtight gas chamber large enough to accommodate one pot each. The top of the gas chamber was outfitted with a rubber septum through which a needle could penetrate. Four evacuated collection vials were needed for each experimental unit, each one representing a time within the 15 minutes of collection (times 0, 1, 2 and 3 represent initial time and 5, 10 and 15 minutes, respectively). Gas samples were pulled for each experimental unit for each of the aforementioned times and results were analyzed using a gas chromatograph. Gas samples tested for were: carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), which represent microbial respiration.. CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O data were analyzed using Tukey's Studentized Range Test in SAS Statistical Software (alpha = 0.05).

## **Results:**

*Overall:* Notation for reporting data will adhere to the following guidelines: PB is pine bark; PPC is poultry protein compost; fertilizer will refer to the Osmocote 19-6-12; and when entire groups of treatments are referenced, the values that follow are given in order of neem rate increase within the group's treatments. The unit for trace gas emission values is  $\mu\text{moles m}^{-2} \text{min}^{-1}$ . All data is presented in Table 3.

First, results will be given for across-substrate comparisons. Three substrates tested in this experiment were: 100% PB + neem; 80% PB + 20% PPC + neem and 80% PB + 20% PPC + 19-6-12 + neem. Secondly, results will be given for within-substrate treatments.

### *Across-substrate results:*

*Carbon Dioxide (CO<sub>2</sub>):* Increasing neem percentage (by volume) as a substrate component appeared to increase CO<sub>2</sub> production (Table 3). However, in the PB + neem treatments, there was no statistical difference among treatments. Within the PB + PPC + neem group, CO<sub>2</sub> production for the 3% neem treatment was statistically larger than the 0% neem treatment. However, there was no statistical difference among treatments in the PB + PC + fertilizer + neem group.

Across all groups, PB + PPC + 3% neem had the highest value for CO<sub>2</sub> production, though not statistically different than PB + PPC + 1 and 2% neem. The PB + 0% neem treatment had the lowest value for CO<sub>2</sub>, but was not statistically different than any of the PB + neem treatments, PB + PPC + 0% neem, or PB + PPC + fertilizer + 0% neem.

*Methane (CH<sub>4</sub>):* Methane's relation to neem percentage does not seem to be as clear-cut as with CO<sub>2</sub>. Three percent neem used in conjunction with PB + PPC had significantly higher CH<sub>4</sub> than 0% neem in the same mixture (Table 3). There was no significant difference in CH<sub>4</sub> levels among treatments within the other two groups tested.

Again, among all groups PB + PPC + 3% neem had the highest CH<sub>4</sub> value, but was not significantly different than PB + 0 and 2% neem; PB + PPC + 1 and 2% neem or PB + PPC + fertilizer + 1, 2 and 3% neem. The PB + PPC + 0% neem had the lowest value for CH<sub>4</sub> across all treatments, but was not statistically different from any treatment other than PB + PPC + 3% neem.

*Nitrous Oxide (N<sub>2</sub>O):* For nitrous oxide there were no statistical differences among treatments within the PB + neem group or the PB + PPC + neem group (Table 3). The PB + PPC + fertilizer + neem group, though, shows that N<sub>2</sub>O emission for 3% neem was significantly higher than 0 and 1% neem.

Across all treatments, N<sub>2</sub>O from 3% neem in PB + PPC + fertilizer was significantly greater than all other treatments, other than 2% neem in PB + PPC + fertilizer. N<sub>2</sub>O from PB + PPC + fertilizer + 2% neem was greater than all treatments from the PB + neem and PB + PPC + neem groups. N<sub>2</sub>O from the 0 and 1% neem treatments within the PB + PB + fertilizer group were also statistically greater than all treatments within the PB + neem and PB + PB + neem groups. Figure 3 also shows a stepwise increase in N<sub>2</sub>O production with the increase of neem within each substrate, though there are negligible results in the pine bark only mixes.

*Within-substrate results:*

*PB + neem:* Three percent neem within the pine bark media had significantly greater CO<sub>2</sub> emission than zero percent neem (Table 4), though neither the 3 nor 0% neem treatments were significantly different than the 1 and 2% neem treatments. There were no significant differences among treatments in this substrate group for CH<sub>4</sub> and N<sub>2</sub>O emission.

*PB + PPC + neem:* The 3% neem treatment within the PB + PPC + neem substrate group had significantly greater CO<sub>2</sub> emission than the 0% neem treatment of the same group (Table 5). Neither the 3 nor 0% neem treatments were significantly different than the 1 and 2% neem treatments. Methane results were similar to CO<sub>2</sub>: 3% neem was significantly greater than 0% neem, though neither the 3 nor 0% neem treatments were significantly different than the 1 and 2% neem treatments. Two percent neem had significantly greater N<sub>2</sub>O production than 0% neem, though neither had significantly different values from 1 and 3% neem.

*PB + PPC + 19-6-12 + neem:* Among all treatments and for all three gas results, CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O, there were no significant differences (Table 6).

**Discussion:**

Across substrates, there were few differences among treatments. Three percent neem in both the PB + PPC + neem and PB + PPC + 19-6-12 + neem groups had greater CO<sub>2</sub> production than 0-2% neem in the PB + neem group. Three percent neem in the PB + PPC + neem produced more CO<sub>2</sub> than all PB + neem treatments as well as 0% neem within its own group and 0% neem in the PB + PPC + 19-6-12 + neem. Methane

comparisons show no notable results across substrates. The PB + PPC + 19-6-12 + neem group, as a whole, had significantly greater N<sub>2</sub>O production than any other treatments.

Within-group comparisons show that 3% neem within the PB + neem and PB + PPC + neem groups had significantly greater CO<sub>2</sub> production than 0% neem within their groups. Three percent neem within the PB + PPC + neem group also had significantly greater CH<sub>4</sub> production than 0% neem within the same group. PB + PPC + 2% neem was significantly greater in N<sub>2</sub>O emission than 0% neem, as well. Across all treatments and for all three gases tested (CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O), the PB + PPC + 19-6-12 + neem group had no significant differences.

Studies to determine the fate of urease when neem is added are ongoing, with some supplemental data not having been analyzed yet. It seems reasonable to conclude that based on the presented data, neem does have an effect on soil respiration, though more testing to prove the extent to which this occurs is currently underway. Current testing includes the aforementioned acid-coated tubes for ammonia volatilization, pH and EC, as well as nutrient composition of the different treatments.

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Table 3. Gas analysis results for organic urease inhibition substrate amendment.<sup>Z</sup>

Treatment	CO <sub>2</sub> <sup>Y</sup>	CH <sub>4</sub>	N <sub>2</sub> O
Pine Bark + 0% neem <sup>X</sup>	53.61 d <sup>W</sup>	0.01768 ab	0.0008 c
Pine Bark + 1% neem	85.39 cd	0.00102 b	0.0013 c
Pine Bark + 2% neem	97.51 cd	0.00279 ab	0.0003 c
Pine Bark + 3% neem	126.86 bcd	-0.00142 b	0.0006 c
Pine Bark + Compost + 0% neem	125.94 bcd	-0.00494 b	0.0394 c
Pine Bark + Compost + 1% neem	172.01 abc	0.01426 ab	0.0442 c
Pine Bark + Compost + 2% neem	198.01 ab	0.02995 ab	0.1299 c
Pine Bark + Compost + 3% neem	247.27 a	0.04150 a	0.0993 c
Pine Bark + Compost + 19-6-12 <sup>V</sup> + 0% neem	142.82 bcd	-0.00026 b	1.0294 b
Pine Bark + Compost + 19-6-12 + 1% neem	153.03 abc	0.01013 ab	1.0998 b
Pine Bark + Compost + 19-6-12 + 2% neem	181.20 abc	0.00687 ab	1.1539 ab
Pine Bark + Compost + 19-6-12 + 3% neem	218.36 ab	0.01235 ab	1.7349 a

<sup>Z</sup>Study conducted Summer 2010.

<sup>Y</sup>All units for gas are  $\mu$ moles trace gas m<sup>-2</sup> min<sup>-1</sup>.

<sup>X</sup>Neem cake (from *Azadirachta indica* A. Juss).

<sup>W</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test,  $\alpha = 0.05$ ).

<sup>V</sup>Scott's Osmocote Classic 19-6-12.

Table 4. Pine bark + neem within-group gas analysis results.<sup>Z</sup>

Treatment	CO <sub>2</sub> <sup>Y</sup>	CH <sub>4</sub>	N <sub>2</sub> O
Pine Bark + 0% neem <sup>X</sup>	53.61 b <sup>W</sup>	0.01768 a	0.0008 a
Pine Bark + 1% neem	85.39 ab	0.00102 a	0.0013 a
Pine Bark + 2% neem	97.51 ab	0.00279 a	0.0003 a
Pine Bark + 3% neem	126.86 a	-0.00142 a	0.0006 a

<sup>Z</sup>Study conducted Summer 2010.

<sup>Y</sup>All units for gas are  $\mu\text{moles trace gas m}^{-2} \text{ min}^{-1}$ .

<sup>X</sup>Neem cake (from *Azadirachta indica* A. Juss).

<sup>W</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test,  $\alpha = 0.05$ ).

Table 5. Pine bark + Compost + neem within-group gas analysis results.<sup>z</sup>

<b>Treatment</b>	<b>CO<sub>2</sub><sup>y</sup></b>	<b>CH<sub>4</sub></b>	<b>N<sub>2</sub>O</b>
Pine Bark + Compost + 0% neem	125.94 b	-0.00494 b	0.0394 b
Pine Bark + Compost + 1% neem	172.01 ab	0.01426 ab	0.0442 ab
Pine Bark + Compost + 2% neem	198.01 ab	0.02995 ab	0.1299 a
Pine Bark + Compost + 3% neem	247.27 a	0.04150 a	0.0993 ab

<sup>z</sup>Study conducted Summer 2010.

<sup>y</sup>All units for gas are  $\mu$ moles trace gas m<sup>-2</sup> min<sup>-1</sup>.

<sup>x</sup>Neem cake (from *Azadirachta indica* A. Juss).

<sup>w</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test,  $\alpha = 0.05$ ).

Table 6. Pine bark + Compost + 19-6-12 + neem within-group gas analysis results.<sup>Z</sup>

<b>Treatment</b>	<b>CO<sub>2</sub><sup>Y</sup></b>	<b>CH<sub>4</sub></b>	<b>N<sub>2</sub>O</b>
Pine Bark + Compost + 19-6-12 <sup>V</sup> + 0% neem	142.82 a	-0.00026 a	1.0294 a
Pine Bark + Compost + 19-6-12 + 1% neem	153.03 a	0.01013 a	1.0998 a
Pine Bark + Compost + 19-6-12 + 2% neem	181.20 a	0.00687 a	1.1539 a
Pine Bark + Compost + 19-6-12 + 3% neem	218.36 a	0.01235 a	1.7349 a

<sup>Z</sup>Study conducted Summer 2010.

<sup>Y</sup>All units for gas are  $\mu$ moles trace gas m<sup>-2</sup> min<sup>-1</sup>.

<sup>X</sup>Neem cake (from *Azadirachta indica* A. Juss).

<sup>W</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test,  $\alpha = 0.05$ ).

<sup>V</sup>Scott's Osmocote Classic 19-6-12.

## **Chapter IV**

### **Final Discussion**

To facilitate the final discussion chapter, the discussion will be segmented according to the experiments described within this thesis.

#### **Commercially Available Organic Substrate Comparisons:**

It was during this experiment that we originally noticed the presence of different fungi in some of the Jungle Growth<sup>®</sup> products. The fungi's presence did not present a concern, it was almost the opposite – we assumed that increased microorganismal activity within the potting media could very well benefit plant material. It did concern us, though, that presence of the fungi was sporadic. Within any given Jungle Growth<sup>®</sup> product, there was bag-to-bag variation which also showed in the performance of the product.

Also, it was noted that some of the Jungle Growth<sup>®</sup> products continued to undergo a “heat” after stocking. This could have been due to the poultry protein compost continuing to compost after bagging. The implications of this possibility are less than beneficial. If composting is still occurring post-bagging, then the physical and chemical properties of its contents are still changing. Even further, this leads to bag-to-bag differences, which we found in some of our studies. The sometimes “erratic” results came through in some of the studies. While this casts a negative light, it should also be

noted that even though this happened, the Jungle Growth® products still performed as well as or better than their competitors in these two studies. However, further evaluation of changes over time of the poultry protein compost and the effects on nutrition and other parameters would be beneficial for the company.

There were cases of which a particular bag of substrate killed the plant it hosted within three days of planting, but subsequent bags of the same formula or recipe resulted in some of the greatest plant growth. Therefore, quality control should truly be evaluated within the company's production line and within the individual components of the substrate mixes.

#### **Evaluation of Neem Percentages as an Organic Substrate Component:**

Again, as mentioned in the commercially available organic substrate comparison study, the poultry protein compost should be further evaluated, because of the inconsistencies that seem to be apparent when working with the substance. Along the same lines, the neem cake should be evaluated for its longevity. Azadirachtin, the chemical attributed to neem cake's benefits, levels should be looked at over time to determine if the chemical degrades with time, or in certain conditions that may be present in a bagged substrate, especially one that may continue composting after mixing and bagging.

A better choice of plant material could have been advantageous for this study. Salvia's growth habit is not always easy to measure (as it did not grow vertically, and was quite brittle) not only growth indices, but flower count is more difficult. Because of this, it was difficult to ascertain neem's effects on plant response.

### **Use of Neem Cake as an Organic Substrate Component:**

While this experiment seems to have had few obvious shortcomings, the initial experimental plan should have been set up factorially. Because the study took place in a glass greenhouse, there became a concern after a few data-taking days that our own respiratory gases were influencing the samples collected from the chambers – the gas chromatograph showed an unusually high carbon dioxide value. Therefore, we moved collection to outside of the greenhouse. This raised a concern, of sorts, in that the environment outside was much more variant than in the greenhouse, so this may have cause some fluctuation (i.e. temperature differences) in the substrate respiration and subsequent gas sample values. Using a covered area could have benefitted greatly, taking out the variable of sun exposure and radiant heat.

Also, this particular project gives an incomplete answer to the question posed at the beginning. This study took a secondary look at neem's effects on urease activity. We can assume a conclusion based on the collected gas values, but a future study heavily steeped in microbiology and soil pathology is necessary to determine the true interaction between neem and the bacteria producing urease as well as the urease itself.

## Appendix A

### Commercially Available Soilless Media Comparisons

#### Significance to Industry:

With “green-mindedness” steadily gaining popularity, the organic movement is beginning to hit the horticultural market. The effects of eleven commercially available substrates on plant growth were evaluated using four species of plants. In experiment one, Jungle Growth<sup>®</sup> organic substrates outperformed other commercial potting substrates and were only slightly inferior in performance to the best-ranking substrate, Miracle-Gro<sup>®</sup> Moisture Control<sup>®</sup>. In the second study, where different, the Jungle Growth<sup>®</sup> substrates outperformed all other substrates tested (though not statistically greater than Sta-Green<sup>®</sup> Flower and Vegetable mix when used on tomatoes and petunias).

#### Introduction:

The 1970's horticulture industry had a “breakthrough” in using pine bark as an invaluable resource, however present-day demands for this commodity have also increased and, with the growth of the horticulture sector, our industry is feeling the pressure to find some alternatives to exclusive pine bark usage (Avent, 2003). While greenhouse plants are almost exclusively produced in peat-substrates, 75-100%, by volume, of container substrates in the eastern US are comprised of pine bark (Lu et al., 2006). Future projections project the rising cost of pine bark combined with less availability to the horticulture industry (Lu et al., 2006).

Numerous organic and inorganic substances are being used to develop varying substrates. Clean chip residual (the material left on the forest floor following timber harvest) can be a realistic alternative to pine bark-exclusive substrates for ornamental plant production (Boyer et al., 2008). Fain et al. (2008) successfully used WholeTree (whole pine tree shoots) in varying percentages as suitable greenhouse media for growing marigolds and petunias. With additional fertilizer, Jackson and Wright (2007) were able to grow plants in 100% pulverized pine wood. Cotton gin compost was used as a viable substrate component by Cole et al. (2002). Spent tea grinds can be successfully used in horticultural substrates (Wells, 2008). Poultry litter was a suitable amendment for substrates comprised primarily of either pine bark, WholeTree (created by chipping, then further milling entire pine trees – log, limbs, needles and bark), or clean chip residual for growing plants in containers with favorable results and is a readily available source of nitrogen (Fain et al., 2008; Marble et al., 2010; Mitchell and Donald, 1995).

Parboiled fresh rice hulls can be used successfully as a substrate amendment comparable to perlite (Evans and Gachukia, 2007). Using substrates from peanut hulls, pine bark, peatmoss and combination thereof, Bilderback et al. (1982) reported success in growing azaleas. In searching for an ecologically-friendly alternative to rockwool, Urrestarazu et al. (2005) concluded that almond shells can be an effective and beneficial soilless media. Coconut coir can be a viable substrate for plant production, however its success depends on particle size (Noguera et al., 2003). Peat-vermiculite substrates had beneficial results when growing chrysanthemums (Paul and Lee, 1976). Mineral substrates have the potential to perform similarly to peat-based mixes (Smith et al.,

1995). Smith and Hall (1994) determined that a perlite-based substrate can be comparable in management and productivity to peat-based potting mixes.

While potential alternative substrates seem to abound, there are requirements that must be met of the material in order for it to be usable and effective. Chang and Lin (2007) report that basic requirements for a successful and beneficial plant-growing medium include: excellent chemical resistance properties, light weight, inexpensiveness, absence of pests and diseases and availability. Nkongolo and Caron (2006) noted that particle size, specifically in peat and pine bark-based substrates, influences plant response. Handreck (1983) reports the importance of particle size and advises that substrate formulators heed the “fines” fraction of a substrate, especially particle size smaller than 0.5mm, as this size controls the physical properties of pine bark-based substrates. Shrinkage is a physical property of organic soils (such as peat) that must be managed (Schwärzel et al., 2002). Nemati et al. (2002) note that insufficient aeration of artificial growing media is a common problem in nursery and greenhouse production.

*Azadirachta indica* A. Juss, or the neem tree, is known as the “village dispensary” in India and Southeast Asia, where it is native (Biswas et al., 2002). This is due to the results of both science and tradition about the medicinal and agricultural uses neem provides. The neem tree is an evergreen tree belonging to the mahogany family, Meliaceae, of which *Melia azedarach* Chinaberry is also a member (Biswas et al., 2002). Steeped in Indian tradition and lore, the neem tree continues to play roles in their traditions, medicine and agriculture and now the rest of the world is beginning to pay more attention to neem, which has been somewhat controversial to the traditionalists in Asia (Marden, 1999).

Neem has been used as a soil amendment in many studies in India. Neem seed residue provided a nitrogen value, after oil extraction, of 7% and at a release rate fast enough to satisfy maize nutrition (Agbenin et al., 1999). Neem also enhanced plant growth when incorporated into soil at 2.5, 5.0 and 20 ppm, though 10 ppm decreased plant growth (Bhaskar and Charyulu, 2005). Likewise, Agyarko et al. (2006) reported that soil nutritional levels increased with increasing levels of neem leaves and poultry manure incorporation. Bhalla and Devi Prasad (2008) reported higher vegetative growth in plants than reproductive growth (both showing higher growth than the control) when neem was incorporated into the soil.

The objectives of this experiment were to compare commercially-available soilless potting media to determine which is most effective in growing selected annuals and tomatoes.

### **Materials and Methods:**

*Experiment 1:* Installed on September 16, 2009, seven commercially available potting substrates were used, with each being its own treatment and each treatment containing twelve replicates. The seven substrates were Jungle Growth<sup>®</sup> products: Flower and Vegetable, Professional Mix and WaterWise<sup>®</sup>; and Scott's products: Miracle-Gro<sup>®</sup> Organic Choice<sup>®</sup>, Miracle-Gro<sup>®</sup> Moisture Control<sup>®</sup>, Miracle-Gro<sup>®</sup> Potting Mix and Scott's<sup>®</sup> Premium Potting Mix. Plant material used in the study included *Petunia xhybrida* and *Tagetes patula* which were transplanted on September 16, 2009, from 288-cell trays into Dillen Products 6" Azalea pots (item #DIL60ATW) and grown in a double-layer poly greenhouse located at Auburn University's Paterson Greenhouse Complex. All plants were hand-watered as needed and the thermostat was set at min/max

temperatures of 65/78°F. Pots were arranged in a randomized complete block design according to replicate number. Data were taken at termination of the 8-week study (56 days after planting, DAP) and included: growth indices (taken as  $[(\text{height} + \text{width}_1 + \text{width}_2) / 3]$  in centimeters), SPAD-502 readings (measures chlorophyll levels in the plants), flower count, pH and electrical conductivity (EC). All data were analyzed using Waller-Duncan k-ratio t-test. Also, it is important to note that no supplemental fertilizer was used for the duration of the study, only what was premixed by the manufacturer. Termination date was November 11, 2009.

*Experiment 2:* This study was a repeat of the first study with a few modifications. On January 29, 2010, four species: *Petunia xhybrida* ‘Dreams Midnight’, *Tagetes patula* ‘Durango Gold’, *Dianthus plumarius* ‘Floral Lace’ and *Lycopersicon lycopersicum* ‘Early Girl’ were transplanted from 288-cell trays into Dillen Products 6” Azalea pots (item #DIL60ATW). For dianthus and marigold, the same seven substrates (treatments) from the first experiment were used. Petunia and tomato received four additional treatments; Scott’s<sup>®</sup> Seeding Soil and three Sta-Green<sup>®</sup> products: Flower and Vegetable, Tree and Shrub and All Purpose Potting Mix. Each treatment was replicated 12 times. Again, all pots were placed in a randomized complete block design inside a greenhouse at the Paterson Greenhouse Complex. Plants were hand-watered as needed and the greenhouse thermostat was set at min/max temperatures of 65/78°F. Data taken was the same as the first experiment and again analyzed using Waller-Duncan k-ratio t-test. Also, no supplemental fertilizer was applied during the 8-week study. Termination date was March 26, 2010.

## Results:

*Experiment 1: Petunia:* Petunias grown in MG Moisture Control<sup>®</sup> had the greatest growth (as indicated by growth indices, Table 7), followed by JG Professional Mix and JG Flower & Vegetable. MG Organic Choice<sup>®</sup>, MG Potting Mix and Scott's<sup>®</sup> Premium Potting Mix produced the least growth in petunias, though plants in JG WaterWise<sup>®</sup> Mix were not different from Scott's<sup>®</sup> Premium Potting Mix.

JG Flower & Vegetable and MG Potting Mix had the highest pH measurements, though they were not different from JG Professional Mix and JG WaterWise<sup>®</sup> Mix (Table 7). MG Moisture Control<sup>®</sup> had the lowest pH, but was not different from MG Organic Choice<sup>®</sup> or Scott's<sup>®</sup> Premium Potting Mix. JG WaterWise<sup>®</sup> Mix had the highest EC measurement. MG Moisture Control<sup>®</sup> had the next highest EC value, but was not different from JG Flower & Vegetable. MG Organic Choice<sup>®</sup> had the lowest EC value, but was not different from MG Potting Mix or Scott's<sup>®</sup> Premium Potting Mix.

Petunias grown in MG Moisture Control<sup>®</sup> had the highest SPAD measurement, but were not different from JG Flower & Vegetable, JG Professional Mix, or JG WaterWise<sup>®</sup> Mix (Table 7). Petunias grown in MG Potting Mix had the lowest SPAD measurement, but were not different from Scott's<sup>®</sup> Premium Potting Mix.

*Marigold:* Marigolds grown in MG Moisture Control<sup>®</sup> had the greatest growth (Table 8). JG WaterWise<sup>®</sup> Mix produced the next greatest growth, but was not different from Scott's<sup>®</sup> Premium Potting Mix, JG Flower & Vegetable, or JG Professional Mix. Marigolds in MG Organic Choice<sup>®</sup> produced the least growth.

There was no statistical difference in pH measurements among all of the substrates (Table 8). JG WaterWise<sup>®</sup> Mix had the highest EC value, but was different

from JG Flower & Vegetable, MG Moisture Control<sup>®</sup>, or MG Potting Mix. Scott's<sup>®</sup> Premium Potting Mix had the lowest EC value, but was only different from JG WaterWise<sup>®</sup> Mix.

Marigolds grown in JG Professional Mix had the highest SPAD value, but were not different from JG WaterWise<sup>®</sup> Mix, JG Flower & Vegetable, or MG Moisture Control<sup>®</sup> (Table 8). Marigolds grown in MG Organic Choice<sup>®</sup> had the lowest SPAD value.

*Experiment 2: Dianthus:* Plants grown in JG Professional Mix had the largest growth indices and were not different from those in JG Flower & Vegetable Mix (Table 9). Dianthus grown in MG Potting Mix had the next greatest growth, but was not different from JG WaterWise<sup>®</sup> Mix, or Scott's<sup>®</sup> Premium Potting Mix.

The only difference in pH measurements among the substrates was between JG WaterWise<sup>®</sup> Mix and MG Moisture Control<sup>®</sup> (Table 9). MG Moisture Control<sup>®</sup> had the lowest pH, but was only different from JG WaterWise<sup>®</sup> Mix (with the highest pH). JG WaterWise<sup>®</sup> Mix had a higher EC value than all other mixes. There were no other differences in EC among the substrates.

Plants grown in JG Professional Mix had the highest flower count, followed by JG Flower & Vegetable (Table 9). MG Potting Mix had the next highest flower count, but was not different from JG WaterWise<sup>®</sup> Mix and Scott's<sup>®</sup> Premium Potting Mix. MG Moisture Control<sup>®</sup> had the lowest flower count and was not different from MG Organic Choice<sup>®</sup>.

Dianthus grown in JG Professional Mix had the highest SPAD value and were not different from those in JG Flower & Vegetable. The other substrate yielded lower SPAD

values than JG Professional Mix and JG Flower & Vegetable and there were no differences among the other treatments.

*Marigold:* Plants grown in JG Professional Mix had the greatest growth and were not different from marigolds grown in JG Flower & Vegetable (Table 10). Marigolds grown in MG Organic Choice<sup>®</sup> had the least<sup>®</sup> growth and were not different from those grown in MG Moisture Control<sup>®</sup>. There were no differences among plants grown in the other media.

There were no differences among treatments in pH (Table 10). JG WaterWise<sup>®</sup> Mix had the highest EC measurement. There were no differences among the other treatments in EC.

Marigolds grown in JG Flower & Vegetable had the highest flower count and were not different from those grown in JG Professional Mix and JG WaterWise<sup>®</sup> Mix (Table 10). Plants grown in MG Organic Choice<sup>®</sup> had the lowest flower count and were not different from marigolds grown in MG Moisture Control<sup>®</sup> or Scott's<sup>®</sup> Premium Potting Mix.

Plants grown in JG Flower and Vegetable and JG Professional Mix had the greatest SPAD values (no difference between the two treatments) (Table 10). Marigolds grown in MG Organic Choice<sup>®</sup> had the lowest SPAD value and were not different from those grown in MG Moisture Control<sup>®</sup>. There were no differences among the other treatments.

*Petunia:* Plants grown in JG Flower & Vegetable had the greatest growth and were not different from those grown in JG Professional Mix or Sta-Green<sup>®</sup> Flower and

Vegetable (Table 11). Petunias grown in Scott's<sup>®</sup> Seeding Soil had the least growth and were not different from those in MG Moisture Control<sup>®</sup>.

Sta-Green<sup>®</sup> All Purpose Potting Mix had the highest pH, but was not different from Scott's<sup>®</sup> Seeding Soil, JG WaterWise<sup>®</sup> Mix, Sta-Green<sup>®</sup> Flower and Vegetable, or Sta-Green<sup>®</sup> Tree and Shrub (Table 11). MG Organic Choice<sup>®</sup> had the lowest pH and was not different from JG Professional Mix. JG WaterWise<sup>®</sup> Mix had the highest EC measurement. There were no differences among the other treatments in EC.

Marigolds grown in Sta-Green<sup>®</sup> Flower and Vegetable had the highest flower count (Table 11). Plants grown in JG Flower & Vegetable had the next highest flower count and were not different from those in JG Professional Mix or JG WaterWise<sup>®</sup> Mix. Marigolds grown in MG Moisture Control<sup>®</sup> had the lowest flower count and were not different from those in Scott's<sup>®</sup> Seeding Soil, MG Organic Choice<sup>®</sup>, or Sta-Green<sup>®</sup> All Purpose Potting Mix.

Plants grown in JG Flower & Vegetable had the highest SPAD value and were not different from those grown in JG Professional Mix, Sta-Green<sup>®</sup> Tree and Shrub, or Sta-Green<sup>®</sup> Flower and Vegetable (Table 11). Marigolds grown in JG WaterWise<sup>®</sup> Mix had the lowest SPAD value and were not different from those in MG Organic Choice<sup>®</sup>, MG Moisture Control<sup>®</sup>, MG Potting Mix, Scott's<sup>®</sup> Premium Potting Mix or Scott's<sup>®</sup> Seeding Soil.

*Tomato:* Plants grown in JG Flower & Vegetable had the greatest growth and were not different from those grown in Sta-Green<sup>®</sup> Flower and Vegetable or JG Professional Mix (Table 12). Tomatoes grown in MG Moisture Control<sup>®</sup> had the least growth and were not different from those in MG Organic Choice<sup>®</sup>.

Sta-Green<sup>®</sup> All Purpose Potting Mix had the highest pH, but was not different from Scott's<sup>®</sup> Seeding Soil, Sta-Green<sup>®</sup> Flower and Vegetable, JG WaterWise<sup>®</sup> Mix, or Sta-Green<sup>®</sup> Tree and Shrub (Table 12). MG Organic Choice<sup>®</sup> had the lowest pH and was not different from JG Professional Mix, MG Moisture Control<sup>®</sup>, Scott's<sup>®</sup> Premium Potting Mix, MG Potting Mix, or JG Flower & Vegetable. There were no differences among treatments for EC.

Flower count was highest for tomatoes grown in JG Professional Mix, and plants grown in JG Flower & Vegetable and Sta-Green<sup>®</sup> Flower and Vegetable were not different (Table 12). Tomatoes grown in MG Moisture Control<sup>®</sup> had the lowest flower count and were not different from those in MG Organic Choice<sup>®</sup>, MG Potting Mix, Scott's<sup>®</sup> Premium Potting Mix, Scott's<sup>®</sup> Seeding Soil, or Sta-Green<sup>®</sup> All Purpose Potting Mix. Tomatoes grown in JG Flower & Vegetable had the highest fruit count and were not different from those in JG Professional Mix, JG WaterWise<sup>®</sup> Mix, or Sta-Green<sup>®</sup> Flower and Vegetable. Fruit count was lowest for plants grown in MG Moisture Control<sup>®</sup> and tomatoes grown in MG Organic Choice<sup>®</sup>, MG Potting Mix, Scott's<sup>®</sup> Premium Potting Mix, Scott's<sup>®</sup> Seeding Soil, and Sta-Green<sup>®</sup> All Purpose Potting Mix were not different.

Tomatoes grown in JG WaterWise<sup>®</sup> Mix had the lowest SPAD value and were not different from MG Moisture Control<sup>®</sup> or MG Potting Mix (Table 12). Disregarding plants in JG WaterWise<sup>®</sup> Mix, there were no differences among all other treatments for highest SPAD values.

## **Discussion:**

*Experiment 1:* In reference to growth indices, plants grown in Miracle-Gro<sup>®</sup> Moisture Control<sup>®</sup> outperformed all other treatments in both the petunia and marigold studies. For the petunias and marigolds, Jungle Growth<sup>®</sup> Flower & Vegetable and Professional Mix produced the second largest plants. Jungle Growth<sup>®</sup> WaterWise<sup>®</sup> and Scott's<sup>®</sup> Premium Potting Mix, though, were not different from Jungle Growth<sup>®</sup> Flower & Vegetable or Jungle Growth<sup>®</sup> Professional Mix in marigolds. For both petunia and marigold, plants grown in Miracle-Gro<sup>®</sup> Moisture Control<sup>®</sup>, Jungle Growth<sup>®</sup> Flower & Vegetable, Jungle Growth<sup>®</sup> Professional Mix and Jungle Growth<sup>®</sup> WaterWise<sup>®</sup> Mix had the highest SPAD value and were not statistically different.

*Experiment 2:* For the purposes of this discussion, dianthus and marigolds are addressed in one section and the petunias with the tomatoes in another, followed by overall conclusions based on the substrates used: 7 products were used with dianthus/marigold and 11 products were used with petunia/tomato.

*Dianthus/Marigold:* In both cases (dianthus and marigold), Jungle Growth<sup>®</sup> Flower & Vegetable and Professional Mix outperformed the other potting mixes in plant growth. Jungle Growth<sup>®</sup> Professional Mix produced the greatest flower count in dianthus (with the second greatest number being from Jungle Growth<sup>®</sup> Flower & Vegetable). Marigold flower count was also greatest in the Jungle Growth<sup>®</sup> products. It is also important to note that the EC for Jungle Growth<sup>®</sup> WaterWise<sup>®</sup> were significantly higher than the other products in both the dianthus and marigold cases.

*Petunia/Tomato:* Again, Jungle Growth<sup>®</sup> Flower & Vegetable and Professional Mixes resulted in the greatest growth for both petunia and tomato; however, Sta-Green<sup>®</sup>

Flower and Vegetable is not different. Flower count for petunias was highest Sta-Green® Flower and Vegetable followed secondly by the three Jungle Growth® products (with no difference among the Jungle Growth® treatments). Tomato flower count was greatest among Jungle Growth® Flower & Vegetable, Jungle Growth® Professional Mix and Sta-Green® Flower and Vegetable (with non difference among the 3 treatments). Fruit count in tomatoes yielded the same results as flower count, with the exception of Jungle Growth® WaterWise® Mix being included among the three highest flower count treatments. Final EC results also show that Jungle Growth® WaterWise® Mix, in the case of the petunia study, was statistically greater than all other mixes (there is no statistical difference among any of the mixes in the tomato study).

*Overall:* Jungle Growth® Flower & Vegetable and Professional Mix, as well as Sta-Green® Flower and Vegetable are most beneficial for plant growth, for the four species tested in this experiment. Growth indices, flower count and fruit count (in the case of tomatoes) reveal that Jungle Growth® outperforms all other treatments tested, other than Sta-Green® Flower and Vegetable when used, for all four species of plants.

While Experiment 1 indicated that Miracle-Gro® Moisture Control® produced the greatest plant performance, followed by the Jungle Growth® products, the same results did not occur in Experiment 2. Miracle-Gro® Moisture Control® was not a competitor in this case.

It should be noted that one component of the Jungle Growth® products that was not part of any other treatment is neem cake powder. Isolating this difference drew us to pursue further research on neem cake powder's activity within the soil and its subsequent effect on plants.

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Table 7. Growth Indices, pH, EC and SPAD on petunias in a 2009 commercial bagged substrate study.<sup>z</sup>

Treatment	Growth Indices <sup>y</sup>	pH <sup>x</sup>	EC <sup>w</sup>	SPAD <sup>v</sup>
Jungle Growth <sup>®</sup> Flower & Vegetable	14.0 b <sup>u</sup>	6.57 a	1.30 cd	41.7 a
Jungle Growth <sup>®</sup> Professional Mix	14.6 b	6.14 ab	1.01 de	39.9 a
Jungle Growth <sup>®</sup> WaterWise <sup>®</sup> Mix	12.3 c	6.15 ab	2.44 a	43.6 a
Miracle-Gro <sup>®</sup> Organic Choice <sup>®</sup>	10.1 d	5.71 bc	0.46 f	32.6 b
Miracle-Gro <sup>®</sup> Moisture Control <sup>®</sup>	17.1 a	5.54 c	1.46 bc	43.8 a
Miracle-Gro <sup>®</sup> Potting Mix	10.7 d	6.40 a	0.86 def	24.5 c
Scott's <sup>®</sup> Premium Potting Mix	11.2 cd	5.72 bc	0.71 ef	28.9 c

<sup>z</sup>Data presented from substrate study 2009; all data taken 56 days after planting.

<sup>y</sup>Measurements taken as [(height + width<sub>1</sub> + width<sub>2</sub>) / 3] in centimeters.

<sup>x</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>w</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>v</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter.

<sup>u</sup>Means within column followed by the same letter are not significantly different (Waller-Duncan K-ratio t test  $p \leq 0.05$ ,  $n=12$ ).

Table 8. Growth Indices, pH, EC and SPAD on marigolds in a 2009 commercial bagged substrate study.<sup>z</sup>

Treatment	Growth Indices <sup>y</sup>	pH <sup>x</sup>	EC <sup>w</sup>	SPAD <sup>v</sup>
Jungle Growth <sup>®</sup> Flower & Vegetable	16.8 b <sup>u</sup>	6.44 a	1.35 ab	54.3 a
Jungle Growth <sup>®</sup> Professional Mix	16.4 b	6.53 a	0.61 b	59.7 a
Jungle Growth <sup>®</sup> WaterWise <sup>®</sup> Mix	18.1 b	6.37 a	1.58 a	58.2 a
Miracle-Gro <sup>®</sup> Organic Choice <sup>®</sup>	5.6 d	6.45 a	0.69 b	13.2 c
Miracle-Gro <sup>®</sup> Moisture Control <sup>®</sup>	21.4 a	6.03 a	1.05 ab	54.2 a
Miracle-Gro <sup>®</sup> Potting Mix	14.2 c	6.20 a	0.84 ab	30.0 b
Scott's <sup>®</sup> Premium Potting Mix	17.8 b	6.29 a	0.60 b	31.2 b

<sup>z</sup>Data presented from substrate study 2009; all data taken 56 days after planting.

<sup>y</sup>Measurements taken as [(height + width<sub>1</sub> + width<sub>2</sub>) / 3] in centimeters.

<sup>x</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>w</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>v</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter.

<sup>u</sup>Means within column followed by the same letter are not significantly different (Waller-Duncan K-ratio t test  $p \leq 0.05$ ,  $n=12$ ).

Table 9. Growth Indices, pH, EC, and SPAD on dianthus in a commercial bagged substrate study.<sup>Z</sup>

Treatment	Growth Indices <sup>Y</sup>	pH <sup>X</sup>	EC <sup>W</sup>	Flower Count	SPAD <sup>V</sup>
Jungle Growth <sup>®</sup> Flower & Vegetable	26.0 a <sup>U</sup>	6.73 ab	2.12 b	32.0 b	48.8 a
Jungle Growth <sup>®</sup> Professional Mix	26.8 a	6.38 ab	2.07 b	42.6 a	49.6 a
Jungle Growth <sup>®</sup> WaterWise <sup>®</sup> Mix	18.0 b	7.12 a	15.60 a	12.9 c	25.1 b
Miracle-Gro <sup>®</sup> Organic Choice <sup>®</sup>	13.0 c	6.37 ab	1.62 b	3.5 d	28.1 b
Miracle-Gro <sup>®</sup> Moisture Control <sup>®</sup>	13.0 c	6.18 b	1.49 b	3.3 d	29.8 b
Miracle-Gro <sup>®</sup> Potting Mix	19.6 b	6.50 ab	1.84 b	16.2 c	30.1 b
Scott's <sup>®</sup> Premium Potting Mix	16.9 bc	6.58 ab	1.59 b	10.3 c	32.0 b

<sup>Z</sup>Data presented from substrate study, spring 2010; all data taken 56 days after planting.

<sup>Y</sup>Measurements taken as [(height + width<sub>1</sub> + width<sub>2</sub>) / 3] in centimeters.

<sup>X</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>W</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>V</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter.

<sup>U</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test, p≤0.05, n=12).

Table 10. Growth Indices, pH, EC, and SPAD on marigold in a commercial bagged substrate study.<sup>Z</sup>

Treatment	Growth Indices <sup>Y</sup>	pH <sup>X</sup>	EC <sup>W</sup>	Flower Count	SPAD <sup>V</sup>
Jungle Growth <sup>®</sup> Flower & Vegetable	21.1 a <sup>U</sup>	7.00 a	1.24 b	11.3 a	48.6 a
Jungle Growth <sup>®</sup> Professional Mix	21.4 a	6.62 a	1.29 b	10.6 a	47.5 a
Jungle Growth <sup>®</sup> WaterWise <sup>®</sup> Mix	15.5 b	7.32 a	11.78 a	9.5 a	30.8 b
Miracle-Gro <sup>®</sup> Organic Choice <sup>®</sup>	9.1 c	6.57 a	1.37 b	1.6 c	15.8 c
Miracle-Gro <sup>®</sup> Moisture Control <sup>®</sup>	9.1 c	6.62 a	1.77 b	1.8 c	16.9 c
Miracle-Gro <sup>®</sup> Potting Mix	14.9 b	6.93 a	1.75 b	4.3 b	33.0 b
Scott's <sup>®</sup> Premium Potting Mix	12.9 b	7.03 a	1.74 b	3.7 bc	28.9 b

<sup>Z</sup>Data presented from substrate study, spring 2010; all data taken 56 days after planting.

<sup>Y</sup>Measurements taken as [(height + width<sub>1</sub> + width<sub>2</sub>) / 3] in centimeters.

<sup>X</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>W</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>V</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter.

<sup>U</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test, p≤0.05, n=12).

Table 11. Growth Indices, pH, EC, and SPAD on petunia in a commercial bagged substrate study.<sup>Z</sup>

Treatment	Growth Indices <sup>Y</sup>	pH <sup>X</sup>	EC <sup>W</sup>	Flower Count	SPAD <sup>V</sup>
Jungle Growth <sup>®</sup> Flower & Vegetable	27.9 a	6.86 cd	1.29 b	12.7 b	42.5 a
Jungle Growth <sup>®</sup> Professional Mix	25.1 a	6.41 ef	1.68 b	10.6 b	39.7 ab
Jungle Growth <sup>®</sup> WaterWise <sup>®</sup> Mix	17.6 bc	7.21 abc	7.78 a	11.3 b	21.2 d
Miracle-Gro <sup>®</sup> Organic Choice <sup>®</sup>	11.6 de	6.04 f	1.60 b	1.3 e	25.6 cd
Miracle-Gro <sup>®</sup> Moisture Control <sup>®</sup>	8.0 ef	6.55 de	1.61 b	0.9 e	24.2 cd
Miracle-Gro <sup>®</sup> Potting Mix	20.1 b	6.94 bcd	1.55 b	5.3 cd	29.7 cd
Scott's <sup>®</sup> Premium Potting Mix	15.9 bc	6.95 bc	1.65 b	6.0 c	29.9 cd
Scott's <sup>®</sup> Seeding Soil	6.2 f	7.27 ab	2.08 b	1.1 e	23.7 cd
Sta-Green <sup>®</sup> All Purpose Potting Mix	15.7 cd	7.45 a	3.72 b	2.3 de	31.4 bc
Sta-Green <sup>®</sup> Flower and Vegetable	25.6 a	7.06 abc	1.90 b	16.2 a	32.8 abc
Sta-Green <sup>®</sup> Tree and Shrub	20.0 b	7.15 abc	3.18 b	6.6 c	33.1 abc

<sup>Z</sup>Data presented from substrate study, spring 2010; all data taken 56 days after planting.

<sup>Y</sup>Measurements taken as [(height + width<sub>1</sub> + width<sub>2</sub>) / 3] in centimeters.

<sup>X</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>W</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>V</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter.

<sup>U</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test, p≤0.05, n=12).

Table 12. Growth Indices, pH, EC, and SPAD on tomato in a commercial bagged substrate study.<sup>Z</sup>

Treatment	Growth Indices <sup>Y</sup>	pH <sup>X</sup>	EC <sup>W</sup>	Flower Count	Fruit Count	SPAD <sup>V</sup>
Jungle Growth <sup>®</sup> Flower & Vegetable	50.5 a	6.78 bcd	2.64 a	7.6 ab	2.3 a	31.4 a
Jungle Growth <sup>®</sup> Professional Mix	46.2 a	6.32 d	1.46 a	7.9 a	2.2 a	30.2 a
Jungle Growth <sup>®</sup> WaterWise <sup>®</sup> Mix	37.7 b	6.95 abc	5.14 a	6.0 b	1.8 abc	18.4 b
Miracle-Gro <sup>®</sup> Organic Choice <sup>®</sup>	20.7 ef	6.29 d	1.29 a	1.8 cd	0.6 de	26.9 a
Miracle-Gro <sup>®</sup> Moisture Control <sup>®</sup>	14.8 f	6.51 cd	1.67 a	1.0 d	0.3 e	25.6 ab
Miracle-Gro <sup>®</sup> Potting Mix	37.2 bc	6.68 cd	1.79 a	1.8 cd	0.8 de	25.2 ab
Scott's <sup>®</sup> Premium Potting Mix	29.7 cd	6.63 cd	1.54 a	2.0 cd	0.9 de	27.0 a
Scott's <sup>®</sup> Seeding Soil	24.4 de	7.29 ab	2.18 a	1.9 cd	0.7 de	26.6 a
Sta-Green <sup>®</sup> All Purpose Potting Mix	32.7 bc	7.38 a	3.43 a	2.8 cd	1.1 cde	28.5 a
Sta-Green <sup>®</sup> Flower and Vegetable	49.6 a	7.02 abc	2.30 a	6.5 ab	1.9 ab	30.5 a
Sta-Green <sup>®</sup> Tree and Shrub	34.4 bc	6.93 abc	3.77 a	3.1 c	1.2 bcd	28.7 a

<sup>Z</sup>Data presented from substrate study, spring 2010; all data taken 56 days after planting.

<sup>Y</sup>Measurements taken as  $[(\text{height} + \text{width}_1 + \text{width}_2) / 3]$  in centimeters.

<sup>X</sup>pH measured by Accumet XL50 pH and conductivity meter.

<sup>W</sup>Electrical Conductivity measured by Accumet XL50 pH and conductivity meter.

<sup>V</sup>SPAD measured by Konica Minolta SPAD-502 Chlorophyll Meter.

<sup>U</sup>Means within column followed by the same letter are not significantly different (Tukey's Studentized Range Test,  $p \leq 0.05$ ,  $n=12$ ).